

THE EFFECT OF TIMING OF ORAL MELOXICAM ADMINISTRATION ON
PHYSIOLOGICAL RESPONSES IN CALVES AFTER DEHORNING WITH LOCAL
ANESTHESIA

by

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Abstract

Dehorning is a painful husbandry procedure that is commonly performed in dairy calves. Parenteral meloxicam combined with local anesthesia mitigates the physiological and behavioral effects of dehorning in calves. The purpose of this study was to determine the influence of timing of oral meloxicam administration on physiological responses in calves after dehorning. Thirty Holstein bull calves 8- 10 weeks of age (28-70 kg) were randomly assigned to one of three treatment groups: placebo-treated control group (CONT) (n=10), calves receiving meloxicam administered orally (1 mg/kg) in powdered milk replacer 12 h prior to cautery dehorning (MEL-PRE) (n=10) and calves receiving meloxicam administered as an oral bolus (1 mg/kg) at the time of dehorning (MEL-POST) (n=10). Following cautery dehorning, blood samples were collected to measure cortisol, substance P (SP), haptoglobin, ex-vivo prostaglandin E₂ (PgE₂) production after lipopolysaccharide stimulation and meloxicam concentrations. Maximum ocular temperature (MOT) and mechanical nociceptive threshold (MNT) was also assessed. Data were analyzed using non-compartmental pharmacokinetic analysis and repeated measures ANOVA models. Mean peak meloxicam concentrations were 3.61 ± 0.21 µg/mL and 3.27 ± 0.14 µg/mL with average elimination half-lives of 38.62 ± 5.87 h and 35.81 ± 6.26 h in the MEL-PRE and MEL-POST groups respectively. Serum cortisol concentrations were lower in meloxicam-treated calves compared with control calves at 4 h post-dehorning (P=0.0004). SP concentrations were significantly higher in control calves compared with meloxicam-treated calves at 120 h after dehorning (P=0.038). PgE₂ concentrations were lower in meloxicam-treated calves compared with control calves (P=0.001). MNT was higher in control calves at 1 h after dehorning (P=0.02) but meloxicam-treated calves tended to have a higher MNT at 6 h after dehorning (P=0.07). There was no effect of timing of meloxicam administration on plasma cortisol concentrations (P= 0.69), SP concentrations (P=0.86), haptoglobin concentrations (P=0.86), MOT (P=0.90), or MNT (P=0.99). However, PgE₂ concentrations in MEL-PRE calves were similar to CONT calves after 12h post-dehorning, while MEL-POST calves had lower PgE₂ concentrations for 3 d post dehorning. These findings suggest that meloxicam reduced cortisol, SP and PgE₂ after dehorning but only PgE₂ production was significantly affected by the timing of meloxicam administration.

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Chapter 1 - Literature Review

Current Pain Management in Cattle

Emphasis on pain management in livestock is increasing primarily due to increasing concerns surrounding animal welfare (George, 2003). In fact, a survey conducted by Animal Industries Foundation revealed that 70% of the public feels strongly that animals deserve equal considerations in matters of pain and suffering (AIF, 1989). These feelings are particularly significant considering a 2010 consumer survey showed the vast majority of residents of the United States eat meat, with vegans and vegetarians making up only 4% of the population (Norwood et al., 2010). With 96% of the population consuming meat on a regular basis, the issue of animal welfare has a direct impact on most Americans. In fact, the same consumer survey showed 81% of respondents believe animals and humans have the same ability to feel pain. Furthermore, most consumers ranked the act of receiving treatment for injury and disease (including pain management), high in important livestock production practices, second only behind ample food and water (Norwood et al., 2010). So as concerns about animal welfare have continued to increase in the past few decades, pain management in particular has come to the forefront as critical for the improvement in animal welfare.

While the majority of the public believe animals, including livestock, should be relieved of pain and suffering, there is no real consensus on what is considered painful and what is considered adequate analgesia for the pain. A survey conducted by Heleski (2004), was directed at defining opinions on welfare and pain management in cattle in those whom have influence on animal production. The survey targeted animal science faculty from different land grant institutions in the United States. Most respondents (90%), supported general principles of animal welfare, such as keeping animals free from unnecessary fear and distress. However, 87% of respondents agreed that the predominant methods already used to produce various types of animal products provided appropriate levels of animal welfare (Heleski, 2004). The survey also presented welfare issues as proposed by the United Kingdom's Farm Animal Welfare Council. The council determined that assurance of animal welfare requires adherence to the concept of the "5 freedoms." These freedoms include 1) freedom from hunger and thirst; 2) freedom from

discomfort; 3) freedom from pain, injury, or disease; 4) freedom to express normal behavior; and 5) and freedom from fear and distress (FAWC, 2009). Of particular interest is when asked if they agree or disagree with each freedom, 97% of the animal science faculty agreed agricultural animals should have freedom from unnecessary pain or discomfort. However, when asked specifically about the welfare concerns surrounding certain agricultural practices, such as castration without anesthetic, only 32% were concerned that the practice elicited an unnecessary level of distress, and only 47% saw welfare concerns when dehorning without anesthetic. When asked to clarify the answers the most common response was: how do we know the animal is experiencing pain (Heleski, 2004). This survey demonstrated that while the majority of livestock industry leaders believe livestock should be free of pain, there are conflicting opinions on what is actually considered painful in the animal.

Similar conflicting opinions of what is painful in livestock exist among veterinarians as well. A survey within the American Association of Bovine Practitioners (AABP) was conducted to rank the pain thought to be experienced by cattle during common husbandry procedures. Castration of calves <6 months of age was ranked the least painful, with castration of calves >6 months of age slightly more painful. Dehorning was ranked more painful than castration regardless of age and dehorning calves >6 months of age was ranked the most painful overall. (Fajt et al, 2011). While all procedures were determined to cause some kind of pain, no procedure was ranked to cause extremely severe pain. The survey also determined most veterinarians agree they have a moral obligation to provide analgesia to animals with painful medical or surgical conditions, and additionally, there is a quicker recovery time in animals that receive analgesia than in animals that do not. However, only 30% of veterinarians surveyed administered analgesics while castrating calves < 6 months old (the procedure deemed to cause the least pain), while 72% of respondents administered analgesics when dehorning calves > 6 months old (the procedure ranked the most painful) (Fajt, 2011). From this survey, it can be concluded that the administration of analgesia by veterinarians closely reflects the pain perception of the procedure.

Common livestock procedures, such as castration and dehorning, have been recognized by producers and consumers alike to inflict pain in cattle. Castration, according to the American Veterinary Medical Association (AVMA), inflicts pain on the animal and causes a period of slow growth rate and reduced feed efficiency. However, castration is a necessary management

practice for cattle because it reduces aggressiveness and therefore, improves safety for animal handlers, as well as promoting desirable carcass characteristics (AVMA 2009). Physical methods of castration in cattle include, surgical removal of testicles, constricting elastic band at the base of the scrotum, or Burdizzo clamp. Chemical and hormonal methods have also been described; however, surgical castration is the most common method used in the United States (Coetzee, 2010). All methods of physical castration are associated with pain which may persist for several days or weeks. Surgical castration may cause the least long term pain (when compared to constricting elastic band); however, pain is still displayed for 8 hours following castration, according to cortisol concentrations (Stafford and Mellor, 2005a). Similar to castration, spaying heifers is a management tool that involves the surgical removal of the ovaries. The AVMA states a flank ovariectomy performed without anesthesia is inhumane, and pain and discomfort should be minimized as much as possible before, during, and after spaying (AVMA, 2009). A study on 24 two year old Brahman heifers, found a significant cortisol response to flank spaying for 6 hours post-procedure, indicating a significant amount of pain and distress was experienced during that time frame (Petherick et. al., 2011). Finally, dehorning is a common livestock procedure that involves the removal of horns in cattle. In young cattle with an intact horn bud, a procedure known as disbudding is performed to prevent the calf from forming horns. Disbudding involves destroying the horn producing cells in the horn bud and thus prevents the horn from forming. Older calves with horns already formed from the horn bud are dehorned by physically removing the horns. As with castration, dehorning is considered painful but necessary because of the benefits of safety for the animal handler and less carcass wastage due to bruising (AVMA, 2010).

While the AVMA has suggested minimizing pain and discomfort as much as possible during these husbandry procedures, castration and dehorning methods are not currently regulated in the United States. The lack of regulation, has allowed for a large variation in surgical and pain management techniques among veterinarians. For example, a survey of members of the Academy of Veterinary Consultants (AVC) and AABP was conducted to define the current common methods of castration in the US. The survey determined more producers castrated light weight calves, while veterinarians were more likely to castrate heavy weight bulls. Surgical castration is the most common method of castration used and most veterinarians dehorn calves at the same time as castration. Twenty percent of veterinarians use a local anesthetic or analgesic

prior to surgical castration; however 57% of these veterinarians do not administer it to perinatal calves. Also, 64% of these anesthetic users wait less than 5 minutes after lidocaine blocking to castrate and only 21% administer additional systemic analgesia (Coetzee, 2010). Similar to the castration survey, 113 dairies in the upper Midwest and eastern states were surveyed to define the current dehorning methods in US dairies. The results showed the majority of dairy calves are dehorned by hot iron with only 12% of dairies using anesthetics and only 2% report using systemic analgesics (Fulwider et. al., 2008). Both surveys indicate pain management is minimal for most US cattle operations and when anesthetics are used, they may not be used properly. Dissimilar to the US, regulations for common husbandry procedures have been put in place in many other countries. Some of the strictest regulations for dehorning is in Sweden where dehorning without local anesthetics and sedation is banned for any age (Bengtsson *et al.*, 1996). In the UK, calves less than 1 week old may be dehorned with caustic paste without local anesthetics, but all other methods require concurrent local anesthetic use (Kent *et al.*, 1999). Also the UK suggests calves older than 2 months should always be castrated with local anesthesia (FAWC 2009). In Canada, the Canadian code of practice for dairy cattle recommends the use of a local anesthetic when dehorning dairy calves (CVMA). However, while the code of practice was developed by organizations involved with the industry and is intended to promote the highest standard of welfare, they are only voluntary guidelines. A survey of dairy producers and veterinarians in Ontario, Canada revealed that most veterinarians, 92%, used a local anesthetic when dehorning dairy calves, however, veterinarians only dehorned a collective 22% of the dairy calves. So most dairy producers (78%) dehorn their own calves and only 22% of dairy producers surveyed use a local anesthetic (Misch *et al.*, 2007). This survey reveals that while most Canadian veterinarians follow the Canadian code of practice and use a local anesthetic when dehorning, equal pain management consideration is not used for the majority of calves dehorned by producers.

When considering livestock, welfare is a balance between ethics and economics and the use of pain management is no exception. While surveys have shown both the public as well as the industry is concerned about painful husbandry procedures (Norwood *et al.*, 2010 and Helweski, 2004), there is concern with what party will absorb the price of the use of pain management. The cost of local anesthesia and analgesics is constantly cited as a reason to forgo pain management when producers and veterinarians are surveyed (Misch *et al.*, 2007, Fajt,

2011). However, a nationwide consumer survey shows that many Americans are willing to pay for improved animal well-being, with 70% agreeing that farmers should be compensated if they adopt stricter animal welfare standards. Also 76% of those polled agreed low meat prices are not more important than the well-being of farm animals (Norwood *et al.*, 2010). While economics remain an obstacle, the growing concerns in animal welfare have encouraged recent research into the assessment and alleviation of pain associated with both castration and dehorning.

Measuring Pain in Cattle

Pain, as defined by the International Association for the Study of Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (IASP, 2011). Evaluation of pain in many veterinary species is hindered because of the animal's inability to verbally communicate. However, it is widely recognized that the lack of verbal communication does not automatically rule out the possibility the animal is experiencing pain and may require pain management (Muir and Woolfe, 2001). Pain is a subjective state that can only be measured indirectly; however, pain inducing stimuli can elicit a range of physiological and behavioral responses that can be directly measured (Heinrich *et al.*, 2009). Physiological responses include changes in heart rate, blood pressure, rectal and skin temperature, plasma levels of stress hormones and related metabolites, and brain electrical activity. Although there are many pain induced responses recognized, a lack of validated diagnostic techniques and pain scoring systems in veterinary species makes it challenging for veterinarians to assess and alleviate pain. In addition to the absence of a pain scoring system for cattle, a lack of familiarity with current pain management strategies, concerns for drug induced adverse effects or toxicity, and the need to comply with federally mandated regulations; all contribute to the frequent mismanagement of pain in cattle. Additionally and most significantly, pain is often considered to be temporary or even beneficial and pain management is thought to cause an economic burden, so consequently pain management is most often minimal or completely avoided (Muir and Woolfe, 2001).

The pathway of pain begins with nociception, which is the detection of a noxious stimulus and the transmission of that information to the brain. In turn, pain is the perception of the sensation induced by a noxious stimulus. Induction of pain involves the activation, modulation, and modification of primary sensory and central neurons. Pain is often coupled with

some tissue damaging stimuli that inevitably results in an acute pain. Initial acute pain enables healing and tissue repair and so serves as a protective response. Acute pain can transition to an intense chronic (clinical) pain, which is not a protective response and has little beneficial effects (Muir and Woolfe, 2001).

Acute (physiologic) pain occurs when a stimulus that induces minimal or no tissue damage activates high-threshold sensory nerve fibers. It is well localized, temporary, and plays a role in normal defense mechanisms by initiating a protective reflex (Muir and Woolfe, 2001). Acute pain activates the sympathetic division of the autonomic nervous system (ANS) to release catecholamine from the adrenal medulla as part of the fight or flight response. When the sympathetic nervous system (SNS) is activated, the catecholamines released promote energy mobilization and vasodilation which will allow for an increase in muscle contractibility and later cardiac output and respiratory rate (Stewart *et al.*, 2010). Consequently, heart rate increases and blood flow is redirected to the skeletal and heart muscles to prepare the animal for the fight or flight response (Stewart *et al.*, 2010). Methods used to measure SNS activity include plasma catecholamine concentrations, heart rate variability, eye pupil diameter, skin resistance, and peripheral blood flow. Acute pain, however, is not the only stimulator of the SNS. Stress not associated with acute pain has also been shown to cause a catecholamine release. When measured, an increase in catecholamine concentration was found as a result of many common livestock processes including branding, isolation, simulated transportation, dehorning and ring castration in cattle (Mellor, 1991). These findings indicate stress and acute pain are both responsible for stimulating the SNS. This finding is significant because current measurement techniques cannot readily differentiate the origin of the sympathetic nervous system stimulation. Chronic pain occurs when excessively intense or prolonged stimuli induce tissue damage that results in extended discomfort and abnormal sensitivity. Chronic pain is characterized by low threshold to additional noxious stimuli known as hypersensitivity and an exaggerated response to additional noxious stimuli known as hyperalgesia. Chronic pain can be caused by inflammation associated with the tissue damage (inflammatory pain) or central or peripheral nerve injury (neuropathic pain). Surgical procedures, trauma, and ischemia cause inflammatory pain, whereas nerve transection and compression cause neuropathic pain (Muir and Woolfe, 2001).

Acute and chronic pain is demonstrated in many livestock husbandry procedures including castration and dehorning. Acute pain is generated by the initial act of damaging the

tissue and by subsequent chemical changes within those tissues. Acute pain is linked to behavioral and physiological responses that are used to measure the presence of pain. Chronic pain is usually less intense than acute pain, although it lasts for days to weeks following the initial insult (Molony *et al.*, 1995). When using dehorning as a model, pain elicited at the time of dehorning is acute and the pain derived from the insult to the tissue results in a chronic inflammatory pain. Cortisol measurements in particular, demonstrate both phases of pain after dehorning. The acute phase of pain after dehorning is marked by a rapid rise in cortisol concentration that peaks within several minutes (acute pain) and then declines to a plateau above the starting baseline and remains at that level for several hours (chronic pain) (Duffield, 2008).

