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Comparison of oral non-steroidal anti-inflammatory drugs in cautery dehorned calves

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Comparison of oral non-steroidal anti-inflammatory drugs in cautery dehorned calves

by

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DEDICATION

To my parents who told me to never stop learning and always work hard. To my brother and his wife, Cam and Veronika, who remind me every day to be better and that perseverance has its rewards. To my wife, Laura, who always anchored me when adrift.

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NOMENCLATURE

A   Y-intercept for the distribution phase

A   distribution slope

ALP   alkaline phosphatase

AST   aspartate aminotransferase

AUC   area under the curve extrapolated to infinity

B   Y-intercept for the elimination phase

B   elimination slope

BUN   blood urea nitrogen

C_{max} maximum plasma concentration

CL   plasma clearance

CL/F   plasma clearance per bioavailability

COX   cyclooxygenase

F   bioavailability

GGT   γ-glutamyltransferase

IC_{50} concentration producing 50% of the maximum inhibition effect

IC_{80} concentration producing 80% of the maximum inhibition effect

K_{01} absorption constant for central compartment

K_{10} elimination constant for central compartment

K_{12} intercompartmental distribution rate constant from central to peripheral

compartment

K_{21} intercompartmental redistribution rate constant from peripheral to central

compartment NSAID: non-steroidal anti-inflammatory drug

PK/PD Pharmacokinetic / Pharmacodynamic

PSI   Pain-Stress Index

SEM   standard error of the mean

$T_{1/2\ K10}$ elimination half-life

$T_{1/2\alpha}$ distribution half-life

$T_{1/2\beta}$ terminal half-life

T_{max} time to maximum plasma concentration

V_{ss} volume of distribution in a steady state

V_c volume of distribution of the central compartment

V/F   volume of distribution per bioavailability

V_{area} volume of distribution during the elimination phase

ABSTRACT

Dehorning is a commonly performed husbandry procedure in cattle to limit injury and conform to modern facility design. Prior research provides evidence that dehorning results in increased nociception and stress through changes in behavioral, neuroendocrine, and physiological responses. Alterations in these actions allow investigators to evaluate pain and stress reducing practices. In addition to improved welfare strategies including institution of polled breeding programs and conducting the procedure on young animals, calves may benefit from the administration of analgesics including local anesthetics, non-steroidal anti-inflammatory drugs (NSAIDs), and α_2-agonists. Reviews of the literature have indicated a multimodal approach including local anesthetics and NSAIDs may be the optimal strategy to mitigate the negative response following dehorning. Since no analgesic products are currently labeled for cattle in the United States, providing an appropriate and effective analgesic remains challenging. As such, we have studied the pharmacokinetics and clinical efficacy of different NSAIDs in calves undergoing cautery dehorning. Initially, we evaluated the pharmacokinetics and clinical efficacy of firocoxib following cautery dehorning in calves. Although this NSAID was well absorbed orally in calves and inhibited prostaglandin for 48 h compared to placebo treated controls, minimal analgesic effects were observed using a study dose of 0.5 mg/kg. Subsequently we evaluated the pharmacokinetics and clinical efficacy of carprofen in a similar cautery dehorning study. Using the approved anti-inflammatory dose of 1.4 mg/kg in the European Union, oral carprofen was well absorbed and moderately inhibited prostaglandin for up to 96 h, however minimal analgesic effects

were observed. Following descriptions of the pharmacokinetics and effects of oral

carprofen and firocoxib, a comparison of four NSAIDs (carprofen, flunixin meglumine,

firocoxib, and meloxicam) orally administered at 2.0 mg/kg was conducted as a field

trial. Although responses indicative of pain and stress reduction varied among the

treatment groups, evidence from the field trial indicate meloxicam may have superior

potency compared to the other evaluated NSAIDs. Moreover, the use of oral meloxicam

provides optimal analgesia for 24 h following a one-time dose of 2.0 mg/kg.

CHAPTER 1

GENERAL INTRODUCTION

Introduction section modified from a manuscript published in *Veterinary Clinics of North America: Food Animal Practice*[1]

Dissertation organization

The following dissertation is constructed using the alternate thesis organization guidelines. The second chapter is a literature review of pertinent methods used to evaluate pain and stress in calves as well as a summary of previously studied analgesics following cautery dehorning. Its content is primarily derived from a previously published chapter in *Veterinary Clinics of North America: Food Animal Practice*, "Bovine dehorning: Assessing pain and providing analgesic management." Additional contributions to this chapter can be found in another *Veterinary Clinics of North America: Food Animal Practice* chapter, "Clinical Pharmacology of analgesic drugs in cattle." The third chapter describes the pharmacokinetics of oral and intravenous firocoxib in calves, which is published in the *Journal of Veterinary Pharmacology and Therapeutics*. The fourth chapter details the analgesic effects of oral firocoxib following cautery disbudding, which has been accepted for publication in the *Journal of Dairy Science*. Chapter 5 describes both the pharmacokinetics and clinical efficacy of carprofen administered orally and subcutaneously immediately prior to cautery dehorning. This paper has been submitted to the *Journal of Animal Science* for review. In chapter 6, we

[1] Stock ML, Coetzee JF. Clinical pharmacology of analgesic drugs in cattle. Vet Clin North Am Food Anim Pract. 2015;31:113-38.

compared the clinical efficacy of carprofen, flunixin meglumine, firocoxib, and meloxicam on pain and stress associated with cautery dehorning in a field trial. This chapter has been prepared for submission to the *Journal of Animal Science*. Chapter 7 details the derivation of a pain-stress index, which compiles and analyzes the responses reported in chapter 6 using one summary variable. A summary of the results including future areas of research are proposed in chapter 8.

Many contributions have been made by co-authors. Johann (Hans) F. Coetzee is the major professor of this dissertation and was involved in all aspects of the study including study design and subsequent analysis. Suzanne Millman was involved in study design, data collection and analysis. In addition to overall guidance on laboratory sample analysis, immunoassay development and analysis were derived by Walter Hsu. Laura Barth provided expertise in data collection, study design, and study logistics. Chong Wong provided the statistical model for statistical analysis. Larry Wulf provided sample analysis using LC-MS and guidance on additional laboratory analysis. Nick Van Engen, Erica Voris, Rebecca Parsons, Lea Labeur conducted sample collection and input in study design. Sarah Baldridge and Dee Griffin assisted in the construction of the literature review including in figure and table preparations.

Introduction

Pain states in cattle are common. Both iatrogenic pain due to livestock management procedures such as dehorning or castration and disease-associated pain including lameness, abdominal disorders, or sepsis are frequently encountered. Regardless of the origin, a noxious insult is typically translated into a chemical and

electric signal where it is modulated in the dorsal horn of the spinal cord and perceived in the brain (Muir and Woolf, 2001; Gottschalk and Smith, 2001). This initial phase is often associated with acute pain; however, a second prolonged and diffuse phase often results in local hypersensitivity (Gottschalk and Smith, 2001). Persistency of this delayed response may cause systemic hypersensitivity known as central sensitization ("wind-up" pain)(Gottschalk and Smith, 2001; Kissin, 2000) clinically manifesting as hyperalgesia (i.e. increased pain from a painful stimulus) and allodynia (i.e. pain from a non-painful stimulus) (Ochroch et al., 2003). Analgesics are provided, if possible, to mitigate both the acute and prolonged phases of pain associated with the noxious stimuli.

When evaluating pain relievers such as non-steroidal anti-inflammatory drugs (NSAID) for clinical analgesia, the Food and Drug Administration's Center for Veterinary Medicine (FDA-CVM) recommends effectiveness be determined by the control of clinical signs of pain associated with a disease (Guidance for Industry #123, 2006). Although the pain state is iatrogenically induced, evaluation of an analgesic in a post-dehorning model may conform to the recommendation by the FDA-CVM due to the frequency of the procedure performed in the dairy industry. Furthermore, demonstrating an analgesic effect using a pain state induced from a common industry practice will support its adoption and use in clinical practice compared to more controlled model of inflammation such as a subcutaneous deposition of formalin. It is important to note that in order to determine analgesic efficacy, GFI #123 recommends the use of validated methods of pain assessment in the target species.

Pain associated with dehorning results in both the described acute and delayed responses. Following the initial noxious insult either from a surgical incision (e.g. Barnes

dehorners) or cautery, stress as determined by cortisol concentrations peaks approximately 30 minutes post procedure (Stafford and Mellor, 2005a,b) Subsequently, a delayed response occurs as evidenced by increased sensitivity and behavioral, physiological, immunological changes that may persist for up to 44 hours (Coetzee, 2013; Stafford and Mellor, 2011; Heinrich et al, 2010). Recently, both Ballou and colleagues (2013) and Sutherland and colleagues (2013) reported that amputation dehorning results in a more acute painful response compared to surgical castration as determined by cortisol concentrations obtained under the same experimental conditions. Furthermore, dehorning 2 to 3 weeks post-castration resulted in an increased stress response and decreased ADG compared to animals that were amputation dehorned first and later castrated (Mosher et al., 2013).

Providing analgesia to cattle is not without its challenges. Primarily, from a regulatory perspective, no analgesic drugs are specifically approved for the alleviation of pain in livestock (Smith et al., 2008). The Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994 permits extra-label drug use (ELDU) in order to relieve suffering in cattle (FDA, 1994). As such, analgesics would be permitted under AMDUCA given the criteria for ELDU are followed. In addition to these regulations, pain medications can be costly, difficult to administer, short-acting requiring frequent administration, and controlled substances necessitate a veterinary license (Coetzee, 2013). Moreover, cattle do not overtly demonstrate signs of pain, making it difficult for some producers to observe the value in providing pain relief especially given a lack of support for economic gain (Coetzee, 2013).

In spite of these challenges, analgesics have demonstrated benefits to cattle during pain states. Following dehorning, analgesics have reduced the physiologic, behavioral and neuroendocrine changes that occur subsequent to the noxious stimuli (Stock et al., 2013). Although, translation of these responses into economic gains continue to be difficult, activation of the sympathetic nervous system has demonstrated immunosuppressive effects that may incite and progress systemic infections (Wong et al., 2011). Although this relationship has not been elucidated in cattle, it may offer an explanation of the reduced pull-rate and bovine respiratory disease morbidity for cattle receiving meloxicam following castration upon arrival to a feedlot (Coetzee et al., 2012).

Previous comprehensive reviews of the literature have reported that effective analgesia in cattle is achieved through a multimodal approach (Stafford and Mellor, 2005b; Stock et al., 2013). Administering different pharmaceuticals to antagonize or attenuate the transmission, modulation and perception of the pain signal optimizes pain relief (Muir and Woolf, 2001). Furthermore, the provision of a combination of compounds with different onsets and duration of action addresses both the acute and delayed phases of pain. With a multimodal approach, effective drug concentrations may be optimized to coincide with the occurrence of pain. Analgesia is maximized by combining local anesthesia, nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, α2-agonists, *N*-methyl *D*-aspartate (NMDA) receptor antagonists, and neuropathic pain antagonists. These analgesic options are reviewed in Chapter 2 with special attention towards evidence-based compounds investigated with randomized, placebo controlled trials (RCT).

The conclusions from reviews strongly support the use of both an NSAID and local anesthetic at the time of or prior to dehorning to mitigate the associated distress and nociception (Stafford, and Mellor, 2011; Stock et al., 2013). Given the positive results of a study treating dehorning pain with meloxicam, an NSAID with a prolonged half-life in cattle, I worked under the hypothesis that a persistently acting NSAID would be the most useful in providing pain relief to calves immediately post-dehorning (Heinrich et al., 2010). Moreover, oral medications were investigated as their potential to be the most practical on-farm method of analgesic administration.

Scant information is publically available evaluating oral NSAIDs in cattle. As such, I investigated NSAIDs available in the United States with oral formulations in other species with reported persistent concentrations or effects. Firocoxib is a COX-1 sparing NSAID available in both dogs and horses to treat osteoarthritis with once daily dosing as a result of a lengthy half-life in these species. To determine if similar pain relieving effects and prolonged concentrations were observed in cattle, the pharmacokinetics were determined and described in chapter 3 and the analgesic efficacy results of a preliminary dehorning study are detailed in chapter 4.

In addition to firocoxib, the pharmacokinetics of intravenous and subcutaneous carprofen in calves indicates a half-life greater than 40 hours (Delatour et al., 1996). In addition, it is approved for the treatment of pain associated with osteoarthritis in dogs. Given the reported pharmacokinetic properties in calves and analgesic effects in dogs, oral carprofen was compared with a subcutaneous dose in a preliminary dehorning trial detailed in chapter 5. Included in this chapter is a description of the pharmacokinetics of subcutaneous and oral carprofen.

Following the preliminary studies of firocoxib and carprofen in calves, I compared the clinical efficacy of carprofen, firocoxib, flunixin meglumine, and meloxicam to placebo treated controls following dehorning in a field trial. This study is presented in Chapter 6. Previous studies have evaluated the oral pharmacokinetics of meloxicam and flunixin meglumine in calves (Mosher et al., 2012; Odensvik, 1995). Moreover, the analgesic effects of meloxicam have been reported in prior dehorning studies (Henrich et al., 2010; Allen et al., 2013). The primary purpose of this study was to potentially determine the optimal NSAID administered orally to control pain and distress following cautery dehorning in a field setting.

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CHAPTER 2

LITERATURE REVIEW

Modified from a combination of two manuscripts published in *Veterinary Clinics of North America: Food Animal Practice*[1][2]

Matthew L. Stock

Abstract

Dehorning or disbudding in cattle is performed for a variety of reasons including: safety for handling, decreased incidence of carcass wastage due to bruising, less feeding trough space needed, decreased risk of injury to other cattle, increased value of the animal, and fewer aggressive behaviors exhibited. There are a variety of dehorning methods employed to effectively remove cornual tissue from cattle including: amputation employing a cutting tool, cautery using a hot iron, and chemical application of a caustic paste. Pain associated with this procedure has been mostly evaluated through behavioral, physiological, and neuroendocrine changes following dehorning. In general, following dehorning, an immediate acute stress or painful response is observed during the first 30 min which plateaus for approximately 6 hours before returning to baseline 7 to 8 hours following the procedure; however additional behavior data has suggested a more prolonged period of pain. Analgesics including local nerve blockades, anti-inflammatories, and opioids have demonstrated an effective attenuation of the cortisol

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[1] Stock ML, Baldridge SL, Griffin D, Coetzee JF. Bovine dehorning: assessing pain and providing analgesic management. Vet Clin North Am Food Anim Pract. 2013;29:103-33.
[2] Stock ML, Coetzee JF. Clinical pharmacology of analgesic drugs in cattle. Vet Clin North Am Food Anim Pract. 2015;31:113-38.

response. Although local anesthesia aids in the reduction of a cortisol response in the immediate post-dehorning period, a delayed cortisol increase is typically observed most likely associated with the loss of anesthetic effect. Non-steroidal anti-inflammatory drugs have demonstrated continued effective analgesia following this period of acute cortisol rise as evidenced by the moderation of a prolonged cortisol response. Additionally, sedatives with analgesic properties have demonstrated a transient attenuation of the acute phase of pain associated with dehorning. Following a literature review, and similar to pain management of other routine procedures performed in cattle such as castration, a multimodal approach to analgesia is recommended for dehorning procedures including the use of a local anesthetic and anti-inflammatory.

Literature Review

Dehorning is a commonly performed practice in both beef and dairy cattle industries. Dehorning or disbudding in cattle is performed for a variety of reasons including: safety for handling, decreased incidence of carcass wastage due to bruising, less feeding trough space needed, decreased risk of injury to other cattle, increased value of the animal, and fewer aggressive behaviors exhibited (AVMA, 2012). Disbudding is a method of removing horns in calves up to around 8 weeks old when horn buds are 5 – 10 mm long (Stafford and Mellor, 2005). Once horns grow longer, they become attached to the underlying frontal sinus. For the purpose of clarity, all disbudding and dehorning will be collectively referred to as dehorning throughout the chapter.

Although cattle are naturally horned for protective purposes, modern commercial industries decrease the necessity of these defenses. Within these production systems, for

reasons listed above, cattle without horns can be more desirable. Horn growth is a genetically heritable autosomal recessive trait (Medugorac et al., 2012). Polled cattle, which are hornless animals, result from an autosomal dominance pattern, which has been shown recently to be a result of allelic heterogeneity of the polled locus (Medugorac et al., 2012). Since the polled genetic inheritance reflects that of an autosomal dominant inheritable trait, artificial genetic selection could result in the decline of this undesirable characteristic of intensively raised cattle. This artificial selection for polled cattle has been used in the beef industry to reduce horned calves by 58% from 1992 to 2007 due to producers breeding for polled animals (USDA, 2009a). However, this breeding selection has not translated into the dairy industry with a reported 94% of dairy operations in the United States still dehorning calves (USDA, 2009b). Breeding strategies have recently been modeled to determine the effect of increasing polled genetics within a dairy herd while evaluating the impact on genetic merit (Spurlock et al., 2014).

An increased societal concern and awareness for food animal welfare has influenced discussions regarding the humane treatment of livestock (Rollin, 2012). Routine procedures in cattle such as dehorning can have a negative public perception. Consequently, several countries including those belonging to the European Union, Australia and New Zealand have created dehorning welfare legislation (Stafford and Mellor, 2005). In North America, The Canadian Code of Practice for Dairy Cattle recommends the use of a local anesthetic combined with analgesia and sedation for dehorning calves; however there are no current regulations for analgesic use in the United States (Bradley and MacRae, 2011). Although it should be noted that the AVMA "supports the use of procedures that reduce or eliminate the pain of dehorning and

castrating of cattle" and suggests that "available methods of minimizing pain and stress include application of local anesthesia and the administration of analgesics" (AVMA, 2008). Moreover, the American Association of Bovine Practitioners has recently released guidelines, which include the use of local anesthesia and systemic pain relief to enhance the welfare of cattle undergoing dehorning (AABP, 2014).

Survey evidence in the United States suggests that dehorning and castration are often performed together (92%) and usually completed without the use of analgesics (Coetzee et al., 2010). A survey of North-Central and North-Eastern United States dairy producers indicated that 12.4% use a local anesthetic nerve block and only 1.8% use systemic analgesia at the time of dehorning (Fulwider et al., 2008). Additionally, in another survey of United States veterinarians, 49% reported administering an analgesic to beef cattle less than 6 months of age during dehorning whereas 63% of the respondents used analgesics in dairy cattle at dehorning calves of the same age (Fajt et al., 2011). A Canadian survey indicated that approximately 72% of veterinarians provided analgesia at the time of dehorning calves (Hewson et al., 2007). Interestingly, additional positive influences for providing analgesia to calves at dehorning included geography where significant public outreach for animal welfare has occurred (Hewson et al., 2007).

The use of analgesics such as local anesthesia, systemic anti-inflammatories, and sedatives have been investigated by several studies following dehorning using behavioral, physiological, and neuroendocrine responses for pain assessment (Stafford and Mellor, 2011). Generally, when using cortisol concentrations as an indicator of stress, evidence exists of a rapid cortisol increase following dehorning peaking within the first 30 minutes. Cortisol concentrations then plateau from 1-6 h and then decline returning to

baseline 7 to 8 h following dehorning (Figure 1). Local anesthetics mitigate the cortisol response for their respective duration of action (i.e. lidocaine: 2 h; bupivacaine: 4 h) following the procedure but a delayed cortisol response is observed presumably once sensitivity returns to the anesthetized area (Petrie et al., 1996; McMeekan et al., 1998; Sutherland et al., 2002). Anti-inflammatories have aided in the reduction of this delayed cortisol response (Sutherland et al., 2002; McMeekan et al., 1998; Stilwell et al., 2009; Milligan et al., 2004; Allen et al, 2013; Glynn et al., 2013). Additionally, the use of sedatives may contribute to the reduction of the initial cortisol response; however the delayed inflammatory response is unaffected (Stafford et al., 2003; Stilwell et al., 2010). Most studies evaluating stress and pain responses in dehorned cattle investigate the acute response; however very few studies have examined chronic pain or stress responses following dehorning.

Sufficient challenges to accurately assess and manage pain in food animals exist within the United States. The following review will assess tools specifically utilized in the evaluation of the effectiveness of pain relief following dehorning. Additionally, supportive evidence evaluating analgesics will be detailed. Studies included in this analysis were identified as those that addressed the pain associated with dehorning using either analgesic-treated or placebo-treated controls. Pain responses determined in these studies were used to determine a percent change associated with a drug treatment (Table 1 and 2). The numerical values of the biomarker used for comparison included: maximum concentration (C_{max}), area under the effect curve (AUEC), and specific time point concentrations. Additionally, these values were further summarized following categorization by analgesic regimen when applicable (Figure 2 and 3). Of the nineteen

dehorning studies included in these summary graphs, seven trials were explicitly stated as masked. Additional discussions of studies with an undocumented masking status have been included as descriptive data.

Following this summary analysis, a multimodal approach using local anesthetics and non-steroidal anti-inflammatory drugs is recommended for the most effective reduction of pain response in cattle following dehorning. These recommendations are similar with other reviews concerning the management of pain in cattle following dehorning (Stafford and Mellor, 2011).

Methods of assessment

The study of pain and stress in animals is complex. Investigations into the treatment of a noxious event remain challenging due to our continued poor assessment of the pain response (Flecknell, 2008). Furthermore, studies of analgesics may prove circular as "analgesics are those substances that eliminate signs of pain which are those signs eliminated by analgesics" (Bateson, 1991). Nevertheless, evaluation of several pain indices has been suggested to improve pain assessment in animals (Molony and Kent, 1997). Through a combination of responses evaluating the behavior, autonomic nervous system (heart rate, ocular temperature), the hypothalamic-pituitary-adrenal-axis (cortisol), and tissue sensitization (mechanical nociception threshold) leading to release of pain neurotransmission (substance P), several indices can be measured to collectively assess the pain in animals following dehorning. Evaluating the combination of multiple pain-related indices potentially allows the induced pain state (i.e. cautery dehorning) to be examined not merely as reflexive nociception but as a response involving cerebral and

emotional perception. In addition, the use of a variety of responses may help to account for the individual variations of responses typically observed in pain and stress studies.

Behavior

Behavior changes are often monitored and recorded in studies involving pain and stress. The observed changes have been suggested as a more sensitive marker for pain compared to other physiological markers such as cortisol (Anil et al., 2002). Behavior indices have been recorded using videography (Graf and Senn, 1999; Milligan et al., 2004; Morisse et al., 1995; Faulkner et al., 2000; Vickers et al., 2005; Heinrich et al, 2010; Doherty et al., 2007; Duffield et al, 2010), chute behavior (Baldridge et al.,2011), accelerometers (Theurer et al.,2012), and remote triangulation devices (Theurer et al., 2012). Head shaking, ear flicking, head rubbing, transition between standing and lying, inert lying, vocalization, and grooming are all behavioral changes frequently recorded in an ethological evaluation of cattle following dehorning (Grøndahl-Nielsen et al., 1999; Stilwell et al., 2009; Morisse et al., 1995; Faulkner and Weary et al., 2000; Vickers et al., 2005).

Although behavioral responses can be objective and collected with reliable quantitative methods, differences may exist in regard to interpretation. In the case of dehorning, reliable behavioral indicators of pain to assess cattle following dehorning with and without analgesia have been examined (Faulkner and Weary, 2000; Heinrich et al., 2010; Duffield et al., 2010). For example, it has been suggested that head rubbing may be a result of increased nociception or indicative of irritation, itching or healing (Duffield et al., 2010). A correlation between behavior responses and changes to cortisol

concentrations has been reviewed supporting a strong association (Stafford and Mellor, 2005).

Recently, Heinrich and colleagues evaluated the behavior changes following cautery dehorning (Heinrich et al., 2010). Based on behavioral changes, the authors determined that pain may be present for up to 44 h following the procedure, which corresponded to the end of the study period. Other studies have indicated a continuation of painful or decreased normal behaviors for up to 72 h after dehorning (Stafford and Mellor, 2011; Faulkner and Weary, 2000). The duration of pain observed in these studies beyond other physiologic or neuroendocrine parameters further supports the necessity to provide long-lasting effective analgesia for cattle following dehorning. Additional research should include investigations into chronic pain responses associated with dehorning.

Physiology

Changes in physiology response are frequently observed following dehorning. Serum cortisol, heart rate, respiratory rate, and average dairy gain (ADG) are often used in studies evaluating the efficacy of analgesics in painful or stressful procedures such as dehorning. Cortisol concentrations should be interpreted with caution due to variations in cortisol response following a stressor as well as a wide variety of inciting causes that can activate the hypothalamus-pituitary-adrenal (HPA) system responsible for cortisol release (Molony and Kent, 1997; Mellor and Stafford, 1997). However, cortisol changes over time have been used frequently as a parameter assessing stress in cattle following

dehorning (Graf et al., 1999; Petrie et al., 1996; Sutherland et al., 2002; Stilwell et al., 2008; Allen et al., 2013).

In cattle dehorned without analgesia, most studies indicate an initial peak in cortisol is observed within the first 30 minutes and subsequently plateaus at an elevated concentration until returning to baseline approximately 7 to 8 h following the procedure (Sylvester et al., 1998; Schwartzkopf-Genswein et al, 2005; Stafford and Mellor, 2011). It has been hypothesized that the initial peak in cortisol is a result of a significant noxious nociception due to the removal of the horn tissue whereas the observed plateau results from pain associated with inflammation (McMeekan et al., 1998). The anti-inflammatory potential of cortisol has been suggested to result in the attenuation of the inflammatory mediated pain response (McMeekan et al., 1998; Sutherland et al., 2002).

Cortisol responses may vary with age. In a study investigating the analgesic effects of ketoprofen in dehorned calves 2 days to 2 weeks of age, older calves within the study had significantly lower serum cortisol concentrations pre and post dehorning (Milligan et al., 2004). In contrast, Dockweiler and colleagues (2013) determined that cortisol concentrations were elevated in 6 month old calves compared to $\leq$ 8 week old calves following castration. Although the insult differed, age may be of consideration in evaluating studies using different aged subjects.

Heart rate has been monitored and recorded in dehorning studies as an indicator of physiological stress (Grøndahl-Nielsen et al., 1999; Heinrich et al., 2009; Stewart et al., 2008; Stewart et al., 2009; Coetzee et al., 2012). Compared to either sham dehorned calves, calves included in the study, which were not dehorned, or baseline values, heart rate remained elevated for at least 3 h in dehorned calves receiving no analgesia

(Grøndahl-Nielsen et al., 1999; Stewart et al., 2009; Heinrich et al., 2009). Additional studies have indicated a decreased heart rate acutely following treatment with an analgesic compared to dehorned calves treated with a placebo (Stewart et al., 2009; Coetzee et al., 2012).

Previous literature has provided equivocal data for an increased average daily gain (ADG) following dehorning with analgesia. Studies have indicated an increased time spent at the grain feeder and an increase ADG following the use of a non-steroidal anti-inflammatory at the time of dehorning when compared to those not treated with any analgesia (Faulkner and Weary, 2000; Heinrich et al., 2010; Baldridge et al., 2011; Coetzee et al., 2012; Glynn et al., 2013). Although Grøndahl-Nielsen and colleagues (1999) did not observe any difference in ADG or feed intake in the 7 days following dehorning, there was a significant difference in animals treated with analgesics at the time of dehorning initiating rumination more quickly than those without analgesics provided. This improved rumination has also been reported in other studies with extended observation periods (Sylvester et al., 2004).

Other physiologic parameters have been evaluated in cattle following dehorning indicating a pain response (Table 3). Plasma adrenocorticotropic hormone (ACTH), vasopressin, noradrenaline, and adrenaline concentrations increased acutely following dehorning and remained elevated for up to 1 h (Graf et al., 1999; Mellor et al., 2002).

Neuroendocrine response

Neuroendocrine changes have been assessed in many studies evaluating nociception following dehorning including: substance P (Coetzee et al., 2012),

electrodermal activity (Baldridge et al., 2011), infrared thermography (Stewart et al., 2009), heart rate variability (Stewart et al., 2009), and electroencephalography (EEG) (Gibson et al., 2007).

Substance P is a neuropeptide expressed within portions of the neuroaxis involved with pain, stress, and anxiety (Coetzee, 2011). Increased concentrations are found in cattle following castration compared to those sham castrated, thus potentially supporting its use as biomarker of a pain response (Coetzee et al., 2008). A study investigated substance P concentrations following scoop dehorning in 16 to 20 week old calves treated with an anti-inflammatory at the time of dehorning (Coetzee et al., 2012). Animals treated with meloxicam had a significant reduction in mean substance P concentrations compared to the placebo treated controls following scoop dehorning. In addition, there were no significant differences observed in cortisol concentrations between the two groups, thereby suggesting an improved sensitivity of using substance P as a biomarker of pain in comparison with cortisol. It should be noted that substance P is a very unstable peptide requiring immediate processing which may be difficult to complete in field designed trials (Mosher et al., 2014).