Physiological changes that can be measured in the blood include cortisol, haptoglobin, Substance P and PgE₂. Historically, plasma cortisol concentrations have been the primary measure of pain. Activation of the hypothalamic-pituitary-adrenal axis (HPA) is a primary physiological response to stress in mammals. Cortisol response that is mediated by the HPA has a slow onset, is persistent, and is easily measured (Stewart *et al.*, 2010). Increases in HPA activity (cortisol levels) in response to painful husbandry procedures in calves have been well documented (Stewart *et al.*, 2008). For example, researchers reliably describe an increase in cortisol concentration immediately following dehorning without pain management associated with acute pain from amputation (Stafford and Mellor, 2005a). Furthermore, when dehorning was performed with a corneal nerve block, the absence of a cortisol response indicated the cortisol response was primarily pain related rather than some other stress (Heinrich *et al.*, 2010). Unlike cortisol, substance P is a relatively new measure that may be more specific to pain. Substance P is a neuropeptide that has been identified as the regulator of excitability of nociceptive neurons and like cortisol, is detected in times of pain and stress. In a study of 10 calves which were castrated or sham castrated, there was a significant increase in plasma concentration of substance P after castration compared to the sham castration. This difference is especially significant when compared to the cortisol concentrations that showed no significant difference between the castrated and control calves (Coetzee *et al.*, 2008). This study indicates that substance P may be a more specific measurement of stress associated with pain rather than other stresses such as handling.

Other measurements of pain include: average daily gain (ADG), pressure algometry, thermography, and behavior and electroencephalography. Significant for measuring pain in

cattle is pressure algometry which has been identified as an objective and accurate tool for assessing pain associated with dehorning (Heinrich *et al.*, 2010). Although historically the algometer has been used to measure chronically lame cattle, recent research has utilized the algometer to measure pain after dehorning. The use of this instrument involves exerting and measuring pressure on the affected region, around the horn bud for the case of dehorning, and the changes in sensitivity to the pressure or the mechanical nociceptive threshold (MNT) can be used to interpret the pain threshold. The pain threshold as defined by the International Association for the Study of Pain is the minimum intensity of a stimulus that is perceived as painful (IASP, 2011). A 2010 study conducted by Heinrich defined the methodology to accurately measure pain sensitivity associated with dehorning using a pressure algometer. To control bias, the same trained researcher should perform each measurement, as well as, the same round rubber tip should be used for every measurement. The first measurement taken is used to determine the baseline mechanical nociceptive threshold and simply measures the amount of pressure the calf will tolerate without manipulation. After dehorning, measurements are taken to determine the sensitivity of the dehorning wound. To take the measurement the calf should be restrained with a halter and the researcher should place a hand lightly on the poll of the calf until it is habituated to being touched and stands in a relaxed position. The hand is slowly removed and replaced with the algometer tip placed directly beside the horn bud such that the rubber tip covers the cautery wound and the edge of normal tissue. Pressure should be applied perpendicular to the poll at a rate of 1 kgf/s until the calf withdraws its head. Three to four sites around each horn bud should be measured in the same order for each horn and the algometer automatically will read the highest level of pressure applied. In the Heinrich study, the validity of the algometer was tested by sham dehorning 4 calves instead of cautery dehorning. The sham dehorning was done to prove the response of the calves when touched by the tip of the algometer was due to pain and not due to being frightened or some other stimulus. The results of the study showed all calves dehorned, regardless if they received an anesthetic or analgesic, were more sensitive to the pressure after dehorning. However, those calves that were sham dehorned did not show an increase in sensitivity when measured by the algometer. Because the sensitivity for the sham dehorned calves did not increase, it suggests that the increase sensitivity in the dehorned calves was caused by pain and fear associated with the measurement taking process (Heinrich *et al.*,

2010). The study demonstrated that the algometer can measure pain sensitivity due to dehorning in the form of a decrease in MNT mechanical pressure is applied.

Additionally, recent research has suggested the temperature of the eye in the region of the lachrymal caruncle, when measured by infrared thermography may be a practical, noninvasive means to measure sympathetic nervous system (SNS) activity in cattle (Stewart *et al.*, 2008 and 2009). The lachrymal caruncle, the small area around the ventromedial border of the eyelid, has many capillary beds innervated by the sympathetic system and thus responds to changes in blood flow. An initial study utilizing eye temperature as a measure of pain observed a decrease in eye temperature following disbudding of calves without local anesthetic. The decrease was attributed to a sympathetic mediated shift of blood flow in capillary beds. The SNS stimulation that caused the decrease in eye temperature was supported by corresponding changes in heart rate variability, which is also associated with increased SNS activity (Stewart *et al.*, 2008). Although this initial study showed a decrease in eye temperature, subsequent studies have shown the opposite response.

A 2010 study by Stewart investigated eye temperature response to changes in the SNS activity using surgical castration of calves as the model. The study found that surgically castrated calves had a transient decrease in eye temperature followed by a rapid increase and 5 minutes after castration eye temperature was greater than baseline for all calves castrated and stayed increased for the entire 20 minute post observation period. Eye temperature increased post treatment in control calves that were sham castrated, although the extent of the change was less than that of the castrated calves. Therefore, the change in eye temperature indicates a response to the acute pain experienced with surgical castration. This observation is supported by an increase in cortisol and catecholamine concentration in the castrated animals, which also indicates a response to the acute pain experienced with surgical castration (Stewart *et al.*, 2010).

Temperature increase of the eye was also observed following cautery disbudding of calves. The study was able to determine the increase in eye temperature was not due to the heat of the cautery or the physical struggle of the animal and so the increase in eye temperature is also thought to be a result of SNS stimulation due to acute pain (Stewart *et al.*, 2008). The complete mechanism as to why the eye temperature increases after acute pain is unknown; however it is thought to be associated with vasodilation caused by SNS stimulation. However, it is important to note that the SNS mediated stimulation of the HPA axis alone is not enough to cause the

increase in eye temperature, as shown by Stewart *et al.* (2007). However, it is known that SNS stimulated endothelial cells release nitric oxide to relax smooth muscle, resulting in vasodilation and increased blood flow. The increase in blood flow through the eye could explain as increase temperature in the eye, as measured by the infrared camera. Although the mechanism is unknown, thermography has proven to be a noninvasive measure of SNS activity in livestock that can be used to assess acute pain.

Other means of measuring pain in cattle include EEG and behavior analysis. Electroencephalographic response to noxious stimulation has been used in many species of mammals. EEG has long been known to reflect changes in central nervous system (CNS) function. Pain perception demonstrated by certain cerebral cortex play an important role. EEG analysis is different from other measures of pain in that EEG measures the degree of pain perceived by an animal rather than the magnitude of the noxious stimulus presented to that animal (Johnson, 2007). Because pain is subjective and therefore, perceived differently for each individual, the EEG offers an objective but individualized approach to measuring pain. The EEG also has the advantage to give insight into pain without actually subjecting animals to pain. Animals can be anesthetized and therefore, not experience pain; however, the brain perceives noxious stimulation the same as if the animal were not anesthetized. Because pain is subjective by nature, objective means of measurement do not always correlate well with the perceived level of pain. Behavior analysis has become a means to measure pain and give a complete picture of the pain felt by groups of animals. In general, any behavior that increases in frequency after noxious stimulus but decreases when analgesia is provided is a potential pain related behavior (Johnson, 2007). The most common behavior in calves that experience pain is foot stomping. Although there have been many potential behaviors identified, including vocalization, rearing, kicking, falling, head shakes, head rubs, ear flicks, and tail flicks (Duffield, 2008). Specific behaviors related to dehorning include: ear flicking, tail flicking, head shaking, rearing and falling down (Faulkner and Weary, 2000). However, large numbers of animals, tedious statistical analysis, and specific behaviors to specific species are some limitations to the use of behavior as a measurement of pain.

Several techniques, both objective and subjective, have been described for measuring and quantifying the degree of pain experienced by cattle. However, it is important to note there is no standard for measuring pain in cattle that has been validated. While research in the management

of pain will continue, pain management techniques will not be approved until a standard for measuring pain has been validated.

Analgesic Use in Cattle

Several reports suggest veterinarians use little to no analgesics in surgical procedures or painful disease states, and furthermore when analgesics are used, it is often inadequate (Heweson *et al.*, 2005, Fajt, 2011, and Misch *et al.*, 2007). A 2005 survey of Canadian veterinarians found that while 90% of veterinarians used local anesthetics for C-section, claw amputation, and omentopexy, the analgesia achieved was often inadequate because only 50% administered some type of analgesia to address the ensuing chronic pain. Furthermore, at castration, the survey revealed only 6.9% of beef calves and 18.7% of dairy calves less than 6 months of age received analgesia; while 19.9% of beef calves and 33.2% dairy calves greater than 6 months of age received analgesia. The phenomena of significantly less analgesia offered to younger calves can also be further explained by the survey. It revealed that safety was one of the main reasons for analgesics to be administered, and because there are other means to adequately restrain younger calves and keep handlers safe, non-analgesic users believe that young animals generally do not require analgesia for dehorning or castration. (Heweson *et al.*, 2005) Surgical castration and dehorning results in acute pain and prolonged chronic pain, however, several surveys have revealed analgesia is not adequately employed (Heweson *et al.*, 2005, Fajt, 2011, and Misch *et al.*, 2007). The minimal use of analgesia seems to be a direct reflection of a lack of a long acting, cost effective analgesic available for use in livestock. As a result, some countries including Canada suggest that at the very least, a local anesthetic should always be given to calves undergoing dehorning or disbudding (CVMA). Local anesthetics are the most common type of pain management used for dehorning, and a local lidocaine block will reduce pain during the 2 to 4 hours immediately following surgical procedure. However, there is evidence that local anesthetics have little effect in alleviating stress and may just delay the stress response to dehorning (Petrie *et al.*, 1995). While a local lidocaine block may be adequate analgesia for the acute pain associated with dehorning, additional analgesia is required to cover the inevitable chronic pain associated with the procedure.

Because local anesthetics alone have shown to be inadequate in mitigating chronic pain, research has been prompted to define adequate pain management in cattle. Research from New Zealand has shown that providing a nonsteroidal anti-inflammatory drug (NSAID), in addition to local anesthetics can reduce plasma cortisol concentrations to baseline levels for up to 6 hours after scoop dehorning in calves 3-4 months of age (McMeekan et al., 1998a). Interestingly, a recent survey of veterinarians revealed, flunixin meglumine (an NSAID) is the most commonly administered systemic analgesia followed by alpha 2 agonists, opioids, and aspirin (Coetzee *et al.*, 2010). The efficacy of flunixin meglumine in the bovine species was considered in a 2009 study by Meyers. The study comprised of six Holstein cows in various stages of lactation and looked at the impact of NSAIDs, flunixin meglumine, compared to dexamethasone on prostaglandin E₂ (PGE₂) production and COX-2 mRNA expression in bovine whole blood cultures stimulated by lipopolysaccharides (LPS). The study concluded that bovine blood cells respond to NSAID (flunixin meglumine in particular) therapy like other mammalian cells with respect to inhibitions of PGE₂ production and suppression of TNF α mRNA induction, but neither NSAID inhibited the induction of COX2 mRNA (Myers *et al.*, 2009).

While NSAIDs have the means to mitigate the chronic pain associated with castration and dehorning in cattle, there are currently no approved drugs labeled for use of pain management in the United States. Therefore, all current use of NSAIDs for use of pain management in cattle constitutes extra label drug use (ELDU). When a drug is used in an extra label manner, AMDUCA regulations require that there be a sufficiently extended withdrawal interval (WDI) period so that no residues are found in meat or milk products. NSAIDs are considered drugs of high concern in food animals because of the potential for harm to humans consuming food and food by-products containing residues and are therefore highly regulated. The only NSAID for which a tolerance has been established in the US is flunixin meglumine; therefore any other residues found, despite the concentration, would be considered illegal.

Flunixin meglumine is an NSAID indicated for the control of pyrexia associated with bovine respiratory tract disease and mastitis as well as for the control of inflammation associated with endotoxemia when given at the approved dose and by the approved route, IV, in cattle. The meat WDI is 4 days and milk WDI is 36 hours. Flunixin meglumine is labeled for IV administration only, and therefore, IM or SQ injections are considered illegal under AMDUCA. While the IV administration is inconvenient for most producers, convenience of route of

administration is not considered a valid reason for ELDU. Additionally, flunixin meglumine was found to cause significant muscle damage when administered outside of the vein, due to the propylene glycol vehicle. The FDA has recently investigated several cases of violative residues of flunixin meglumine in cattle and most have been attributed to administration via extra label routes. Therefore, an extended WDI for meat and milk is recommended when IM administration has already occurred (Smith *et al.*, 2008).

Ketoprofen is another NSAID that has been used in ruminants for alleviating some of the clinical signs associated with endotoxemia; however, ketoprofen is known to be more expensive. Ketoprofen is rapidly eliminated by the kidneys following IV or IM administration and is substantially less irritating to tissues than flunixin meglumine when injected IM. FARAD recommends a meat WDI of 7 days and milk WDI of 24 hours following dosages of up to 3.3 mg/kg every 24 hours for 3 days (Smith *et al.*, 2008). A study by Duffield assessed the efficacy of ketoprofen for mitigating pain following dehorning with an electric cautery iron. Forty Holstein heifer calves, 4 to 8 weeks of age, were randomized to receive a lidocaine corneal nerve block and either an injection of ketoprofen or physiologic saline. Calf behavior was video recorded for frequency of ear flicks, head shakes, head rubs, lying, standing, feeding, and grooming. The results showed ketoprofen treated calves displayed less ear flicks and total head behavior. The study concluded ketoprofen is effective for mitigating behavioral effects of postsurgical pain following dehorning in 4-8 week old calves (Duffield *et al.*, 2010). The results of the study support administration of ketoprofen in addition to local anesthetics for a complete reduction of pain in response to cautery dehorning when compared to local anesthetic alone.

Other analgesics that have been used to control pain in cattle in an ELDU manner include gabapentin and xylazine. Gabapentin is a GABA analogue indicated for treatment of neuropathic pain and is used in the management of chronic pain in human medicine. It has been suggested that gabapentin is also effective for the management of chronic pain of inflammatory or neuropathic origin. A study by Coetzee showed that gabapentin concentrations in calves were maintained for up to 15 hours, which would imply gabapentin offers analgesia significantly longer than a local anesthetic alone. Furthermore, administration of gabapentin capsules to ruminant calves in combination with meloxicam resulted in plasma concentrations above the minimum therapeutic concentrations previously reported for meloxicam or gabapentin (Coetzee *et al.*, 2011b). Recently, a study was conducted to determine if gabapentin truly works

synergistically with NSAIDs to mitigate hyperalgesic effects in ruminant calves. The study compared the analgesic effects of oral meloxicam, oral gabapentin, oral meloxicam and gabapentin, and intravenous flunixin meglumine after scoop dehorning 6 month old dairy calves. Physiologic parameters including: plasma drug concentrations, serum cortisol concentrations, plasma PGE₂ concentrations, plasma haptoglobin concentrations, plasma substance P concentrations, ocular thermography, mechanical nociceptive threshold (MNT) as measured through pressure algometry, and average daily gain (ADG) were evaluated. Results of the study showed no analgesic effect of oral gabapentin or oral gabapentin with meloxicam (Glynn et al., 2013). Xylazine is also a common drug, used in veterinary medicine as a tranquilizer or adjunct to surgical anesthesia. Xylazine, an alpha 2 adrenergic agonist, is a potent sedative, analgesic and muscle relaxant; however remains unapproved for use as long term pain management in the US (Chamberlain *et al.*, 1998). A 2003 Stafford study further disproved xylazine as appropriate long term analgesia. This study used 100, 3 month old calves, half of which underwent amputation dehorning. There were 5 treatment groups in this study: control, local anesthesia and ketoprofen, local anesthesia and xylazine, local anesthesia, xylazine and tolazoline, and xylazine alone. Plasma cortisol concentration was measured for 8 hours post dehorning. The results of this study showed that while xylazine treated calves decreased the initial cortisol spike, the cortisol did spike at 3 hr post dehorning and was at baseline values until 8 hr post dehorning. In contrast calves give ketoprofen and a local anesthetic completely blocked the initial cortisol spike and remained unchanged for the entire 8 hr post dehorning (Stafford et al., 2003). The results of this study suggest an NSAID, such as ketoprofen, is a more appropriate choice for long term analgesia when compared to xylazine.