Heart rate variability has been suggested to reflect a measurement of the autonomic nervous system through the assessment of sympathetic and parasympathetic activity, thus potentially providing an evaluation of distress (von Borell et al., 2007). Using heart rate variation, the control of the intervals between consecutive beats is increased through vagal tone (increased HRV, high frequency (HF) power) or sympathetic (decreased HRV, low frequency (LF) power). In 4-5 week old calves dehorned using a cautery dehorning unit, changes in heart rate variation illustrated a

sympatho-vagal imbalance coinciding with reported pain associated with dehorning (Stewart et al., 2009).

Additionally, a decreased ocular temperature has been suggested as a neuroendocrine response mediated by sympathetic vasoconstriction of conjunctival blood vessels upon the induction of pain (Stewart et al., 2008). Stewart and colleagues (2009) investigated ocular temperature concurrently with heart rate variability and reported a decrease in ocular temperature during the same time period as the changes in heart rate variations further supporting the sympatho-vagal imbalance. However, this response has not been reported by other dehorning studies using scoop or cautery methods (Glynn et al., 2013; Allen et al 2013; Stock et al., 2015)

Analysis of an EEG following a painful procedure has been validated for detection of acute pain in dehorning (Gibson et al., 2007). Acute noxious sensory stimuli produce changes within EEG frequencies reflecting the cerebral cortical electrical activity perceiving the nociception (Gibson et al., 2007). Mean EEG frequencies were evaluated in calves 24 to 36 weeks of age following amputation via scoop dehorning (Gibson et al., 2007). EEG frequencies were recorded following the induction of minimal anesthesia in the calves using intravenous ketamine and propofol. Although the use of injectable anesthesia may have confounded the results, specific wavelengths were significantly altered following dehorning indicative of noxious nociception. Animals in which a local nerve block was administered had significantly less changes in EEG frequencies potentially supporting the reported decreased nociception following dehorning compared to dehorned controls (Gibson et al., 2007).

Mechanical Nociception Threshold (MNT)

Following an acute injury, inflammatory mediators such as prostaglandins, bradykinin, serotonin, and histamine will accumulate at the site of injury. These molecular changes result in the increased nociceptor sensitivity associated with allodynia and hyperalgesia in pain states. This change in nociceptor sensitivity has been evaluated using different methods and techniques including pressure algometry for mechanical threshold determination or CO_2 laser or concentrated areas of heat for thermal threshold determination. Mechanical nociception thresholds have been investigated in rodent burn models indicating a prolonged recovery of up to 28 days to return to baseline nociception thresholds (Summer et al., 2007).

In cattle dehorning studies, MNT has been previously measured to evaluate an analgesic effect of NSAIDs (Heinrich et al., 2010; Glynn et al., 2013; Allen et al., 2013; Stock et al., 2015) and local anesthetics (Tapper et al., 2011). Heinrich and colleagues (2010) reported a 31% increase in MNT following administration of IM meloxicam. Moreover, Tapper and colleagues (2011) indicated up to 83 h of increased MNT following administration of an ethanol nerve block. Minimal effects on MNT were observed following administration of oral meloxicam (Glynn et al., 2013; Allen et al., 2013), intravenous flunixin (Glynn et al., 2013), and oral firocoxib (Stock et al., 2015). The tested dose or data collection, which included repeat handling and blood collection may have heightened the avoidance response during MNT determination potentially explaining the incongruity between studies.

Dehorning analgesic strategies and their effects on pain responses

Dehorning methods

Management practices have been adopted to dehorn animals in order conform to modern facility designs. There are three primary methods to dehorn cattle: 1) Amputation using equipment such as Barnes, Keystone, gauges, saws and gigli wire; 2) Cautery using a hot iron electrically, gas, or battery powered and 3) Chemical application of caustic paste usually consisting of a strongly alkaline agent such as sodium hydroxide or calcium hydroxide.

Several studies have evaluated the different dehorning techniques on relative changes in pain responses. Sylvester and colleagues (1998) compared the differences in cortisol concentrations in calves dehorned by 4 different methods of dehorning: Barnes scoop dehorning, guillotine shears, a butcher's saw, and embryotomy wire. This study found no differences among treatment groups during the 36 hours post dehorning for cortisol, except calves dehorned by guillotine shears had a significantly lower cortisol at 2 to 2.5 hours post procedure. The cortisol C_{max} and integrated cortisol response was not statistically different among treatment groups. Another study investigated differences in cortisol response to variations in performing the technique of scoop dehorning (McMeekan et al., 1997). Shallow scoop dehorning versus deep scoop dehorning were performed in 14 to 16 week old Friesian calves and no significant difference was found between rises in cortisol concentrations or the integrated cortisol response from 0.25 hours after dehorning to 5 hours after dehorning. The only difference noted was cortisol concentrations in calves undergoing shallow scoop dehorning returned to control values by 8 hours while deep scoop dehorning calves returned by 6 hours.

Several studies have investigated cautery as a means to destroy or remove tissue. In lambs, an attenuated cortisol response was observed following tail docking using a thermocautery device compared with a knife (Lester et al., 1991). It was suggested that the tissue damage caused by the heat from the hot iron destroyed the nociceptors adjacent to the wound, thus mitigating the cortisol response (Stafford and Mellor, 2005). This reported cortisol variation was also observed while comparing cautery dehorning with amputation. A study using scoop versus cautery dehorning by Petrie and colleagues (1996) using 6 to 8 week old Friesian calves found scoop dehorning without the provision of anesthesia or analgesia produced a significantly higher cortisol AUC from -70 minutes to 2 hours post procedure as compared to cautery dehorning. The examination of two different electric cautery dehorning methods using 3 to 4 week old Holstein calves indicated no difference in C_{max} (Wohlt et al., 1994).

Chemical dehorning methods have also been recently evaluated (Morisse et al., 1995; Vickers et al., 2005; Stilwell et al., 2008). Using behaviors such as head shaking, head rubbing, and lying to standing transitions, Vickers and colleagues (2005) determined that caustic paste resulted in less behavior changes compared with a hot iron in 10 to 35 d old calves treated with a sedative and a local anesthetic. However, in an earlier study, 4 week old calves dehorned with caustic paste had increased plasma cortisol concentrations in comparison with 8 week old calves dehorned using a hot iron (Morisse et al., 1995). It is reported that the application of the caustic paste is not painful; however within an hour both cortisol and behavioral changes indicate a pain or stress response persisting for up to 24 h following paste application.

Without the provisions of analgesics, it has been recommended to dehorn cattle with cautery rather than amputation or chemical methods (Stafford and Mellor, 2005). This conclusion was recommended as a result of an extensive review of the published literature indicating a decreased cortisol response in cautery dehorning; however it was suggested more research needs to be completed comparing the cautery and chemical dehorning methods (Stafford and Mellor, 2005).

Local anesthetics

The most commonly used analgesics in cattle are local anesthetics. By blocking sodium channels within nerve cells, the conduction and transmission of the pain signal is inhibited (Webb and Pablo, 2009). As such, the region where the anesthetic is deposited is devoid of sensation. Of benefit to the patient experiencing a noxious stimulus, stimulated nerves are more sensitive to local anesthesia. Moreover, nerves responsible for pain and temperature are blocked prior to those fibers involved with touch, pressure, and motor activity (Catterall and Mackie, 2011).

Although the observed effect is reversible, its duration of action depends on the length of contact time with the nerve (Catterall and Mackie, 2011). Therefore, innate chemical properties that determine rates of absorption and tissue distribution as well as metabolism are heavily involved in determining the length of desensitization. Moreover, the addition of vasoconstrictor compounds (e.g. epinephrine) to increase contact time through reduction of both absorption and metabolism may be beneficial (Catterall and Mackie, 2011). However, as local environment is crucial to the activity of a local anesthetic, infected tissues with a decreased pH may reduce the effect.

Most commonly performed nerve blocks consist of infiltrating the perineural space surrounding the cornual nerve, a branch of the zygomaticotemporal portion of the ophthalmic division of the trigeminal nerve, with a local anesthetic; however, other local nerve blocks have been used in dehorning studies such as ring blocks or caudal horn blocks in efforts to increase the likelihood the effective anesthesia (Figure 4) (Skarda, 1986; Graf and Senn, 1998; Doherty et al., 2007; Faulkner and Weary, 2000). The cornual nerve block has been described by several studies and textbooks. Briefly, a 2.5 cm 18 or 20 gauge needle is inserted lateral to the palpable temporal ridge of the frontal bone and 2.5 cm cranial to the base of the horn (Figure 4). Following a negative aspiration confirming the needle is placed subcutaneously, five to ten milliliters of 2% lidocaine is injected directing the needle towards the horn for desensitization of the area (Skarda, 1986). In cattle with larger horns, cutaneous branches of the second cervical nerve will need to be desensitized using a local anesthetic infiltration caudal to the horn (Skarda, 1986). Proper restraint is necessary in order to deliver the local anesthetic to the correct location for complete cessation of nociception (Figure 5). This procedure can be performed using plastic disposable syringes or automatic syringes for multiple animals (Figure 5).

Several administration routes are available for local anesthetics including injection via a needle or needle-free techniques and topical. When injected, the onset of activity for lidocaine is fairly rapid, occurring within 2 to 5 minutes and persisting for approximately 90 minutes (Coetzee, 2013). This duration is prolonged (median 304 min; range: 107-512 min) with the addition of epinephrine (0.01 mg/ml) (Fierheller et al., 2012). Additional injectable anesthetics such as bupivacaine may provide superior

duration of activity (5-8 h); however onset may be delayed (20-30 min) (Webb and Pablo, 2009). Recently, a gel-based topical local anesthetic consisting of lidocaine, bupivacaine, adrenaline (epinephrine) and cetrimide (Tri-Solfen, Bayer Animal Health, Australia) demonstrated desensitization of the dehorning area (Espinoza et al., 2013). Regional anesthesia via epidural administration of lidocaine (0.2 mg/kg) produces fairly rapid (5 min) desensitization of the perineal region for a time interval of 10-115 min (Muir et al., 1995).

The administration technique may contribute to the duration of activity. A recent study using 2-month-old dairy calves examined the efficacy of lidocaine with epinephrine using four local anesthetic delivery techniques including: cornual nerve block, ring block, percutaneous injection via a needle free drug delivery system (JET), and a topical eutectic mixture of local anesthetics containing 2.5% lidocaine and 2.5% prilocaine (EMLA) (Fierheller et al., 2012). Although the calves in the study were not dehorned, a peripheral variable output nerve stimulator was used to evaluate anesthetic efficacy. There was no difference in onset time between the cornual nerve block (2 min) compared to a ring block (3.25 min); however, the mean duration of the cornual nerve block was approximately 2.5 hours longer than that of the ring block (304 min vs 147 min, respectively). Both the JET delivery system and the EMLA cream failed to provide consistent, effective local anesthesia.

Sufficient evidence supports the use of a local anesthetic in reducing the stress response observed immediately following dehorning (McMeekan et al., 1998; Sutherland et al., 2002; Heinrich et al., 2009). Local anesthetics mitigate the cortisol response for their respective duration of action (i.e. lidocaine: 2 h; bupivacaine: 4 h) following the

dehorning procedure but a delayed cortisol response is observed presumably once

sensitivity returns to the anesthetized area (McMeekan et al., 1998; Sutherland et al.,

2002; Heinrich et al., 2009). In addition to cortisol changes, imbalances of the autonomic

nervous system are also reported. Heart rate variability and ocular temperatures indicate

an imbalance 2 – 3 hrs post dehorning with lidocaine administration, coinciding with the

expected loss of anesthetic activity (Stewart et al., 2009). Notably, average daily gain

(Grøndahl-Nielsen et al., 1999), heart rate (Grøndahl-Nielsen et al., 1999), immune

function (Doherty et al., 2007), and lying time (Morisse et al., 1995) were minimally

affected by administration of a only a local anesthetic

Lidocaine

Two percent lidocaine is the most commonly used analgesic in dehorning studies

(Table 1 – 3). Pharmacokinetics studies following an inverted L nerve block using a local

lidocaine infusion in mature cattle indicated a serum elimination half-life of 4.19 ± 1.69 h

(Sellers et al., 2009). Clinically, studies on assessing the analgesic duration of lidocaine

conclude an approximate 2 h duration based on both behavioral and physiological

changes (Stafford and Mellor, 2005). Although integrated cortisol concentrations are

typically not significantly different between cattle dehorned using local anesthesia

compared to non-treated controls, consistent cortisol changes are significantly reduced or

eliminated during the acute phase of the pain response (McMeekan et al., 1998;

Sutherland et al., 2002). Generally, once the desensitization associated with lidocaine

local infusion has diminished, cortisol concentrations significantly increase compared

with those dehorned without lidocaine (McMeekan et al., 1998; Sutherland et al., 2002).

Many studies look at the effects of nerve blocks on cortisol response to dehorning (Tables 1 - 3). Although few studies have indicated no difference in the pain or stress response following the provision of a local anesthetic prior to dehorning, most studies support the use due to a near elimination of the acute behavior and physiological changes that are typically observed (Stafford and Mellor et al., 2005). Graf and Senn (1999) found a cornual nerve block with 2% lidocaine significantly diminished the cortisol response in 4 to 6 week old calves as compared to those injected with saline from 20 to 90 minutes post dehorning. A study investigated the use of cautery following amputation dehorning and local lidocaine anesthesia in 20 to 24 week old calves (Sylvester et al., 1998b). The integrated cortisol response over a 9 h period indicated a significant reduction using lidocaine local anesthesia prior to amputation dehorning. In addition to the use of lidocaine anesthesia, cautery following amputation dehorning significantly diminished the cortisol response by 75% (Table 1).

The effects of scoop dehorning versus scoop dehorning with cautery, both with and without the addition of local anesthesia have been evaluated (Sutherland et al., 2002b). This study found calves undergoing dehorning had significant elevations in cortisol compared with control calves from 0.5 h to 6 h and then again at 13 to 15 h. Interestingly, while administering local anesthesia with lidocaine 15 minutes prior to the procedure and then with bupivacaine again at 1 h and 45 min post-procedure, a rise in cortisol concentrations from 0 to 5 h was abolished and calves experienced a significant increase in cortisol response that was greater than calves dehorned without anesthesia at 6 and 7 h. Calves receiving local anesthesia plus cautery in addition to scoop dehorning had almost no change in cortisol concentrations throughout the 24 h period measured.

Doherty and colleagues (2007) found that 10 to 12 week old Holstein calves experienced a significantly lower cortisol response at 30 and 60 minutes post dehorning after a cornual nerve block of either 10 mL of 5% lidocaine or 10 mL of 2% lidocaine administered 30 minutes prior to dehorning as compared to untreated, dehorned calves. No significant difference was noted between the 5% and 2% lidocaine solutions on cortisol response.

Interestingly, studies involving chemical dehorning indicated a decreased duration of analgesia efficacy using local lidocaine anesthesia alone (Stilwell et al., 2009; Vickers et al., 2005). Following dehorning using a caustic paste and local lidocaine anesthesia in 3 to 5 week old calves, behavioral signs of distress were attenuated for the first hour but then became evident for the next 5 h (Stilwell et al., 2009). It was hypothesized that the alkalotic paste may have increased the pH of the surrounding tissue thus affecting the equilibrium of the anesthetic solution and disrupting its function (Vickers et al., 2005).

Bupivacaine

In addition to lidocaine, bupivacaine has been used in several dehorning studies mostly due to a prolonged clinical duration of analgesia compared with lidocaine (McMeekan et al., 1998a; Sutherland et al., 2002a; Sutherland et al., 2002b). Clinical analgesic efficacy in one study was reported to be approximately 4 h as confirmed by a lack of behavioral reaction to a needle-prick of the skin adjacent to the horn (McMeekan et al., 1998a).

McMeekan and colleagues (1998a) evaluated the effect of timing of cornual nerve block administration using 0.25% bupivicaine on cortisol response in 3 to 4 month old

calves. They found calves administered a cornual nerve block at 20 min prior to

dehorning and then again 4 h post dehorning experienced a significantly lower cortisol

AUC than control calves dehorned without analgesia, calves administered the cornual

nerve block only at 20 min prior, and calves administered the cornual nerve block

immediately prior. Another study by McMeekan and colleagues (1998b) found that

calves undergoing scoop dehorning with a cornual nerve block using bupivacaine

administered 20 min prior and 4 h post had a significantly lower AUC from 0 to 9.33 h

for cortisol as compared to the calves dehorned with only a cornual nerve block 20 min

prior, immediately prior, or with no analgesia (McMeekan et al., 1998b). However for

the first 3.83 h, all calves receiving a cornual nerve block experienced a significantly

lower AUC cortisol response as compared to scoop dehorning without treatment.

Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs primarily inhibit cyclo-oxygenase (COX) isoenzymes subsequently

reducing the production of prostaglandins (PG) from arachidonic acid (Ochroch et al.,

2003). In addition to contributing to the inflammatory response through vasodilation and

inflammatory cell recruitment, PG, in concert with other cytokines and neuropeptides

decrease the action potential threshold in nociceptors and propagate the pain signal

(Grosser et al., 2011). Peripherally, this causes a local hyperalgesia or peripheral

sensitization. Moreover, COX isoenzymes present in the spinal cord produce excess PG

following acute noxious stimuli leading to central sensitization and chronic pain (Grosser

et al., 2011). This is clinically indicated by hyperalgesia and allodynia. Both isoenzymes,

COX-1 and COX-2, are thought to be responsible for the inflammatory response, with the

initial effects a result of COX-1 derived PG and the delayed effects due to upregulation

of COX-2 expression (Svensson and Yaksh, 2002). As such, NSAIDs that target both

isoenzymes may be advantageous for both immediate and prolonged pain; however,

increased inhibition of COX-1 is associated with renal and gastrointestinal adverse

effects (Grosser et al., 2011).

NSAIDs are typically weak acids with a low pK_a resulting in good oral

bioavailability in monogastrics (Lees, 2009). With the exception of firocoxib, NSAIDs

commonly used in veterinary species generally have a low volume of distribution most

likely due to the high plasma protein binding, which may impact overall tissue

distribution (Lees, 2009). Elimination can occur through the renal system following

metabolism in the liver; however, reports of biliary secretion leading to fecal elimination

is observed in other species (Lees, 2009; Rubio et al., 1980). Compounds known as

COX-2 selective or COX-1 sparing were developed to reduce the potential for adverse

effects associated with COX-1 inhibition; although evidence for an improved safety

profile in veterinary medicine is lacking. These molecules were designed with side chains

too large to bind to the smaller COX-1 active site, therefore only physically able to bind

COX-2 (Grosser et al., 2011). Table 4 summarizes the unique properties and

pharmacokinetics of NSAIDs available in the United States.

An extensive review of the literature suggests, in general, NSAIDs mitigate the

overall AUEC cortisol response by a greater magnitude than their ability to reduce peak

cortisol concentrations following dehorning (Figure 2 and 3). Moreover, NSAIDs alone

may not be adequate enough to control the associated distress post-dehorning (Figure 2

and 3); although this conclusion should be interpreted with caution as it is drawn from a

small number of studies. The analgesic effect of NSAIDs is amplified with the use of a local anesthetic for dehorning (Figure 2 and 3). Primarily, NSAIDs provide analgesia during the delayed phase of pain observed post-operatively in dehorning procedures.

Carprofen

Carprofen is an NSAID in the propionic acid class (Lees, 2009) and is administered as a racemic (50:50) mixture of RS± enantiomers (Delatour et al., 1996). For cattle in the European Union, carprofen is indicated as an adjunct to antimicrobial therapy associated with respiratory disease and mastitis. Prior pharmacodynamic studies evaluating analgesia in an inflammatory model in non-ruminants indicated carprofen to have a greater anti-inflammatory and analgesic potential compared to phenylbutazone and aspirin (Strub et al., 1982). Pharmacokinetics are presented in Table 4. Unique pharmacokinetic properties of carprofen in cattle include a prolonged half-life, slow clearance, and possibly biliary drug secretion as observed in dogs (Rubio et al., 1980). Interestingly, the pharmacokinetics of carprofen are age dependent with a prolonged half-life in younger animals (<10 weeks) most likely due to the decreased clearance common to neonates (Delatour et al., 1996).

Stilwell and colleagues (2012) report carprofen (1.4 mg/kg) in combination with a lidocaine block administered 15 minutes prior to cautery disbudding resulted in an attenuation of the acute cortisol concentration at 1 hour post-disbudding in comparison to placebo treated calves. However, this reduction of cortisol was transient as untreated controls had a reduced cortisol at 24 hours compared to calves receiving carprofen (Stilwell et al., 2012). A study conducted by our group evaluating 6 to 8 week old calves

either sham dehorned or cautery dehorned following administration of carprofen (1.4

mg/kg) subcutaneously, orally, or a placebo in a randomized controlled trial did not

indicate overall group treatment differences in measured analgesic response variables

including cortisol, substance P, mechanical nociception threshold, and ocular temperature

(Stock et al., 2014). However, calves receiving carprofen regardless of route tended to

tolerate more pressure around the horn bud for the 96 hour study duration compared to

placebo-treated controls (P=0.09) (Stock et al., 2014).

Firocoxib

Firocoxib is an NSAID of the coxib class. This is a newer group of NSAIDs

demonstrating COX-2 selectivity in dogs and horses, thereby potentially limiting adverse

effects caused by COX-1 inhibition (Lees, 2009). Currently, firocoxib is indicated for the

treatment of pain and inflammation associated with osteoarthritis for dogs and horses in

the United States. Limited information is known about firocoxib in cattle with only one

study conducted in preweaned calves (Stock et al., 2015). Pharmacokinetic parameters

for firocoxib are presented in Table 4. Unique pharmacokinetic properties in preweaned

calves include high oral bioavailability, prolonged terminal half-life, and an extensive

tissue distribution (high volume of distribution).

A RCT was conducted on 4 to 6 week old calves evaluating oral firocoxib (0.5

mg/kg) administered in combination with a lidocaine cornual nerve block administered

10 minutes prior to cautery dehorning (Stock et al, 2015). Although the acute effects of

cautery dehorning as determined by physiologic and nociception changes were unaffected

by treatment, firocoxib calves tended to have an overall reduced integrated cortisol

response compared to placebo-treated controls suggesting a potential role for firocoxib in the control of the delayed distress response.

Flunixin Meglumine

Derived from nicotinic acid in the anthranilic acid NSAID class, flunixin is the only FDA approved NSAID for cattle in the United States (Smith et al., 2008). Currently, flunixin is indicated for the control of fever associated with respiratory disease or mastitis, and fever and inflammation associated with endotoxemia. Pharmacokinetic properties are presented in Table 4. Given its anti-inflammatory properties, it has been evaluated as a pain reliever.

Flunixin administration provided benefits to calves during the acute distress and painful phase following dehorning. Flunixin meglumine (2 mg/kg) administered to calves following a cornual nerve block reduced cortisol concentrations for 3 and 6 hrs compared to untreated controls in calves undergoing chemical (Stilwell et al., 2009) and amputation dehorning (Ballou et al., 2013), respectively. Moreover, in addition to a local anesthetic, flunixin (2.2 mg/kg) administered to calves preoperatively and again 3 hours post cautery dehorning had a significantly reduced integrated cortisol concentration over an 8 hour study period with significant reductions at 0.5 and 2 hours compared to dehorned controls (Huber et al., 2013).   However, heart and respiratory rates were unaffected. The alleviation of the initial pain response is consistent with flunixin concentrations that suppress ex-vivo PGE_2 concentrations up to 12 hrs in calves surgically dehorned (Fraccaro et al., 2013).

In addition to analgesic effects observed in the acute pain period, cortisol concentrations and average daily gains were significantly improved over 7 days for flunixin (2.2 mg/kg) treated calves compared to untreated calves (Glynn et al., 2013). With IV administration, analgesic concentrations may have been rapidly achieved potentially reducing central sensitization; however, evidence for this effect in cattle is scarce (Stilwell et al., 2008). One study reported equivocal analgesic response in calves receiving an NSAID 12 hours prior to versus immediately before dehorning, indicating the pain response was not alleviated with pre-emptive analgesia (Allen et al., 2013). Interestingly, flunixin (2 mg/kg) combined with a cornual nerve block was protective against the observed leukocytosis post-amputation dehorning for up to 24 hours compared to untreated controls (Ballou et al., 2013). The persistence of effect may be due to an IM route of administration or as a result of a hysteretic response of certain immune mediators.

Ketoprofen

Ketoprofen, an NSAID of the propionic acid class, has EU and Canada approval as an adjunctive therapy for fever, pain, and inflammation associated with mastitis and inflammatory and painful conditions of bones and joints (USP, 2004). Pharmacokinetic properties are presented in Table 4. Ketoprofen is administered as a racemic mixture (50:50) with chiral RS± enantiomers. Interestingly, the R(-) enantiomer will undergo chiral inversion to S(+) which is clinically relevant since the S(+) enantiomer is a more potent PGE_2 inhibitor (Aberg et al., 1995). Given the short half-life (0.42 h) due to rapid metabolism and elimination, efforts to sustain analgesia may require multiple doses.

Nevertheless, many studies have investigated the analgesic potential of ketoprofen (Faulkner and Weary, 2000; Sutherland et al., 2002; McMeekan et al., 1998b; Milligan et al., 2004; Duffield et al., 2010).

Ketoprofen (3 mg/kg) administration in combination with a local anesthetic resulted in the amelioration of the acute cortisol response with effects persisting up to 5 hours compared to untreated controls (Sutherland et al., 2002; McMeekan et al., 1998b; Milligan et al., 2004; Duffield et al., 2010). With ketoprofen (3 mg/kg) administration without local anesthesia, the typically observed cortisol plateau was attenuated; however, peak cortisol concentrations were only mildly reduced (McMeekan et al., 1998). A tendency for improved weight gains following multiple doses and increased starter consumption has also been reported over a 24-hour period in ketoprofen treated calves compared to control calves (Duffield et al., 2010; Faulkner and Weary, 2000); although it is noteworthy that the analgesic regimen in these trials also included local anesthesia (Duffield et al., 2010; Faulkner and Weary, 2000) and xylazine (Faulkner and Weary, 2000) for all calves dehorned.

Meloxicam

Meloxicam is a member of the oxicam class of NSAIDs. It has approval for use in the European Union and Canada for adjunctive therapy of acute respiratory disease, diarrhea, acute mastitis, and as an analgesic to relieve pain following dehorning in calves. Moreover, meloxicam is approved in the United States to control pain associated with osteoarthritis in humans, dogs, and cats. The pharmacokinetics of meloxicam are presented in Table 4. Notably, the pharmacokinetics of meloxicam in cattle indicates a

prolonged half-life and a high bioavailability when administered orally (Coetzee et al., 2009). Due to these favorable pharmacology properties for providing practical analgesia in cattle, many studies have recently investigated oral meloxicam as an analgesic.

Several recent studies have provided support for the analgesic effect of meloxicam in cattle after dehorning or disbudding. Significant reductions in cortisol concentrations have been reported in cattle receiving a local anesthetic in combination with IM meloxicam (0.5 mg/kg) compared to placebo-treated cattle only receiving a lidocaine cornual nerve block (Heinrich et al., 2009). This effect persisted for up to 6 hrs post cautery dehorning (Heinrich et al., 2009). In contrast, another study reported no effects on cortisol concentrations following administration of only IV meloxicam (0.5 mg/kg) compared to placebo-treated controls immediately prior to surgical dehorning (Coetzee et al., 2012) Taken together, this provides additional support for the use of a multimodal approach to reduce the distress associated with dehorning. Furthermore, physiologic variables including heart rate (Heinrich et al., 2009; Coetzee et al., 2012), respiratory rate (Heinrich et al., 2009), and time spent standing (Theurer et al., 2012) were elevated in placebo-treated calves compared with those administered meloxicam. Maintenance of the autonomic nervous system including heart rate variability and reduced changes to ocular temperature was observed in 4-5 week old dairy calves receiving IV meloxicam (0.5 mg/kg) compared to placebo following cautery dehorning (Stewart et al., 2009). In addition, placebo treated calves are reported to be nearly twice as sensitive post-cautery disbudding compared with calves treated with IM meloxicam (0.5 mg/kg) (Heinrich et al., 2010). A reported reduction in substance P, a pain neurotransmitter, provides further support for the reduced pain sensitivity following

scoop dehorning in 4 month old calves treated with IV meloxicam (0.5mg/kg) compared with untreated controls. Production parameters were also improved in meloxicam treated cattle compared to placebo treated controls with an increased average daily gain (Coetzee et al., 2012) potentially due to increased feed consumption or time spent near the feeder (Heinrich et al, 2010; Theurer et al., 2012). In summary, IV or IM meloxicam (0.5 mg/kg) may effectively attenuate dehorning pain and distress.