In order to achieve complete pain management, veterinarians must prevent the onset of pain, prevent the perception of noxious stimulus, and limit the altered behavior and deviation from normal physiologic states (Anderson and Muir, 2008). Pre-emptive analgesia is a type of pain management which may control all three criteria. Pre-emptive analgesia is the initiation of an analgesic regimen before the onset of the noxious stimulus to prevent central sensitizations and limit the subsequent pain experience. Surgery induced central sensitization has two phases; an immediate incisional phase and a prolonged inflammatory phase due to tissue damage. An initial painful experience can imprint permanently on the nervous system during central sensitization, amplifying the response to subsequent noxious stimuli (hyperalgesia) and causing

typically painless sensations to be experienced as pain (allodynia) (George, 2003). However if the central sensitizations are blocked with pre-emptive analgesia, hyperalgesia and allodynia are not achieved either. A pre-emptive analgesia study showed that opiates administered before the first phase of central sensitization and reversed with an opiate antagonist before the expected onset of the second phase were still capable of preventing the late stage of pain response. Thus the initial neural cascade was blocked, leading to long term pain relief by eliminating the hypersensitivity produced by noxious stimuli (Gottschalk and Smith 2001). While pain is thought to be inadequately managed in one half of all surgical procedures mostly due to inconvenience, pre-emptive analgesia may prove to be a convenient way to achieve complete pain management in cattle (Anderson and Muir, 2008).

It is recognized that the combination of local or regional anesthesia with analgesic drugs controls pain after surgery more efficiently than the use of an NSAID alone (Stillwell *et al.*, 2008). In a study which used mulesing as its model for pain, the administration of NSAIDs alone provided less behavioral responses but did little to reduce plasma cortisol concentration in sheep?. In comparison, the administration of the topical anesthetic alone had benefits in terms of reduced cortisol immediate after mulesing and less hunched standing compared with no treatment. Greatest pain relief, as indicated by the behavioral and cortisol response to mulesing, was provided by the combined administration of the commercially available topical local anesthetic formulation and the long acting NSAID carprofen. In fact, this treatment showed that the combination of a local anesthetic with a systemic analgesic can provide complete pain management (Fisher *et al.*, 2007). Recommendations of the European Commission are that protocols to control pain (local anesthesia and analgesia) should be used when calves are castrated. Even where legislation is not specific, analgesia should be maintained for the period during which pain is confirmed or probable. However, under field conditions it is unlikely that 2 injections of an analgesic drug would be given. Therefore, because inflammation and pain associated with most common husbandry procedures will last greater than 24 hours it would be advantageous to study longer lasting ways to reduce pain.

Meloxicam Use in Cattle

When considering long lasting pain reduction, meloxicam has been identified as an effective drug choice for this purpose. Meloxicam is a NSAID that is considered to bind

preferentially to cyclooxygenase-2 (COX-2) to inhibit prostaglandin synthesis. It has been approved for use in cattle in several European countries for acute respiratory disease, diarrhea, and acute mastitis in cattle at a single IV or SC dose of .5 mg/kg with a WDI of 15 days for meat and 5 days for milk. Additionally, meloxicam is approved in Canada for pain relief after disbudding calves (Smith *et al.*, 2008). While IV or SC meloxicam has been shown to be effective, recently oral meloxicam is of particular interest. The difference is two-fold: availability in the United States, since the injectable form is not approved in food animals, and financial. The cost of administering IV meloxicam to calves is approximately \$58/100 kg compared to the cost of oral meloxicam which is \$0.30/100 kg (Coetzee *et al.*, 2009).

Therefore, a study consisting of six Holstein calves was conducted to determine the pharmacokinetics of oral meloxicam in ruminant calves. The study found the bioavailability of oral meloxicam after corrected for dose was 100%, additionally; no adverse effects were noted after either IV or oral meloxicam administration. Meloxicam administered IV demonstrated a relatively low volume of distribution and a slow clearance which resulted in a relatively long mean plasma $t_{1/2}$ of 20.35 hours. Comparably, meloxicam administered orally resulted in a mean max concentration of 3.1 ug/ml and an approximate plasma $t_{1/2}$ of 27.54 hours. These findings indicate that oral administration of meloxicam is an efficient route for providing long-lasting analgesia to cattle. Furthermore, when considering pre-emptive analgesia, the pharmacokinetic profile of meloxicam described suggests that oral preemptive analgesia should be administered 12 hours before surgery so that surgery coincides with peak plasma drug concentrations. Oral meloxicam can provide effective analgesic concentrations for several days after surgery based on the mean plasma $t_{1/2}$ of approximately 28 hours (Coetzee *et al.*, 2009). These results suggest that oral meloxicam administration may offer a long-acting, safe, and practical alternative to injectable preemptive analgesia.

While the pharmacokinetics of meloxicam in ruminants has been identified, the pharmacokinetics of meloxicam in preruminant calves cannot be assumed to be the same. Recently, a study was performed to identify the pharmacokinetics of meloxicam in preruminant calves as compared to ruminant calves when administered orally and orally with milk replacer (Mosher *et al.*, 2011). Because meloxicam is a weak acid which is nearly insoluble in water, the absorption of the drug is favored in relatively acidic areas of the GI tract. In the preruminant calf that is on a primarily milk diet, the abomasal pH is variable depending on time of feeding.

Immediately following suckling, the abomasal pH quickly rises to approximately six, depending upon the pH of the ingested milk. Thereafter, the pH remains relatively constant for up to 2 hours. Then, as the milk curd is digested, gradually the pH declines over 7-9 hours to reach the pre-prandial values in the pH range of 1-2 (Church 1993). The results of the study showed no significant differences between the bioavailability of ruminant and preruminant calves.

Although plasma concentration profiles are more variable in preruminant calves, the total drug exposure as indicated by the area under the curve (AUC) is similar to that of ruminant calves. Meloxicam can be successfully administered to preruminant calves through feeding a suspension of drug powder in the usual ration of milk replacer, though peak-plasma concentrations may be reduced as a result of possible interaction with curd forming proteins. These results suggest that an adjustment in meloxicam dose may be necessary when administering with milk replacer (Mosher *et al.*, 2011). Nevertheless, the oral delivery of meloxicam with milk replacer may be useful in administering analgesia prior to surgical procedures such as dehorning and castration of calves.

Meloxicam has been identified as a drug capable of managing pain associated with common husbandry procedures, in particular dehorning calves. A study was conducted to measure the impact of meloxicam on postsurgical stress associated with cautery dehorning (Heinrich *et al.*, 2009). Sixty Holstein heifer calves were randomly assigned to receive an IM injection of meloxicam or a placebo. Also, all calves were given a lidocaine corneal nerve block 10 minutes before dehorning. Results of the study showed dehorning was associated with elevated serum cortisol and heart rate in both groups for 24 h and elevated respiratory rates in both groups for 6 hours. Although still elevated, meloxicam calves were found to have lower serum cortisol than controls until 6 hours after dehorning and the changes in heart rate and respiratory rate were lower for the meloxicam group. These findings compared to the control group indicate that meloxicam was capable of reducing the physiological stress response associated with dehorning. Results of the study also showed that only control calves demonstrated a peak in cortisol at 1.5 hours post-dehorning, which is associated with the local block wearing off. Because no meloxicam calves exhibited the characteristic peak in cortisol and calves were assumed to be in pain, the study concluded that meloxicam was effective at managing the pain associated with dehorning (Heinrich *et al.*, 2009).

Beyond pain management, meloxicam has also been studied to identify the effects on health and productivity in cattle. Recently, a study evaluated the effects of meloxicam on performance and health of calves surgically castrated on arrival in a feedlot compared with steers. The study was comprised of 145 bulls and 113 steers and treatments consisted of a lactose placebo or meloxicam suspended in water. All treatments were administered per os, 24 hours prior to castration. Meloxicam was administered 24 hours prior to castration in the study to ensure the peak plasma drug concentrations were achieved prior to the onset of tissue damage. Results of the study showed castration regardless of treatment reduced pen ADG on days 1 – 14, but no effects were apparent by day 28. DMI increased with days on feed throughout the study for all calves, but the increase was less in castrated calves than steer calves. Finally, meloxicam treated calves reduced the first pull rate at the pen level and reduced respiratory disease morbidity rate of treated calves (Coetzee et al., 2011a). The results of the study were able to show meloxicam administered prior to castration in post-weaning calves reduced the incidence of respiratory disease at the feedlot.

Dehorning in Cattle

The removal of horns in cattle most commonly occurs in calves through the process of disbudding. Hot iron cauterization is the preferred method for disbudding and is on average performed before one month of age. According to several surveys, very few farmers use local anesthetic before cauterization and even less provide postoperative analgesia (Gottardo, *et al.*, 2011, Fajt, 2011, and Misch *et al.*, 2007). While dehorning is considered necessary because of the benefits of safety for the animal handler and less carcass wastage due to bruising, it is widely accepted to cause pain (AVMA, 2010). The use of anesthetics or analgesics is not mandated in the United States; however, recent concerns with animal welfare have sparked research into evaluating techniques to manage the pain associated with dehorning.

Cautery dehorning is the most common method of dehorning; however, research concerning the pain associated with other methods of dehorning including caustic paste and cryosurgery has been conducted. In a 2005 study 36 Holstein heifer calves were enrolled in a three tier study to: determine the postoperative pain response to caustic paste, determine if a local anesthetic block is effective in blocking any associated pain, and comparing the pain response of caustic paste dehorning with cautery dehorning and a local block (Vickers et al.,

2005). Results of the study showed pain related behaviors increased in calves that were dehorned with caustic paste compared with calves that were sham dehorned. However, calves that were dehorned with a hot iron and local block showed more pain related behaviors when compared with those dehorned with caustic paste. These results indicate that dehorning with caustic paste causes pain, but the pain is less than that caused by the hot iron, even when using a local block. Furthermore, results showed calves dehorned with caustic paste that were also treated with lidocaine showed no evidence of reduced pain response in the hours after the caustic paste dehorning. The study concluded that while caustic paste dehorning resulted in a pain response, local anesthesia with lidocaine provides no pain relief in the hours that follow application of the paste. However, the pain response in calves with caustic paste is less than that following dehorning with a hot iron using a local anesthetic (Vickers et al., 2005).

Another alternative dehorning method is cryosurgery. In this method of dehorning, the tissue to be removed is frozen to below -20 degrees Celsius. The freezing causes formation of intracellular ice crystals leading to cell destruction and eventually after some weeks, the dead tissue sloughs off. A rapid freeze and a slow thaw and several freeze/thaw sequences gives the most reliable results. Discomfort in cryosurgery has long been thought to be minimal, for example treatment of superficial skin lesions in humans generally requires no anesthesia. In a 1996 study, two types of equipment were evaluated for the amount of pain caused when dehorned. Biokry equipment, where freezing is induced by holding a metal cryoprobe cooled by nitrous oxide from a gas bottle against the tissue to be treated and Erbocryo equipment which uses liquid nitrogen applied directly on to the tissue were used in the study. Results of the study showed, calves dehorned with Biokry equipment bellowed, struggled, and had many movements of the head and legs. Calves dehorned with Erbocryo equipment behaved similarly immediately after dehorning however, these calves were quieter than the other group for the two days following cryosurgery (Bengtsson *et al.*, 1996). While, the Erbocryo equipment may have caused less chronic pain when dehorning calves, there are several disadvantage to the cryosurgical method. The completeness of dehorning is unreliable and the time needed to dehorn a calf (10 minutes) is longer than with cautery dehorning and the method is known to be more awkward to use in the field than the hot iron. Further research comparing the pain elicited for cautery dehorning and cryosurgery would help indicate if the disadvantages of cryosurgery are worth the pain relief it is reported to provide.

Pain associated with dehorning has been studied in all ages of calves. To assess the pain elicited by amputation dehorning in older calves, the behavior of 6 month old Friesian calves subjected to dehorning or dehorning after local anesthesia treatment was monitored in a 2004 study (Sylvestere *et al.*, 2004). The behavior of all study calves was recorded during the first 10 hours and between 26 and 29 hours after dehorning. The results of the study showed dehorned calves were restless for the first 6 hours after dehorning and showed an increase in behaviors such as tail flicking, head shaking, and ear flicking and a decrease in rumination. By contrast control calves, which were not dehorned, were more likely to stand still and ruminate. The behavior of calves given a local anesthetic prior to dehorning was similar to that of the control calves for the first 2 hours, but thereafter, the calves began to show behavior more similar to that of the dehorned calves. The results of this study suggest that dehorning in 6 month old calves is a painful experience that lasts at least 6 hours and that local anesthetic alleviates that pain for only 2 hours (Sylvestere *et al.*, 2004).

Dehorning has also been shown to cause pain in younger calves. A study that measured behavior, plasma cortisol and heart rate in 4-6 week-old calves during and after dehorning found a significant difference in calves that received a corneal nerve block and those that did not (Grondahl *et al.*, 1999). Dehorning in the young calves was performed through hot iron cauterization. The results showed head and leg movements during dehorning were significantly reduced when the corneal nerve was blocked in the calves. Furthermore, the behavior of calves with a corneal nerve block continued to differ from those in the group without anesthesia for 4 hours after dehorning. Additionally, the corneal nerve block also prevented the short-term increase in plasma cortisol concentration and the long-term increase in heart rate which was seen in the group without anesthesia (Grondahl *et al.*, 1999). The study concluded that the use of hot iron cauterization does cause pain in young calves and local anesthesia in the form of a corneal nerve block may improve the welfare of these young calves.

Local anesthetic with 2% lidocaine has previously been shown to cause perioperative relief to pain caused by dehorning of approximately 2 hours (Petrie *et al.*, 1995). A 2007 study was conducted to determine whether a corneal nerve block with a concentrated solution of lidocaine (5%) would further reduce the degree of pain associated with dehorning. The study consisted of 32, 10-12 week old female Holstein calves that were randomly allocated to 4 treatments which consisted of: 5% lidocaine followed by dehorning, 2% lidocaine followed by

dehorning, saline followed by dehorning, or 5% lidocaine followed by sham dehorning. Plasma cortisol concentration was measured and feeding, drinking, scratching, grooming, rubbing, licking, and inactivity behaviors were observed. The frequency of vocalization, kicking, and lying in the chute during the dehorning procedures were also assessed. The results of the study showed all calves exhibited a spike in cortisol concentration within 30 minutes of treatment. The saline and 2% lidocaine-treated calves, however, displayed the greatest magnitude of cortisol increase. In contrast to the 2% lidocaine treatment, no further increase in cortisol occurred in the 5% lidocaine-treated group after 30 minutes post dehorning. Additionally, the control and 5% lidocaine treated calves responded similarly with regard to behavior during dehorning. Most notably calves injected with 5% lidocaine and control calves demonstrated less kicking than both the saline and 2% lidocaine group. This is an important observation, suggesting that calves, injected with 5% lidocaine rather than 2% lidocaine, are capable of achieving greater pain relief. However, no behavioral differences were noted among treatments in the post-dehorning period. Thus, injection of 5% lidocaine may not provide any added pain relief after dehorning but may decrease the overall stress response during the procedure (Doherty *et al.*, 2007).

While perioperative pain has been shown to be managed with a local lidocaine block, postoperative chronic pain is still experienced (Petrie *et al.*, 1995). Consequently, systemic analgesia is necessary to provide complete relief from the pain experienced from dehorning. Although there is no drug approved to provide pain relief after dehorning in cattle, historically, NSAIDs have been the most common systemic analgesia in cattle. Ketoprofen has been used in recent research to assess the effectiveness of decreasing the pain response during the 24 hour period after hot-iron dehorning. Ketoprofen is known to clear quickly from the body, due to a half-life of 0.42 hour, but can persist in inflamed tissues at higher concentrations (Duffield *et al.*, 2010). A 2000 study measured behavioral response after dehorning with the drug and after a sham procedure in 20 Holstein calves aged 4-8 week. All calves received a sedative (xylazine) and local anesthetic (lidocaine) before dehorning and behavior was observed for 24 hours after dehorning. While, the ketoprofen had no discernible effect on the calves after sham dehorning, the ketoprofen was shown to have a pronounced effect on head shaking and ear flicking after actual dehorning; control calves engaged in these behaviors frequently, but ketoprofen-treated calves rarely performed these. The study results showed, after hot-iron dehorning calves treated with ketoprofen demonstrated little head shaking or ear flicking but control animals

demonstrated a much higher frequency of these behaviors and both responses peaked 6 hours after dehorning. Calves treated with ketoprofen also tended to gain more weight during the 24 h after dehorning than did control calves. These results indicate that ketoprofen mitigates pain after hot-iron dehorning in young dairy calves. In conclusion, ketoprofen reduced behavioral evidence of pain after hot-iron dehorning in dairy calves for the 24 hours post dehorning (Faulkner and Weary, 2000). The conclusions of this study indicate the combination of a local anesthetic and a NSAID will minimize the response to the pain both during dehorning and in the hours that follow dehorning.

While substantial research suggest the combination of a local anesthetic and NSAID is necessary to relieve pain, a study has been conducted to try to minimize the amount of anesthesia and analgesia used in relieving pain associated with dehorning. This study evaluated the effect on plasma cortisol concentration when using ketoprofen on calves dehorned with and without local anesthesia. One hundred calves were divided into 10 groups: 4 control and 6 dehorned and plasma cortisol concentrations were measured before and after dehorning. The results of the study showed the groups that were given ketoprofen before dehorning had a marked rise in plasma cortisol concentration for the first 1.3 hours after dehorning but then the plasma cortisol concentration returned to pretreatment levels. When animals were give a local anesthetic and ketoprofen the plasma cortisol concentrations were similar to control animals which had not been dehorned, with only a small increase in plasma cortisol concentration for the first hour after dehorning and then a rapid return to pretreatment values (McMeekan *et al.*, 1998.) The results of this study suggest that a systemic analgesic should be combined with local anesthesia to relieve all pain associated with dehorning. In addition to behavior and plasma cortisol levels other measurements of pain have been used to demonstrate the effect combining a local anesthetic and NSAID has on mitigating the onset of pain when the local anesthetic wanes. Eye temperature and heart rate variability were used to measure pain response in a 2009 study. The results support the hypothesis that administration of an NSAID abolishes the pain responses caused when the effects of the local anesthetic wane between 2 to 3 hours after hot-iron dehorning as determined by changes in the eye temperature and cardiac responses following the procedures. Calves dehorned with a local anesthetic in the study had an increase in heart rate except when they had received an NSAID. This suggests that there was an onset of pain during this time, which was most likely caused by the effects of the local anesthetic wearing off. The study also

demonstrated that eye temperature not only drops in response to acute/immediate pain, but also to the onset of pain after 2-3 hours when a NSAID was not given. Neither the control group nor the group treated with both local anesthetic and NSAID has such a drop in eye temperature, suggesting that this response was associated with the onset of pain. In conclusion an NSAID successfully mitigated physiological responses between 2 and 3 h after hot-iron dehorning when the pain-relieving effects of a local anesthetic would normally diminish (Stewart et al., 2009).

Conclusion

There have been several biomarkers of pain suggested including: plasma cortisol concentrations, PGE₂ concentrations, algometer measurements, and thermography. Although several analgesic regimens have been studied, meloxicam with a local anesthetic block has come to the forefront as a fast, effective, and inexpensive means to providing complete pain relief post dehorning. However, until the drug obtains a label for use in cattle for analgesia, continued research will need to be performed in identifying a validated biomarker of pain in cattle. The study in Chapter 2 will compare the effectiveness of oral meloxicam offered 12 hours prior to dehorning in milk replacer with oral meloxicam given at the time of dehorning as a bolus.

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Chapter 2 - The effect of timing of oral meloxicam administration on physiological responses in calves after cautery dehorning with local anesthesia

Introduction

Consumer interest in animal welfare, especially pain associated with routine livestock management procedures such as castration and dehorning, is increasing (Rollin, 2004). The purpose of dehorning is to minimize the risk of injuries to caretakers and other animals and to decrease the incidence of carcass downgrading due to bruising and hide damage (Stewart et al., 2009). Although there are many benefits to dehorning, the procedure is considered painful in calves (AVMA, 2011).

In a survey of dairies in the North Central and Northeastern United States, hot-irons were identified as the most common dehorning method used. However, local anesthesia was only provided by 12.4% of dairy owners and systemic analgesics are used by only 1.8% of dairy owners (Fulwider et. al, 2008). The American Veterinary Medical Association (AVMA) (2011) has stated the importance of minimizing pain associated with dehorning in order to reduce distress and changes in behavioral and physiological states. While it has been reported that the combination of a local anesthetic and a nonsteroidal anti-inflammatory drug (NSAID) can mitigate the onset of pain associated with dehorning (Heinrich et al., 2009; Stewart et al., 2009; Coetzee., 2011), there are currently no FDA approved NSAIDs labeled for pain management in food animals in the USA (Coetzee, 2013). Flunixin meglumine is the only NSAID approved for use in beef and dairy cattle, but it is only labeled for IV administration for the control of pyrexia associated with bovine respiratory disease and mastitis and for the control of inflammation associated with endotoxemia (Smith et al., 2008; FDA, 2006).

Several methods have been used to quantify the pain associated with dehorning (Stock et al, 2013). Although there are many pain-induced responses recognized, there are none currently validated for scoring pain in cattle (Muir and Woolfe, 2001 and Smith, 2013). Indicators of physiological change that can be measured in the blood include plasma cortisol concentration (Heinrich et al., 2009), haptoglobin concentration (Earley and Crowe, 2002), and plasma

Substance P (SP) concentration (Coetzee et al., 2008, Coetzee et al., 2012). Other measures of pain include: assessment of mechanical nociceptive threshold (MNT) using pressure algometry (Heinrich et al., 2010), ocular temperature (Stewart et al., 2008), behavioral measures, including ear flicks and head rubs (Heinrich et al., 2010 and Faulkner and Weary, 2000), and heart and respiratory rates (Stewart et al., 2008, Heinrich et al., 2009, Coetzee 2012).

Studies indicate that NSAIDs, such as ketoprofen and sodium salicylate mitigate responses to pain associated with hot-iron dehorning in young dairy calves (Faulkner and Weary, 2000; Baldrige et al., 2011). Recent studies found that meloxicam administration ameliorates the physiological stress response to dehorning following intramuscular or intravenous injection (Heinrich et al., 2009; Coetzee et al. 2012). Meloxicam is a NSAID in the oxicam class that is considered to bind preferentially to cyclo-oxygenase-2 enzymes in cattle. Meloxicam has been approved for use in bovine in European countries as well as Canada as a single IV or SC dose of 0.5 mg/kg but is currently not on label in the U.S to use in cattle (EMEA, 1999 and Health Canada 2009). Oral meloxicam tablets can be legally used to alleviate pain in cattle in the U.S. if used in accordance with the Animal Medicinal Drug Use Clarification Act, 1994 (AMDUCA). The specific conditions of AMDUCA include: 1. ELDU is permitted only by or under the supervision of a veterinarian; 2. It is allowed only for FDA-approved animal and human drugs; 3. It is permitted only when the health of the animal is threatened and not for production purposes; 4. ELDU in feed is prohibited; and 5. It is not permitted if it results in a violative food residue (US FDA, 2009). A recent study determined that oral administration of meloxicam provides similar plasma drug concentrations to those achieved following parenteral administration (Coetzee et al., 2009). The study also concluded the plasma $T_{1/2}$ of oral meloxicam is approximately 28 h and therefore, meloxicam should provide effective analgesia for several days after oral administration (Coetzee et al., 2009). However, studies investigating the effect of oral meloxicam in calves experiencing dehorning with local anesthesia are currently deficient in the published literature.

Pre-emptive analgesia involves the administration of an analgesic compound prior to the onset of the noxious event thus limiting the pain-stress-distress cascade that results when pain is induced (Anderson and Muir, 2005). When considering pre-emptive analgesia, it is hypothesized that meloxicam should be administered at least 12 hours prior to dehorning so that the onset of the painful stimulus corresponds with peak plasma drug concentrations (Coetzee et al., 2009).

Meloxicam may be conveniently administered in milk replacer 12 h before dehorning to provide pre-emptive analgesia as shown in Mosher and others (2011). This study reported a similar plasma meloxicam concentration profile following co-administration of meloxicam tablets with powdered milk replacer as was observed following administration of an oral bolus of meloxicam (Mosher et al., 2011). The purpose of the present study was to determine the overall effect of timing of oral meloxicam administration on physiological responses in calves after dehorning. There were two objective of this study: (1) to assess the effect of oral meloxicam on pain response after dehorning with local anesthesia and (2) to determine if oral meloxicam is clinically more effective when given 12 hours prior to a surgical procedure than when given at the time of the procedure.

Materials and Methods

All experimental procedures in this study were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC) under the supervision of the university veterinarian (Protocol # 2898).

Animal Husbandry and Housing

Thirty Holstein bull calves were obtained from a dairy in Boulder, Colorado in May 2011. On arrival, calves were processed with a single subcutaneous dose of oxytetracycline 300mg/mL (NoromycinTM 300 LA, Norbrook Inc., Lenexa, KS), a single subcutaneous dose of an eight-way clostridial vaccine (Covexin[®] 8, Intervet/Schering-Plough Animal Health, Summit, NJ), and a single subcutaneous dose of a 4-way bovine respiratory disease vaccine (Bovi-Shield GOLD[®] 5, Pfizer Animal Health, New York, NY). A topical pour-on comprising of 5% permethrin and 5% piperonyl butoxide (Ultra Boss[®] Pour-On Insecticide, Intervet/Schering-Plough Animal Health, Summit, NJ) was applied to all calves and repeated as needed for fly control. Calves were castrated approximately 2 weeks after arrival and given a further 2 week acclimation prior to study commencement. Calves were approximately 8-10 weeks old at the time of dehorning. Housing consisted of two identical barns composed of twenty 9.29 m² individual stalls with concrete floors, per industry standard. One-half of each stall was shaded by a tin roof. Stalls were randomly assigned to calves upon arrival. Calves were fed a bottle consisting of 350g of powdered milk replacer (Land O'Lakes Instant Amplifier Max, Land O'Lakes Inc., Shoreview, MN) with 2.18 L of water every 12 h. Calves were also fed 0.68 kg of

calf starter diet (KSU feedmill) per day. The diet consisted of rolled corn, soybean hulls, dehydrated distillers grains, soybean mill, corn steep liquor, and a vitamin/mineral supplement. The diet was 87% dry matter, with 16% crude protein, 3.4% crude fat, and 10.4% crude fiber. Water was offered in buckets ad libitum.

Study Procedure

The study design was a randomized complete block design with two periods 24 hr apart. Calves were ranked by ascending weight and assigned a random number using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA). Calves were approximately 8-10 weeks of age at the time of this study commencement and were blocked by weight that was obtained at castration four weeks earlier. Each period consisted of 15 calves that were assigned, using random numbers, to one of three treatments (n=5 calves/ treatment/ period).

Jugular catheterization. Approximately 12 h prior to dehorning for each period, calves were restrained in pens with a halter for catheter placement. The area of the left jugular vein was clipped and disinfected using povidone iodine and 70% isopropyl alcohol swabs. Prior to catheter placement, 0.5 mL of 2% lidocaine hydrochloride (Lidocaine HCl 2% (20mg/mL), Hospira Inc., Lake Forest, IL) was injected subcutaneously over the jugular vein to provide analgesia before a one cm incision with a #22 scalpel blade was made only through the skin. Next, a 14G x 140 mm jugular catheter (Abbocath-T (305 mL/min), Abbott Ireland, Sligo, Republic of Ireland) was inserted. Catheters were sutured in place and patency was maintained with 5 mL of heparin saline flush (4 U/mL). Catheters remained patent a total of 36 hours and were removed after the 24 h blood sample was collected.

Drug Administration. After randomization, meloxicam was administered to calves 12 h before dehorning (MEL-PRE), immediately after dehorning (MEL-POST), or a placebo control was administered (CONT). All calves were fed an individually prepared bottle approximately 12 h before dehorning. Calves assigned to the MEL-PRE treatment group received meloxicam (Meloxicam Tablets USP 15mg [NDC 98300-125-01], Unichem Pharmaceuticals USA Inc. Rochelle Park, NJ; Lot GMMH10108) at a dose of 1 mg/kg and 25 g of whey powder (Spray Dried Pasteurized Whey, Kraft, Harford, CA) suspended in the bottle immediately prior to feeding. All other calves received only 25 g of whey powder as a placebo suspended in the bottle immediately prior to feeding. Calves assigned to the MEL-POST treatment were given

gelatin capsules containing 15 mg meloxicam tablets administered at a dose of 1 mg meloxicam/kg bodyweight and 12 g of whey powder at the time of dehorning. All other calves were given a bolus consisting of only 12 g of whey powder as a placebo at the time of dehorning.

Dehorning. Approximately 15 min before dehorning, calves were restrained in individual stall with a rope halter. A cornual nerve block was performed with 5 mL of 2% lidocaine hydrochloride (Lidocaine HCl 2% (20mg/mL), Hospira Inc., Lake Forest, IL) on both sides as previously described (Stock et al, 2013). Ten minutes after the application of local anesthetic, horn but sensitivity was tested with a needle prick using an 18 gauge needle around the horn bud. Both horn buds were then removed via a hot iron dehorning as previously described (Heinrich et al. 2010). To maintain consistency, all nerve blocks were performed by the same veterinarian (JFC) and all dehorning was performed by the same technician who were masked to treatment group.

Data Collection

Blood sampling. Blood samples were collected using the aforementioned catheters at baseline (approximately 2 h before dehorning), immediately prior to dehorning (-1 min) and at 5, 30, 60, 120, 240, 360, 480 and 720 min after dehorning. A blood sample drawn from the jugular vein using an 18 gauge needle was collected every 24 h thereafter for 7 days. Additionally, a blood sample was taken at time of catheterization and before treatment administration to obtain a -12 h drug concentration sample. Catheter patency was maintained using 5 mL of heparin saline flush both before and after blood was drawn and 3 mL of blood was discarded before the sample was taken. The samples were collected while the animals were haltered and tied in individual shaded stalls with access to water. Cortisol, SP, and drug concentrations were measured at every time point while haptoglobin and PgE₂ concentrations were analyzed at baseline (approximately 2 h before dehorning), 5, 360, 720 min, and every 24 h for 3 days. Cortisol and haptoglobin analysis required 6 mL of blood collected into two separate serum clot activator tubes, blood for SP analysis was collected into one EDTA K3 tube, and drug concentration and PgE₂ blood samples were collected into two separate lithium heparin tubes. The EDTA K3 tubes and lithium heparin tubes were stored on ice and the serum clot activator tubes were stored at room temperature prior to centrifugation.

Algometer measurements. Mechanical nociceptive threshold (MNT) was measured by an algometer (Wagner Force One FDIX, Wagner Instruments, Greenwich, CT) at baseline (approximately 2 h before dehorning), -1, 60, 120, 240, 360, 480, 720 min, as well as at the 24 hour blood sample for 7 days after dehorning as previously described (Heinrich et al., 2010). Each calf was restrained with a halter and a hand was placed lightly on the poll until the calf habituated to being touched and stood in a relaxed posture. The hand was then slowly removed and replaced with the algometer rubber tip placed directly beside the horn wound, such that the rubber tip covered the cautery wound and the edge of normal tissue. Pressure was applied perpendicular to the poll at a rate of approximately 1 kgf/s until the calf withdrew its head. The right horn was measured first and the three sites around each horn bud were measured in the same order for each horn. The technician was masked to the algometer reading until after the calf withdrew its head and, to maintain consistency, the same technician took every algometer reading.