More recently, oral (PO) meloxicam (1.0 mg/kg) has been evaluated in 8 to 10 week old calves at the time of cautery dehorning demonstrating reduced cortisol at 4 hours and substance P at 120 hours compared to placebo-treated controls (Allen et al., 2013). Furthermore, 6 month old calves undergoing amputation dehorning demonstrated improved ADG when treated with oral meloxicam (1.0 mg/kg) compared with placebo treated controls (Glynn et al., 2013). However, pain sensitivity, ocular temperature, and haptoglobin were not affected (Glynn et al., 2013; Allen et al., 2013). Oral meloxicam administration may exert a persistent analgesic effect as evidenced by concentrations significantly inhibiting ex-vivo production of prostaglandin E_2 for 48 hours compared to placebo-treated controls (Allen et al., 2013). This effect is more prolonged when meloxicam is administered at the time of dehorning compared with 12 hours prior (Allen et al., 2013).

Phenylbutazone

Although phenylbutazone is not approved for cattle in the United States and is illegal to use in dairy cattle greater than 20 months of age, historically, it has been used as an analgesic in cattle (Smith et al., 2008). Given its weak anti-inflammatory properties in

comparison with ketoprofen, there is little evidence to suggest its efficacy to reduce

dehorning pain (Lees et al., 2004; Lees et al., 2004). Calves treated with phenylbutazone

(4.0 – 5.3 mg/kg) in addition to local anesthesia did not show a significant attenuation of

the delayed cortisol response following return of sensitivity to the anesthetized area

(Sutherland et al., 2002a).

Salicylic Acid Derivatives

Both aspirin (acetylsalicylic acid) and sodium salicylate have historically been

used in cattle as anti-inflammatory, anti-pyretic and analgesic agents. Despite its common

use and label claims, the FDA Center for Veterinary Medicine has never formally

approved the drug (USP, 2004). As such, the use of salicylic acid derivatives should be

used with caution due to the lack of tissue residue studies, which provide withdrawal

intervals. The pharmacokinetics of salicylic acid derivatives (Table 4) are associated with

limited tissue distribution, slow oral absorption, and rapid elimination (Smith, 2013). Due

to the availability and previous practices, studies have evaluated its use as an analgesic.

Sodium salicylate metered in water (2.5-5 mg/ml) initiated 3 days prior to

simultaneous castration and dehorning of calves and continued for 2 more days, resulted

in improved average daily gains for 13 days as well as a decreased integrated cortisol

concentrations from 1 to 6 hours post-procedure compared to untreated controls

(Baldridge et al., 2011). These acute stress reduction effects did not persist past 6 hours;

however ADG was increased over a 13 day period (Baldridge et al., 2011). It is

noteworthy that water palatability may have been affected as cattle receiving salicylate

had decreased water consumption.

Sedative-analgesic drugs

Pharmaceutical agents such as α-2 agonists and opioids have been investigated to determine the potential effects on pain biomarkers following dehorning. Potential benefits of these analgesics including the attenuation of the acute cortisol response can aid in the reduction of prolonged handling stress associated with dehorning; however, there is no evidence of continued analgesia following this initial period (Figure 2 and 3) (Grøndahl-Nielsen et al., 1999; Stafford et al., 2003; Stilwell et al., 2010).

Alpha-2 Adrenergic Agonists

Alpha-2-adrenergic agonists produce a dose-dependent analgesic effect by inhibiting the amplification of norepinephrine (NE) release from the presynaptic nerve in the brainstem and spinal cord (Posner and Burns, 2009). Analgesia is achieved through this reduction of NE thereby inhibiting the afferent pain pathway (Posner and Burns, 2009). Adverse effects can include decreased cardiac output, a centrally mediated decreased respiratory rate, and depressed gastrointestinal motility. Pharmacokinetics of xylazine are detailed in Table 4. Given its common use in cattle, previous studies have evaluated its potential as an analgesic.

The response of xylazine administered perioperatively to calves being dehorned is temporary (Stafford et al., 2003). Compared to dehorned controls, the acute peak cortisol response is reduced in calves treated with IV xylazine (0.1 mg/kg); however, this effect does not persist past 3 hours (Stafford et al., 2003). With the addition of lidocaine to calves, the cortisol peak was further mitigated but still the same cortisol profile was

observed over time (Stafford et al., 2003). In contrast, Stillwell and colleagues (2010) reported no difference in cortisol concentrations in calves treated with IM xylazine (0.2 mg/kg) for the first hour post-disbudding compared to saline treated controls. Furthermore, in combination with local anesthesia and an opioid, no treatment effect was observed 10 minutes after dehorning in 4 to 6 week old calves (Grøndahl-Nielsen et al., 1999). The use of xylazine alone should be avoided for the development of dehorning analgesic protocols.

It is noteworthy that the use of an α-2 antagonist to reverse the sedative effects of an α-2 agonist administered 5 minutes after dehorning resulted in a significant increase in cortisol concentrations (Stafford et al., 2003). This response was greater than concentrations of those animals dehorned without analgesia and persisted for 8 hours; however, the AUEC was not significantly different (Stafford et al., 2003).

Tramadol

Tramadol (1RS, 2RS)-2[(dimethylamino-methyl]-1-(3-methoxyphenyl)-cyclohexanol is a centrally acting multimodal analgesic primarily used in humans and companion animals to treat mild to moderated pain (Reeves and Burke, 2008; Kukanich and Papich, 2011). Analgesia is suggested to be a result of a dual mechanism involving both opioid receptor activation and increased serotonin and norepinephrine transmission (Reeves and Burke, 2008). Although pharmacokinetic and pharmacodynamics values have not been determined in cattle, one study evaluated its anti-nociceptive potential following chemical dehorning in 3 week old dairy calves using an intravenous dose of 4 mg/kg or a rectal dose of 200 mg (Braz et al., 2012). Following an evaluation of pain

associated behaviors while using a numerical rating scale, tramadol administered at the investigated dosages and routes did not provide adequate analgesia for controlling pain related to chemical dehorning.

Neuropathic pain analgesic drugs

Gabapentin

Gabapentin is a γ-aminobutyric acid (GABA) analogue historically used as an anti-seizure medication. In addition, an improved management of chronic, neuropathic pain is reported. This is likely due to a decreased excitatory neurotransmitter release as a result of modulation of voltage-gated calcium channels (Taylor et al., 2009). Furthermore, analgesic activity can be enhanced with the addition of an NSAID due to a reported synergism (Hurley et al., 2002; Picazo et al., 2006). Pharmacokinetic properties are listed in Table 4. As control of chronic pain is challenging, gabapentin has been investigated to address this concern.

Gabapentin (15 mg/kg) alone or in combination with meloxicam (1 mg/kg) has demonstrated an increased average daily gain in 6 month-old cattle following scoop dehorning; however additional physiologic responses were not different compared with placebo-treated controls (Glynn et al., 2013).

Conclusions

The literature focusing on pain management in cattle during dehorning is plentiful. As demonstrated, there have been several studies looking at the effects of dehorning on plasma cortisol concentrations. Additionally, several analgesic regimens

have been used in efforts to relieve pain during these procedures, with varying results.

Following this review, the authors suggest a multimodal approach using local anesthetics

and NSAIDs to best provide analgesia to cattle following dehorning (Tables 1 – 3). Local

anesthetics aid in the attenuation of the immediate cortisol response and NSAIDs mitigate

the observed inflammation associated pain. As with all extra label drug use in food

producing species, valid Veterinary-Client-Patient-Relationships must be maintained and

appropriate withdrawal times must be followed. Further research should be implemented

to determine safe, long-lasting, and cost effective analgesics to food animals following

noxious procedures.

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Table 1. Summary of the scientific literature examining the effect of analgesic drug administration on plasma cortisol response in dehorned calves.

Reference	Procedure	Study Population	Analgesic Regiment	Outcome parameter	Percent change in cortisol (%)	Significance
Boandl, 1989	Cautery (electric) dehorning	7 -16 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml/horn), 5 min prior to dehorning	Cortisol (30 m)	-13.41	NS
Morisse, 1995	Chemical paste dehorning	4 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 4 ml/horn), 15 min prior to dehorning	Cortisol (1 h)	-19.34	NR
				Cortisol (4 h)	-57.26	NR
				Cortisol (24 h)	-6.45	NR
	Cautery (electric) dehorning	8 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 4 ml/horn), 15 min prior to dehorning	Cortisol (1h)	-18.03	NR
				Cortisol (4h)	234.48	NR
				Cortisol (24h)	101.56	NR
Petrie, 1996	Amputation (Scoop) dehorning	6-8 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 3 ml/horn), 20 min prior to dehorning	Cortisol (AUEC:0-2 h)	-53.64	<0.05
				Cortisol (AUEC:2-9.5 h)	53.94	NS
				Cortisol (AUEC:0-9.5h)	10.92	NS
	Cautery (gas) dehorning		Lidocaine local anesthesia (cornual nerve, 3 ml/horn), 20 min prior to dehorning	Cortisol (AUEC:0-2 h)	-23.37	NS
				Cortisol (AUEC:2-9.5 h)	22.78	NS
				Cortisol (AUEC:0-9.5 h)	2.28	NS

Table 1 cont.

McMeekan, 1998[18]	Amputation (Scoop) dehorning	12-16 weeks Dairy	Bupivacaine local anesthesia (cornual nerve, 6 ml/horn), 20 minutes prior to dehorning	Cortisol (AUEC:0-3.83 h)	-61.00	<0.05
				Cortisol (AUEC:3.84-9.33 h)	109.71	NS
				Cortisol (AUEC: 0-9.33 h)	-10.24	NS
			Bupivacaine local anesthesia (cornual nerve, 6 ml/horn),immediately prior to dehorning	Cortisol (AUEC:0-3.83 h)	-72.82	<0.05
				Cortisol (AUEC: 3.84-9.33 h)	47.35	NS
				Cortisol (AUEC: 0-9.33 h)	-37.07	NS
			Bupivacaine local anesthesia (cornual nerve, 6 ml/horn),20 min prior to dehorning & 4 h later	Cortisol (AUEC:0-3.83 h)	-80.69	<0.05
				Cortisol (AUEC: 3.84-9.33h)	31.97	<0.05
				Cortisol (AUEC: 0-9.33h)	-47.18	<0.05

Table 1 cont.

Sylvester , 1998[54]	Amputation (Scoop) dehorning	20-24 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 6 ml/horn), 30 min prior to dehorning	Cortisol AUEC	-50.48	<0.05
			Lidocaine local anesthesia (cornual nerve, 6 ml/horn), 30 min prior to dehorning, cauterize wound following amputation	Cortisol AUEC	-75.24	<0.05
Sutherland, 2002b	Amputation (scoop) dehorn	12-16 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 6 ml/horn) 15 min prior to dehorning; bupivacaine (cornual nerve, 6 ml/horn) 2 hours following lidocaine injection	Cmax	-6.57	NS
Sutherland, 2002a	Amputation (scoop) dehorn	12-16 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 6 ml/horn) 15 min prior to dehorning; bupivacaine (cornual nerve, 6 ml/horn) 2 hours following lidocaine injection	Cortisol AUEC (0-24 h)*	-13.14	NS
				Cmax	-6.57	NR
			Lidocaine local anesthesia (cornual nerve, 6 ml/horn), phenylbutazone 4.0-5.3 mg/kg, IV 15 minutes prior to dehorning; bupivacaine (cornual nerve, 6 ml/horn) 2 hours following initial lidocaine injection	Cortisol AUEC (0-24 h)*	-8.89	NS
				Cmax	2.92	NR
			Lidocaine local anesthesia (cornual nerve, 6 ml/horn), ketoprofen 3.0-3.75 mg/kg, IV 15 minutes prior to dehorning; bupivacaine (cornual nerve, 6 ml/horn) 2 hours following initial lidocaine injection	Cortisol AUEC (0-24 h)*	-21.41	NS
				Cmax	-52.55	NR

Table 1 cont.

Mellor, 2002	Amputation (scoop) dehorn	10 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml/horn) 20 minutes prior to dehorning	Cortisol (30 min)	-36.90	NR
Stafford, 2003*	Amputation (scoop) dehorn	12 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml/horn) & Ketoprofen 3mg/kg IV 15 minutes prior to dehorning	Cortisol AUEC	-55.17	NS
			Xylazine 0.1 mg/kg IV, 20 min prior to castration	Cortisol AUEC	3.45	NS
			Xylazine 0.1 mg/kg IV, 20 minutes prior to dehorning & lidocaine local anesthesia (cornual nerve, 5 ml/horn) 15 minutes prior to dehorning	Cortisol AUEC	6.90	NS
			Xylazine 0.1 mg/kg IV, 20 minutes prior to dehorning & lidocaine local anesthesia (cornual nerve, 5 ml/horn) 15 minutes prior to dehorning; tolazoline 2 mg/kg 5 min after dehorning	Cortisol AUEC	81.03	NS
Doherty, 2007*	Cautery (electric) dehorning	10-12 weeks Dairy	Lidocaine (2%) local anesthesia (cornual branch of zygomatic-temporal n., 3 ml/horn; cornual branch of infratrochlear n.; 4 ml/horn rostral to horn base)	Cortisol (30 min)	-25.00	<0.05
			Lidocaine (5%) local anesthesia (cornual branch of zygomatic-temporal n., 3 ml/horn; cornual branch of infratrochlear n.; 4 ml/horn rostral to horn base)	Cortisol (30 min)	-56.25	<0.05

Table 1 cont.

				Cortisol (1h)	0.37	NS
Stilwell, 2008	Chemical paste dehorning	1.5 - 6 weeks Dairy	Flunixin meglumine 2 mg/kg IV, 5 min prior to dehorning	Cortisol (3h)	-58.47	NS
				Cortisol (6h)	-52.08	NS
				Cortisol (24 h)	37.70	NS
			Flunixin meglumine 2 mg/kg IV, 60 min prior to dehorning	Cortisol (1h)	-8.08	NS
				Cortisol (3h)	-2.18	NS
				Cortisol (6h)	21.83	NS
				Cortisol (24 h)	96.48	NS
Stilwell, 2009	Chemical paste dehorning	3-5 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml/horn) 5 min prior to dehorning	Cortisol (10 m)	-25.18	NS
				Cortisol (30 m)	-59.63	<0.05
				Cortisol (50 m)	-65.19	<0.05
				Cortisol (1 h)	-47.51	<0.05
				Cortisol (3h)	-5.50	NS
				Cortisol (6h)	7.89	NS
				Cortisol (24h)	34.68	NS
			Flunixin meglumine 2.2 mg/kg IV & Lidocaine local anesthesia (cornual nerve, 5 ml/horn), 5 min prior to dehorning	Cortisol (10 m)	-9.40	NS
				Cortisol (30 m)	-50.06	<0.05
				Cortisol (50 m)	-53.21	<0.05
				Cortisol (1 h)	-77.68	<0.05
				Cortisol (3h)	-67.85	<0.05
				Cortisol (6h)	-24.64	NS
				Cortisol (24h)	-25.61	NS
			Lidocaine local anesthesia (cornual nerve, 5 ml/horn) 5 min prior to dehorning	Cortisol (90 m)	-42.47	<0.05
				Cortisol (120 m)	-51.26	NS
				Cortisol (150 m)	39.80	NS
				Cortisol (180 m)	59.19	<0.05

Table 1 cont.

				Cortisol (Cmax)	1.60	NS
Baldridge, 2011	Amputation (scoop) dehorning followed by surgical castration	2 - 4 month Dairy	Sodium salicylate at 2.5 to 5 mg/mL in the drinking water (13.62 to 151.99 mg of salicylate/kg bodyweight)	Cortisol AUEC (0 - 1h)	-9.27	NS
				Cortisol AUEC (1 - 6h)	-36.90	p<0.05
				Cortisol AUEC (6 - 24h)	-22.83	NS
			0.025 mg/kg butorphanol, 0.05 mg/kg xylazine, 0.1 mg/kg ketamine co-administered IM immediately prior to castration	Cortisol (Cmax)	-12.00	NS
				Cortisol AUEC (0 - 1h)	-28.90	p<0.05
				Cortisol AUEC (1 - 6h)	-5.82	NS
				Cortisol AUEC (6 - 24h)	-0.01	NS
			Sodium salicylate at 2.5 to 5 mg/mL in the drinking water (13.62 to 151.99 mg of salicylate/kg bodyweight) and 0.025 mg/kg butorphanol, 0.05 mg/kg xylazine, 0.1 mg/kg ketamine co-administered IM immediately prior to castration	Cortisol (Cmax)	-3.46	NS
				Cortisol AUEC (0 - 1h)	-20.89	NS
				Cortisol AUEC (1 - 6h)	-24.19	NS
				Cortisol AUEC (6 - 24h)	-15.69	NS
Coetzee, 2012	Amputation (scoop) followed with cautery (electric) dehorning	16-20 weeks Dairy	Meloxicam 0.5 mg/kg IV immediately prior to dehorning	Cortisol AUEC	-7.95	NS
				Cortisol (Cmax)	3.66	NS

Table 1 cont.

Ballou, 2013	Amputation (scoop) followed by cautery	3 mo Dairy	Lidocaine local anesthesia (cornual nerve and ring, 12 ml/horn) + flunixin meglumine 2 mg/kg IM immediately prior to dehorning	Cortisol (Cmax)	-42.97	$p<0.05$
Sutherland, 2013	Amputation (scoop) followed by cautery	3 mo Dairy	Lidocaine local anesthesia (cornual nerve and ring, 12 ml/horn) + flunixin meglumine 2 mg/kg IM immediately prior to dehorning	Cortisol (AUEC)*	-70.59	$P<0.05$

Percent change in cortisol was calculated using the formula [(Mean of analgesic group/ Mean of control group) – 1]*100

AUEC: Area under the effect curve for cortisol. Cmax: Maximum plasma concentration; * indicates values were estimated from

published graphical representation of the data; NR: values not reported

Table 2. Summary of the scientific literature examining the effect of analgesic drug administration on plasma cortisol response in dehorned calves using local anesthesia in the control group.

Reference	Procedure	Study Population	Control Analgesic Regimen	Analgesic Regimen	Outcome Parameter	Percent change in cortisol (%)	Significance (P-value)
Milligan, 2004	Cautery (gas) dehorning	2d - 2 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml) 10 min prior to dehorning	Ketoprofen 3 mg/kg IM, 10 min prior to dehorning	Cortisol (3 h)	-24.91	NS
					Cortisol (0-3 h)	-224.02	<0.05
					Cortisol (6 h)	14.55	NS
					Cortisol (3-6 h)	336.79	NS
Heinrich, 2009	Cautery (electric) dehorning	6-12 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml) 10 min prior to dehorning	Meloxicam 0.5 mg/kg IM, 10 minutes prior to dehorning	Cortisol (0-6 h)	-80.88	<0.01
					Cortisol (24 h)	0.86	NS
Duffield, 2010	Cautery (electric) dehorning	4-8 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml) 10 min prior to dehorning	Ketoprofen 3 mg/kg IM, 10 min prior to dehorning	Cortisol (3h)	4.62	NS
					Cortisol (6 h)	10.45	NS
Stilwell, 2010	Cautery (gas) dehorning	5-6 weeks Dairy	Xylazine 0.2 mg/kg IM, 10 min prior to dehorning	Lidocaine local anesthesia (cornual nerve, 5 ml) 8 min prior to dehorning	Cortisol (10 m)	-8.94	NS
					Cortisol (25 m)	5.07	NS
					Cortisol (40 m)	17.41	NS
					Cortisol (60 m)	53.65	NS

Table 2 cont.

Huber, 2013	Cautery (electric) dehorning	5-9 weeks	Procaine local anesthesia (cornual nerve, 10 ml) 20 min prior to dehorning	Flunixin meglumine 2.2 mg/kg, IV, 20 min prior to dehorning	Cortisol (AUEC)	-26.62	NS
				Flunixin meglumine 2.2 mg/kg, IV, 20 min + 3 h prior to dehorning	Cortisol (AUEC)	-43.46	P<0.05
Glynn, 2013	Amputation (scoop) followed by cautery	6 mo Dairy	Lidocaine local anesthesia (cornual nerve, 3 ml) 10 min prior to dehorning	Flunixin meglumine 2.2 mg/kg IV, 1 minute prior to dehorning	Cortisol (overall)	-50.83	P<0.05
Stock, 2015	Cautery (gas) dehorning	4-6 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml) 10 min prior to dehorning	Firocoxib 0.5 mg/kg PO, 10 minutes prior to dehorning	Cortisol (AUEC)	-32.20	P=0.09

Percent change in cortisol was calculated using the formula [(Mean of analgesic group/ Mean of control group) – 1]*100

AUEC: Area under the effect curve for cortisol; NS: not significant

Table 3. Summary of scientific literature examining the effect of analgesic drug administration on other outcomes in dehorned calves

Reference	Procedure	Study Population	Analgesic Regiment	Outcome parameter	Percent change (%)	Significance
Morisse, 1995	Chemical paste dehorning	4 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 4 ml/horn), 15 min prior to dehorning	Lying time	3.50	NS
	Cautery (electric) dehorning	8 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 4 ml/horn), 15 min prior to dehorning	Lying time	7.88	NS
Graf, 1999*	Cautery (electric) dehorner	4-6 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml; caudal horn bud SQ, 5 ml; medial horn bud, 3 ml), 20 min	Vasopressin (Cmax)	-90.00	<0.05
				ACTH (Cmax)	-72.50	<0.05
			Saline injection (cornual nerve, 5 ml; caudal horn bud SQ, 5 ml; medial horn bud, 3 ml), 20 min	Vasopressin (Cmax)	125.00	NS
				ACTH (Cmax)	62.50	NS

Table 3 cont.

Grøndahl-Nielsen, 1999[*]	Cautery (electrical) dehorner	4-6 weeks Dairy	Lidocaine local anesthesia (cornual nerve, unknown amount), 20 minutes prior to dehorning	ADG (0-7d)	NR	NS
				Feed intake (0-7d)	NR	NS
				Heart rate (0-4h)	NR	NS
				Rumination (0-4h)	NR	NS
				Rumination latency	-58.33	<0.05
			Xylazine 0.2 mg/kg & butorphanol 0.1 mg/kg, IM, 20 min prior to dehorning	ADG (0-7d)	NR	NS
				Feed intake (0-7d)	NR	NS
				Heart rate (0-4 h)	NR	NS
				Rumination (0-4h)	NR	NS
				Rumination latency	-37.50	<0.05
			Xylazine 0.2 mg/kg & butorphanol 0.1 mg/kg, IM, 20 min prior to dehorning; Lidocaine local anesthesia (cornual nerve, unknown amount), 15 minutes prior to dehorning	ADG (0-7d)	NR	NS
				Feed intake (0-7d)	NR	NS
				Heart rate (0-4h)	NR	NS
				Rumination (0-4h)	NR	NS
				Rumination latency	-46.67	<0.05

Table 3 cont.

Faulkner and Weary, 2000	Cautery (electrical) dehorner	4-8 weeks Dairy	Ketoprofen 3 mg/kg PO 2 h prior to dehorning, 2 h post dehorning, & 7 h after dehorning	Weight gain (0-24 h)	500.00	NS (p=0.07)
			[Analgesic control: Xylazine 0.2 mg/kg IM 20 min prior to dehorning & Lidocaine local anesthesia (cornual nerve & ring block 4.5 ml/side) 10 min prior to dehorning]			
Mellor, 2002*	Amputation (scoop) dehorn	10 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml/horn) 20 minutes prior to dehorning	Noradrenaline (Cmax)	-16.00	NS
				Adrenaline (Cmax)	-9.09	NS

Table 3 cont.

				Neutrophil %	9.53	NS
Doherty, 2007	Cautery (electric) dehorning	10-12 weeks Dairy	Lidocaine (2%) local anesthesia (cornual branch of zygomatic-temporal n., 3 ml/horn; cornual branch of infatrochlear n.; 4 ml/horn rostral to horn base)	Lymphocyte %	-10.04	NS
				N:L %	-19.83	NS
				Fibrinogen	NA	NS
				α_1-acid glycoprotein	NA	NS
			Lidocaine (5%) local anesthesia (cornual branch of zygomatic-temporal n., 3 ml/horn; cornual branch of infatrochlear n.; 4 ml/horn rostral to horn base)	Neutrophil %	0.23	NS
				Lymphocyte %	1.70	NS
				N:L %	-21.49	NS
				Fibrinogen	NA	NS
				α_1-acid glycoprotein	NA	NS
Heinrich, 2009	Cautery (electric) dehorning	6-12 weeks Dairy	Meloxicam 0.5 mg/kg IM given 10 min prior to dehorning; Analgesic control: lidocaine local anesthesia (cornual nerve, 5 ml/horn)	Heart Rate	-20.43	<0.05
				Respiratory rate	-100.00	<0.05

Table 3 cont.

				Eye temperature (2 - 3 h post dehorning difference)	-610.00	<0.001
Stewart, 2009*	Cautery (gas) dehorning	4-5 weeks Dairy	Lidocaine local anesthesia (cornual nerve, 5 ml/horn & ring block, 3-4 ml/horn) 10 minutes prior to dehorning	Heart Rate (0-5 min)	-22.22	<0.05
				HRV: LF Power (2-3h)	13.16	<0.05
				HRV: HF power (2-3h)	-35.90	<0.05
				HRV: LF:HF ratio (2-3h)	500.00	<0.05
			Meloxicam 0.5 mg/kg IV, 55 min prior to dehorning & Lidocaine local anesthesia (cornual nerve, 5 ml/horn & ring block, 3-4 ml/horn) 10 minutes prior to dehorning	Eye temperature (2 - 3 h post dehorning difference)	-.00	NS
				Heart Rate (0-5 min)	-26.98	<0.05
				HRV: LF Power (2-3h)	7.89	<0.05
				HRV: HF power (2-3 h)	-15.38	<0.05
				HRV: LF:HF ratio (2-3 h)	166.67	NS
Heinrich, 2010	Cautery (electric) dehorning	6-12 weeks Dairy	Meloxicam 0.5 mg/kg IM given 10 min prior to dehorning; Analgesic control: lidocaine local anesthesia (cornual nerve, 5 ml/horn)	Accelerometer (0-5 h)	-10.26	<0.05
				Pressure algometry	31.48	<0.05
				Feed Consumption	300.00	NS (p=0.07)

Table 3 cont.

Baldridge, 2011	Amputation (scoop) dehorning after surgical castration	2 - 4 month Dairy	Sodium salicylate at 2.5 to 5 mg/mL in the drinking water (13.62 to 151.99 mg of salicylate/kg bodyweight)	ADG (0- 13 d)	-1111.22	< 0.05
				Chute exit speed	0.97	NS
			0.025 mg/kg butorphanol, 0.05 mg/kg xylazine, 0.1 mg/kg ketamine co-administered IM immediately prior to castration	ADG (0- 13 d)	-729.98	NS
				Chute exit speed	-77.81	< 0.05
			Sodium salicylate at 2.5 to 5 mg/mL in the drinking water (13.62 to 151.99 mg of salicylate/kg bodyweight) and 0.025 mg/kg butorphanol, 0.05 mg/kg xylazine, 0.1 mg/kg ketamine co-administered IM immediately prior to castration	ADG (0- 13 d)	-1095.92	< 0.05
				Chute exit speed	-94.97	< 0.05

Table 3 cont.

Theurer, 2012*	Cautery (electric) dehorning	10 weeks Dairy	Meloxicam 0.5 mg/kg PO immediately after dehorning	Lying down % (1-4d)	20.13	<0.05
				Hay feeder % (0-1d)	-40.86	<0.05
				Grain feeder % (0-1d)	-50.00	<0.05
				Grain feeder % (1-2d)	80.00	<0.05
Coetzee, 2012	Amputation (scoop) followed by cautery (electric) dehorning	16-20 weeks Dairy	Meloxicam 0.5 mg/kg IV, immediately before the start of dehorning	Substance P	-37.79	<0.05
				Lying time %	-97.06	<0.01
				Heart Rate (8 & 10 h)	NR	<0.05
				ADG (0-10 d)	162.50	<0.05
Glynn, 2013	Amputation (scoop) followed by cautery	6 month Dairy	Analgesia (Meloxicam 1 mg/kg PO or Gabapentin 15 mg/kg PO or Meloxicam 1.0 mg/kg + Gabapentin 15 mg/kg PO) immediately prior to dehorning); lidocaine local anesthesia to all calves	Substance P	-53.86	<0.05
				Haptoglobin	NA	NS
				Ocular temperature	NA	NS
				Mechanical Nociception Threshold	NA	P=0.074
				ADG (7d)	275	P<0.0001

Table 3 cont.

Allen, 2013	Cautery dehorning	8- 10 weeks	Meloxicam 1.0 mg/kg PO immediately after dehorning or 12 hrs prior to dehorning; lidocaine local anesthetic	Substance P	NA	NS; Decreased @ 120 h P<0.05
				Haptoglobin	NA	NS
				Ocular temperature	NA	NS
				Mechanical Nociception Threshold	NA	NS; decreased @ 1 h P<0.05; increased @ 6 h P=0.073
				ADG (7d)	NA	NS
Huber, 2013	Cautery (electric) dehorning	5-9 weeks	Flunixin meglumine 2.2 mg/kg, IV, 20 min or 20 min + 3h prior to dehorning; Procaine local anesthesia (cornual nerve, 10 ml) 20 min prior to dehorning	Heart rate	NA	NS
				Respiratory Rate	NA	NS

Table 3 cont.

				TNF-α	31.94	P<0.05
				Neutrophil oxidative burst	16.51	P<0.05
				Neutrophil L-selectin (0.5h)	-5.33	NS
				Neutrophil L-selectin (24h)	32.26	NS
				Total Leukocytes (0.5h)	-18.18	NS
				Total Leukocytes (6h)	-36.67	P<0.05
			Lidocaine local anesthesia (cornual nerve and ring, 12 ml/horn) + flunixin meglumine 2 mg/kg IM immediately prior to dehorning	Total Leukocytes (24h)	-29.79	P<0.05
Ballou, 2013*	Amputation (scoop) followed by cautery	3 month Dairy		Total Leukocytes (72h)	-2.13	NS
				Neutrophil:Lymphocyte (0.5h)	-20.00	NS
				Neutrophil:Lymphocyte (6h)	-60.53	P<0.05
				Neutrophil:Lymphocyte (24h)	-36.36	NS
				Neutrophil:Lymphocyte (72h)	10	NS
				Haptoglobin (24h)	-38.18	NS
				Haptoglobin (72h)	0	NS
Sutherland, 2013	Amputation (scoop) followed by cautery	3 month Dairy	Lidocaine local anesthesia (cornual nerve and ring, 12 ml/horn) + flunixin meglumine 2 mg/kg IM immediately prior to dehorning	Eating	187.93	P<0.05
				Drinking	42.31	NS
				Lying	-4.57	NS
				Standing	4.8	NS

Table 3 cont.

Stock, 2015	Cautery (gas) dehorner	4-6 week Dairy	Firocoxib 0.5 mg/kg PO, 10 minutes prior to dehorning; Lidocaine local anesthesia (cornual nerve, 5 ml) 10 min prior to dehorning to all calves	MNT	19.79	NS
				OT	0.53	NS
				Substance P	9.13	NS
				Heart Rate	-0.27	NS
				ADG	0	NS

Percent change in cortisol was calculated using the formula [(Mean of analgesic group/ Mean of control group) – 1]*100

ADG: Average daily gain in bodyweight; HRV: heart rate variability; LF: Low frequency; HF: High frequency; * indicates values were estimated; NR: values were not reported

Table 4. Analgesic compounds available for use in cattle.

Drug	Approved Species	Indications	Dose (mg/kg)	Route	T ½ (h)	F (%)	Tmax (h)	Withhold time	Comments
NSAID									
Flunixin meglumine	Cattle horses pigs	Antipyretic, Anti-inflammatory	2.2	IV	3-8 h			Meat- 4 d Milk- 36 h	• IV only approved route • PO / IM: Prolonged withhold • IM: tissue necrosis
			2.2	PO	6.2 h	60%	3.5± 1.0	Not approved route for cattle	
Phenyl-butazone	Horses and dogs	Anti-inflammatory	4	IV ONLY!	40 – 55 h			Not approved in cattle in the USA	• ELDU prohibited for dairy cattle ≥20 mo • Use strongly discouraged
			4 – 8	PO	57.9±6.5	54-69%	8.9-11.7		

Table 4 cont.

Ketoprofen	Horses and dogs; EU approval in EU and Canada	Anti-inflammatory; adjunctive therapy of fever, pain, and inflammation associated with mastitis (EU)	3	IV, IM	0.42 h			Not approved in cattle in the USA	• Concentrates in inflammatory exudates • Consists of racemic RS ± enantiomers • $S(+) > R(-)$ inhibiting PGE_2 • Multiple doses may be required to maintain analgesia
Aspirin / sodium salicylate	No formal FDA approval cattle and horses	Reduction of fever Relief of minor muscle aches and joint pain	50 – 100	PO	3.7±0.4 h	<20%		No formal FDA approval Not for use in lactating cattle	• PO: Rumen acts as reservoir for slow absorption • Limited tissue distribution (low V_d) • Not associated with clotting deficits in cattle
				IV	0.5				

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Table 4 cont.

					Half-life	Bioavailability	T_{max}	Approval	Notes
Carprofen	Dogs; EU approval in cattle	Anti-inflammatory, Antipyretic; Adjunctive therapy of acute respiratory disease and mastitis	1.4	IV, SC	<u><10 weeks:</u> R(-): 49.7±3.9 h S(+): 37.4±2.4 h <u>Adult:</u> RS± 30.7±2.3 h			Not approved in cattle the USA	• Consists of racemic RS ± enantiomers • S(+) > R(-) inhibiting PGE_2
Meloxicam	Dogs and cats; EU and Canadian approval in cattle	Adjunctive therapy of acute respiratory disease; diarrhea and acute mastitis; pain associated with dehorning (Canada)	0.5	IV, SC	22 ± 3 h			Not approved in cattle in the USA	• Both injectable and oral formulations available
			0.5 – 1.0	PO	27 h (20 – 43 h)	100%	11.6 h		
Firocoxib	Horses and dogs	Anti-inflammatory	0.5	PO	18.8 h (14.2 – 25.5 h)	98%	4 h	Not approved in cattle in the USA	• Evaluated in preweaned calves

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Table 4 cont.

Opioids									
Butorphanol	Dogs, cats, horses	Analgesia; Sedation	0.025	IM	71±8 min		9.5±0.5 m	Not approved in cattle in the USA	• PK data following administration with ketamine and xylazine
Nalbuphine	No known veterinary-labeled product	Analgesia	0.4	IV	41m (32-47 m)			Not approved in cattle in the USA	• Plasma samples undetectable after 3h
Alpha2 agonist									
Xylazine	Dogs, cats, horses, deer, and elk; EU approval in cattle	Sedation; Analgesia	0.05 – 0.3	IM	96±20 m		9.5±0.5 m	Not approved in cattle in the USA	• PK data following administration with ketamine + butorphanol • Dose-dependent response: Higher doses result in recumbency • Fast to prevent rumen tympany; aspiration of rumen contents

Table 4 cont.

NMDA antagonist									
Ketamine	Cats	Sedation; Analgesia	0.1	IM	67±11m		10±1m	Not approved in cattle in the USA	• PK data following administration with xylazine alone (IV) or with xylazine and butorphanol (IM) • Metabolite norketamine may contribute to analgesia
				IV	29.4±4.5m				
Neuropathic pain analgesic									
Gabapentin	No known veterinary-labeled product	Neuropathic analgesia	15	PO	7.9 h (6.9-12.4h)		7.2h (6-10h)	Not approved in cattle in the USA	• Plasma concentrations above those reported as therapeutic in humans for up to 15 hrs
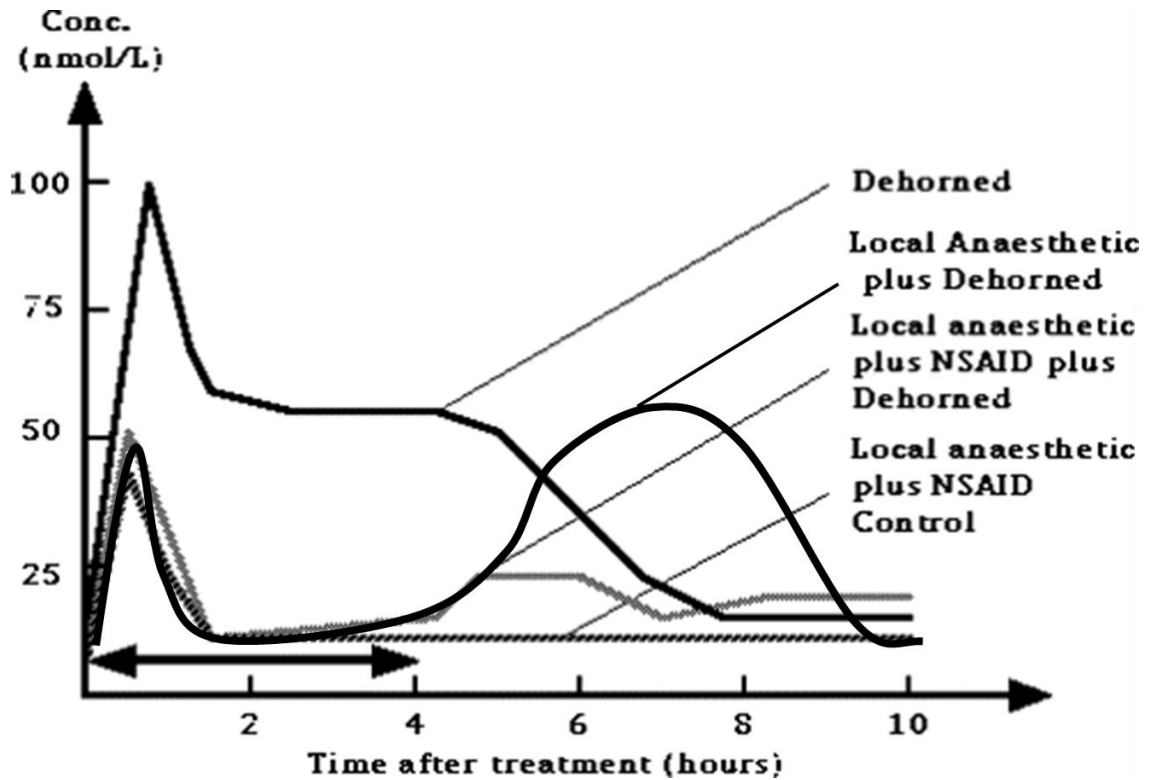
Figure 1. Constructed graphic representation of cortisol change over time in cattle following amputation (scoop) dehorning (derived from Stafford and Mellor, 2005 and currently published in Stock et al., 2013). Local anesthesia (bupivacaine) with NSAID (ketoprofen) administration provides a reduction in measured cortisol concentrations, although a delayed cortisol response is evident without the addition of an anti-inflammatory. The double headed arrow along the x-axis represents the duration of the local anesthesia provided by bupivacaine.
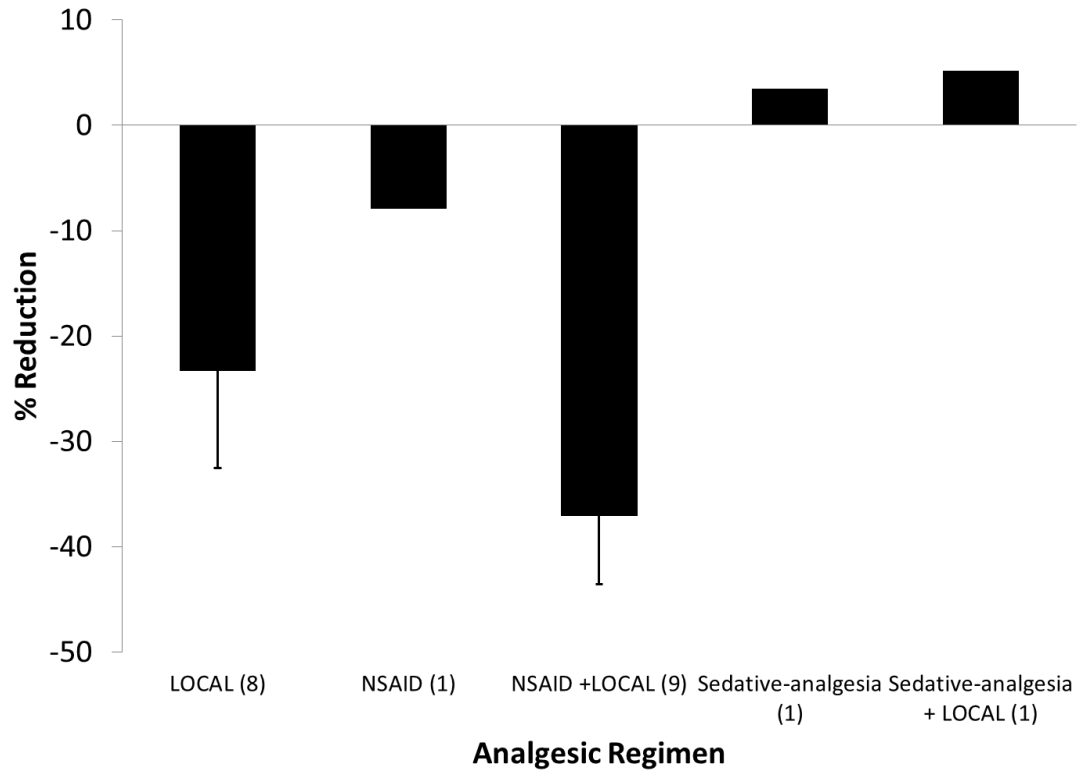
Figure 2. Mean (±SEM) percent reduction in AUEC or overall mean cortisol concentrations following dehorning in analgesic treated calves compared with a control group. Number in parentheses indicates studies reviewed. Percent change in cortisol was calculated using the formula [(Mean of analgesic group/Mean of control group)-1]*100.
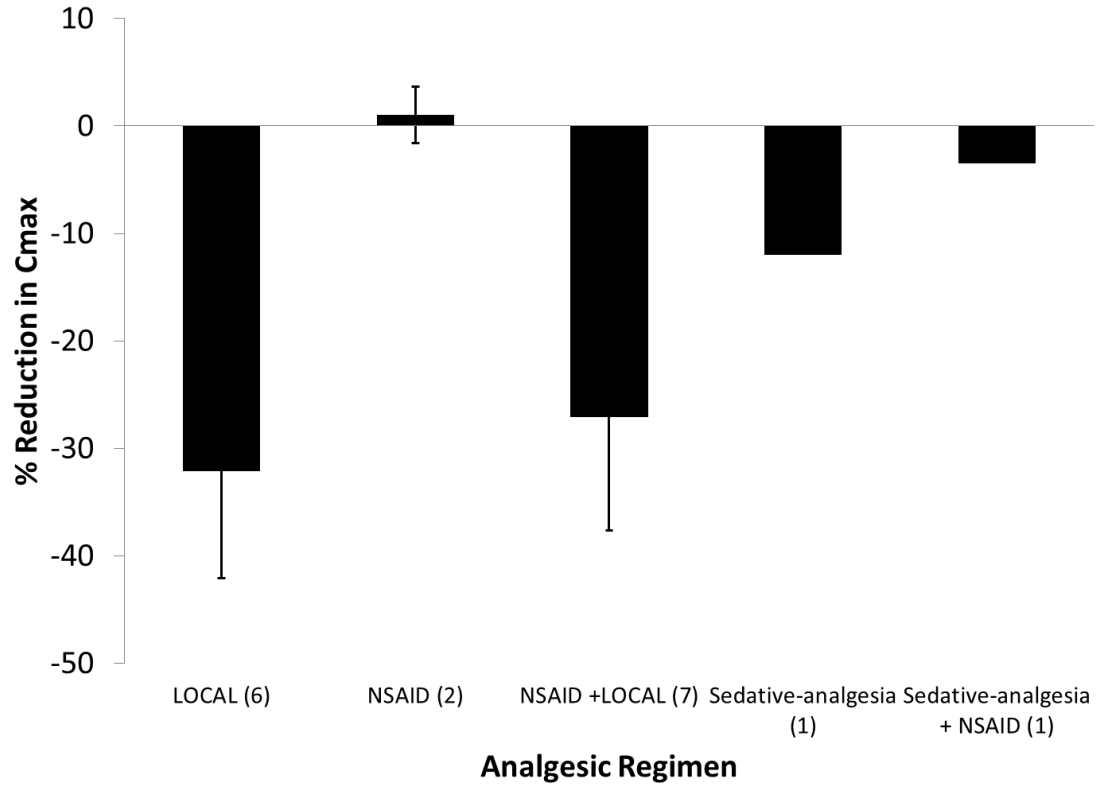
Figure 3. Mean (±SEM) percent reduction in peak plasma cortisol concentrations following dehorning in analgesic treated calves compared with a control group. Number in parentheses indicates studies reviewed. Percent change in cortisol was calculated using the formula [(Mean of analgesic group/Mean of control group)-1]*100.
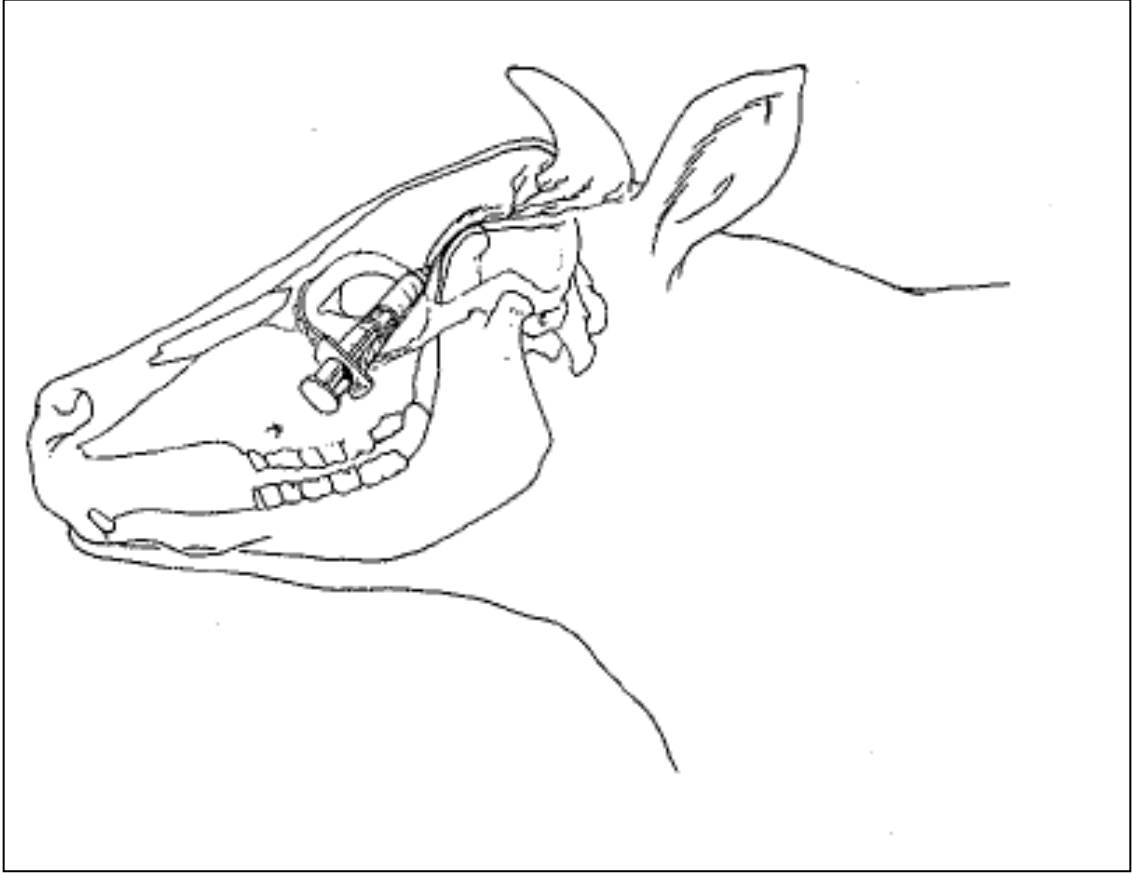
Figure 4. Diagram of cornual nerve anatomy including approximate locations for local anesthetic injection as illustrated by Skarda, 1986.
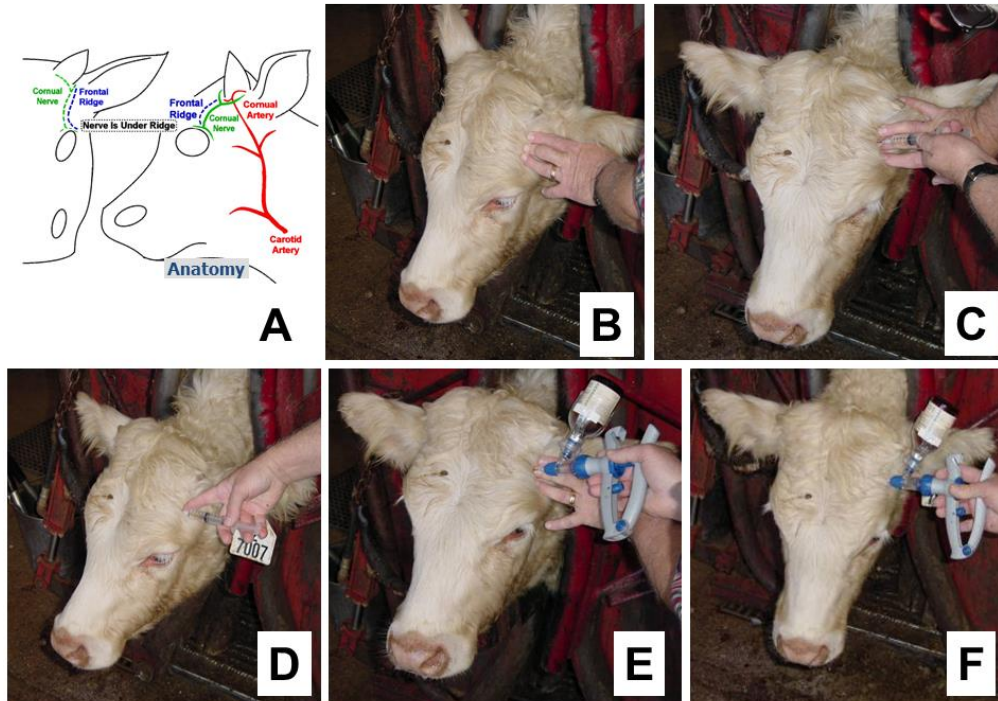
Figure 5. Steps for providing local anesthetic for dehorning using a cornual nerve block: (A) Anatomy of the cornual innervation; (B) Palpation of the temporal ridge; (C) Insert the needle below the ridge and aspirate; (D) Inject 5- 10 mL of lidocaine; Automatic syringe: (E) Palpate the frontal ridge and insert needle attached to automatic syringe; (F) Inject 5 – 10 ml of lidocaine using an automatic syringe.

CHAPTER 3

PHARMACOKINETICS OF FIROCOXIB IN PREWEANED CALVES AFTER ORAL AND INTRAVEOUS ADMINISTRATION

Modified from a manuscript published in the *Journal of Veterinary Pharmacology and Therapeutics*

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Abstract

The objective of this study was to determine the pharmacokinetics of intravenous and oral firocoxib in 10 healthy preweaned calves. Firocoxib (0.5 mg/kg) was initially administered IV to calves and following a 14-day washout period, animals received firocoxib orally prior to cautery dehorning. Firocoxib concentrations were determined by liquid chromatography tandem mass spectrometry. Changes in hematology and plasma chemistry were determined using automated methods. Computer software was used to estimate pharmacokinetic parameters best described with a two-compartment model for IV administration and a one-compartment model for PO administration. Following IV

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dosing, the geometric mean (range) $T_{1/2K10}$ and $T_{1/2\beta}$ were 6.7 (4.6 – 9.7) and 37.2 (23.5 – 160.4) hours, respectively, V_{ss} was 3.10 (2.10 – 7.22) L/kg, and CL was 121.6 (100.06 – 156.7) mL/h/kg. Following oral administration, geometric mean (range) C_{max} was 127.9 (102.5 – 151.3) ng/mL, T_{max} was 4.0 (2.6 – 5.6) hours, and $T_{1/2K10}$ was 18.8 (14.2 – 25.5) hours. Bioavailability of oral firocoxib was calculated using the AUC derived from both study populations to be 98.4% (87.1% – 117.6%). No adverse clinical effects were evident following firocoxib administration. Pharmacokinetic analysis of IV and PO firocoxib indicates high bioavailability and a prolonged terminal half-life in preweaned calves.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) [6] in veterinary medicine have been effectively used to control pain associated with conditions such as post-surgical procedures and osteoarthritis (Davila et al., 2013; Orsini et al., 2012). More recently, a newer class of NSAIDs known as the coxibs, have been evaluated as selective inhibitors of cyclooxygenase-2 (COX-2)[7] isoenzyme in horses and dogs (McCann et al., 2002; McCann et al., 2004). This specificity is mediated by steric hindrance to the COX-1 isoform and provides anti-inflammatory benefits with a potentially reduced risk for gastrointestinal irritation (Bergh et al, 2005). As young animals are at risk for developing gastric ulcers with a multifactorial etiology, NSAIDs demonstrating COX-2 selectivity and thus a COX-1[8] sparing effect may be ideal for use in calves requiring analgesic and

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[6] NSAID: non-steroidal anti-inflammatory drugs
[7] COX-2: cyclooxygenase-2
[8] COX-1: cyclooxygenase-1

anti-inflammatory therapy (Marshall, 2009). However, pharmacokinetic profiles are species dependent requiring studies involving target species (Lees, 2004).

Currently, firocoxib is formulated as an intravenous injectable solution and oral paste in horses or oral tablet in dogs to control osteoarthritis pain and inflammation. Pharmacokinetics of firocoxib following a one-time oral dose indicates bioavailability and clearance values similar to other NSAIDs of veterinary use (Kvaternick et al, 2007; McCann et al., 2004). Conversely to other NSAIDs, a large volume of distribution is reported for firocoxib, potentially attributed to its lipophilic property and neutrality charge at physiologic pH (Kvaternick et al., 2007; McCann et al., 2004; Letendre et al., 2008). Consequently, firocoxib has a long half-life. In conjunction with this prolonged half-life, pharmacodynamic data support once daily dosing in horses, dogs, and cats (McCann et al., 2002; McCann et al, 2004; McCann et al., 2005)

Routine husbandry procedures including dehorning and castration as well as painful disease states such as septic arthritis often affect calves. As such, analgesic treatment may be helpful in management and control of the pain associated with these conditions. Although the pharmacokinetics has been described in horses, dogs and cats, there is a gap in knowledge regarding the pharmacokinetic profile in calves. The objective of this study was to determine the pharmacokinetics of firocoxib in preweaned calves following IV and PO administration dosed at 0.5 mg/kg.

Materials and Methods

Animals

Ten Holstein calves (males and females) with a mean ± SD weight of 44.5± 4.3 kg and age of 20.7 ± 4.1 days for period 1 and 55.5 ± 5.3 kg and age of 34.7 ± 4.2 days for period 2 were included in this study. Calves were obtained from the Iowa State University Dairy. All calves were determined healthy by a physical examination and normal findings on a complete blood count and serum chemistry. This study protocol was approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC #: 10-12-7443-B).

Calves were individually housed with other preweaned dairy calves in an enclosed facility on the Iowa State University Dairy. Study animals were maintained in individual three sided pens (1.22 m x 1.82 m) bedded with straw added daily. Pasteurized waste milk collected from mature cattle was fed to the calves at 3 L twice daily for the length of the study. Water and grain, consisting of primarily pelleted corn, oats, molasses and protein/vitamin/mineral supplement, were fed to the calves ad libitum. Animals were examined daily by a veterinarian including the monitoring of milk consumption and a subjective assessment of grain and water consumption throughout the study period.

Study Design

A parallel design using the same animals (n=10) with 2 treatment periods separated by a 14-day washout period was utilized for this investigation. During the first period, study animals received firocoxib (0.5 mg/kg) intravenously. Following the

washout period, during the second study period animals were given firocoxib orally at the same dose (actual dose 0.5 mg/kg; 0.48-0.52 mg/kg) and subsequently cautery dehorned.

In both periods, a jugular catheter was placed for the purpose of blood sample collection. In addition, during the first period, a second jugular catheter was placed in the contralateral jugular vein for intravenous drug administration. Placement of the jugular catheter occurred approximately 12 hours prior to the start of the investigation. Calves were restrained by a handler during the process of catheter placement. The area over the jugular vein was clipped and surgically prepared with alternating scrubs of 70% isopropyl alcohol and povidone iodine. The catheter site was infiltrated with 2% lidocaine injection, 1 mL subcutaneously (VetOne®, Boise, ID) (NDC: 13985-222-04) (Lot #: 120200). Using sterile technique, an 18 G x 55 mm intravenous catheter (SURFLO®, Terumo Medical Corp., Somerset, NJ) with injection plug (Hospira Inc, Lake Forest, IL) was inserted into the vein and sutured to the skin using #3 nylon suture (Ethilon™, Ethicon, San Lorenzo, PR). Catheter patency was maintained by flushing with 2 mL of a heparin saline solution containing 3 USP units heparin sodium/mL saline (Heparin Sodium Injection, Baxter Healthcare, Deerfield, IL). The catheter port was disinfected with an alcohol swab prior to sample collection.

During the first period of the study, animals were administered firocoxib (0.5 mg/kg) (Equioxx® Injection, 20 mg/mL; NADA 141-313, Merial LLC, Lot #: 0VP03, Exp. date: 11/2013) via an intravenous catheter designated for drug administration. The dose was rounded to the nearest tenth milliliter and administered in a three milliliter

syringe. Following drug injection, the catheter was flushed using 10 mL of 0.9% saline (Sterile Saline, Vet One®, Boise, ID) and then removed.