Thermography imaging. Changes in maximum ocular temperature (MOT) were measured using a commercially-available infrared inspection system (ThermaCAM® P65HS, FLIR Systems, Wilsonville, OR) as previously described, all images were taken on the left side, at a 90 degree angle, and approximately 0.5 m away from the subject. (Stewart et al., 2009). Maximum temperatures within the lacrimal caruncle were recorded. Images were obtained at baseline (approximately 2 h before dehorning), -1, 1, and 720 min after dehorning. Images were analyzed for changes in maximum ocular temperature using research grade software (Thermacam Researcher Pro 2.8 SR-1, FLIR Systems, Wilsonville, OR).

Weight measurements. Weights were obtained at time of catheterization and at time of the seven day blood draw post dehorning. Calves were weighed in a hydraulic squeeze chute (Daniels Manufacturing Co., Ainsworth, NE).

Sample Analysis

All sample analysis was conducted by technicians that were masked to treatment group allocation.

Cortisol analysis. For every time point, 6 mL of blood was collected into serum clot activator tubes and centrifuged at 1500g for 10 min within 30 min of collection. The serum was collected with transfer pipettes and stored in 2 mL cryogenic vials and frozen at -80°C prior to

cortisol analysis. Serum cortisol concentrations were determined using solid-phase competitive chemiluminescent enzyme immunoassay and an automated analyzer system (Immulite® 1000 Cortisol, Siemens Medical Solutions, Los Angeles, CA). This method has been validated in the Kansas State Veterinary Diagnostic Laboratory (Coetzee et al., 2007).

Haptoglobin analysis. For selected time points, 6 mL of blood was collected into serum clot activator tubes and centrifuged at 1500 g for 10 min within 30 min of collection. The serum was then pipetted off using 3 mL transfer pipettes into 2 mL cryogenic vials and stored at -80°C prior to haptoglobin analysis. The method of analysis depends on the peroxidatic activity of hemoglobin. Hemoglobin added in excess to serum samples binds to haptoglobin and becomes resistant to acid inactivation. In contrast, the peroxidatic activity of free hemoglobin is lost. The plasma haptoglobin concentration is calculated using a standard curve prepared by incubating known amounts of hemoglobin with a serum sample containing a concentration of haptoglobin greater than 150 mg/dL.

PgE₂ analysis. Blood samples were collected into lithium heparin tubes and stored on ice for no longer than 30 min before incubation and analysis for plasma PgE₂ concentrations. For each time point, 3 mL of whole blood was incubated in glass tubes for 24 h at 37°C with the exception of baseline sample, which was divided into two different aliquots: 3 mL incubated with lipopolysaccharide (LPS) (K+) and other 3 mL incubated without LPS (K-) used as positive and negative control of basal levels of PgE₂. LPS (diluted in PBS and used at 10 µg/ml) was added to the samples to stimulate ex-vivo PgE₂ production by monocytes. At the end of incubation, all samples were centrifuged at 400 g for 10 min to obtain plasma. The resulting supernatant was taken and stored at -80°C until the determination of plasma PgE₂ levels using an ELISA kit (Prostaglandin E₂ Kit, Cayman Chemical Company, Ann Arbor, MI) previously validated in our laboratory (Fraccaro et al, 2013).

Substance P analysis. Blood samples were collected into EDTA K3 tubes containing the protease inhibitor, benzamidine hydrochloride (Benzamidine Hydrochloride, Santa Cruz Biotechnology Inc., Santa Cruz, CA). A 20 mM solution of benzamidine was prepared in water and 300 µL was added to each tube for a final concentration of 1 mM benzamidine in whole blood to act as a protease inhibitor. These tubes were stored on ice for no more than 30 minutes before being centrifuged at 1500 g for 10 min. The plasma was pipetted off with 3 mL transfer

pipettes and stored in 2 mL cryogenic vials and stored at -80°C until analysis. Analysis of substance P was performed as previously validated (Coetzee et al., 2008).

Plasma drug analysis. Plasma concentrations of meloxicam (m/z 352.09 \rightarrow 114.90) were determined with high-pressure liquid chromatography (Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, MD, USA) and mass spectrometry (API 2000, Applied Biosystems, Foster City, CA, USA). Plasma samples or standards (50 μL) were added to 200 μL of internal standard (piroxicam 0.5 $\mu\text{g}/\text{mL}$ in methanol, m/z 332.12 \rightarrow 95.10 and pregabalin 5 $\mu\text{g}/\text{mL}$, m/z 160.00 \rightarrow 142.00) in methanol with 0.1% formic acid to precipitate the proteins. The samples were vortexed for 5 s and centrifuged for 10 mins at 10 000 x g. The supernatant, 200 μL , was transferred to an injection vial with the injection volume set to 10 μL . The mobile phase consisted of A: acetonitrile and B: 0.1% formic acid at a flow rate of 0.4 mL/min. The mobile phase consisted of 100% B from 0 – 0.5 mins with a linear gradient to 50 % B at 2.5 mins which was maintained until 5 mins, followed by a linear gradient to 100 % B at 5.5 mins with a total run time of 8 mins. Separation was achieved with a C18 column (Supeclo Discovery, 50 mm x 2.1 mm x 5 μm , Sigma, St Louis, MO, PA, USA) maintained at 40 C. The standard curve was linear from 0.025 to 5 $\mu\text{g}/\text{mL}$ for meloxicam and the standard curves were accepted if the correlation coefficient exceeded 0.99 and predicted values were within 15 % of the actual values. The accuracy of the assay was 102 %, 99 %, 100 % of the actual value at 0.025, 0.5, and 5 $\mu\text{g}/\text{mL}$, respectively, on replicates of 5 for each concentration. The coefficient of variation was 6 %, 3 %, and 3% at 0.025, 0.5, and 5 $\mu\text{g}/\text{mL}$, respectively, on replicates of 5 for each concentration.

Data Analysis

Pharmacokinetic analysis. Non-compartmental pharmacokinetic analyses were performed with computer software (WinNonlin 5.2, Pharsight Corporation, Mountain View, CA, USA). The variables calculated included the area under the curve from time 0 to infinity ($\text{AUC}_{0-\text{INF}}$) using the linear trapezoidal rule, area under the first moment curve from time 0 to infinity ($\text{AUMC}_{0-\text{INF}}$), plasma clearance per fraction of the dose absorbed (Cl/F), apparent volume of distribution (area method) per fraction of the dose absorbed (V_z/F), first-order rate constant (L_z), terminal half-life ($\text{T}_{1/2}$), and mean residence time extrapolated to infinity ($\text{MRT}_{0-\text{INF}}$). The percent of the AUC extrapolated to infinity (AUC extrapolated) was determined. The maximum serum

concentration (C_{\max}) and time to maximum serum concentration (T_{\max}) were determined directly from the data.

Statistical Analysis. Models were fitted using the GLIMMIX procedure of SAS (Version 9.2, SAS Institute, Cary, NC). Model assumptions were considered to be appropriately met using externally studentized residuals. Differences in means of the response variables between analgesia (the combination of MEL-PRE and MEL-POST) vs. no analgesia (CONT) and the mean difference interactions with time were assessed using F-tests in the repeated measures ANOVA models. Effects of timing of treatment administration and time on response variables were similarly analyzed using repeated measures analysis of variance (ANOVA) models. Treatment, time and their interaction were used as fixed effects, whereas calf was the subject of the repeated measures. Plasma cortisol concentration, plasma haptoglobin concentration, and algometer measurements were log-transformed before analyses to stabilize the distribution variances. Relevant pairwise comparisons were conducted using Tukey-Kramer adjustments to avoid inflation of Type I error rate due to multiple comparisons. A significance level of $\alpha = 0.05$ was used to determine statistical differences, as well as $\alpha \leq 0.10$ for assessing effects with marginally significant differences especially with respect to time by treatment interactions.

RESULTS

No calves were removed from this study.

Cortisol Concentration

Overall, there was evidence of an effect of time on mean cortisol concentrations across treatment groups ($P < 0.0001$) and a time by analgesia treatment interaction ($P = 0.047$). To clarify the time by treatment interaction, the mean cortisol concentration was graphed versus time (Figure 1a and 1b). Calves that received meloxicam had significantly lower serum cortisol concentrations compared with placebo-treated controls at 4 h after dehorning ($P = 0.0004$). Comparison between individual treatment groups revealed that mean cortisol concentration was significantly lower in both the MEL-PRE ($P = 0.015$) and MEL-POST ($P = 0.002$) compared with the CONT calves at this time point.

Substance P Concentration

There was evidence of a time by treatment interaction on SP concentrations after dehorning. This interaction is illustrated in Figure 2a and 2b which compares mean SP concentration against time for the analgesia group and placebo-treated control group. At 120 h post dehorning, calves given analgesia had a significantly lower substance P concentration than those not given analgesia (P=0.0386).

Haptoglobin Concentration

Mean haptoglobin concentration did increase significantly over time across all treatment groups (P=0.0046). The comparison of mean haptoglobin concentrations post dehorning and time are summarized in Figure 3 for all three treatment groups. It is noteworthy that there was a significant increase in haptoglobin concentrations between 12 h and 72 h post dehorning as well as between 24 h and 72 h post dehorning (P=0.0077 and P=0.0297 respectively) across all treatment groups.

Maximum Ocular Temperature

There was evidence of an effect of time on MOT across all three treatment groups (P<0.0001). The mean MOT at baseline, 1 min pre dehorning, and 1 min post dehorning and at 12 h post dehorning for all three treatment groups is summarized in Figure 4. The graph demonstrates that, compared to baseline (-120 min) values, MOT was significantly decreased at 1min pre dehorning and 1 min post dehorning (P<0.0001 for both) across all treatment groups.

PgE₂ Concentration

The administration of meloxicam had a significant effect on plasma PgE₂ concentrations when compared to the placebo-treated control group. Specifically, the data revealed that the PgE₂ concentration in calves that received meloxicam were an average of 309.68 ± 84.4 pg/mL lower than calves that did not receive meloxicam analgesia (P=0.0011). Additionally, an effect of time (P<0.0001) on PgE₂ concentration across all treatment groups was also observed. Specifically, at 24 h post dehorning, calves in all treatment groups had a PgE₂ concentration that was significantly above baseline concentrations (P=0.0140).

A significant interaction between time and meloxicam administration was evident (P=0.0489). These differences are further illustrated in Figure 5 showing PgE₂ concentrations

against time for all three treatment groups. Specifically, at 6 h post dehorning, both MEL-POST and MEL-PRE had a significantly reduced PgE₂ concentrations when compared to placebo-treated control calves (P=0.0036 and P=0.0053 respectively). Similarly, at 12 h post dehorning, the PgE₂ concentrations of both MEL-POST and MEL-PRE treatment groups were significantly lower than the concentrations of the placebo-treated control calves (P=0.0024 and P=0.0240). Accordingly, the PgE₂ concentrations of the MEL-POST calves (P=0.0160) and the placebo-treated control calves (P=0.0147) was significantly higher than the MEL-PRE calves. These finding suggest a significant effect of timing of meloxicam administration on PgE₂ concentrations at 24 h post dehorning.

Mechanical nociceptive threshold (MNT)

There was an effect of time on MNT (P<0.0001). In particular, MNT was significantly elevated above baseline values for 2 h post dehorning (P= 0.0004). In contrast, at 4 h post dehorning, MNT significantly less than baseline measurements (P<0.0001). There was evidence of a significant interaction of time and meloxicam analgesia administration (P=0.0143) on amount of force tolerated by calves post dehorning. This significant difference is illustrated in Figure 6a and 6b which displays MNT versus time for all three treatment groups. Specifically, at 1 h post dehorning the MNT was 0.2160 ± 0.09 kgf/s higher in the placebo-treated control group than the group that received meloxicam analgesia (P=0.0168). This difference can more specifically be attributed to the MEL-PRE calves, where the mean pressure tolerance of the placebo-treated control calves tended to be higher when compared with the MEL-PRE calves (P=0.0588) as opposed to MEL-POST calves, which only revealed a slight increase in MNT between the placebo-treated control calves and the MEL-POST calves (P=0.1484). In contrast, calves that received analgesia had a MNT that was 0.1619 kgf higher than those calves that did not receive meloxicam analgesia (P=0.0728) at 6 h post dehorning.

Average Daily Gain

The average weight of the calves at baseline was 65.3 kgs while the average weight of calves at day 7 was 67.6 kgs. There was no evidence of an effect of the use of analgesia on average daily gain (P=0.8689). Additionally, the timing of meloxicam administration was also not found to cause a significant difference on average daily gain (P=0.9542).

Meloxicam Pharmacokinetic Parameter Estimates

Pharmacokinetic parameter estimates are summarized in Table 1 and include mean \pm SEM. The plasma drug concentration time curve is also characterized in Figures 7 and 8. The terminal half-life of MEL-POST and MEL-PRE was determined to be 35.81 ± 6.26 hr and 38.62 ± 5.87 hr respectively. The volume of distribution (V_z/F) was very similar between the two treatments, MEL-POST was 0.28 ± 0.04 and MEL-PRE was 0.27 ± 0.02 L/kg. The AUC was also very similar between the groups as MEL-POST was 217.12 ± 38.20 (hr·ug/mL) and 216.12 ± 40.05 (hr·ug/mL) for the MEL-PRE group. Although not statistically significant ($P=0.1775$), the C_{max} of the MEL-PRE group was numerically higher at 3.61 ± 0.21 ug/mL than the MEL-POST group at 3.27 ± 0.14 ug/mL. Furthermore, the λ_z Upper was significantly ($P=0.0015$) greater in the MEL-PRE group, compared with that in the MEL-POST group.

DISCUSSION

The purpose of this study was two-fold: (1) to assess the effect of oral meloxicam on the pain response and (2) to determine if oral meloxicam is clinically more effective when given 12 hours prior to a surgical procedure than when given at the time of the procedure. The present study supports the hypothesis that oral meloxicam administration ameliorates the physiological response to dehorning similar to what has recently been reported following intramuscular or intravenous injection (Heinrich et al., 2009; Coetzee et al. 2012). Furthermore, while the co-administration of a local anesthetic (LA) and a NSAID has been proven to mitigate the pain response after the effect of the LA is diminished (about 2-3 h post dehorning), the effect of timing of NSAID administration before the onset of pain has not been studied (Stewart et al., 2009 and McMeekan et al., 1998). The present study found no clinically significant difference between meloxicam administered in the milk replacer 12 h before dehorning compared with meloxicam administered as a bolus at the time of dehorning. These results provide support for the use of oral meloxicam as an analgesic compound administered at the time of dehorning in preweaning Holstein calves.

The pharmacokinetics of oral meloxicam administered to calves as a bolus or in milk replacer has been described (Mosher et al., 2011). In the present study, T_{max} was in agreement with what has previously been reported. However, the C_{max} for both MEL-PRE (3.61 ug/mL) and MEL-POST (3.27 ug/mL) treatment groups was approximately twice the C_{max} of the above

mentioned study (Mosher et al., 2011). This outcome was anticipated given that a dose of 1 mg/kg as opposed to a 0.5mg/kg dose was administered in the present study. The reason for increasing the dose in this was to add an additional half-life to the plasma drug concentration vs. time curve which we hypothesized would extend the duration of analgesia following oral administration.

Regardless of treatment, cortisol concentrations were above baseline concentrations for 30 minutes following dehorning suggesting that the surgical procedure caused some distress in calves despite the use of local anesthesia. However, at 4 hours post dehorning, plasma cortisol concentrations in CONT calves were significantly higher than either MEL-PRE or MEL-POST treated calves. A similar increase in cortisol concentration in calves dehorned without systemic analgesia has been previously reported at 1.5 hours post dehorning and has been attributed to the effects of the LA decreasing (Stafford and Mellor, 2005; Heinrich et al., 2009). Although the cortisol response in the CONT calves in the present study occurred later than in other trials, this response likely coincides with the return of sensitivity to the previously anesthetized horn bud. The absence of a cortisol response in meloxicam-treated calves at 4 h post-dehorning can likely be attributed to the analgesic effect of the drug as has previously been described following parenteral meloxicam administration (Heinrich et al, 2009). The results of the present study therefore provide support for the hypothesis that oral meloxicam has a similar physiological effect to parenteral meloxicam.

Haptoglobin has been suggested as an alternative to plasma cortisol to determine the extent of tissue damage and the effect of analgesic compounds in calves (Earley and Crowe, 2002). However, the results of the present study did not find any significant differences in haptoglobin concentration associated with meloxicam administration. Haptoglobin is an acute phase protein which increases in association with an inflammatory response. Earley and Crowe (2000) found that ketoprofen administration either alone or in combination with local anesthetic reversed the increase in haptoglobin concentrations in surgically castrated calves at 24 h after castration. Furthermore, on day 3 post-castration, concentrations of haptoglobin were lower in ketoprofen treated calves when compared with calves that did not receive ketoprofen (Earley and Crowe, 2002). One explanation for the observed difference in haptoglobin concentrations is that Earley and Crowe (2002) used 5.5 month old castrated bull calves whereas the present study used 8-10 week old calves that were subjected to hot-iron dehorning. It is reasonable to assume

that differences in age and procedure influenced the outcome with respect to haptoglobin concentrations (Mellor et al., 2000).

Substance P is a neuropeptide that regulates excitability of the dorsal horn nociceptive neurons and may be stimulated in times of pain, stress, and anxiety (Coetzee et al., 2011). It has previously been reported that despite similar plasma cortisol concentrations, castrated calves had a significantly elevated plasma SP concentration when compared with simulated-castrated beef calves (Coetzee et al., 2008). Furthermore, the same research group recently reported that an intravenous injection of meloxicam significantly reduced plasma SP concentrations and that an inverse relationship exists between plasma meloxicam concentrations and SP concentrations in calves after dehorning (Coetzee et al., 2012). The present study provides further evidence to support the finding that meloxicam suppresses plasma SP concentrations after dehorning and that oral meloxicam dosing has similar effects to those observed following parenteral injection.

There was no effect of timing of meloxicam administration on the mechanical nociceptive threshold measured by algometer. These results are mostly likely due to the LA administered to all calves, including CONT calves. This conclusion is supported by the observation that all groups of calves increased their force tolerance above baseline in the 4 hours post dehorning. The observation that the effect of the local anesthesia lasted approximately 4 h in this study is further supported by the presence of a cortisol concentration spike in CONT calves at 4 h post-dehorning as reported in Figure 1. However, at 48 h post-dehorning calves given meloxicam, regardless of timing, had a significantly increased MNT when compared with CONT calves. The association between increased MNT and analgesia in the present study is supported by the presence of quantifiable meloxicam concentrations in the plasma of treated calves at this timepoint. This observation supports the finding of Heinrich and others (2012) who first reported that meloxicam-treated calves had a greater tolerance to force at the disbudding site at 24h post-dehorning compared with control calves. This suggests that the effect of oral meloxicam is similar to parenteral meloxicam in alleviating pain following disbudding.

There were no significant differences in maximum ocular temperature detected between treatments. However, the ocular temperature did decrease in all calves from baseline for the 1min pre and 1 min post dehorning. This finding can most likely be attributed to epinephrine release following handling which causes sympathetic stimulation and constriction of the vasculature supplying the eye resulting in a decrease in ocular temperature. However, because

the temperature had dropped before dehorning, it is probably indicative of stress not directly associated with pain. While these results are in accordance with previous work that has reported exogenous stimulation of the sympathetic nervous system tended to cause a decrease in ocular temperature, these results did not show a unique response to pain (Stewart et al., 2007). Further research is needed before ocular temperature determination can be recommended as an objective method of pain assessment in cattle.

A key finding in this study was that PgE₂ concentrations, following ex-vivo stimulation of whole blood with LPS, were significantly lower in MEL-PRE and MEL-POST treated calves compared with CONT calves over 24 h post-dehorning. However, these results were expected because NSAIDS reduce prostaglandin synthesis by inhibiting cyclooxygenase (COX). PgE₂ is a prostaglandin generally associated with inflammation and fever and believed to have a significant effect on pain modulation (Myers et al., 2009). Meloxicam is a COX-2 selective NSAID that reduces the production of prostaglandins, in particular PgE₂, by inhibiting COX-2 and thus reducing pain associated with inflammation (Anderson and Muir, 2005). The findings of the present study demonstrated that meloxicam in the plasma of treated calves suppressed PgE₂ synthesis ex-vivo because meloxicam treated calves had a significantly lower PgE₂ than CONT calves. These findings support the results of a previous *in vitro* study that showed that NSAIDs inhibit the formation of PGE₂ (Myers, 2009). Interestingly, meloxicam administration 12 h prior to dehorning only suppressed PgE₂ synthesis for 12 h post dehorning and then spiked to concentrations equivalent to CONT calves. However, meloxicam administration at the time of dehorning suppressed PgE₂ concentrations for 3 days post dehorning. This was the only significant finding in relation to timing of meloxicam administration. While post-emptive administration of meloxicam did show a longer suppression of PgE₂ concentration indicating a longer duration of action than the pre-emptive administration of meloxicam, no other biomarker supported this finding. There are currently no verified biomarkers of pain in cattle, so further research is necessary to determine if PgE₂ is an appropriate measure of pain and to determine the reason for the differences in timing of meloxicam administration and PGE₂ suppression observed in the present study. To our knowledge, this is the first published report that compares the effects of meloxicam on PGE₂ synthesis when administered preemptively vs. post-procedure. Further investigation is necessary to evaluate the potential benefits of preemptive analgesia using more sensitive outcome measures.

Meloxicam is not currently approved for use in food animals in the US (Coetzee, 2011). Therefore, all current use of meloxicam for pain management in cattle is considered to be extra label drug use (ELDU) and such use must comply with the Animal Medicinal Drug Used (AMDUCA) regulations. Flunixin meglumine is an approved NSAID for use in cattle; however when used for relief of pain, it is considered an ELDU and must also comply with AMDUCA regulations. Significant differences between flunixin meglumine and meloxicam, including the purported COX-2 selectivity of meloxicam and the 4x longer plasma elimination half-life of meloxicam compared with flunixin meglumine (Coetzee et al., 2009), may make meloxicam a more attractive choice in pain management. Although oral meloxicam offered in the milk replacer 12 hours prior to dehorning may be preferable because of ease of administration and convenience, it is important to note that AMDUCA regulations state that ELDU in the feed is illegal.

The present study found no significant difference between the routes of meloxicam administered when comparing administration in the milk 12 h before dehorning and as a bolus at the time of dehorning. It is noteworthy that there was no significant difference in any of the pain biomarkers measured in this trial between calves that received pre-emptive vs. post-emptive meloxicam. This may be because all animals also received local anesthesia prior to dehorning. Previous studies involving parenteral meloxicam administration have typically reported few differences in the biomarkers assessed after 24 h. In the present study the combined effect of local anesthesia and the use of biomarkers that remain unchanged after 24 h may have been too insensitive to detect benefits of pre-emptive vs. post-emptive analgesia. Therefore, meloxicam administered in milk replacer cannot be justified based on these results. Due to AMDUCA regulations that state ELDU in the feed is illegal, the use of meloxicam in the milk 12 h before dehorning for the relief of pain is discouraged until an FDA-approval can be obtained. Taken together, the results of the present study have confirmed that meloxicam, regardless of time of administration, suppresses a pain response following dehorning. This outcome is significant, considering that no previous study has reported the effects of oral meloxicam following dehorning. As dehorning is a common practice in the U.S and there are currently no approved methods for extended pain relief following this procedure, the present study can impact the development of analgesic drug regimens on livestock production systems. Furthermore, we provide evidence to suggest that PgE₂ concentrations may be a sensitive measure of NSAID

effects in cattle. Meloxicam, irrespective of the time of administration, mitigated PgE₂ response for 12 hours post dehorning; however, only meloxicam given at the time of dehorning reduced PgE₂ concentrations for an additional 3 days after dehorning. The findings in the present study not only suggest that oral meloxicam is capable of adequate analgesia following dehorning but also suggest that PgE₂ concentration is a sensitive biomarker for measuring the effect of NSAIDs that warrants further investigation.

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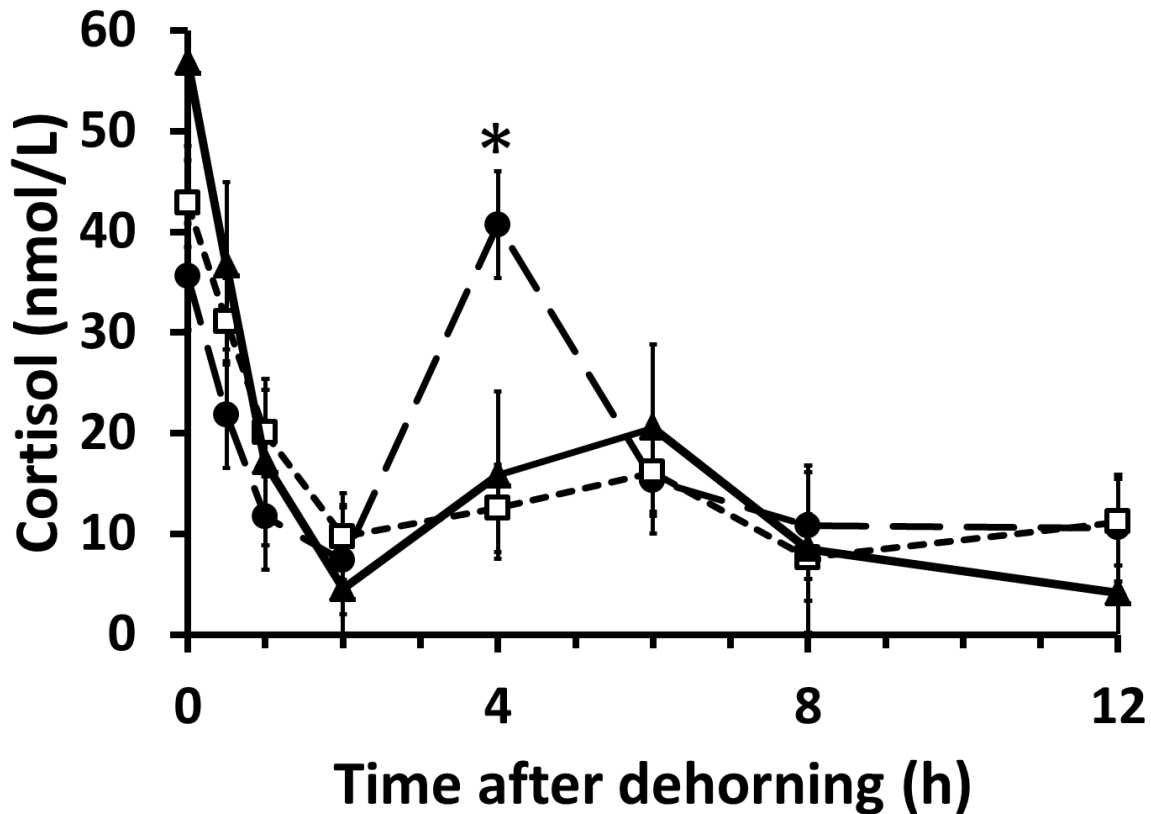
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Figures

Figures 2.1a and 21.b. Mean \pm SEM serum cortisol concentrations in dairy calves dehorned (n=10 calves/group) with meloxicam administration 12 h before dehorning (black triangle), meloxicam administered at the time of dehorning (white square) and control group with no meloxicam administered (black circle). Time of dehorning was designated at time 0. Serum cortisol concentrations were lower in meloxicam-treated calves compared with control calves at 4 h post-dehorning ($P=0.0004$) (designated with a *).



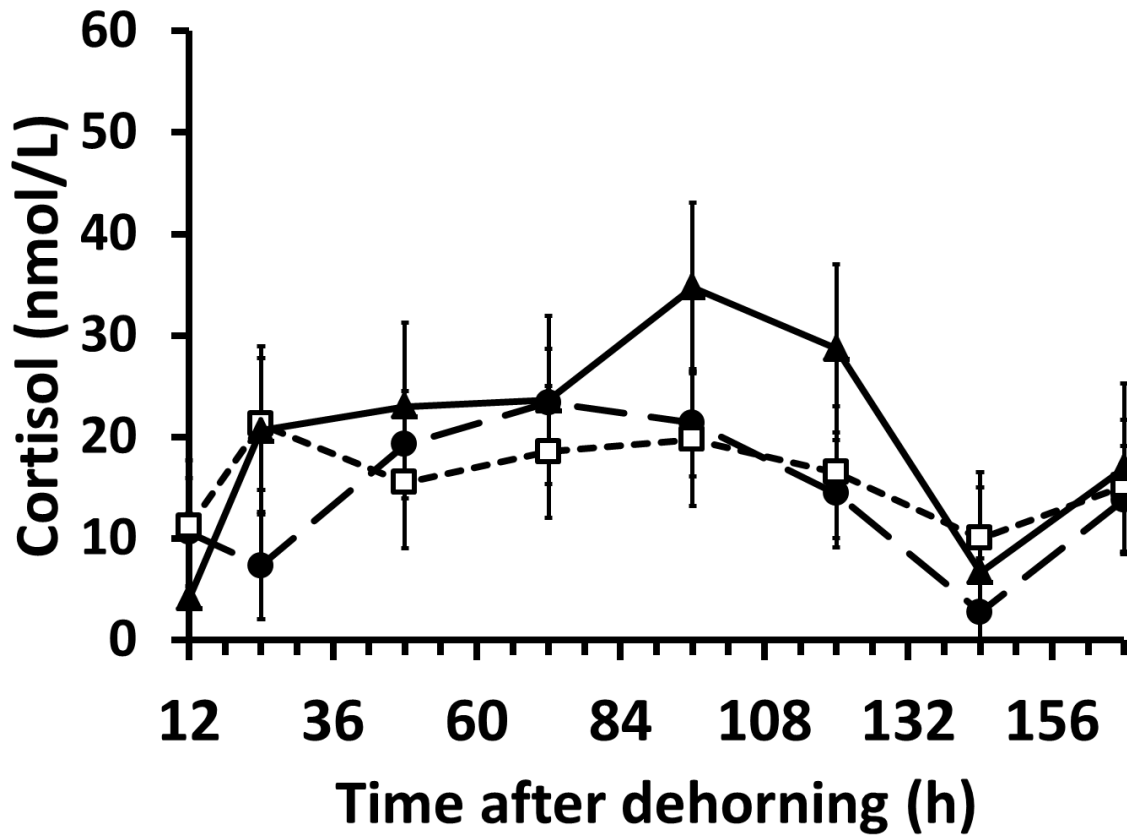
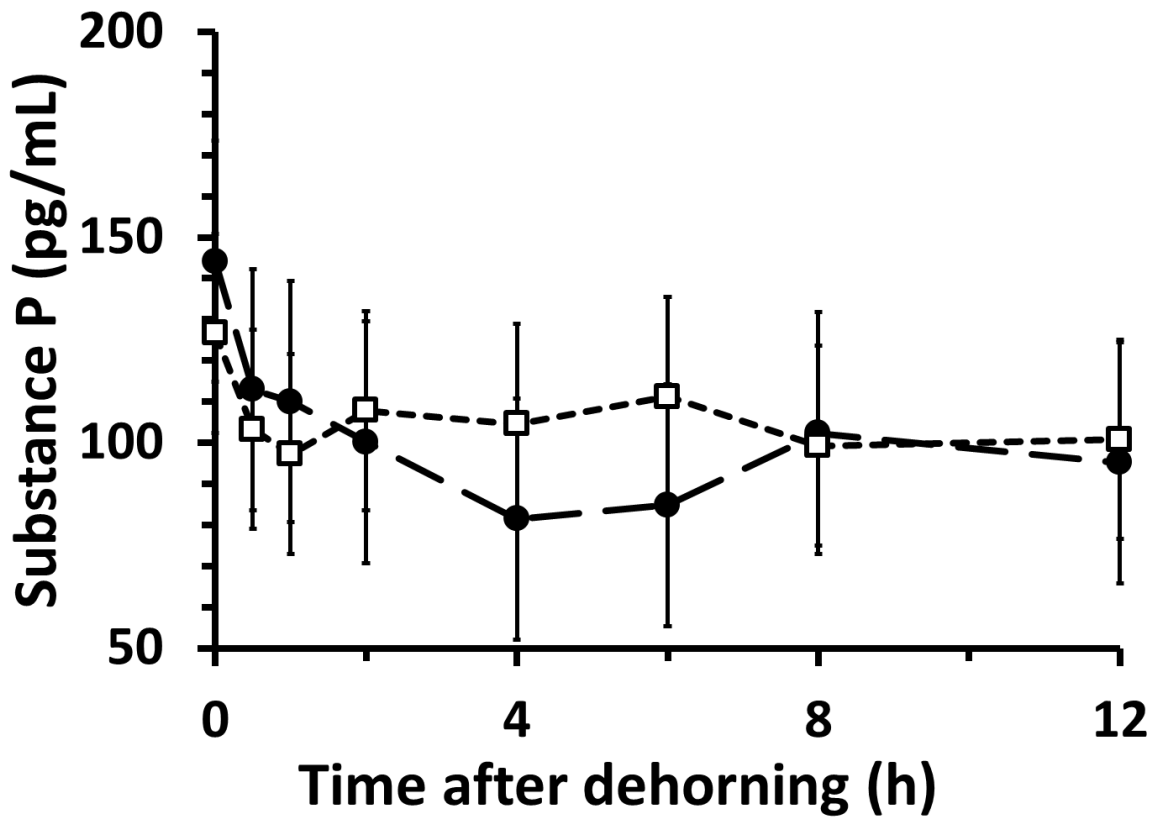


Figure 2.2a and 2.2b. Mean \pm SEM plasma Substance P concentration in dairy calves dehorned (n=10 calves/group) with meloxicam administration either 12 h before dehorning or at the time of dehorning (black square) and control group with no meloxicam administered (black circle). Time of dehorning was designated at time 0. SP concentrations were significantly higher in placebo-treated control calves compared with meloxicam-treated calves at 120 h after dehorning (P=0.038) (designated with a *).