Following a 14 day washout period, calves previously receiving intravenous firocoxib were administered oral firocoxib (Equioxx® Oral Paste, NADA 141-253,Merial LLC, Lot #: AB015/12, Exp. date: 06/2015) at a calculated dose of 0.5 mg/kg prior to cautery dehorning. Oral firocoxib was administered in a commercially provided dosing syringe, which was weighed before administration and after to confirm the amount of firocoxib administered. The dose was rounded to the nearest increment provided on the syringe, which was equivalent to 10 pounds (4.54 kg) based on the target dose. Using a concentration of 0.82% firocoxib, a dose of 0.5 mg/kg (range: 0.48 – 0.52 mg/kg) was administered to the calves as an oral paste in the dosing syringe.

Dehorning

All calves were dehorned in this study during the second period, 10 minutes following administration of PO firocoxib. Additionally, all calves received a local anesthetic cornual nerve block in both periods of the study to reduce the potential for confounding interactions between the periods. Desensitization of the cornual tissue was provided via a cornual nerve block using 2% lidocaine (VetOne®, Boise, ID) (NDC: 13985-222-04) (Lot#120200) (5 ml / site) as described by Stock et al. (2013). This was performed immediately following drug administration. Cautery dehorning was initiated 10 minutes following administration of the local anesthetic and drug delivery by one experienced veterinarian and one handler to minimize variation as detailed in Glynn et al (2013).

Blood sample collection

Animals were restrained during blood collection by trained handlers. Baseline samples were obtained immediately prior to drug administration. During period 1, blood samples were collected for animals receiving intravenous firocoxib or placebo via the catheter at 3, 6, 10, 20, 30, 45 minutes and 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours. During period 2, blood samples were collected at 15, 30 minutes and 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, and 96 hours following oral firocoxib administration and dehorning. Samples were immediately transferred to heparinized blood collection tubes (Vacutainer®, BD Diagnostics) and stored on ice before processing. Blood samples were centrifuged for 10 minutes at 1,500g. Collected plasma was placed in cryovials and frozen at -70 °C until analysis.

Plasma chemistry and complete blood count data

Plasma chemistry was performed by the Iowa State University Clinical Pathology Laboratory by automated methods using a Vitros® 5.1 FS Chemistry System (Ortho Clinical Diagnostic, Johnson & Johnson Co., Rochester, NY). Additionally, a complete blood count was determined by automated methods using an ADVIA® 120 hematology system (Siemens Medical Solutions USA, Inc., Malvern, PA) with a program for bovine blood.

LCMS-MS analysis

Plasma concentrations of firocoxib were measured with high-pressure liquid chromatography –tandem mass spectrometry utilizing a TSQ Quantum Discovery Max triple quadrupole mass spectrometer coupled to a Surveyor Pump and Autosampler (Thermo Scientific, San Jose, CA, USA). Plasma samples or plasma standards were prepared as detailed in Letendre et al. 2007 with slight modifications: Briefly, frozen samples or standards were thawed at room temperature and rigorously vortexed once completely thawed. A 200 µL plasma sample was added to 400 µL 5% (v/v) acetic acid. The entire diluted sample was applied to a solid phase extraction (SPE)[9] cartridge, Strata X (60 mg/3 mL, Phenomenex, Torrance, CA, USA) which was preconditioned prior with methanol (2 ml) and equilibrated with water (2 ml). The sample was then gravity filtered through the SPE tubes and subsequently washed with 1 ml 5% (v/v) acetic acid, followed by 1 ml of 25% (v/v) methanol in water. Firocoxib was eluted with 2 x 0.75 mL portions of acetonitrile into a glass test tube. Samples were evaporated to dryness at 48°C under a stream of nitrogen, reconstituted with 200 µL 25% (v/v) acetonitrile in water and transferred into an injection vial for LC-MS/MS analysis with the injection volume set to 10 µL. The mobile phases consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile at a flow rate of 0.25 mL/min. The mobile phase began at 40% B with a linear gradient to 95% B at 6 minutes, which was maintained for 1.5 minutes, followed by re-equilibration to 40% B. Separation was achieved with an Intersil ODS-4 column (75 mm x 2.1 mm, 3 µm particles, GL Sciences, Torrance, CA, USA) maintained at 40°C.

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[9] SPE: solid phase extraction

External standard calibration with matrix matched calibrates were used as no suitable internal standard was found following exploration of piroxicam and celecoxib. The standard curve of firocoxib concentration determined using bovine plasma was linear from 0.005 to 5.0 µg/mL and was accepted when the correlation coefficient exceeded 0.99 and measured values were within 15% of the actual values. The lower limit of quantification, defined as the lowest concentration on a linear standard curve with predicted concentrations within 15% of the actual concentration, was 0.005 µg/mL. Three SRM transitions were used for quantitation of firocoxib and piroxicam, respectively. The three ions (m/z) 130, 237, and 283 were monitored for analysis of firocoxib. Intraday accuracy of the assay for firocoxib in calf plasma was $99 \pm 3\%$ of the actual concentration, whereas the intraday coefficient of variation was 2%, as determined through replicates of 5 for each of the following firocoxib concentrations: 0.015, 0.150, and 1.50 µg/mL.

Pharmacokinetic analysis

Firocoxib time concentration data were analyzed using a commercially available computer software program (Phoenix®, Pharsight Corporation, NC, USA). Using the value of Aikake's Information Criterion and visual inspection of predicted versus observed data, the best models were selected for each route of administration. A two compartment model with first-order elimination best fit IV firocoxib administration whereas a one compartment model with first-order elimination best fit the PO route. Standard equations were used to calculate pharmacokinetic parameters (Gibaldi & Perrier 1982).

Statistical analysis

Statistical analysis was performed using JMP® Pro 10.0.2 analytical software (SAS Institute, INC, Cary, NC, USA). Distribution curves were examined for normality. Pharmacokinetic values are reported as geometric means, medians, and ranges due to log-normal distributions. Plasma chemistry and complete blood count data were statistically analyzed using paired t-tests or Wilcoxon signed rank tests for normal or non-normal distributions, respectively. Significance was designated *a priori* as p<0.05.

Results

Plasma chemistry and complete blood count analysis

No adverse clinical effects including changes in appetite, water intake, or physical exam parameters were observed during either study period. Significant differences were observed in both plasma biochemistry and hematology parameters obtained prior to and 72 hours after firocoxib administration for both IV and PO routes (Table 1). Changes determined as significant observed during intravenous administration include minor decreases in hematocrit and γ-glutamyltransferase (GGT) and increases in total protein and albumin. Reported significant changes following oral administration include decreases in BUN, Creatinine, and GGT and increases in red blood cells, total protein, albumin, and AST. Although these changes were significant, values remained either within or slightly below the reference ranges provided by the analyzing chemistry lab.

Pharmacokinetic analysis

The time-concentration profile following IV administration at 0.5 mg/kg is best described with a 2-compartment model (Figure 1), whereas the profile following PO administration at 0.5 mg/kg (range 0.48 – 0.52 mg/kg) is best described as a 1-compartment model (Figure 2). This is because the absorption phase following PO administration overlaps with the rapid distribution phase, making the latter undetectable. Concentrations were detectable up to the last sampling point for both routes of administration. Oral drug absorption (F) was calculated using the AUC values derived from both IV and PO study populations to be 98.4% (range 83 – 118 %) (Table 2 and 3).

Discussion

The present study is the first to examine the IV and PO pharmacokinetics of firocoxib in preweaned calves. The oral preparation of firocoxib appears to be well absorbed as evidenced by a high bioavailability. Additionally, a prolonged terminal half-life was observed in both routes of administration supporting a once a day treatment. These properties of firocoxib suggest the potential for use in calves.

A limitation of this study was that PO and IV dosing was not randomly distributed between period 1 and 2 of the study. As such, influencing factors including increased weights, age, physiologic effects of dehorning, and environmental conditions were not controlled between periods essentially separating the two study periods into two study populations. This study design was adopted because calves were enrolled in a second trial intended to determine the analgesic effects of oral firocoxib and therefore administration of IV firocoxib would have confounded the second trial. This potential for

increased total variability has been documented and supported in parallel pharmacokinetic studies for drugs having relatively long half-lives (Chow & Wang, 2001). Although factors limiting variability were employed such as using the same analyzing lab and study animals, due to these limitations detailed, the pharmacokinetic parameters have been separated into two non-superimposable populations. The bioavailability calculation was determined using the AUC derived from these two populations notwithstanding the aforementioned limitations.

The dose used in this experiment was based on previous studies in horses (Kvaternick et al., 2007, Letendre et al., 2008). In these studies, a dose of 0.1 mg/kg was given to horses as both a single administration and multiple doses reaching steady state. The goal in this study was to administer a single, higher dose to achieve maximal plasma concentrations that were comparable to those at steady-state in the horse without the need for repeated dosing. This desired increase in plasma concentrations was chosen for an expected single-time dose administration in calves whereas achieving effective steady state concentrations in the horse required multi-day administration (Letendre et al, 2008; Cox et al., 2013). This is comparable to a loading dose, which was recently evaluated in horses (Cox et al., 2013). The investigated 3X loading dose reduced the variability of onset of action and efficacy in horses supporting the use of a higher initial dose. Although linearity was not evaluated in this study, firocoxib has demonstrated linear kinetics following 0.5X and 2X recommended doses in horses (Kvaternick et al., 2007).

Hematologic and plasma biochemical analytes obtained prior to and 72 h following administration primarily remained within the references ranges provided by the

Iowa State University Clinical Pathology Laboratory. The elevated GGT[10] observed in

the IV study population was most likely due to the proximity to colostrum ingestion as

colostrum provides high concentrations of GGT (Perino et al., 1993). Although

significant changes were observed in both plasma biochemistry and hematology analytes,

these differences mostly were within reference ranges provided by the analyzing

laboratory. A significant increase in total protein was observed 72 hrs post administration

in both routes of administration which may indicate volume depletion; however, both

BUN and Creatinine significantly decreased or were not altered suggesting a lack of renal

stress. Moreover, hypoproteinemia is more commonly reported in NSAID toxicity (Kivett

et al., 2013). Although a mild increase of AST is observed following PO administration,

GGT significantly decreased following both routes of administration potentially

suggesting an extrahepatic origin for the mild increase in AST. Previous studies

evaluating the safety of firocoxib in horses indicate the development of ulcers and

nephropathies are associated with multiplicative increases in the recommended dose

administered daily for a prolonged time period. Mild increases in BUN[11] and creatinine

were observed in horses receiving $1 - 5$ X the dose for 42 days whereas additional

increases in AST[12], GGT, and ALT[13] were reported in horses receiving 12.5 X the dose

for 92 days (US FDA, 2005). Further studies evaluating the safety of firocoxib in calves

are required.

A prolonged half-life for both routes was observed in this study which was shorter

than that reported in horses and longer than in dogs (Kvaternick et al., 2007; McCann et

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[10] GGT: γ-glutamyltransferase
[11] BUN: blood urea nitrogen
[12] AST: aspartate aminotransferase
[13] ALT: alanine aminotransferase

al., 2004). As such, administration of firocoxib in preweaned calves potentially supports a once daily administration. In cattle, this dosing strategy related to its long half-life is more similar to meloxicam compared with other commonly administered NSAIDs with shorter half-lives such as flunixin meglumine (6 h) and ketoprofen (2 h) (Mosher et al, 2012; Glynn et al, 2013; Igarza et al., 2004). Taken together, the dosing strategy and ease of administration potentially promote treatment compliance.

The reported half-life was most likely a result of a low clearance observed commonly with NSAIDs as well as the large volume of distribution observed for both PO and IV routes. The large volume of distribution is also reported in horses and dogs and atypical of most NSAIDs due to their high protein binding (Kvaternick et al., 2007; McCann et al., 2004). Although protein binding percentage was not determined in this study, 97% of firocoxib is reported to be protein bound in the horse (Kvaternick et al., 2007). Most likely the large volume of distribution is influenced by the lipophilic property of the compound. Moreover, a larger volume of distribution is observed in young animals potentially due to decreased protein concentrations resulting in increased concentrations of unbound drug (Notarianni, 1990).

Firocoxib was well absorbed following oral administration. This high bioavailability is similarly reported in horses and dogs as well (Kvaternick et al., 2007; McCann et al., 2004). In the present study, the provided milk feedings may have impacted absorption and distribution of the PO administered firocoxib due to altered protein-feed binding in the digestive tract as observed with other NSAIDs (Mosher et al, 2012; Toutain et al, 2004).

A mean C_{max}[14] of 128 ng/mL with a mean T_{max}[15] of 4 h was observed following

oral administration. A comparison of dose-normalized C_{max} following a single time or the

first dose administered in horses indicates values that are approximately $1.75 - 3$ X

greater than determined in this study in calves (Kvaternick et al., 2007; Letendre et al.,

2008). However, AUC[16] is similar between horses and calves in a dose-normalized

comparison (Kvaternick et al., 2007; Letendre et al., 2008). Taken together, the extent of

absorption appears similar between horses and calves, although the rate of absorption

may be slower in calves.

The suggestive advantages of a selective COX-2 inhibitor would provide

continued mitigation of inflammatory mediator production with a decrease risk of side

effects (Bergh et al., 2005). The highly selective inhibition of COX-2 isoenzyme has

been described in both the dog (384 X) and the horse (265- 643 X) (McCann et al., 2002;

McCann et al., 2004; Kvaternick et al., 2007). Using data generated from an in-vitro

LPS[17]-stimulation of whole equine blood, the IC_{50}[18] and IC_{80}[19] of COX-2 was calculated

to be approximately 30 ng/ml and 67 ng/ml respectively (McCann et al., 2002; Letendre

et al., 2008). Moreover, although these concentrations are achieved following

administration of the labeled PO dose, clinical differences were not detected in

chronically lame horses using a higher than labeled dose (Back et al., 2009). In the

present study, mean concentrations of firocoxib in calves remained above these reported

equine COX-2 IC_{50} and IC_{80} for 48 h and 24 h respectively following PO administration

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[14] C_{max}: maximum plasma concentration
[15] T_{max}: time to maximum concentration
[16] AUC: area under the curve
[17] LPS: lipopolysaccharide
[18] IC_{50}: 50% of maximum inhibitory concentration
[19] IC_{80}: 80% of maximum inhibitory concentration

and 12 h and 24 h, respectively following IV administration. Studies to evaluate both COX selectivity and inhibitory concentrations are needed in bovines.

Given specific required guidelines are followed, extra-label drug use in the United States is permitted under Animal Medicinal Drug Use Clarification Act (AMDUCA)[20] (US FDA, 1994). As there are no labeled pharmaceuticals for the treatment or control of pain in cattle and the currently labeled NSAID for cattle in the US has undetermined analgesic properties following a single administration in preweaned calves, the use of firocoxib would constitute extra-label drug use permitted under AMDUCA. Importantly, no violative tissue residues may result from extra-label administration. Currently, the European Union regulatory agency recommends a slaughter withhold of 26 days in horses administered a dose 5 X less than that administered in this study (European Medicines Agency, 2010). Given the large volume of distribution and lipophilic properties of firocoxib, further studies evaluating tissue residues would need to be performed prior to recommendation of withholding times in preweaned calves.

Acknowledgements

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[20] AMDUCA: Animal Medicinal Drug Use Clarification Act

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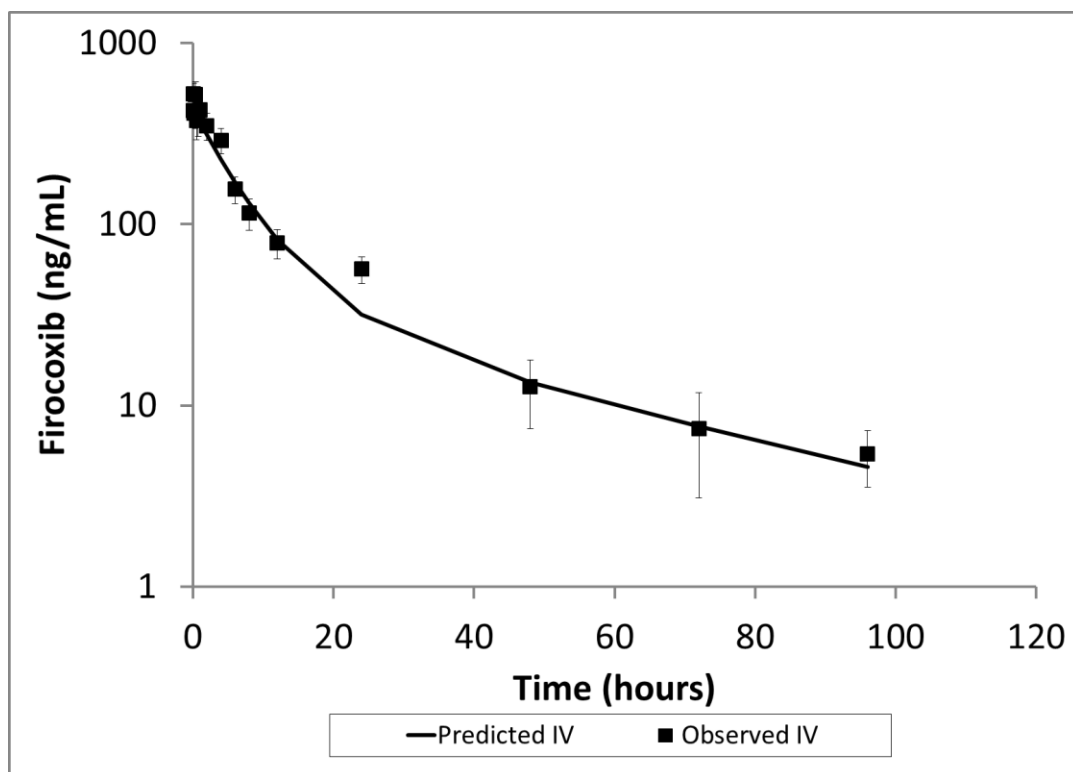
Figure 1. Mean ± SE serum concentrations of firocoxib at various points after IV (0.5 mg/kg) administration of the drug to 10 healthy calves.
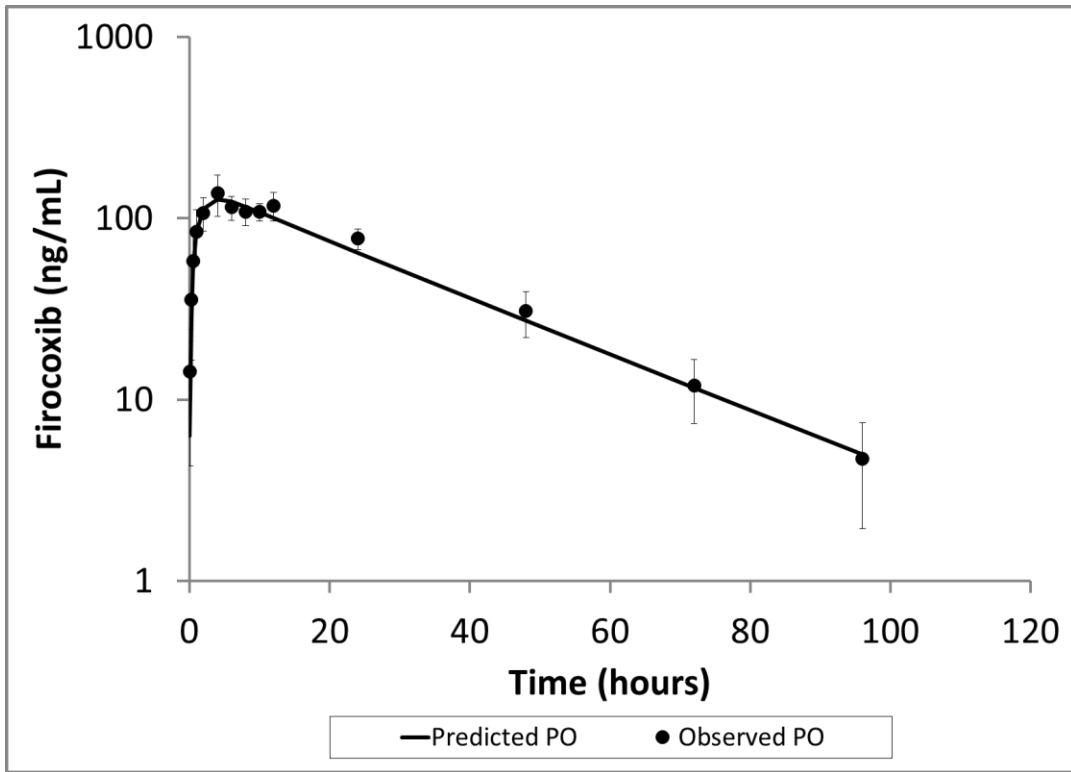
Figure 2. Mean ± SE serum concentrations of firocoxib at various points after PO (mean dose, 0.5 mg/kg; range, 0.48 to 0.52 mg/kg; circles) administration of the drug to 10 healthy calves.

Table 1. Mean ± SEM plasma biochemistry and hematology analytes obtained prior to (PRE) and 72 h following (POST) both IV and PO firocoxib administration in study calves (n=10).

Parameter	Reference Range	IV		PO	
		Mean ± SEM (PRE)	Mean ± SEM (POST)	Mean ± SEM (PRE)	Mean ± SEM (POST)
White Blood Cell (x10^3/ul)	4.0- 12.0	7.9 ± 0.7	7.2 ± 0.6	8.2 ± 1.0	8.7 ± 1.0
Neutrophils (x10^3/ul)	0.6- 4.0	3.0 ± 0.6	1.8 ± 0.3	3.0 ± 0.9	3.1 ± 0.6
Lymphocyte (x10^3/ul)	2.5-7.5	4.2 ± 0.4	4.5 ± 0.3	4.3 ± 0.2	4.6 ± 0.2
Red Blood Cells (x10^6/ul)	5.0 – 10.0	7.2 ± 0.5	7.0 ± 0.4	6.8± 0.3[a]	7.3 ± 0.3[a]
Hematocrit (%)	24 – 46	25 ± 2[a]	24 ± 2[a]	20 ±2	21 ± 2
BUN (mg/dl)	10 – 25	12 ± 0.5	11 ± 0.6	7 ± 0.7[a]	5 ± 0.6[a]
Creatinine (mg/dl)	0.5 – 2.2	1.1 ± 0.04	1.1 ± 0.04	1.0 ± 0.04[a]	0.9 ± 0.03[a]
Total Protein (gm/dl)	6.7 – 7.5	6.5 ± 0.1[a]	6.8 ± 0.1[a]	6.5 ± 0.1[a]	6.9 ± 0.1[a]
Albumin (gm/dl)	2.5 – 3.8	2.6 ± 0.04[a]	2.7 ± 0.05[a]	2.7 ± 0.04[a]	2.8 ± 0.05[a]
AST (IU/L)	55 – 125	53 ± 4	54 ± 3	44 ± 3[a]	50 ± 2[a]
ALP (IU/L)	25 – 250	178 ± 14	166 ± 14	211 ± 21	244 ± 25
GGT (IU/L)	0 – 74	236 ± 33[a]	176 ± 22[a]	88 ± 8[a]	70 ± 6[a]

[a] – P<0.05; BUN – blood urea nitrogen; AST – aspartate aminotransferase; ALP – alkaline phosphatase; GGT – γ-glutamyltransferase

Table 2. Pharmacokinetic parameters for firocoxib administered IV (0.5 mg/kg) to 10 healthy calves. Data were fit to a 2-compartment model with first-order elimination.

Parameter	IV			
	Geometric mean	Minimum	Median	Maximum
AUC (h•ng/mL)	4109.5	3190.8	4248.9	4996.9
A (ng/ml)	388.8	325.3	367.0	498.3
B (ng/ml)	30.6	5.8	32.6	88.8
K_{10} (l/h)	0.104	0.072	0.110	0.152
K_{12} (1/h)	0.049	0.020	0.049	0.124
K_{21} (1/h)	0.031	0.006	0.034	0.069
$T_{1/2\ K10}$ (h)	6.7	4.6	6.3	9.7
α (1/h)	0.17	0.10	0.18	0.26
$T_{1/2\alpha}$ (h)	4.0	2.7	3.9	7.2
β (1/h)	0.019	0.004	0.022	0.029
$T_{1/2\beta}$ (h)	37.2	23.5	31.8	160.4
V_c (L/kg)	1.17	0.95	1.16	1.43
V_{ss} (L/kg)	3.10	2.10	2.93	7.22
V_{area} (L/kg)	6.54	4.01	5.38	23.66
CL (mL/h/kg)	121.7	100.0	117.7	156.7

AUC – area under the plasma drug concentration curve; A – Y-intercept for the distribution phase; B – Y-intercept for the elimination phase; K_{10} – elimination rate constant for central compartment; K_{12} – intercompartmental distribution rate constant from central to peripheral compartment; K_{21} – intercompartmental redistribution rate constant from peripheral to central compartment; $T_{1/2\ K10}$ – elimination half-life; α – distribution slope; $T_{1/2\alpha}$ – distribution half-life; β – elimination slope; $T_{1/2\beta}$ – terminal half-life; V_c – volume of distribution of the central compartment; V_{ss} – volume of distribution in a steady state; V_{area} – volume of distribution during the elimination phase; CL – plasma clearance

Table 3. Pharmacokinetic parameters for firocoxib administered PO (mean dose, 0.5 mg/kg; range, 0.48 to 0.52 mg/kg) to 10 healthy calves. Data were fit to a 1-compartment model with first-order elimination.

Parameter	PO			
	Geometric mean	Minimum	Median	Maximum
AUC (h•ng/mL)	4043.8	3190.5	3931.0	5617.0
C_{max} (ng/mL)	127.9	102.5	128.5	151.3
T_{max} (h)	4.0	2.6	4.0	5.6
K_{01} (l/h)	0.815	0.492	0.801	1.473
K_{10} (l/h)	0.037	0.027	0.037	0.049
$T_{1/2\ K10}$ (h)	18.8	14.2	18.9	25.5
V/F (L/kg)	3.36	2.86	3.27	3.91
CL/F (mL/h/kg)	123.6	89.0	127.2	156.7
F (%)	98.4	83	96	118

AUC – area under the plasma drug concentration curve; C_{max} – maximum plasma concentration; T_{max} – time to maximum plasma concentration; K_{01} –absorption rate constant for central compartment; K_{10} – elimination rate constant for central compartment; $T_{1/2\ K10}$ – elimination half-life; V/F – volume of distribution per bioavailability; CL/F – plasma clearance per bioavailability; F – Bioavailability calculated from data collected from both study populations

CHAPTER 4

THE EFFECTS OF FIROCOXIB ON CAUTERY DISBUDDING PAIN AND STRESS RESPONSES IN PREWEANED DAIRY CALVES

Modified from a manuscript accepted for publication in the *Journal of Dairy Science*

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Abstract

Perioperative analgesic effects of oral firocoxib following cautery disbudding were investigated in preweaned calves. Twenty Holstein calves approximately 4-6 weeks old received a single oral dose of firocoxib, a non-steroidal anti-inflammatory, at 0.5 mg/kg (n=10) or placebo (n=10) in a randomized controlled clinical trial. Responses, including ocular temperature determined by infrared thermography, pressure algometry measuring mechanical nociception threshold, and heart rate, were evaluated at 2, 4, 7, 8, and 24 h after cornual nerve block and cautery disbudding. Blood samples were collected

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over 96 h and analyzed for plasma cortisol and substance P concentrations by radioimmunoassay. Additionally, ex-vivo prostaglandin E_2 concentrations were determined over a 72 h study period using an enzyme immunoassay. Data were analyzed using a linear mixed effects model with repeated measures. An inhibition of ex-vivo PGE_2 synthesis was observed from 12 to 48 h following disbudding in calves treated with firocoxib. Cautery disbudding was associated with an increased nociception for the duration of sampling (24 h). During the initial 24 h period following disbudding, there was no difference in response between treatment groups. Following 24 h, mean cortisol concentrations diverged between the two study groups with placebo treated calves having increased cortisol concentrations at approximately 48 h after disbudding. Furthermore, the overall integrated cortisol response as calculated as area under the effect curve tended to be reduced in firocoxib treated calves. The prolonged effects of cautery dehorning require further investigation. Moreover, the effect of firocoxib on cortisol reduction observed in this study requires additional exploration.

Introduction

Dehorning or disbudding cattle is a management procedure commonly performed on nearly 95% of US dairy operations (USDA, 2010). Horns or horn buds are primarily removed to accommodate production practices and prevent economic loss due to carcass bruising. Many techniques have been described with the use of a hot iron reported to be the most commonly employed method in the United States (USDA, 2010). Although the American Veterinary Medical Association (2012) supports methods to minimize pain and distress associated with disbudding and dehorning,

methods to evaluate this response can be challenging given the need to use indirect measures to assess an affect state.

Behavioral, physiologic, and neuroendocrine changes have been reported following disbudding or dehorning (Stafford and Mellor, 2005; Stock et al., 2013). These observed and reported responses are frequently interpreted as being associated with pain and distress (Faulkner and Weary, 2000; McMeekan et al., 1998; Heinrich et al., 2010; Stewart et al, 2008). More specifically, indirect measures such as changes in the hypothalamic-pituitary-adrenal (HPA) axis activation resulting in cortisol release (McMeekan et al., 1998), mechanical nociception threshold (Heinrich et al., 2010), autonomic nervous system response (Stewart et al., 2008), and behavior (Faulkner and Weary, 2000) have been previously used to assess the pain and distress associated with disbudding in calves. The concurrent evaluation of several indices may improve the assessment of pain and distress in animals due to individual response variations (Molony and Kent, 1997).

As a consequence of disbudding or dehorning, acute changes in cortisol release (McMeekan et al., 1998), local nociception (Heinrich et al., 2010), ocular temperature and heart rate (Stewart et al., 2008), and head shakes and ear flicks (Faulkner and Weary, 2000; Stilwell et al., 2009) are reported. Local anesthetics have been useful in mitigating these acute effects following removal of the horns or horn buds (McMeekan et. al, 1998; Stewart et al., 2008; Stilwell et al., 2009); however, these local anesthetics typically demonstrate only temporary reduction of these observed responses once the local anesthetic is no longer effective (Doherty et al., 2007; Heinrich et al., 2009; Allen et al., 2013).

Subsequent to the initial tissue damage involved with horn removal, continued cortisol and behavioral changes are observed which may be due to inflammation-related pain (McMeekan et al., 1998; Stafford and Mellor, 2005). Behavior responses are reported to persist up to 24 to 44 h (Faulkner and Weary, 2000; Heinrich et al., 2010). Non-steroidal anti-inflammatory drugs (NSAIDs) including ketoprofen (Faulkner and Weary, 2000), meloxicam (Heinrich et al, 2009), carprofen (Stilwell, 2012), and flunixin (Glynn et al., 2013; Huber et al., 2013) reduce responses associated with pain and distress following dehorning or disbudding. In order to address both the initial and continued responses, a multimodal approach using analgesics that act both acutely and at length have been proposed (Stafford and Mellor, 2011)

Currently, there are no compounds specifically approved to alleviate pain in livestock in the United States (Coetzee, 2013). Concerns of efficacy and administration frequency necessary to maintain analgesia concentrations have been discussed due to the number of analgesic drugs available in the United States with short elimination half-lives (Coetzee, 2011). Heinrich and colleagues (2010) demonstrated the administration of meloxicam, an NSAID with a long half-life in cattle, reduced both the acute response, which included reductions in cortisol concentrations, local sensitivity as well as the prolonged pain-related behaviors. As such, the use of an NSAID with a long half-life in calves may be the most ideal for pain management. Moreover, since feed is potentially the most practical and pain-free drug delivery method that can be applied in commercial livestock production systems, analgesics administered orally should be explored. Given the diversity of the pharmacologic properties of NSAIDs, continued

investigation for optimal pain relief for disbudding and dehorning pain will benefit

animal welfare.

Firocoxib is an NSAID of the coxib class with a prolonged elimination half-life

and high oral bioavailability in calves (Stock, et al., 2014). As a potential additional

benefit, firocoxib is COX-1 sparing in both the horse and dog, which may reduce adverse

effects (McCann et al., 2002, 2004). The objective of this study is to measure the effects

of firocoxib on pain and distress in pre-weaned dairy calves immediately prior to cautery

disbudding. In addition, we assess the neuroendocrine, nociception, and physiologic

responses observed following cautery disbudding.

Materials and Methods

Animals and Housing

Twenty Holstein calves (11 male and 9 female), 4-6 weeks of age (mean ± SD:

32.9 ± 3.9 days) weighing 55.2 ± 5.8 kg at the time of disbudding were obtained from

the Iowa State University Dairy. All calves were determined healthy following a

physical examination by a veterinarian and normal findings on a complete blood count

and serum chemistry. This study protocol was approved by the Institutional Animal

Care and Use Committee at Iowa State University (Log#: 10-12-7443-B).

Study animals were housed in individual three sided closed pens (1.82 m x 1.22

m) bedded with straw added daily in an enclosed facility at the Iowa State University

Dairy. Calves are placed in these pens at birth and remained within the pens through the

entirety of the study. While in these pens, calves have minimal physical contact with

other calves, although contact to neighboring calves is possible during feeding through

the one open side. Three liters of pasteurized waste milk were fed twice daily for the

length of the study. In order to control for the variation in waste milk components, both

treatment groups were given milk from the same batch pasteurization. Calves were fed

grain, consisting primarily of pelleted corn, oats, molasses and protein/vitamin/mineral

supplement, and offered water ad libitum. Grain was added daily at 0.45 - 0.90 kg.

Daily examinations were conducted by a veterinarian, including the monitoring of milk

consumption and a subjective assessment of grain and water consumption throughout

the study period.

Study Design

A randomized complete block design was used for this investigation with 10

calves enrolled in each treatment group (Figure 1). The study was conducted in two

Periods. Calves were enrolled into a treatment group during Period 1 and remained in

that treatment group for Period 2. Period 1 was conducted two-weeks prior to Period 2.

Disbudding only occurred during Period 2. The purpose of Period 1 was to obtain IV

firocoxib concentrations in calves used in a subsequent pharmacokinetic analysis

(Stock et al., 2014). In Period 1, study animals were blocked by age and randomly

assigned to receive either firocoxib (0.5 mg/kg) (n=10; male=7) or a placebo (n=10;

male=4). Randomization of group assignment was mediated by a computer generated

random number (Microsoft Excel 2011, Microsoft Corp., Redmond, WA, USA).

During Period 1, calves assigned to the firocoxib group received intravenous firocoxib

(Equioxx® Injection, NADA 141-313, Merial LLC, Duluth, GA) whereas calves in the

control group received intravenous saline via a preplaced jugular catheter followed by

multiple timed blood collections. As such, calves in both treatment groups were identically handled and experienced placement and maintenance of an indwelling catheter prior to Period 2. Following a 14-day washout period from Period 1, concentrations of firocoxib were not detected at the start of Period 2. Period 2 was conducted in two trials using 10 animals per trial which were balanced for treatment (n=5 calves / treatment / trial). The two trials were initiated in consecutive days. In Period 2, calves previously receiving IV firocoxib now received oral firocoxib (Equioxx® Oral Paste, NADA 141-253, Merial LLC, Duluth, GA) and control calves received an oral whey protein placebo (Body Fortress, Bohemia, NY).

A jugular catheter was used for blood sample collection. Placement of the jugular catheter occurred approximately 12 hours prior to the start of the investigation. A handler manually restrained the calves and the area over the jugular vein was clipped and surgically prepared with alternating scrubs of 70% isopropyl alcohol and povidone iodine. The catheter site was infiltrated with 2% lidocaine injection, 1 mL subcutaneously (Hospira Inc, Lake Forest, IL). Using sterile technique, an 18 G x 55 mm intravenous catheter (SURFLO®, Terumo Medical Corp., Somerset, NJ) was inserted into the vein and sutured to the skin using #3 nylon suture (Ethilon™, Ethicon, San Lorenzo, PR). An injection port (Hospira Inc, Lake Forest, IL) was subsequently attached and disinfected with an alcohol swab prior to sample collection. Catheter patency was maintained by flushing with 3 mL of a heparin saline solution containing 3 USP units heparin sodium/mL saline (Heparin Sodium Injection, Baxter Healthcare, Deerfield, IL).

Calves assigned to receive firocoxib were administered a single oral dose of 0.5 mg/kg. Oral firocoxib was administered in a commercially provided dosing syringe with syringe weights obtained before and after administration to confirm the dose administered. The dose was rounded to the nearest 50 pounds as designated by the increments provided on the syringe. Using a concentration of 0.82% firocoxib, the actual mean dose of 0.5 mg/kg (range: 0.48 – 0.52 mg/kg) was administered to the calves as an oral paste in a dosing syringe. Control animals received an equivalent amount of whey protein mixed with water to a similar consistency as the firocoxib paste and administered via a similar shaped, voided dosing syringe. In order to control for the influence of milk feeding on absorption, all calves were given their milk feeding immediately after disbudding, approximately 10 minutes post-firocoxib or placebo administration. In relation to disbudding, this feeding time was the same for both treatment groups.

Disbudding

Disbudding was performed in 10 minute intervals by a single, experienced veterinarian and the same handler to minimize variation. The disbudding order was randomized between treatment groups to control for variation in start time. Cautery disbudding was initiated 10 minutes following administration of the local anesthetic and oral treatment. All calves received a local anesthetic prior to cautery disbudding. Desensitization of the cornual tissue was provided via a cornual nerve block using 2% lidocaine (VetOne®, Boise, ID) (5 ml / site) as described by Stock and others (2013). Desensitization of the cornual tissue was confirmed using behavior reactions (e.g. ear

flicks, head shaking, strong escape behavior) to a needle prick 5 minutes after administration of the cornual nerve block. Administration of lidocaine (1-2 ml) was repeated in the same manner described above if a response was observed to the initial needle prick. Following confirmation of appropriate desensitization, calves were cautery disbudded by placement of a pre-heated butane hot-iron (approx. 600 °C) (Express dehorner, Guilbert Express, New York, NY) on the horn tissue for approximately 10 seconds.

The disbudding sites were monitored daily for signs of discharge or infection, in addition to an assessment of attitude, posture, appetite, lying time, and peri-operative swelling. A rescue analgesia protocol of flunixin meglumine at 2.2 mg/kg, IV once daily for 3 days was devised if overt pain or distress was evident, such as increased lying time, head pressing, inflammation with major drainage of the disbudding site, dehydration or inappetence.

Blood Sample Collection

Animals were restrained during blood collection by trained handlers. Prior to sample collection, blood was aspirated and flushed back through the catheter to eliminate the heparin dilution before sample collection. Baseline samples were obtained at approximately -1.5 h from disbudding. Plasma drug concentration was confirmed as undetectable at this time. Additional blood samples were collected at 15 and 30 minutes and 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, and 96 h (-10 min) following disbudding. Sampling time points were determined in relation to the time drug or placebo was administered. Post-disbudding cortisol and substance P concentrations were subsequently analyzed in

relation to the time of disbudding. Samples were immediately transferred to a blood

collection tube with either heparin for cortisol and drug concentration or

ethylenediaminetetraacetic acid (EDTA) for substance P (Vacutainer®, BD

Diagnostics, Franklin Lakes, NJ) and stored on ice before processing. EDTA tubes

were spiked with 1 mM of benzamidine (Santa Cruz Biotechnology, Santa Cruz, CA)

prior to blood collection. Blood samples were centrifuged for 15 minutes at 1,500g at

room temperature. Collected plasma was placed in cryovials and frozen at -70 °C until

analysis.

Cortisol

Plasma cortisol samples were determined using a commercial radioimmune assay

kit (Coat-A-Count® Cortisol, Siemens Medical Solutions Diagnostics (formally

Diagnostic Products Corp.), Los Angeles, CA) previously used for bovine plasma

(Stilwell et al, 2008; Rialland et al, 2014). Samples were assayed in duplicate with the

reported concentration equaling the average cortisol concentration between duplicates.

Samples were reanalyzed if there were subjectively large discrepancies between the

duplicates. The average intra- and inter-assay coefficients of variation were 12.8 and

13.2%, respectively. Area under the effect curve (AUEC) was calculated using the

linear trapezoidal rule as previously described (Glynn et al., 2013).

Substance P

Substance P (SP) concentrations were analyzed as described by Van Engen and

others (2013) using non-extracted plasma. Samples were assayed in duplicate with the

reported concentration equaling the average SP concentration between duplicates. The

coefficient of variation for intra-assay variability was at 7.9% and the inter-assay

variability was calculated at 7.7%.

Prostaglandin E_2

Ex-vivo prostaglandin E_2 (PGE_2) synthesis inhibition was determined as described

by Fraccaro and others (2013). Blood collected at 4, 8, 12, 24, 48, and 72 h from calves

was placed into sterile vacuum tubes containing heparin. LPS obtained from *E. coli*

0111:B4 (Sigma-Aldrich, Co. St. Louis, MO) in PBS was added at 10 µg/mL to the

heparinized whole blood and incubated for 24 h at 37°C. Baseline samples were

incubated with and without LPS. At the end of incubation, all samples were centrifuged

at 400 *g* for 10 minutes at room temperature to obtain plasma. Methanol was added to

plasma in a 1:5 plasma to methanol dilution, facilitating protein precipitation. Following

centrifugation at 3,000 g for 10 minutes, the supernatant was collected and stored at -80

°C. A commercial PGE_2 ELISA kit (Cayman Chemical, Ann Arbor, MI) previously

described using methanol precipitated bovine plasma was used for determination of PGE_2

concentration (Donalisio et al., 2013; Fraccaro et al, 2013). The coefficient of variation

for intra-assay variability was at 8.9% and the inter-assay variability was calculated at

12.2%.

Nociception and Autonomic Nervous System Responses

Calves were restrained using a modified calf-restraining device (Easy B-Z

Portable Calf Restraint, eNasco, Fort Atkinson, WI) approximately 10 minutes prior to

determination of ocular temperature (OT), heart rate (HR), and mechanical nociception

threshold (MNT). Additionally, following infrared thermography imaging and heart

rate determination, calves were blindfolded for MNT to avoid withdrawal reflex based

on visual cues. Baseline samples for OT, HR, and MNT were obtained on the same day

for both trials thus 16 and 21 hours prior to initiation of the first trial and 38 and 42

hours prior to initiation of the second trial. Baseline data for each animal were averaged

for use in statistical analysis. In addition to baseline samples, response variables were

collected at approximately 2, 4, 7, 8 and 24 h post-disbudding. All individuals

collecting data were blinded to calf treatment group assignment.

Infrared Thermography.

A thermography camera (FLIR SC 660, FLIR Systems AB, Boston, MA) with a

thermal sensitivity of 0.05 Celsius, 320 x 240 pixel display, precision > 98%, supported

by research grade data analysis software, was utilized to quantify changes in ocular

temperature. The camera was internally calibrated to ambient temperature prior to

image collection; however additional minute adjustments to ambient temperature and

humidity were utilized during software processing. Images were obtained from the left

side of the calf, at an approximately 45° angle, and 0.5 meter distance from the eye.

Maximum temperature (°C) within a circumferential area of the eye including the

medial posterior palpebral border of the lower eyelid and the lacrimal caruncle was

obtained as previously described (Stewart et al., 2008). Images were analyzed using

FLIR Tools (v. 4.1; FLIR Systems Inc, Boston, MA) following collection. At each

timepoint, three images were obtained and averaged for statistical analysis.

Heart Rate.

Heart rate was evaluated via auscultation using a stethoscope (3M™, Littmann®, St, Paul, MN, USA) placed between the 3rd and 5th intercostal space and beats were counted over a 30 second period. The value obtained was used to calculate beats per minute.

Mechanical Nociception Threshold.

Pain sensitivity was measured using a hand held pressure algometer (Wagner Force Ten™ FDX 25 Compact Digital Force Gage, Wagner Instruments, CT, USA) around the disbudding site as previously described with modifications (Tapper et al., 2011, Heinrich et al., 2010; Allen et al., 2013). Two landmark locations around each horn bud as well as a control landmark were used (Figure 2). The mechanical nociceptive threshold (MNT) was determined as the peak applied force resulting in a withdrawal response defined as a directed movement of the head away from the applied pressure (Tapper et al., 2011). Using a rate of approximately 1.0 kgf/second, the stimulus was applied perpendicular to the landmarks. A maximum force ceiling was established at 10 kgf. In order to prevent bias, the mechanical nociceptive threshold output was not visualized by the individual operating the algometer. Moreover, the order of MNT landmarks and the calf side from which the pressure algometrist stood to apply the stimulus was randomized between each calf to control for potential effects on MNT determination. Each algometry site was assessed three times for each timepoint with the values averaged for the statistical analysis.

Average Daily Gain

Animals were weighed using a Way-Pig® 505 (Raytec Manufacturing, Ephrata, PA) scale 24 hours prior to the disbudding (d -1), and 7 days following disbudding (d7). Average daily gains were calculated by dividing the total weight gained between measurements and then dividing by the number of days.

Statistical Analysis

Analyses were performed in SAS 9.3 (SAS Institute, Cary NC) using a linear mixed effects model with repeated measures. Data obtained from response variables including MNT, cortisol, substance P, and PGE_2 were log transformed for normality. Baseline values were used as covariates for all variables analyzed. The fixed effects were treatment (firocoxib, placebo), time, the interaction between treatment and time. The effect of sex was tested in the statistical model and since there was no effect (P>0.1), sex of calf was removed to improve the fit of the statistical analysis. Trial was a random effect and calf was the subject of repeated measures. F-tests were used to test the significance of main effects and interactions. If significant overall differences were identified among levels of a factor, pairwise comparisons were performed using Tukey's t-tests. Additionally, paired t tests were performed to test the differences between response variable baselines as well as LPS-stimulated and unstimulated baseline ex-vivo PGE_2 concentrations. Statistical significance was designated as a *P* value < 0.05; *P* values ranging between 0.05 and 0.1 were discussed as a tendency to significance.

Results

No animals required rescue analgesia or were removed throughout the course of the study. There was no effect of sex observed on any measured responses (P>0.1). As such, sex was removed from the statistical analysis.

Cortisol

Mean cortisol concentrations were not significantly different between treatment groups (P=0.80) (Table 1). Both a time effect (P<0.0001) and time x treatment interaction (P=0.0076) were observed in cortisol concentrations throughout the 96 h sampling period (Table 1). Initially, cortisol concentrations increased following disbudding, peaking, on average, at approximately 20 minutes following the hot-iron procedure. Mean cortisol concentrations decreased thereafter. At 50 minutes post disbudding, placebo-treated calves had a significant reduction (2.2 nmol/L) in cortisol concentrations compared with firocoxib treated calves (P=0.012) (Figure 3a). In contrast, at approximately 48 h post-disbudding, a significant increase in cortisol concentrations was observed in placebo treated calves compared with firocoxib treated calves (22.8±2.9 nmol/L vs. 11.5±1.7 nmol/L) (P=0.0006) (Figure 3b). Furthermore, the integrated cortisol response calculated as the area under the effect curve (AUEC) tended to be reduced in firocoxib treated calves (1157.0±179 nmol.h/L vs. 1610.8±255 nmol.h/L) (P=0.093) (Figure 4).

Substance P

Mean±SE substance P concentrations in firocoxib treated calves (22.7±0.7 pg/ml) were not significantly different compared with calves receiving placebo (20.8±0.4 pg/ml)

(P=0.61) (Table 1). Additionally, there was no effect of time (P=0.42) or a time x treatment interaction (P=0.66) observed for the 96 h sampling period.

Prostaglandin E_2

Due to laboratory error in sample processing, samples collected and processed for ex-vivo PGE_2 synthesis at 4 and 8 h were removed from the statistical analysis. An overall treatment effect was observed with PGE_2 synthesis reduced in firocoxib treated calves (770.4±97.9 pg/ml) compared with placebo treatment (1,249.7±105.5 pg/ml) (P=0.0012). Moreover, an effect of time (P<0.0001) and a time x treatment interaction (P=0.019) was observed. Firocoxib treatment resulting in decreased PGE_2 concentrations at 12 h (P<0.0001), 24 h (P=0.0073), and 48 h (P=0.0005) (Figure 5). At 72 h, no difference was observed between treatment groups (P=0.34). Baseline LPS-stimulated blood resulted in a significant increase in PGE_2 concentrations compared to non-LPS stimulated control samples for both treatment groups (P<0.0001).

Infrared thermography

Mean ocular temperatures were not significant between treatment groups (P=0.85). A time effect was observed in ocular temperature response following disbudding (P<0.0001) (Table 1) (Figure 7). For both firocoxib and placebo treated calves, ocular temperatures numerically decreased 0.12±0.10 °C and 0.15±0.10 °C from 2 to 4 h following disbudding. Ocular temperature significantly increased thereafter with a mean peak temperature recorded at 8 h for both treatment groups. Additionally, a significant temperature reduction was observed 24 h post-disbudding for both treatment

groups in comparison to all previous time points, including baseline (P<0.0001). No

interaction between time and treatment (P=0.98) was observed.

Heart rate

No treatment effects were observed between treatment groups (P=0.96). A time

effect was observed with heart rate altered following disbudding (P=0.019) (Table 1).

There was no time x treatment interaction (P=0.16). Interestingly, heart rate significantly

decreased in firocoxib treated calves 24 hours post-disbudding compared to both 7 h

(P=0.021) and 8 h (P=0.0030). This response was not observed in placebo treated calves.

Mechanical Nociception Threshold (MNT)

Numerically, firocoxib treated calves tolerated more pressure around the horn bud

area (1.15±0.16 kg) compared to placebo treated controls (0.96±0.14), however this

effect was not significant (P=0.56) (Table 1). Overall there was a time effect on MNT

(P<0.0001). Post-disbudding measurements were reduced below baseline values

throughout the 24 hour testing period (Figure 6). There was no evidence of an interaction

of time and treatment (P=0.84).

Average Daily Gain

Initial weights were determined the day prior to disbudding. The mean weights

(LSM±SE) prior to disbudding were 55.5±1.7 kg and 55.0±2.0 for the firocoxib and

placebo groups, respectively. All calves gained weight throughout the study period with

final mean weights (LSM±SE) equaling 59.1±2.0 and 59.0±2.4 for firocoxib and placebo

treated groups, respectively. There was no difference in average daily gain for firocoxib and placebo treated calves (0.5±0.1 kg vs. 0.5±0.1 kg) (P=0.61) (Table 1).

Discussion

Cautery disbudding in calves resulted in an increased cortisol concentrations as well as an increased sensitivity as determined by mechanical nociception threshold. Moreover, changes in nociception persisted throughout the 24 h study period where local pressure tolerated by calves did not return to baseline values. Firocoxib administration did not alter the tested responses observed between treatment groups over the initial 24 h period; however, cortisol concentrations between treatment groups diverged at 48 h post-disbudding where firocoxib treated calves had reduced cortisol concentration. Furthermore, the integrated cortisol response tended to be decreased in firocoxib treated animals compared with calves administered a placebo. These differences observed following the initial 24 h could be attributed to the administration of an NSAID with persistent concentrations; however, additional study is required to determine its long-term effects.

Historically, perioperative analgesics administered to calves prior to dehorning or disbudding have used non-steroidal anti-inflammatories (NSAIDs) that have data, both public and proprietary, to support an anti-inflammatory claim (Stilwell et al. 2009; Heinrich et al, 2010; Duffield et al., 2010; Glynn et al. 2013). This aids in the dose determination for an investigation into its analgesic potential. However, for this study there is no information publicly available concerning the analgesic use of firocoxib in preweaned calves. As such, the dose of 0.5 mg/kg used in this study was based on

pharmacokinetic information available in the horse labeled for use at 0.1 mg/kg. The

equine approved dose demonstrated analgesia in naturally occurring lameness following

multiple daily doses (Orsini et al., 2012). More recently, it has been suggested to use a

loading dose in horses to achieve steady state drug concentrations more quickly (Cox et

al., 2013). As a result, effective concentrations may be reached more quickly. In our

study, a one-time dose administration was anticipated for the practical application of this

analgesic in calves. As such, the increased dose compared with the horse was chosen to

best provide potentially effective concentration of drug as well as remain practical in its

administration. However, we cannot rule out that differences in pharmacokinetic

properties of firocoxib between species may also have contributed to the differences in

analgesic response.

A primary property of most NSAIDs is reducing eicosanoid production via

inhibition of cyclooxygenase isoenzymes. Therefore, a potential method of dose

evaluation involves an investigation into a drugs ability to decrease PGE_2 concentrations,

a major eicosanoid metabolite associated with inflammation and nociception signaling

threshold reduction (Basbaum et al., 2009). An investigation of the PGE_2 in this study

indicates the administered dose reduced ex vivo PGE_2 synthesis from 12 to 48 h

compared to placebo treated controls. Although further evaluation is required to

determine concentrations necessary to achieve analgesia, it should be noted that the

administration of firocoxib at the study dose attained concentrations necessary to

significantly inhibit ex-vivo PGE_2 concentrations for at least 2 days. This is similar to the

findings of Allen and others (2013) who observed a suppression of ex-vivo PGE_2

synthesis for 48 h after oral administration of meloxicam at 1 mg/kg at the time of disbudding.

During the first 24 h, cortisol concentrations were similar over time for both treatment groups. Although placebo treated calves had decreased cortisol concentrations 50 minutes post-disbudding, the effect size was small and the response may be confounded with the desensitization of the local anesthetic. The initial increase in cortisol concentrations peaking at 20 minutes post-disbudding and returning to pre-treatment levels within approximately 1 h is similar to that presented in previous literature (Stafford and Mellor, 2005). Moreover, in combination with a local anesthetic, the use of cautery following amputation dehorning nearly eliminated the cortisol response for 24 h (Sutherland et al, 2002) to 36 h (Sylvester et al, 1998) after dehorning. It should be noted that in the former study, both lidocaine and then bupivacaine 2 h later were used to provide local anesthesia for a duration of 5 h (Sutherland et al., 2002). This significantly reduced cortisol response is also observed in lambs undergoing cautery tail docking, suggesting that cautery may attenuate the nociception signal below the pain threshold for transmission (Lester et al., 1991; Stafford and Mellor, 2011). An attenuation of this systemic response using cautery may contradict the MNT profile observed in this study; however, the pressure algometer evaluates local sensitivity surrounding the horn bud and may not reflect a systemic distress response.

Following 24 h, the mean cortisol response diverges between the two treatment groups with placebo treated calves increasing in cortisol with a peak at approximately 48 h post-disbudding. Using cortisol as a measure of distress, firocoxib administration may have mitigated the distress associated with disbudding. This statement is further

supported with evidence suggesting that firocoxib concentrations between 24 and 48 h continued to reduce ex-vivo PGE_2 synthesis. Prolonged changes in cortisol associated with dehorning and disbudding have been difficult to routinely characterize. Morisse and others (1995) reported elevated cortisol concentrations 24 h post-dehorning compared to control calves 8 wk of age. More recently, in 8 to 10 wk old calves, Allen and others (2013) reported a mean increased cortisol concentration from 96 to 120 h in calves treated with meloxicam 12 h prior to dehorning. Calf behavior, including ear flicks, grazing time, and rumination responses, provide further support that disbudding and dehorning pain may continue for 24 to 48 h (Heinrich et al., 2010, Faulkner and Weary, 2000; Stafford and Mellor, 2005). These changes in cortisol and behavior may be mediated by an increased inflammatory response evidenced by an increased haptoglobin concentration reported 24 to 48 h following amputation dehorning in 6 mo old calves (Glynn et al., 2013). In contrast, other studies report cortisol concentrations return to and are maintained at baseline values 24 h (Sutherland et al., 2002) to 36 h (Sylvester et al., 1998) in 3-4 mo or 5-6 mo old calves, respectively, that were scoop dehorned followed by wound cauterization.

Substance P (SP), a neuropeptide previously indicated as a pain biomarker in cattle, was not different between treatment groups (Coetzee et al, 2008). Moreover, there was no significant change in SP over time. These data differ from other investigations evaluating substance P following dehorning. In 8 to 10 wk old calves, Allen and others (2013) reported a time by treatment interaction with a significant increase in SP concentrations at 120 h post-cautery dehorning in control animals compared with those receiving meloxicam. Furthermore, Coetzee and others (2012) reported a significant

reduction in SP following administration of an NSAID, meloxicam, following scoop

dehorning in 4 to 5 mo old calves. Differences in the length of sample collection time,

disbudding / dehorning method, and analytical method used to measure SP may be the

reason for the observed disagreement with the present study. Moreover, age differences

in these study populations may significantly influence SP response. Dockweiler and

others (2013) reported a decreased response in SP in cattle castrated at ≤8 wk in

comparison to cattle ≥6 mo. The authors suggest a reduced pain response or different

physiological parameters contributing to this difference. Coupled with data collected in

the present study, SP may be a poor indicator of pain in young animals. Both in this

study and demonstrated by Allen and others (2013), no significant differences in SP were

reported for the first 96 h following dehorning. As SP release is thought to be associated

with pain, evidence from this study support the hypothesis that cautery may destroy

nearby nociceptors necessary to reach pain thresholds needed for central hyperalgesia

transmission (Sylvester et al., 1998; Sutherland et al., 2002). It should be noted this

hypothesis was derived from the evaluation of the cortisol response following local

anesthesia and scoop then cautery dehorned calves greater than 3 mo of age.