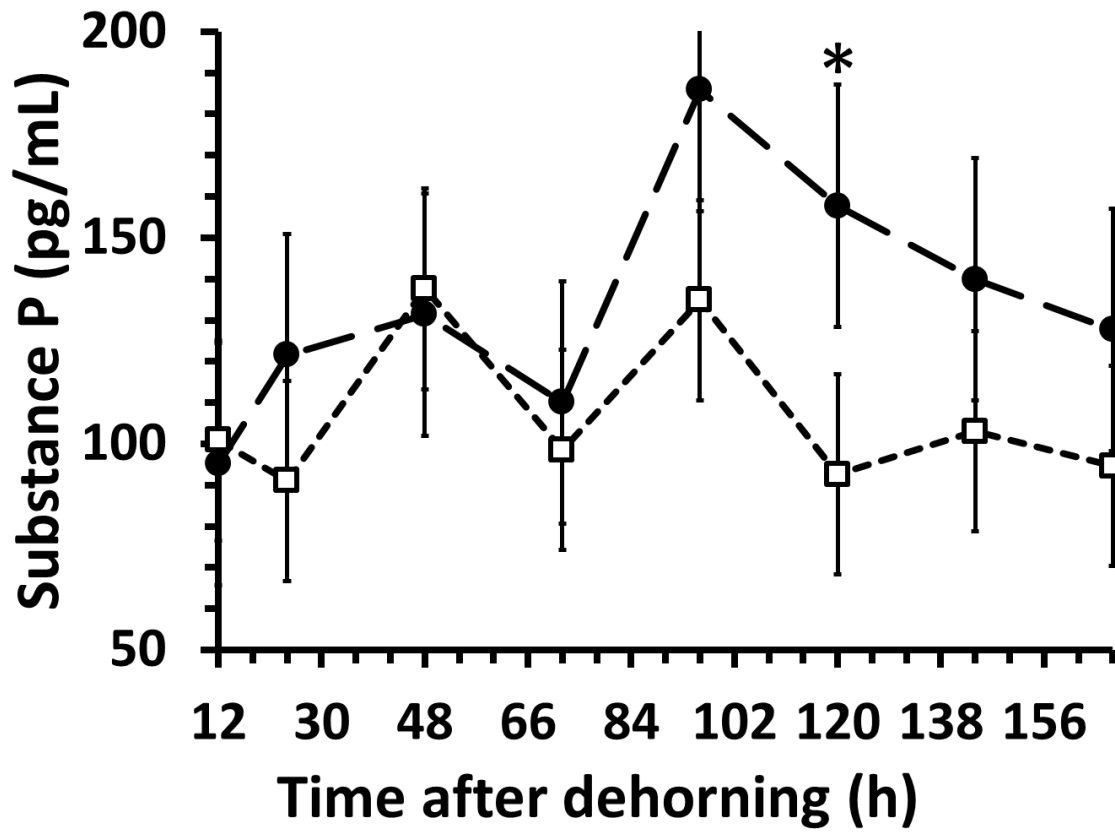


Figure 2.3. Mean \pm SEM haptoglobin concentration in dairy calves dehorned (n=10 calves/group) with meloxicam administration 12 h before dehorning (black triangle), meloxicam administered at the time of dehorning (white square) and control group with no meloxicam administered (black circle). Time of dehorning was designated at time 0. Concentrations did not differ significantly (P=0.8573) between groups.

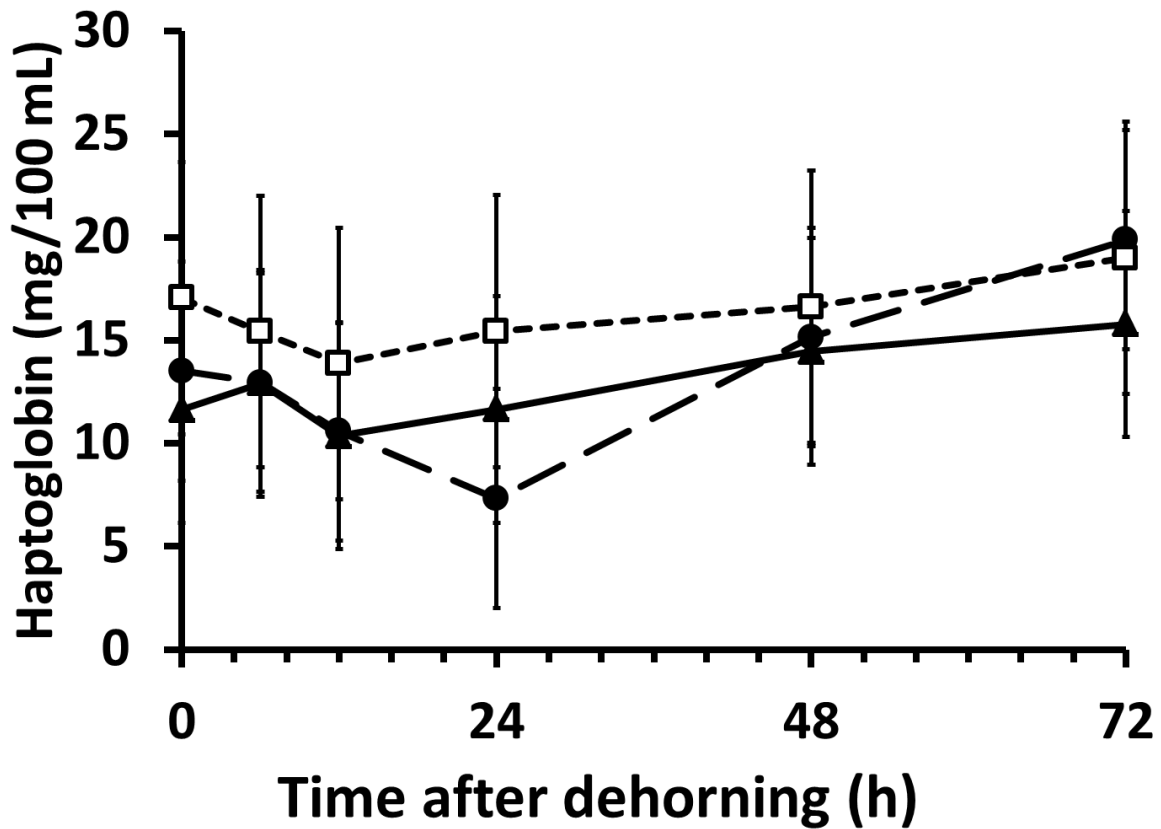


Figure 2.4. Mean \pm SEM maximum ocular temperature in dairy calves dehorned (n=10 calves/group) with meloxicam administration 12 h before dehorning (Straight line bar), meloxicam administered at the time of dehorning (diagonal line bar) and control group with no meloxicam administered (solid black bar). Time of dehorning was designated at time 0. Concentrations did not differ significantly (P=0.9016) between groups.

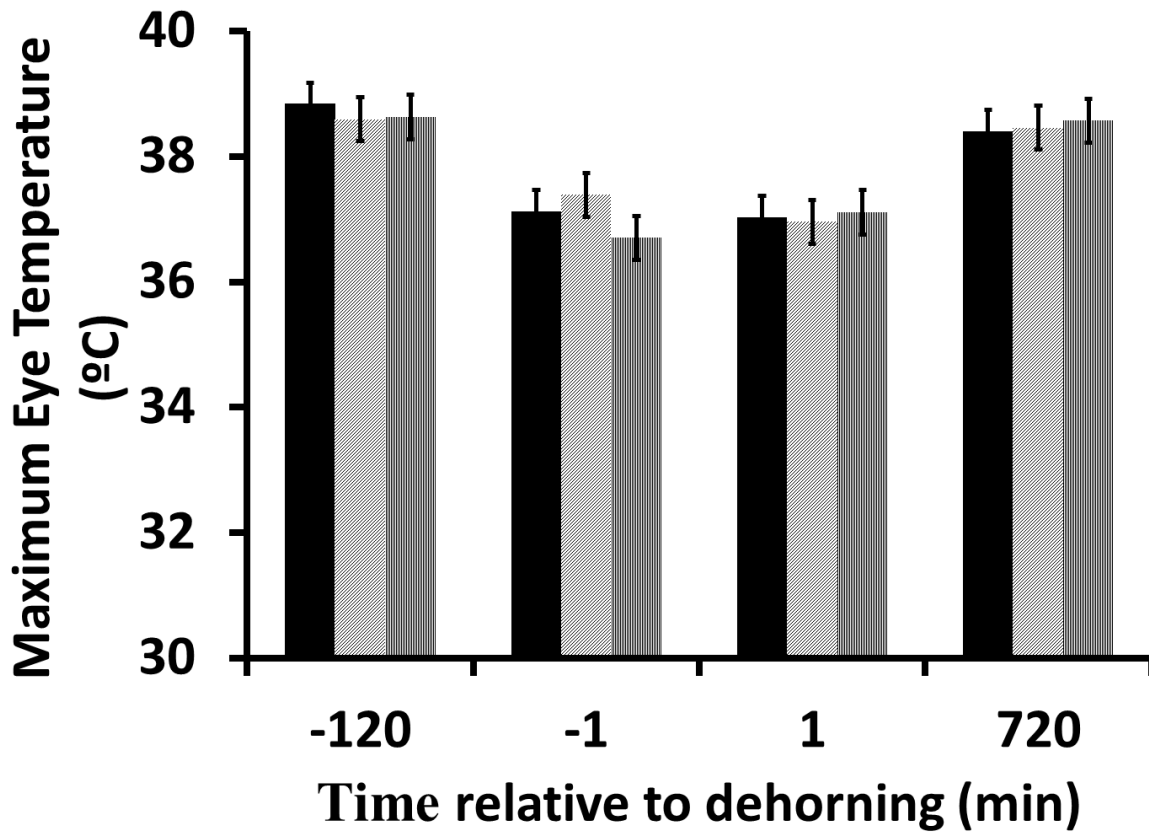


Figure 2.5. Mean \pm SEM plasma PGE₂ concentration in dairy calves dehorned (n=10 calves/group) with meloxicam administration 12 h before dehorning (black triangle), meloxicam administered at the time of dehorning (white square) and control group with no meloxicam administered (black circle). Time of dehorning was designated at time 0. Concentrations were significantly different (P=0.0023) between groups. Further, PGE₂ concentrations in MEL-PRE calves were similar to CONT calves after 12h post-dehorning, while MEL-POST calves had lower PGE₂ concentrations for 3 d post dehorning (designated with *).

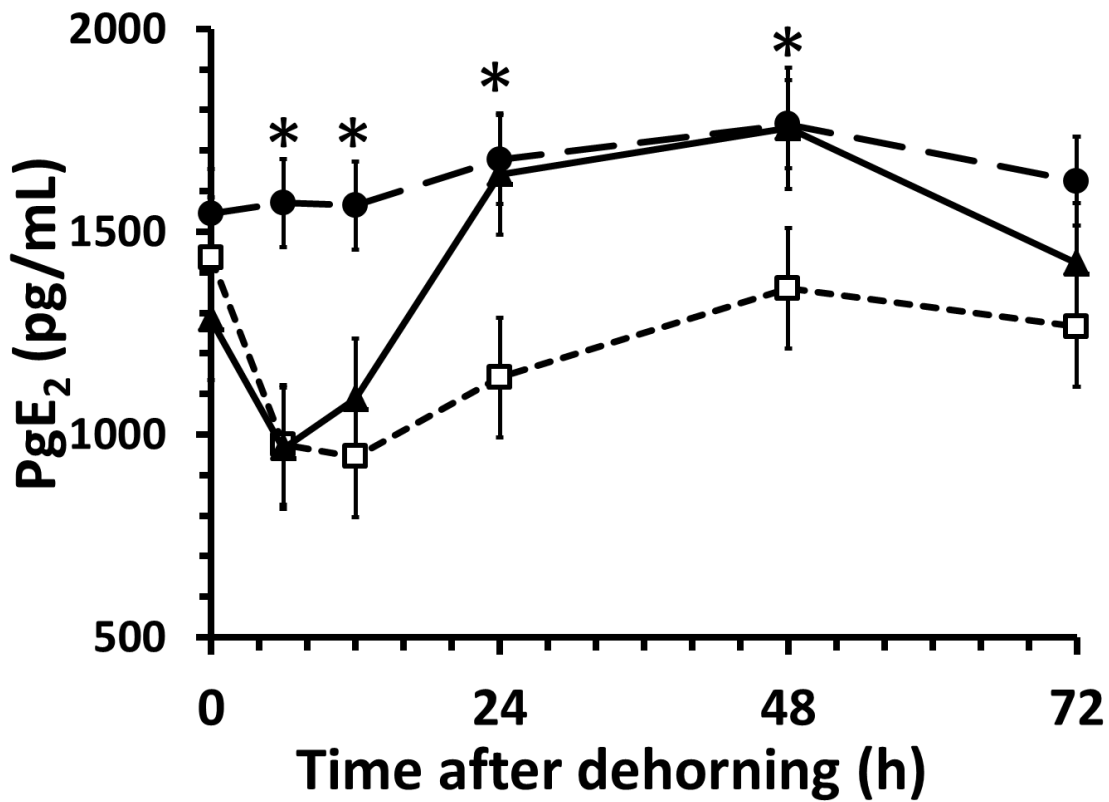
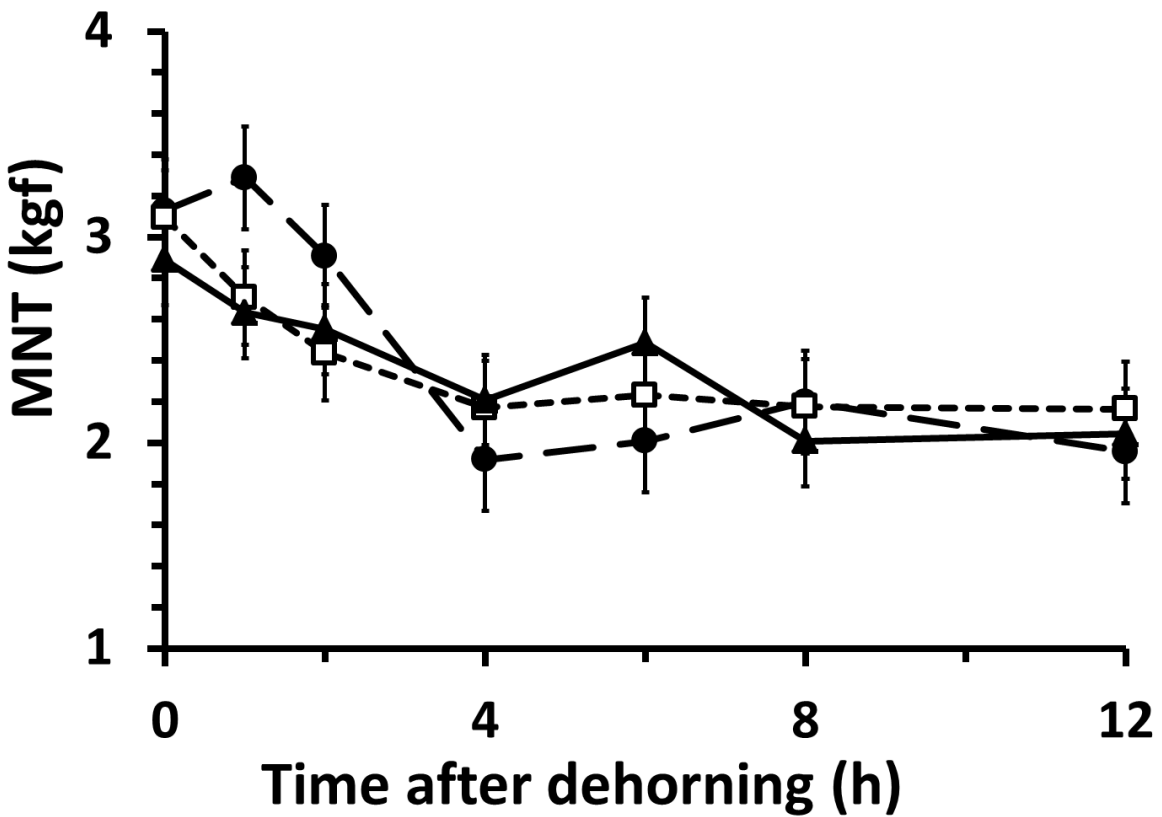