Local nociception changes persisted for 24 h in both treatment groups as observed

in the MNT profiles such that nociception thresholds did not return to baseline values

during the measured response period. This duration of sensitivity appears to be consistent

with other reports indicating a prolonged response following dehorning / disbudding

based on behavior (Faulkner and Weary, 2000; Heinrich et al., 2010) and on nociceptive

thresholds (Tapper et al., 2011). Average MNT profiles were similar between treatment

groups, suggesting firocoxib did not significantly demonstrate anti-nociceptive effects

over the 24 h investigation period post-disbudding. This response differs from MNT values reported following perioperative administration of meloxicam (Heinrich et al., 2010) and ethanol as a local anesthetic (Tapper et al., 2011). Repeated handling for data collection may have heightened the avoidance responses of calves in the current study when compared to the nociception threshold testing performed at one time point by Heinrich and colleagues (2010). Reduction in subtle dehorning pain related behaviors, such as ear flicking and head rubbing, have been associated with perioperative administration of meloxicam (Heinrich et al., 2010), ketoprofen (Faulkner & Weary, 2000; Duffield et al., 2010) and carprofen (Stilwell et al., 2012), but this was not assessed in the current study.

Changes to the autonomic nervous system (ANS) response including heart rate and ocular temperature were observed post disbudding. Although not statistically significant, the decrease in ocular temperature observed between 2 and 4 h most likely was due to the loss of the local anesthetic effect as previously described by Stewart and others (2009). The significant decrease of ocular temperature at 24 h for both treatment groups may also suggest a continued ANS response caused by disbudding; however, changes in temperatures due to diurnal core temperature changes or environment cannot by eliminated (Vickers et al., 2010; Church et al., 2014). Although prior studies have indicated a reduced volatility of the ANS with the use of an NSAID following cautery disbudding or dehorning (Stewart et al., 2009; Heinrich et al., 2009; Coetzee et al., 2012), no treatment differences were observed over the 24 h sampling period for both ocular temperature and heart rate in the present study. Animals were frequently handled prior to disbudding in order to help reduce response variations associated with sample collection.

However, responses of the autonomic nervous system may vary according to novel stimuli and exertion; frequent handling required for blood collection may result in elevated responses regardless of the administration of an analgesic. As no analgesic effects were noted in any response variable during the first 24 h, firocoxib administration at the study dose may be ineffective at managing the acute nociception and distress associated with cautery disbudding.

Conclusion

Evidence provided in the current study indicates cautery disbudding resulted in changes in nociception and cortisol concentrations. The study dose of firocoxib was sufficient to effectively reduce ex-vivo PGE_2 synthesis, however, determination of analgesic concentrations require further investigation as no significant differences in analgesic response variables were observed in the first 24 h. As such, the relationship between response variables and ex-vivo PGE_2 inhibition requires further exploration. Overall, a one-time oral administration of firocoxib reduced cortisol concentrations at 48 h and contributed to the attenuated integrated concentration of plasma cortisol; however the acute response (<24 h) as measured by ocular temperature, heart rate, MNT, cortisol, and substance P was unaffected by treatment. Further research is needed to determine the significance of the prolonged cortisol response following disbudding and the potential for firocoxib to ameliorate this effect.

Acknowledgements

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Table 1. Summary table of untransformed response variables of firocoxib and placebo treated calves post-disbudding

Response	Firocoxib LSM±SE	Placebo LSM±SE	Treatment (P value)	Time (P value)	Time x Treatment (P value)
Cortisol (nmol/L)	13.1 ± 0.9	15.1 ± 1.0	0.80	<0.0001	0.0076
Substance P (pg/mL)	22.7 ± 0.7	20.8 ± 0.4	0.61	0.42	0.66
Ocular temperature (ºC)	37.7 ± 0.1	37.5 ± 0.1	0.85	<0.0001	0.98
Heart rate (bpm)[2]	109.5 ± 1.7	109.8 ± 2.2	0.96	0.019	0.16
MNT[1] (kgf)	1.15 ± 0.16	0.96 ± 0.14	0.56	<0.0001	0.84
ADG (kg/day)	0.5 ± 0.1	0.5 ± 0.1	0.61	NA	NA

[1] Mechanical Nociception Threshold

[2] Beats per minute
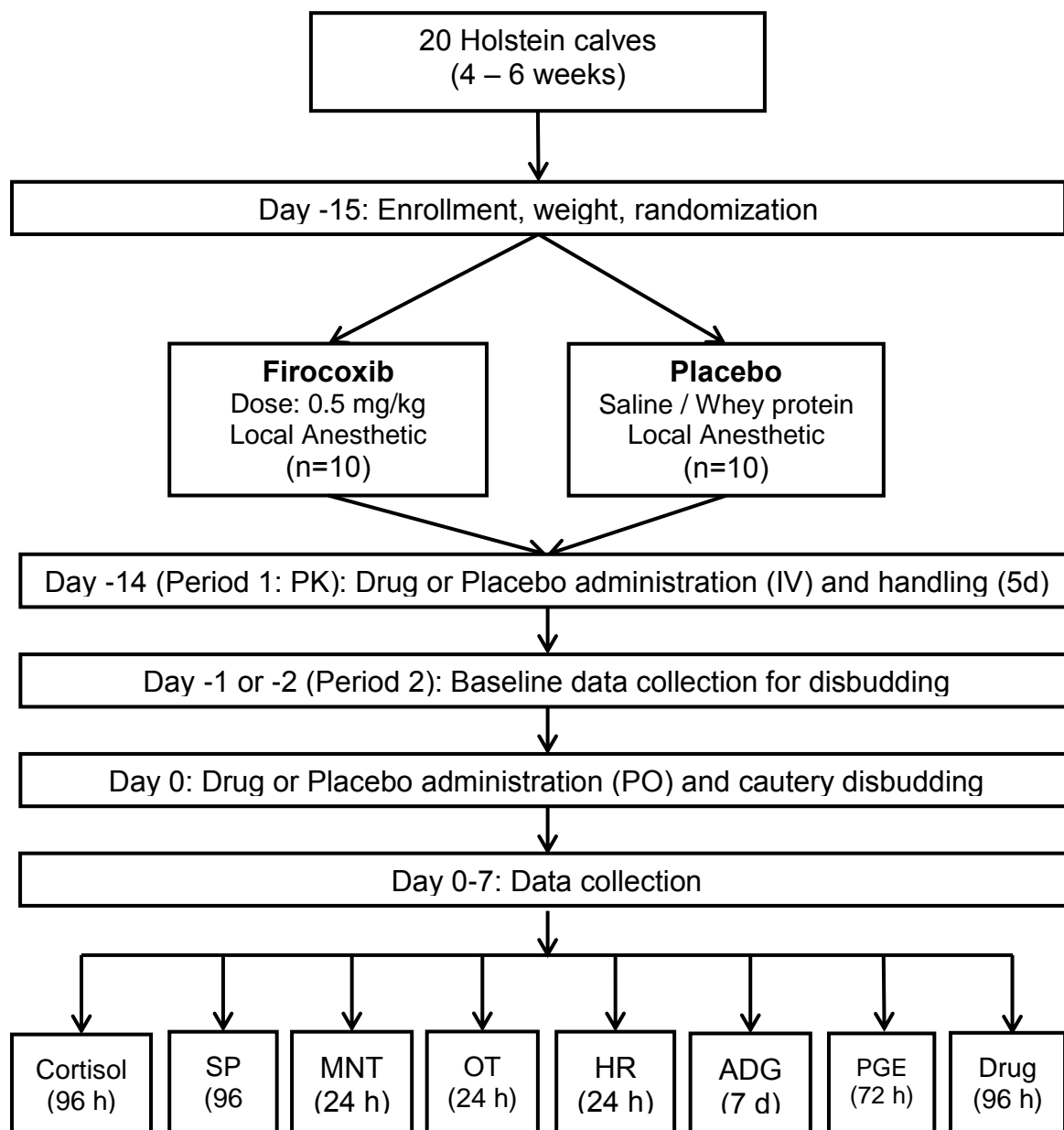
Figure 1. Flow Chart outlining the timing of the study events. During Period 1, firocoxib or placebo was administered IV to calves to provide data to be analyzed for a pharmacokinetic study. In Period 2, calves were disbudded and monitored for cortisol, substance P (SP), mechanical nociception threshold (MNT), ocular temperature (OT), heart rate (HR), average daily gain (ADG), ex-vivo prostaglandin E_2 (PGE), and firocoxib concentration. The times in parentheses represent the duration of data collection for each variable.

Caudal

1   1

2   2

3

L   Cranial   R
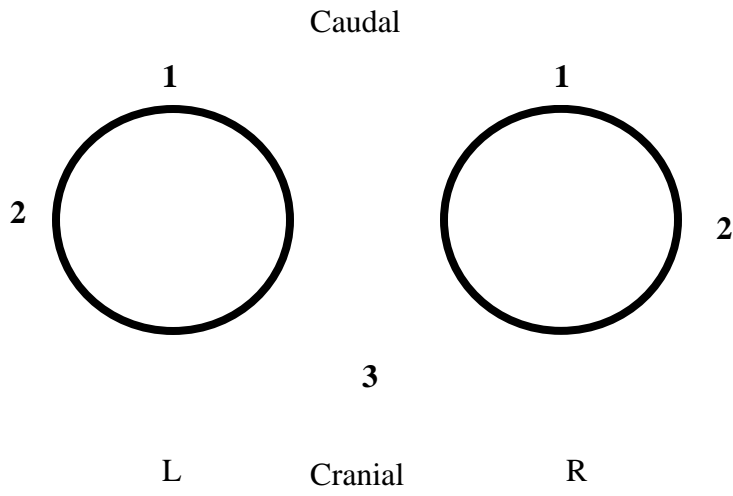
Figure 2. Pressure algometry locations at each horn bud

(a)
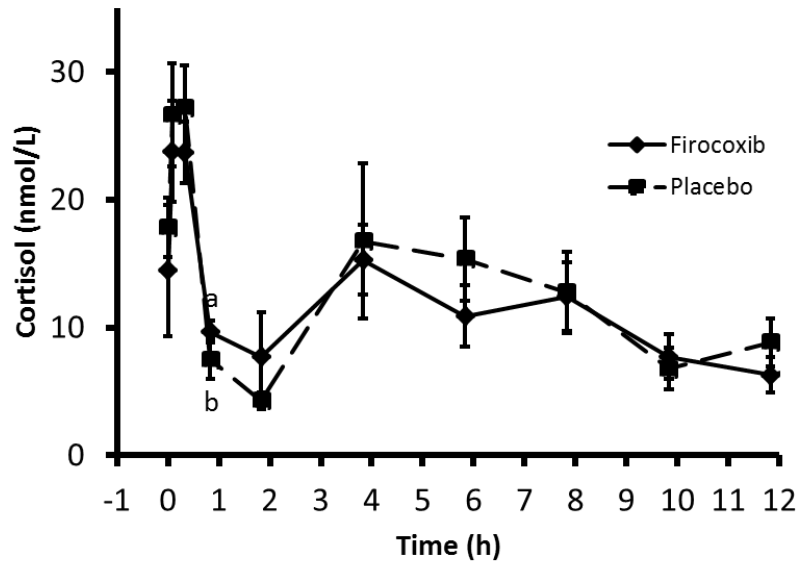
(b)
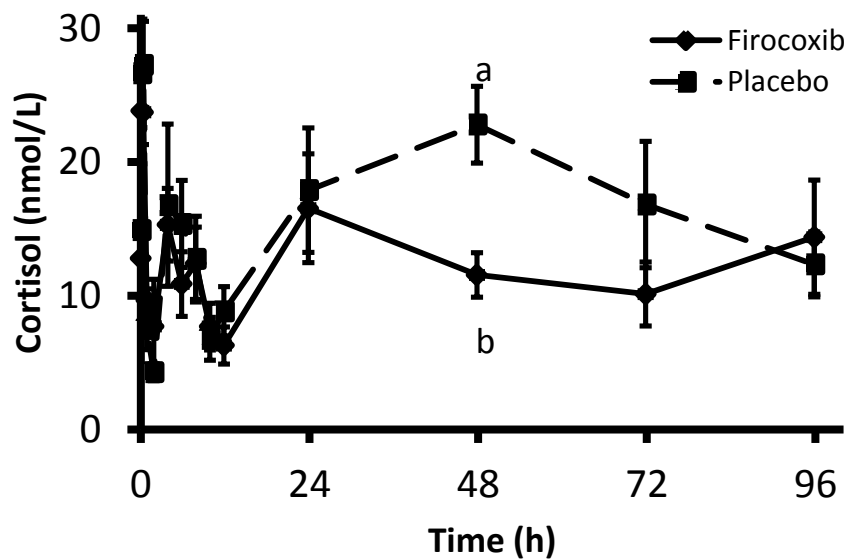
Figure 3. Untransformed cortisol concentrations over 12 h (a) and 96 h (b) for firocoxib and placebo treated calves post cautery disbudding. Baseline values are graphically represented at Time 0, the time of disbudding. Significant differences (P<0.05) between time points are indicated by different letters (a, b).
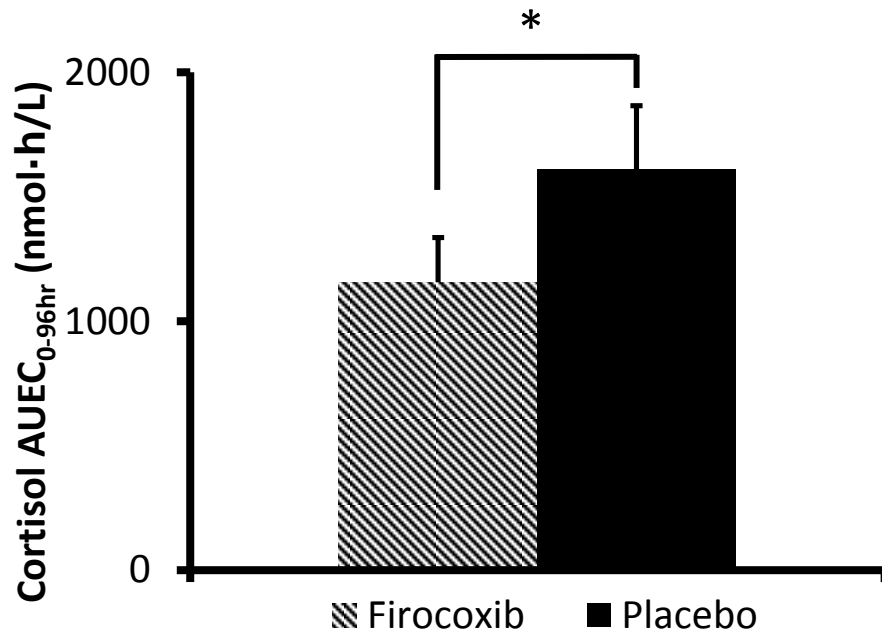
Figure 4. Area under the effect curve (AUEC) of untransformed cortisol concentrations over the 96 h study period for firocoxib and placebo-treated calves post-disbudding. Values were calculated using the linear trapezoid method. * indicates differences between treatment groups (P=0.093).
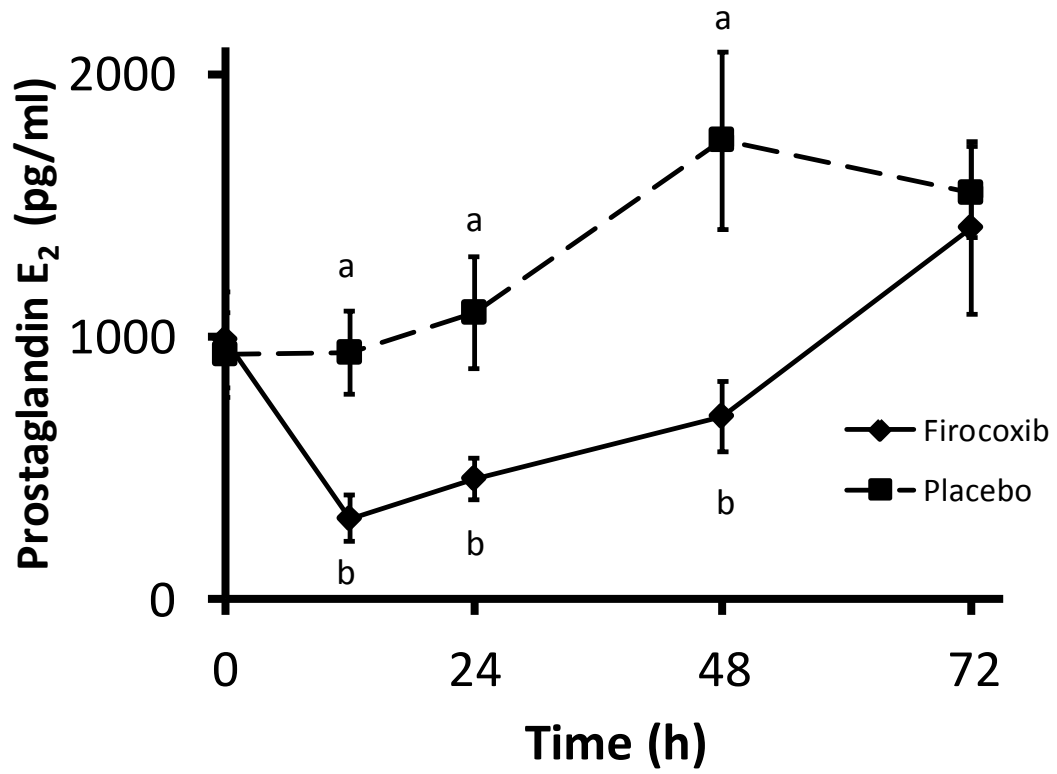
Figure 5. Untransformed ex-vivo prostaglandin E_2 concentrations over 72 h in firocoxib and placebo treated calves post-disbudding. Significant differences (P<0.05) between time points are indicated by different letters (a, b). Baseline values are represented at time 0.
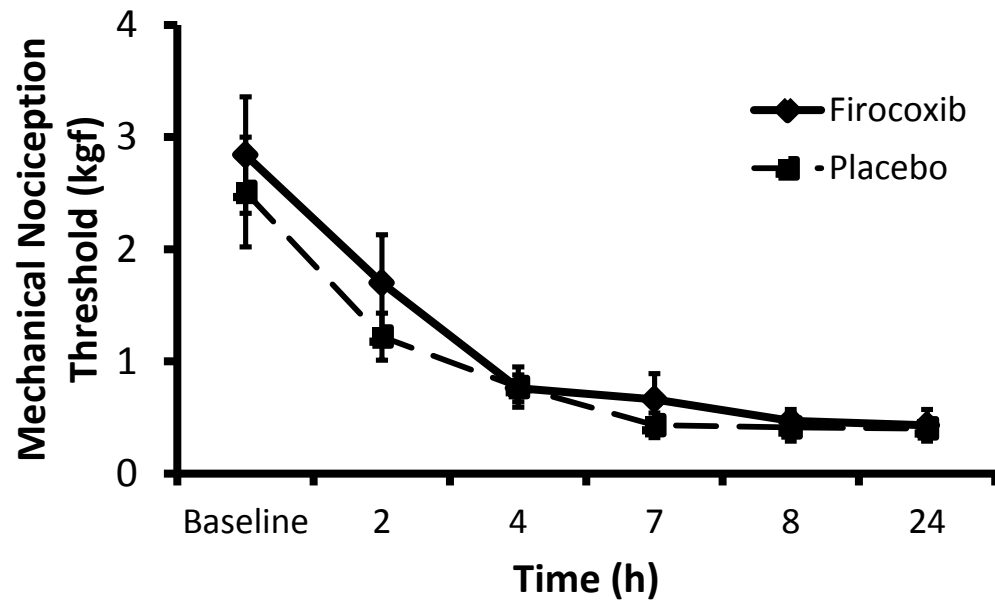
Figure 6. Untransformed Mechanical Nociception Threshold (MNT) as measured through pressure algometry over 24 h for firocoxib and placebo treated calves. Pressure tolerance did not return to baseline values by the conclusion of MNT measurement. Baseline values are indicated.
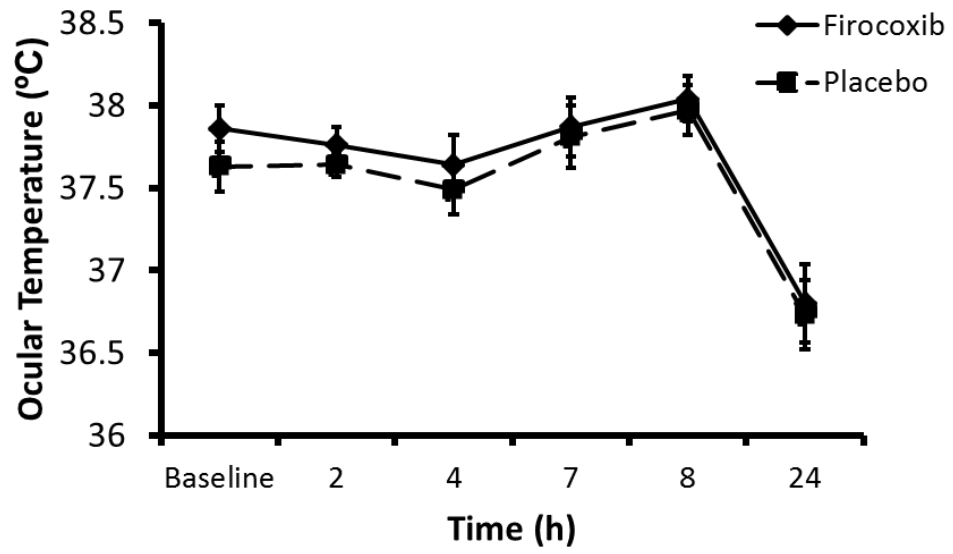
Figure 7. Untransformed ocular temperature as measured through infrared thermography over 24 h for firocoxib and placebo treated calves. Baseline values are indicated.

CHAPTER 5

IMPACT OF CARPROFEN ADMINISTRATION ON STRESS AND NOCICEPTION RESPONSES OF CALVES TO CAUTERY DEHORNING

Modified from a manuscript submitted to the *Journal of Animal Science*

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Abstract

The objective of this study was to investigate the effects of carprofen administered immediately prior to cautery dehorning on nociception and stress. Forty Holstein calves aged approximately 6 to 8 weeks old were either sham dehorned (n=10) or cautery dehorned following administration of carprofen (1.4 mg/kg) subcutaneously (n=10), orally (n=10) or a placebo (n=10) in a randomized, controlled trial. All animals received local anesthesia via a cornual nerve block using lidocaine prior to dehorning.

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Response variables including mechanical nociception threshold, ocular temperature, heart rate and respiratory rate were measured prior to and following cautery dehorning for 96 h. Blood samples were also collected over 96 hours following dehorning and analyzed for plasma cortisol and substance P concentrations by radioimmune assay. Plasma carprofen concentration and *ex-vivo* prostaglandin E2 concentrations were also determined for this time period. Average daily gain was calculated for 7 days post dehorning. Data were analyzed using a linear mixed effects model with repeated measures, controlling for baseline values by their inclusion as a covariate in addition to planned contrasts. Dehorning was associated with decreased nociception thresholds throughout the study and a stress response immediately after dehorning, following the loss of local anesthesia, and 48 h post-dehorning compared to sham dehorned calves. Carprofen was well absorbed after administration and reached concentrations that moderately inhibited ex-vivo prostaglandin E2 concentrations and were reduced for 72 h (SQ) and 96 h (PO) compared with placebo treated calves (P<0.05). Carprofen-treated calves tended to be less sensitive (P=0.096) to nociceptive threshold tests and had a decreased cortisol (P=0.10) and substance P (P=0.016) release at 8 h following dehorning, however, increases in cortisol at 12 h (P=0.069) and respiratory rate at 48 h (P=0.0040) were observed in carprofen treated calves compared to placebo treated calves. Oral carprofen administration resulted in the largest average daily gain, albeit not different than placebo treated calves (P=0.23) but increased compared with calves administered carprofen subcutaneously (P=0.0039). Overall, at the study dose, the effect of carprofen on sensitivity and stress following dehorning was minimal; although given the observed

tendency towards reduction of nociception and stress associated with dehorning as well as the prolonged prostaglandin inhibition, further dose titration studies may be warranted.

Introduction

The pain and distress associated with disbudding and dehorning has been evaluated using changes to physiology, behavior, and the neuroendocrine system (Stafford and Mellor, 2011; Stock et al, 2013). Analysis of affective states such as pain and distress are often achieved through the measurement of indirect measures such as changes in the hypothalamic-pituitary-adrenal axis (McMeekan et al., 1998), autonomic nervous system response (Stewart et al., 2008), and behavior (Faulkner and Weary, 2000). Despite these previously reported changes indicative of pain and distress following a noxious event, there are currently no approved analgesics for cattle in the United States (Coetzee, 2013a).

Several pain mitigating strategies have been evaluated. Through reductions in cortisol, a multimodal approach to analgesia including the use of local anesthesia (McMeekan et. al, 1998), non-steroidal anti-inflammatory drugs (NSAID) (Huber et al., 2013), or sedatives (Stilwell et al., 2010) may provide optimal pain relief (Stafford and Mellor, 2005; Coetzee, 2013b). Furthermore, additional benefits may be observed from use of an analgesic with persistent activity (Heinrich et al, 2010; Coetzee, 2011; Allen et al., 2013).

Carprofen is an NSAID with a prolonged half-life in cattle that is administered as a racemic (RS±) mixture with efficacy primarily attributed to the S(+) enantiomer (Brentnall et al., 2012). Currently, it is approved in countries in Europe and Asia as an adjunctive treatment for inflammation associated with pneumonia in young calves.

Carprofen has demonstrated both COX-dependent (Micelleta et al., 2014) and COX-independent mechanisms including attenuating IL-6, a proinflammatory cytokine in equine synoviocytes and chondrocytes (Armstrong and Lees, 2002). Together with the significance of IL-6 mediating the pain associated with burns in rodents, carprofen may be ideally suited to manage pain associated with cautery dehorning in cattle (Summer et al, 2008).

The objective of our study is to investigate the effect of peri-operatively administered carprofen on nociception and stress following cautery dehorning.

Materials and Methods

Animals and Housing

Forty Holstein calves (18 castrated males and 22 females) with a mean $\pm$ SD age of 50.9 ± 5.3d and mean weight $\pm$ SD of 63.8 ± 8.7 kg at dehorning were included in this study. The Iowa State University Dairy provided the calves used in the study. A veterinarian determined all calves were healthy based on physical examination prior to enrollment. This study protocol was approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC Log # 5-13-7566-B)

Calves were housed on the Iowa State University Dairy in a naturally-ventilated, group housing facility. A total of five groups of eight animals were moved from individual 3-sided pens into group housing 6 days prior to dehorning for environmental and social acclimatization. Group pens (3.7 x 6.4 m) were bedded with straw added daily. Both water and grain were provided to the calves *ad libitum* for the duration of the trial. Grain consisted of primarily pelleted corn, oats, molasses

protein/vitamin/mineral supplement, and monensin (Table 1). The study was conducted in July and August.

Animals were monitored twice daily for health observations. Animals appearing depressed or lethargic were examined by a veterinarian and monitored or treated accordingly. Veterinarian diagnosed respiratory disease was diagnosed based on persistent elevated rectal temperature (>103.5°C), spontaneous cough, and ocular and/or nasal discharge. Respiratory disease was treated using tulathromycin (2.5 mg/kg) (Draxxin®, Pfizer Inc., New York, NY) with resolution of clinical signs following treatment. Overall, one animal was treated in the SHAM group, two animals were treated in the both the PO and PLCBO groups, and 3 animals were treated in the SQ group throughout the length of the study. Calves did not require further treatment for any other disease process.

Study Design

A randomized controlled trial design was used for this investigation. Study animals were randomly assigned to receive carprofen orally (1.4 mg/kg) (PO) (n=10), subcutaneously (1.4 mg/kg) (SQ) (n=10), or sterile saline subcutaneous placebo and oral lactose placebo with actual dehorning (PLCBO) (n=10) or with sham dehorning (SHAM) (n=10) (Figure 1). In addition, an oral and SQ placebo was administered to all calves not administered carprofen such that all animals received an oral bolus and SQ injection. Randomization of group assignments was accomplished using a computer generated random number using calf weight (Microsoft Excel, Redmond, WA, USA) as described by Theurer et al. (2012). In addition, animals were blocked by sex to ensure

equal distribution of sex within the treatment groups. Following randomization and group assignment, calves were enrolled in one of 4 treatment groups (n=10) containing equal number of animals. Five phases of 8 calves per phase were used during a 6 week period of time. Two animals from each treatment group were represented in each phase (n=2 calves / treatment / phase) and were group housed in equivalent pens. Investigators collecting data were masked to the treatment groups.