Figure 2.6a and 2.6b. Mean \pm SEM nociceptive threshold (MNT) (kgf) in dairy calves dehorned (n=10 calves/group) with meloxicam administration 12 h before dehorning (black triangle), meloxicam administered at the time of dehorning (white square) and control group with no meloxicam administered (black circle). Time of dehorning was designated at time 0. Control calves tolerated more force than meloxicam-treated calves at 3 h after dehorning (P=0.02) but meloxicam-treated calves tended to tolerate more force at 8 h after dehorning (P=0.07) (designated with *).



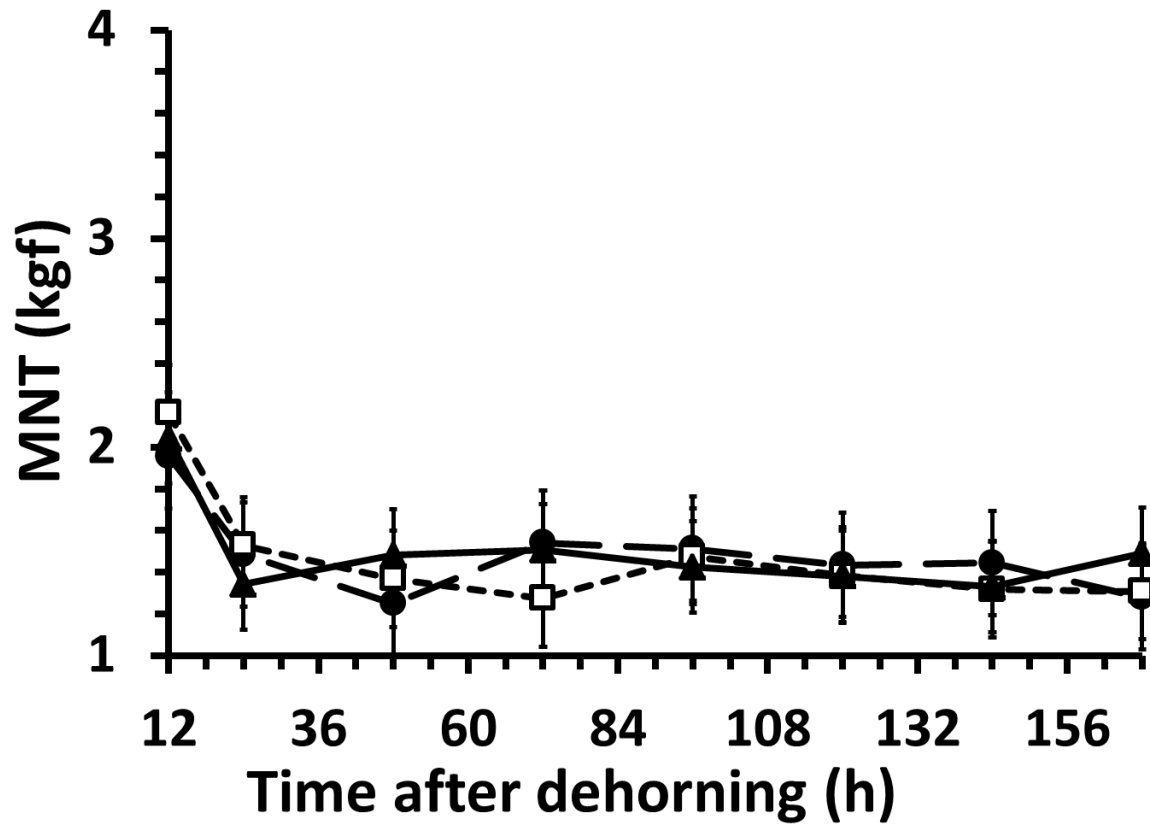


Figure 2.7. Mean \pm SEM of the plasma meloxicam concentration after oral administration of 1 mg/kg dissolved in milk replacer and administered 12 h before dehorning (Arrow) in the MEL-PRE calves.

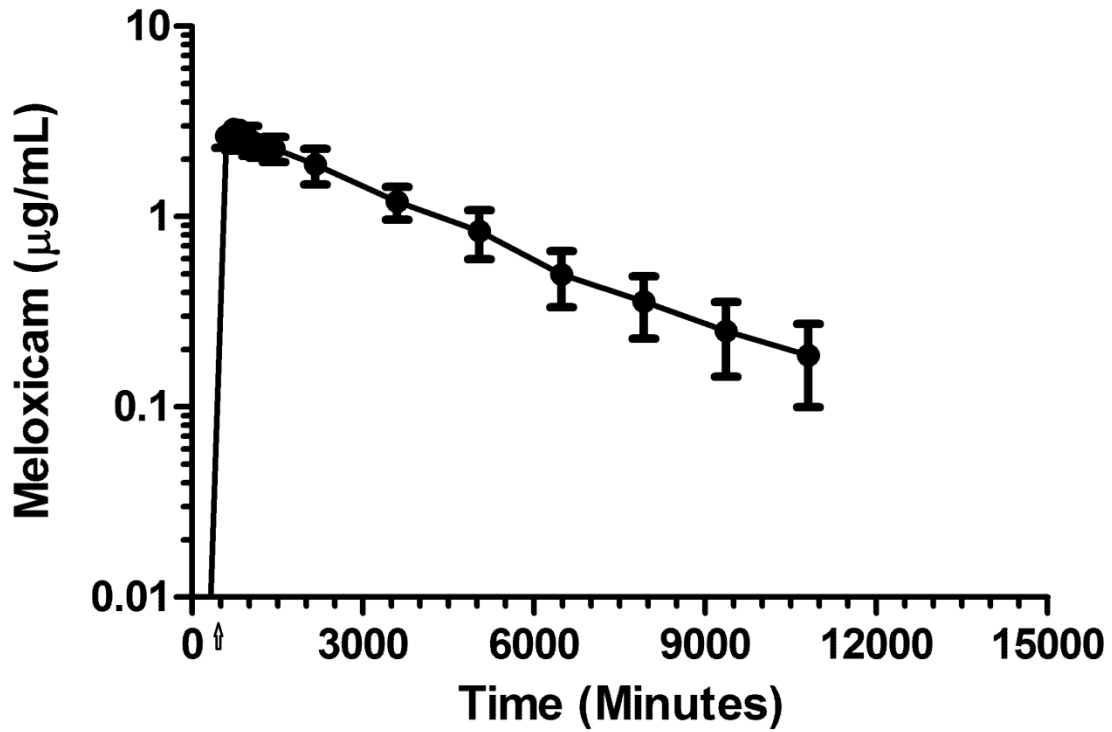
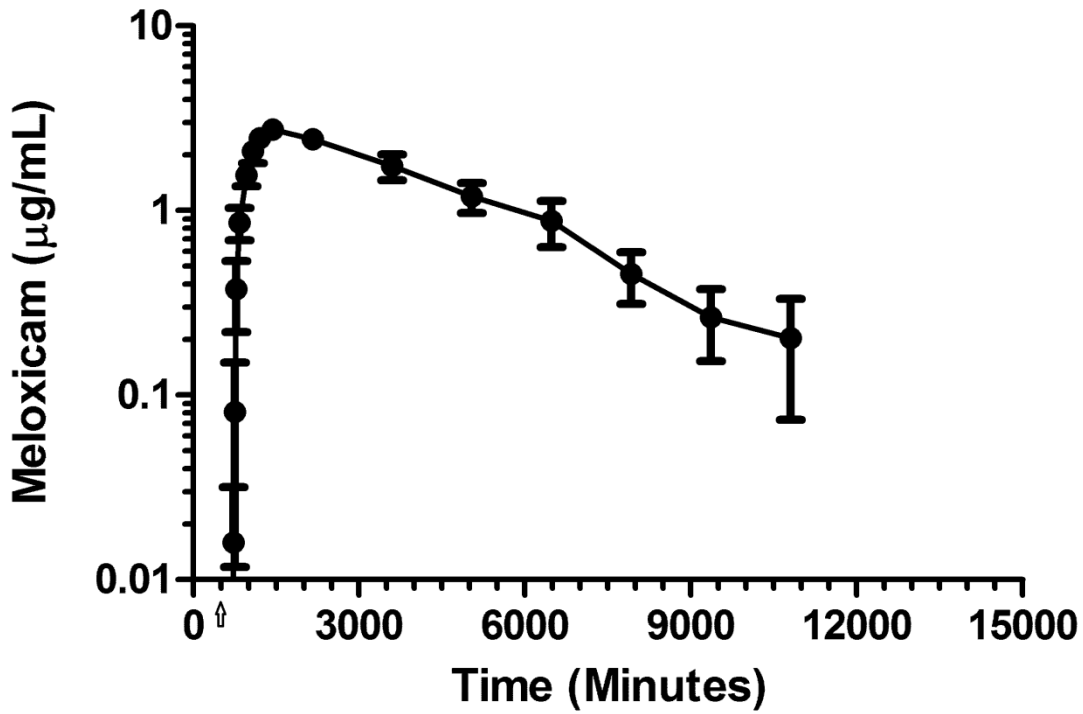


Figure 2.8. Mean \pm SEM of the plasma meloxicam concentration after oral administration of 1 mg/kg administered at the time of dehorning (Arrow) in the MEL-POST calves.



Tables

Table 2.1. Mean \pm SEM meloxicam pharmacokinetic parameters in dairy calves dehorned (n=10/group) with meloxicam administration 12 h before dehorning (MEL-PRE) and meloxicam administered at the time of dehorning (MEL-POST). P-values represent statistical comparison of the MEL-PRE and MEL-POST pharmacokinetic parameters.

| Parameter | Group | Mean \pm SEM | SD | Lower 95% | Upper 95% | P Value |
|--------------------------------------|----------|--------------------|--------|-----------|-----------|---------|
| $T_{1/2\lambda z}$ (h) | MEL-POST | 35.81 \pm 6.26 | 19.79 | 21.65 | 49.97 | P=0.488 |
| | MEL-PRE | 38.62 \pm 5.87 | 17.61 | 25.08 | 52.16 | |
| T_{max} (h) | MEL-POST | 15.0 \pm 2.17 | 6.85 | 10.10 | 19.90 | P=0.838 |
| | MEL-PRE | 13.95 \pm 1.05 | 3.14 | 11.54 | 16.37 | |
| C_{max} (ug/mL) | MEL-POST | 3.27 \pm 0.14 | 0.46 | 2.94 | 3.40 | P=0.191 |
| | MEL-PRE | 3.61 \pm 0.21 | 0.62 | 3.14 | 4.08 | |
| $AUC_{0\text{ to}\infty}$ (hr·ug/mL) | MEL-POST | 217.53 \pm 38.20 | 120.80 | 131.11 | 303.94 | P=0.653 |
| | MEL-PRE | 216.12 \pm 40.05 | 120.16 | 123.75 | 308.48 | |
| V_z/F (L/kg) | MEL-POST | 0.28 \pm 0.04 | 0.13 | 0.19 | 0.38 | P=0.775 |
| | MEL-PRE | 0.27 \pm 0.02 | 0.07 | 0.22 | 0.33 | |
| CL/F (mL/min/kg) | MEL-POST | 0.10 \pm 0.02 | 0.05 | 0.06 | 0.14 | P=0.967 |
| | MEL-PRE | 0.10 \pm 0.01 | 0.03 | 0.06 | 0.12 | |

Chapter 3 - Future Research Implications

Conclusions and Recommendations

There is significant research that suggests that dehorning is painful and further, new research indicating that there are techniques to successfully manage the pain. Still however, pain management is not considered necessary by many producers. With increasing concerns about animal welfare and countries worldwide adopting new rules and bans when dehorning calves, it is logical to question if the US should consider regulating the dehorning of calves. Mellor, however, suggests the banning approach has several disadvantages. First, further research may reveal a need to revise the ban, and thereby call into question the credibility of subsequent bans. Second, in being inflexible, a ban may alienate those who actually want to make improvements but cannot meet fully the new higher standard. Third, a ban becomes discredited if it is ignored and cannot be enforced (Mellor 1991). An alternative approach, making strong recommendations for or against the use of particular procedures and recommending that farmers make the greatest improvement that is economically and practically feasible for them, is likely to recruit more farmers into making some welfare improvements. When farmers cannot meet the highest standard immediately, this approach engages them directly in thinking about the welfare implications of dehorning. Their initially small changes are recognized as a good start and they feel good about them; that makes them more open to take further steps in the future as their own circumstances change and if consumers demand higher welfare standard. The use of polled beef cattle breeds and disbudding by cautery following local anesthetic of all dairy calves will improve the welfare of cattle dramatically. It is important that local anesthetic be used at all times when cattle are dehorned and that systemic analgesia be used whenever possible if wounds are not cauterized. The development of longer lasting and inexpensive systemic analgesics for use in cattle will improve the welfare of cattle significantly in the future (Stafford and Mellor, 2005).

There is scarce literature available focusing on pain management in cattle during dehorning. There have been several biomarkers of pain suggested including: plasma cortisol concentrations, PGE₂ concentrations, algometer measurements, and thermography. Although several analgesic regimens have been studied, meloxicam with a local anesthetic block has come to the forefront as a fast, effective, and inexpensive means to providing complete pain relief post

dehorning. However, until the drug obtains a label for use in cattle for analgesia, continued research will need to be performed in identifying a validated biomarker of pain in cattle. The study in Chapter 2, to the author's knowledge, is the first study to prove the analgesic effects of oral meloxicam post dehorning. As dehorning is a common practice in the U.S and there are currently no approved methods for extended pain relief following this procedure, the study in Chapter 2 can impact the development of analgesic drug regimens on livestock production systems. However, as previously stated, an FDA validated pain scale is necessary before any analgesic drug be approved for use in food animal. The study in Chapter 2 further provides evidence to suggest that PgE₂ concentrations may be a sensitive measure of NSAID effects in cattle. The data suggests that PgE₂ concentration is a sensitive biomarker for measuring the effect of NSAIDs that warrants further investigation.