A jugular catheter was placed for the purpose of blood sample collection as previously described in Stock et al., 2014. Briefly, a trained handler manually restrained the calves during catheter placement. The jugular area to be catheterized was clipped and surgically prepared using 70% isopropyl alcohol and povidone iodine. Local anesthesia consisting of subcutaneously administered 1 mL of 2% lidocaine was provided in the intended catheterized area (Phoenix™, Sparhawk Laboratories, Inc., Lenexa, KS). A 18 G x 55 mm intravenous catheter (SURFLO®, Terumo Medical Corp., Somerset, NJ) with injection plug (Hospira Inc, Lake Forest, IL) was inserted into the vein. The catheter was sutured using #3 nylon suture (Ethilon™, Ethicon, San Lorenzo, PR). Using 3 mL of a heparin saline solution containing 3 USP units heparin sodium/mL saline (Heparin Sodium Injection, Baxter Healthcare, Deerfield, IL), catheter patency was maintained. The catheter port was disinfected with an alcohol swab prior to sample collection.

Study animals (n=10) were administered SQ carprofen (1.4 mg/kg) (Rimadyl® Injectable, 50 mg/mL; NADA 141-199, Pfizer Inc.) under the skin in the cervical muscular region in compliance with Beef Quality Assurance (BQA). The test article was stored according to manufacturer's instructions. A 3 ml syringe was used to

administer the dose which was rounded to the nearest tenth mL. Calves enrolled in the other groups not receiving SQ carprofen (PO, PLCBO, and SHAM) received an equivalent amount of 0.9% saline subcutaneously in the same manner previously described.

Oral carprofen (1.4 mg/kg) administration was completed using tablets of two different concentrations in order to provide the most accurate targeted dose. Either 100 mg tablets (Novox® Caplets, ANADA #200-498, Vedco, Inc.) or 75 mg tablets (Novox® Caplets, ANADA #200-498, Vedco, Inc) were provided to calves in a small gelatin capsule (Torpac®, Fairfield, NJ) administered using an oral balling gun. The test article was stored according to manufacturer's instructions. Using the two different formulations, the dose was rounded to the nearest tablet of the weight determined 24 hrs prior to administration. Using these two tablet formulations, the actual mean dose of 1.40 mg/kg (range: 1.13 – 1.63 mg/kg) was administered to the calves as an oral bolus. Animals not receiving oral carprofen (SQ, PLCBO, SHAM) were identically administered a placebo (lactose powder) encapsulated in the same gelatin bolus.

Dehorning

Dehorning was performed in 10 minute intervals. Calves were restrained during dehorning using a modified calf restraining device (Easy B-Z Portable Calf Restraint, eNasco, Fort Atkinson, WI). Cautery dehorning was initiated 10 minutes following administration of the oral bolus, subcutaneous injection and local anesthetic. A local anesthetic (2% lidocaine; VetOne®, Boise, ID) (5 ml / site) was administered to all calves using a cornual nerve block as described by Stock and others (2015). Effective

local anesthesia of the cornual tissue was confirmed using behavior reactions (e.g. ear flicks, head shaking, strong escape behavior) to a needle prick 5 minutes after administration of the cornual nerve block. Following confirmation of appropriate desensitization, calves were cautery dehorning by placement of a pre-heated electrical hot-iron (approx. 600 °C) (Dehorner X-50, Rhinehart Development Corporation, Spencerville, IN) on the horn tissue for approximately 15-20 seconds until a circumferential copper-colored ring surrounding the horn bud was formed similarly described by Stock and others (2015). A duplicate, non-heated electric dehorner (Dehorner X-50, Rhinehart Development Corporation, Spencerville, IN) was used identically for SHAM animals. Each group of eight study animals was dehorned by the same person to minimize variation within the phase.

The dehorning sites were monitored daily for signs of discharge or infection, in addition to an assessment of attitude, posture, appetite, lying time, and peri-operative swelling. A rescue analgesia protocol of flunixin meglumine at 2.2 mg/kg, IV once daily for 3 days was devised if obvert pain or distress was evident, such as increased lying time, head pressing, inflammation with major drainage of the dehorning site, dehydration or inappetence.

Blood Sample Collection

Trained handlers manually restrained animals for blood collection. Baseline samples were obtained immediately prior to drug administration. Blood samples were collected for animals receiving carprofen or placebo via the catheter at 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, and 96 h (-10 m) following dehorning. Samples were

immediately transferred to a blood collection tube with either sodium heparin or

ethylenediaminetetraacetic (EDTA) (Vacutainer®, BD Diagnostics) and stored in a

cooler with ice packs before processing. Blood samples were centrifuged for 15

minutes at 1,500g at ambient temperature. Collected plasma was placed in cryovials

and frozen at -80°C until analysis.

Calves were restrained using the same head restraint as previously described

approximately 5 minutes prior to nociception threshold detection via pressure

algometry and infrared thermography. Blood collection would occur before calves were

placed in the head restraint. After this brief period of acclimatization in the restraint

device, infrared images were obtained. Following infrared thermography imaging,

calves were blindfolded for pressure algometry nociception threshold testing to avoid

conditioned withdrawal reflex based on visual cues. Baseline samples for heart rate,

respiratory rate, ocular temperature, and mechanical nociception threshold were

obtained 14±2 h prior to the initiation of the study.

Ocular Temperature

A thermography camera (FLIR SC 660, FLIR Systems AB, Boston, MA) with a

thermal sensitivity of 0.05 Celsius, 320 x 240 pixel display, precision > 98%, was used

to quantify changes in ocular temperature similarly described in Stock and others

(2015). The camera was internally calibrated to ambient temperature prior to image

collection; however additional minute adjustments to ambient temperature and

humidity were used during software processing. Images were obtained from the left

side of the calf, at an angle of approximately 45°, and a distance of 0.5 meters distance

from the eye. Maximum temperature (°C) within a circumferential area of the eye

including the medial posterior palpebral border of the lower eyelid and the lacrimal

caruncle was obtained as previously described (Stewart el al., 2008). Images were

analyzed using FLIR Tools (v. 4.1; FLIR Systems Inc, Boston, MA) following

collection. At each time point, three images were obtained and the minimum

temperature recorded was used for statistical analysis.

Mechanical Nociception Threshold (MNT)

MNT, as defined by a maximum force which induces a withdrawal response, was

determined at 4, 8, 12, 24, 48, 72 and 96 h post-dehorning as described in Tapper et al.

2012 and Stock et al., 2015. Briefly, using a hand held pressure algometer (Wagner Force

Ten™ FDX 25 Compact Digital Force Gage, Wagner Instruments, CT, USA), a force

was applied perpendicular at a rate of approximately 1 kg of force per second at 2

locations (lateral and caudal) adjacent to the horn bud. The 1 cm2 rubber tip of the

algometer was placed immediately adjacent to cauterized skin. Additionally, a third

control location between the eyes on the frontal bone was used to evaluate MNT of an

area that was not adjacent to cauterized skin. A withdrawal response was indicated by an

overt movement away from the applied pressure algometer. The obtained pressure value

was recorded by a second researcher prior to observation from the investigator applying

the pressure. A maximum value of 10 kgf was determined *a priori*. Calves were

blindfolded prior to MNT to avoid a response associated with visual cues. Both the order

of locations tested and the side of the calf the researcher stood on was randomized

between each calf. Locations were tested three times in sequential order and the value was averaged for statistical analysis.

Heart and Respiratory Rate

Heart and respiratory rates and heart rates were obtained prior to placement within the restraining device, with respiratory rates obtained by a recorder outside of the group pen and heart rates determined following a brief period of acclimatization of the recorder in the group pen. In this way, the restraining device would not influence respiratory and heart rates.

Heart and respiratory rate was evaluated via auscultation at 3.5, 7.5, 11.5, 23.5, 47.5, 71.5, and 95.5 ± 0.5 hrs. The bell of a stethoscope (3M™, Littmann®, Master Classic II, St, Paul, MN, USA) was placed between the 3rd and 5th intercostal space and beats were counted over a 15 second period. The value obtained was used to calculate beats per minute. Respiratory rate was determined without handling by observing the movements of the thoracic cavity over a 15 second period.

Average Daily Gain

Animals were weighed using a Brecknell digital scale (PS500-36S, Avery Weight-Tronix, Fairmont, MN). Average daily gain was calculated using weights obtained approximately 24 hours prior to the carprofen administration (d -1), and 7 days following dehorning (d7). The scale was calibrated with weights of a known mass immediately prior to obtaining the weight of a calf.

Cortisol

Plasma cortisol samples were determined using a commercial radioimmune assay kit (Coat-A-Count® Cortisol, Siemens Medical Solutions Diagnostics (formally Diagnostic Products Corp.), Los Angeles, CA) previously used for bovine plasma (Stilwell et al., 2008a; Stock et al., 2015). Samples were assayed in duplicate with the reported concentration equaling the average cortisol concentration between duplicates. The average intra- and inter-assay coefficients of variation were 8.2% and 7.8%, respectively. Area under the effect curve (AUEC) was calculated using the linear trapezoidal method as previously described (Glynn et al., 2013).

Substance P

Substance P (SP) concentrations were analyzed as described by Van Engen and others (2013) using non-extracted plasma. Samples were assayed in duplicate with the reported concentration equaling the average SP concentration between duplicates. The coefficient of variation for intra-assay variability was at 8.6% and the inter-assay variability was calculated at 8.4%. Area under the effect curve (AUEC) was calculated using the linear trapezoidal method.

Prostaglandin E2

Ex-vivo prostaglandin E2 (PGE2) synthesis inhibition was determined as described by Fraccaro and others (2013). Blood collected from calves was placed into sterile vacuum tubes containing heparin at 2, 6, 12, 24, 48, 72, 96 h. LPS obtained from E. coli 0111:B4 (Sigma-Aldrich, Co. St. Louis, MO) in PBS was added at 10 µg/mL to

the heparinized whole blood and incubated for 24 h at 37°C. Baseline samples were incubated with and without LPS. The same volume of PBS was added to the negative control baseline samples. Plasma was subsequently collected post-incubation following centrifugation at 400 g for 10 minutes at ambient temperature. Methanol was added to plasma in a 1:5 plasma to methanol dilution, facilitating protein precipitation. Following centrifugation at 3,000 g for 10 minutes, the supernatant was collected and stored at -80 °C. A commercial PGE2 ELISA kit (Cayman Chemical, Ann Arbor, MI) previously described using methanol precipitated bovine plasma was used for determination of PGE2 concentration (Donalisio et al., 2013; Fraccaro et al, 2013). Samples were assayed in duplicate with the reported concentration equaling the average PGE2 concentration between duplicates. The coefficient of variation for intra-assay variability was at 9.8% and the inter-assay variability was calculated at 14.6%. One animal from each of the PLCBO and SQ treatment groups were removed from analysis due to laboratory error.

Carprofen Plasma Concentration

Plasma concentrations of carprofen (R & S enantiomers) were determined using high-pressure liquid chromatography (Agilent 1100 Pump and Autosampler, Agilent Technologies, Santa Clara, CA, USA) coupled with ion trap mass spectrometry detection (LTQ , Thermo Scientific, San Jose, CA, USA).   Plasma samples, plasma spikes, and blanks, 100 µL, were mixed with 400 µL of acetonitrile to precipitate plasma proteins. Diclofenac (10 µL of 5 ng/ µL) was added as an internal standard to all samples.   The samples were vortexed for 5 seconds and centrifuged for 20 minutes at 2,000 x g to

sediment the protein pellet.   The supernatant was poured off into dry down tubes and evaporated at 50°C with a flow of nitrogen in a Turbovap. The contents were reconstituted with 150 µL of 25% acetonitrile in water. The samples were transferred to an autosampler vials fitted with a glass insert and centrifuged at 2,000 x g prior to analysis.

For LC-MS analysis the injection volume was set to 15 µL. The mobile phases consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile at a flow rate of 0.225 mL/min. A solvent gradient beginning at 40% B with a linear gradient to 60% B in 5 minutes was used for analysis. Separation was achieved with a LUX Cellulose-1 chiral column, 50 mm x 2 mm , 3 µm particles (Phenomenex, Inc., Torrance, CA, USA) maintained at 40°C. S+ and R- carprofen eluted at 4.4 and 4.9 minutes, respectively, while diclofenac eluted at 4.5 minutes.   Electrospray ionization (ESI) and full scan MS was used for analyte detection. Carprofen was detected with negative ESI with MS3 fragmentation (272→228). Wideband activation in positive ESI mode was used to aid the loss of water from diclofenac during transition from the parent (m/z 297) to the fragment ions. The sum of the intensities of ions at m/z of 190, 226, and 228 were used for carprofen quantitation. The internal standard, diclofenac, was quantitated with the sum of the fragment ion intensities at m/z of 215, 250, and 252. Sequences consisting of plasma blanks, calibration spikes, and bovine plasma samples were batch processed with a processing method developed in the Xcalibur software (Thermo Scientific, San Jose, CA, USA). The processing method automatically identified and integrated each peak in each sample and calculated the calibration curve based on a weighted (1/X) linear fit. Plasma concentrations of carprofen in unknown samples were calculated by the

Xcalibur software based on the calibration curve. Results were then viewed in the Quan

Browser portion of the Xcalibur software. Twelve calibration spikes were prepared in

blank bovine plasma covering the concentration range of 5 to 20,000 ng/mL. Calibration

curves exhibited a correlation coefficient (r2) exceeding 0.996 across the entire

concentration range. QC samples at 15, 150, and 1500 ng/mL were prepared in bulk and

run with each set of samples/calibrators. The variance of the QC samples was within 2-

10% of the nominal value.

Pharmacokinetic Analysis

A non-compartmental pharmacokinetic analysis was performed using computer

software. The following parameters were evaluated: area under the curve from time 0 to

infinity (AUC0-∞) and time 0 to last collected sample (AUC0-96) using the linear

trapezoidal rule, as well as the percent of the AUC extrapolated to infinity (AUC

extrapolated). The extrapolated portion of the AUC was determined by multiplying the

last measured plasma concentration by the λz, the first-order terminal rate constant (λz).

The range of the λz was determined by visual inspection of the plasma profile and

determined by linear regression of time and natural log (ln) of the plasma concentration.

The volume of distribution per bioavailability Vz/F was determined by diving the dose by

$\lambda z \bullet AUC$. Clearance per bioavailability (Cl/F) was determined by dividing the dose by

AUC. In addition, T½λz, maximum plasma concentration (Cmax) and time to maximum

concentration (Tmax) was determined. The relative F was estimated by dividing the oral

(mean AUC/Dose) by the subcutaneous (mean AUC/Dose).

Statistical Analysis

Analyses were performed in SAS 9.3 (SAS Institute, Cary NC) using a general linear mixed effects model with repeated measures. Data obtained from response variables including cortisol, substance P, MNT, heart rate, respiratory rate, and PGE2 were natural log transformed for normality. Baseline values were used as covariates. The fixed effects were treatment (carprofen PO, carprofen SQ, PLCBO, SHAM), time, and the interaction between treatment and time. The effect of sex was included for all statistical models. Phase was a random effect and calf was the subject of repeated measures. F-tests were used to test the significance of main effects and interactions. If significant overall differences were identified among levels of a factor, pairwise comparisons were performed using Tukey's t-tests. Paired t tests were performed to test the differences between LPS-stimulated and unstimulated baseline ex-vivo PGE2 concentrations. Planned contrasts were performed evaluating the responses of carprofen (PO &SQ) versus PLCBO treated calves following dehorning as well as the response following actual (PO, SQ, & PLCBO) or sham (SHAM) dehorning. These three contrasts are analyzed for the entire study along with at different time points (4, 8, 12, 24, 48, 72, 96).

Results & Discussion

Animal Medicinal Drug Use Clarification Act (AMDUCA) permits the use of medicines to be used in an extra-label manner provided specific requirements are met (FDA, 1994). As no drugs are currently labeled in cattle for analgesia or those that are

labeled as anti-inflammatory have unknown analgesic properties following a single dose, the use of carprofen would constitute extra-label drug use permitted under AMDUCA. Following an IV or SQ dose of 1.4 mg/kg, a 14 d slaughter withhold period is required in the European Union using the approved European formulation. Since there is a zero tolerance on the presence of violative residues under AMDUCA for extra-label drug use, and carprofen demonstrates age-related pharmacokinetics, additional tissue residues studies would be necessary prior to any recommendation for use in young calves.

Carprofen

Carprofen was well absorbed following both subcutaneous and oral administration (Table 2 and 3). Although carprofen is administered as a racemic mixture, the S(+) enantiomer has been reported to be primarily responsible for the observed prostaglandin inhibition (Brentnall et al., 2012). Using AUC0-∞, the observed enantiomer ratio, R:S, was 57:43 for both oral and subcutaneous administration. This ratio is nearly identical to that previously reported following IV administration (Delatour et al., 1996). In the present study, both SQ and PO administration of carprofen demonstrated a small volume of distribution as well as a slow clearance resulting in prolonged median half-lives, S(+) = 52.8 h (range: 38.3 – 180.7 h), S(+) = 49.7 h (range: 38.4 – 79.2 h), respectively. These values are marginally longer in comparison to those previously published in calves of similar age (Delatour et al., 1996). Carprofen-S(+) achieved a mean maximum concentrations of 5.3±0.9 µg/mL at 9 h following SQ administration and 3.9±0.8 µg/mL at 18 h following PO dosing. The

relative bioavailability of PO carprofen in comparison to SQ calculated using mean AUC0-∞ was approximately 70%.

The pharmacokinetics in the present study indicated a marginally longer half-life than previously reported in animals of similar age (Delatour et al., 1996). This may be a result of using a younger population of animals in the present study. Age dependent pharmacokinetics of carprofen has been reported in calves with concentrations persisting longer in younger animals (Delatour et al., 1996; Brentnall et al, 2012). Longer half-lives in younger calves are suggested to be a result of a reduction in clearance which is consistent with our findings (Lees et al., 1996, Delatour et al., 1996). It should be noted that the AUC0-∞ observed in our study should be interpreted with caution due to the large percent of the AUC extrapolated.

Analgesic concentrations of NSAIDs have been previously estimated using a calculated IC_{80} concentration of COX-2 inhibition (Lees et al., 2004; Huntjens et al, 2005). A recent study evaluating in-vitro COX-2 inhibition using a bovine whole blood assay indicted an IC_{80} equal to a mean total carprofen (RS±) concentration of 8.9 µg/mL (range: 1.9 – 42.9 µg/mL) (Miciletta et al., 2013). Interestingly, it was reported that the R(-) enantiomer may attenuate COX inhibitory activity of the S(+) enantiomer (Miciletta et al., 2013). Assuming these concentrations provide pain relief, the use of total carprofen concentrations with a similar enantiomer ratio as derived in our study, SQ administration would provide analgesia from approximately 4 h to 24 h of time; however, a direct comparison between studies should be interpreted with caution given differences in study design. Oral administration did not achieve this target mean concentration throughout the course of the study. These data would potentially support

the current daily regimen of SQ administration to achieve the previously reported IC_{80}; however, the duration above the IC_{80} observed in this study is shorter than that previously reported using the S(+) enantiomer alone (Brentnall et al, 2012). This may due to the interaction of the R(-) enantiomer given the enantioselectivity observed following administration of the racemic mixture in calves as reported by Miciletta and collegues (2013).

Prostaglandin E2

An effect of treatment (P<0.0001) and time (P<0.0001) was observed on prostaglandin E2 (PGE) percent inhibition (Table 4). Moreover, there was an interaction of time and treatment (P<0.0001). Percent inhibition was increased for calves administered carprofen subcutaneously compared to placebo treated controls from the initial collection time point of 2 h through 72 h (P<0.05) (Figure 2). In addition, the percent inhibition was decreased in calves treated orally with carprofen from 6 h through 96 h compared to placebo treated controls (P<0.05)(Figure 2). Prostaglandin percent inhibition was significantly increased in sham dehorning calves compared to calves treated with carprofen for all time points (P<0.05). No difference was observed in prostaglandin inhibition between placebo-treated dehorning calves compared with those sham dehorning (P>0.1). Baseline PGE concentrations of LPS stimulated blood was greater than unstimulated blood (P<0.0001).

NSAIDs inhibit COX isoenzymes responsible for producing prostaglandins which increase nociception thereby increasing the sensitivity for nerve transmission at the site of injury (Basbaum et al. 2009). Carprofen is known to be a weak inhibitor of

cyclcoxygenase (Delatour et al., 1996; Lees et al., 1996). As such the mechanism of action for analgesia is thought to be both COX-dependent and COX-independent. In addition to inhibition of prostaglandins (Miciletta et al., 2013; Brentnall et al, 2012), the effect of carprofen may be mediated through changes in proinflammatory cytokines such as IL-6 (Armstrong and Lees, 2002). In our study, ex-vivo PGE concentrations were persistently decreased for the duration of the study in carprofen treated calves compared to placebo treated controls; however, only a moderate overall reduction was observed in comparison to other NSAIDs demonstrating up to 100% PG inhibition using tissue cage models (Lees et al., 2004).

Multiple studies have used in vitro assays to measure the effect and potency of carprofen on PGE inhibition (Brentnell et al., 2012; Miciletta et al., 2013). However in our study, ex-vivo PGE inhibition was used to determine the duration of effect following a single dose. Determination of ex-vivo PGE concentrations includes the effect of the parent compound in in vivo enantiomer ratios as well as any contribution from circulating metabolites. Although it is unknown if carprofen metabolites are important for producing analgesia, active metabolites are known to contribute to the analgesic effect in humans following administration of morphine (Penson et al., 2000). Moreover, glucuronide and coenzyme A thioester metabolites of ketoprofen, an NSAID of the same 2-arylpropionic acid class as carprofen, demonstrate potent prostaglandin inhibition in vitro (Levoin et al., 2004). Although not measured in this study, active metabolites may explain the observed difference in inhibition of PGE between carprofen and placebo treated calves that persisted longer than 48 h with decreasing parent drug concentrations.

Although no differences were observed throughout the study between placebo treated controls and animals sham dehorned, an insignificant decrease in PGE production in placebo treated controls was observed. This may be due to increased glucocorticoid concentrations associated with dehorning which are reported to down regulate the induction of PG concentrations (Santini et al., 2001).

Cortisol

There was no effect of treatment on cortisol concentrations (P=0.48). Cortisol concentrations were affected over time (P<0.0001); however there was no interaction of time and treatment (P=0.22) (Table 4) (Figure 3). No treatment differences were observed in evaluating the overall AUEC0-96h (P=0.51) (Figure 4). Furthermore, no treatment differences were observed when the AUEC was divided as follows: 0-2 h (P=0.64), 2-8 h (P=0.34) and 8-96 h (P=0.55) (Table 5).

In contrast evaluations, calves that were dehorned compared to sham dehorned had greater cortisol concentrations at 20 m (P=0.042), and approximately 4 h (P=0.0048) following actual or sham dehorning. Additionally, dehorned calves tended to have greater cortisol concentrations at 48 h (P=0.060) compared to those sham dehorned. In comparison with calves receiving carprofen, placebo treated calves tended to have increased cortisol concentrations at 8 h (P=0.10) and decreased cortisol at 12 h (P=0.069).

Cortisol concentrations have been previously described to increase in calves dehorned compared to those sham dehorned (Stafford and Mellor, 2005). Although changes in cortisol concentrations were observed over time in this study, no overall

treatment differences among the groups were observed. Contrast statements reveal that time points at 20 m, 4 h and 48 h post-dehorning appear to be potentially sensitive time points in detecting differences in cortisol concentrations following dehorning compared to those sham dehorned. This is consistent with previous literature reporting increases in cortisol concentrations immediately following dehorning, and after the loss of local anesthesia (McMeekan et al., 1998; Doherty et al., 2004, Allen et al., 2013). Furthermore, changes in both cortisol and behavior are noted to persist up to 24 to 48 h in placebo treated controls compared to calves dehorned with NSAIDs (Faulkner and Weary, 2000; Heinrich et al., 2009; Stock et al., 2014). This response may be due to the delayed amplification of acute phase proteins observed at this time post-dehorning (Glynn et al., 2013).

Carprofen did not have an overall treatment effect on cortisol concentrations compared to placebo treated calves in this study. This differs from a previous study which reported decreased cortisol concentrations at 1 h post cautery disbudding compared to placebo treated controls (Stilwell et al., 2012). These differences may be due to the route of administration as carprofen was administered intravenously in the previous study potentially achieving greater concentrations rapidly.

Administration of carprofen has been reported to decrease cortisol concentrations and pain related behaviors at 24 h and 48 h following non-surgical castration (Pang et al., 2006; Stilwell et al., 2008b). We did not observed this analgesic effect following dehorning which may be due to a muted cortisol response in non-surgical castration or due to the potential of amputation dehorning resulting in a greater stress response in comparison to surgical castration (Robertson et al., 1994; Mosher et al, 2013; Ballou et

al, 2013). Consequently, concentrations necessary to achieve analgesia for calves following cautery dehorning may need to be greater in comparison to castration.

Substance P

There was no effect of treatment (P=0.77), time (P=0.23) or interaction of time and treatment (P=0.53) on substance P (SP) concentrations (Table 4). Area under the effect curve and maximum substance P concentrations were not different among treatment groups (Table 5). Contrasts statements indicated decreased SP concentration at 8 h post dehorning for calves treated with carprofen compared to placebo treated calves (P=0.016). Moreover, SP concentrations for calves treated with carprofen tended to be increased at 20 m (P=0.096) and decreased at approximately 6 h (P=0.082) and 96 h (P=0.093) following dehorning compared to placebo treated dehorned controls (Figure 5). The decreased SP concentrations at approximately 8 h in calves treated with carprofen correlate with decreasing cortisol concentrations at 8 h as well. Taken together, carprofen administration may attenuate the pain and distress associated with cautery dehorning at 8 h.

Interestingly, an effect of gender was observed (P=0.005) with substance P concentrations greater (0.11±0.039 pg/ml) in female calves compared to male calves. Differences in pain responses between sexes have been previously well documented (Fillingim et al, 2009). More recently, SP differences between sexes were reported in a rat model using a formalin-evoked pain model resulting in SP release (Nazarian et al., 2014). Consistent with our study, female rats had an increase in SP release which was determined to be mediated by estradiol concentrations. In our study, although a

difference was noted, the effect size was small. Additional studies are needed to further elucidate the role of sexual dimorphism in SP release in cattle.

Mechanical Nociception Threshold

An effect of treatment was observed (P<0.0001) on mean mechanical nociception threshold (MNT). Furthermore there was an effect of time (P<0.0001) but there was no interaction between treatment and time (P=0.99) (Table 4). Sham dehorned calves had an increased MNT at all collection timepoints (P<0.001) (Figure 6). No treatment differences were observed among treatment groups of dehorned calves; however, there was a tendency for calves treated with carprofen to tolerate more pressure around the dehorning site compared to placebo treated controls (P=0.097).

Previous studies have evaluated MNT differences following actual or sham dehorning of calves (Heinrich et al, 2010; Tapper et al, 2012, Allen et al. 2013, Glynn et al. 2013). In these previous studies, calves were initially sham dehorned prior to actual dehorning to determine baseline MNT information. In this study, in addition to determining baseline MNT values, sham dehorned calves were included throughout the data collection period to help control for the effects of the study on the pressure algometry response. Due to the MNT differences observed between sham and actual dehorned calves throughout the study period, MNT appears to be a reliable measure of increased pain sensitivity associated with cautery dehorning. Although MNT did not return to baseline values following the duration of the study (4 d) for calves dehorned, partial thickness epidermal burns have been noted to induce mechanical hyperalgesia for up to 4 weeks in rat thermal injury models (Summer et al., 2007).

MNT has been useful in detecting nociception differences in dehorned calves treated with an analgesic. Heinrich and others (2010) demonstrated decreased sensitivity as determined by increased MNT following the use of IM meloxicam. Moreover, Tapper et al., (2012) reported an increased MNT in calves administered local anesthesia using ethanol for at least 3d post-dehorning compared to lidocaine. Although, carprofen administration tended to increase MNT throughout the study period, further investigation is warranted to determine the improved sensitivity associated with carprofen administration.

Ocular Temperature

There was no effect of treatment on ocular temperature (P=0.65). An effect of time was observed (P<0.0001), however, there was no interaction of time and treatment (P=0.26) (Table 4). Previous studies have indicated an equivocal response of ocular temperature following dehorning. Stewart and colleagues (2009) reported a decreased ocular temperature associated with the loss of local anesthesia following dehorning. Moreover, administration of meloxicam attenuated the decreased ocular temperature observed in placebo treated calves post-dehorning (Stewart et al., 2009); however, other investigations have not observed this same response (Glynn et al., 2013; Allen et al., 2013; Stock et al., 2014). As this response is hypothesized to result from activation of the autonomic nervous system (ANS) producing peripheral vasoconstriction, brief handling and restraint may confound this observed effect. In our study, calves were placed in a head restraint device prior to obtaining the image. This was to maintain the collection of a consistent image. Calves were allowed to adjust to the restraint device

briefly before the infrared image was collected which may have masked the ANS response on ocular temperatures associated with dehorning. The similar ocular temperature profiles throughout the study of sham and actual dehorned calves might further support this statement.

Heart Rate

There was no effect of treatment on heart rate (P=0.43). An effect of time was observed (P=0.0092), however, there was no interaction between treatment and time (P=0.96) (Table 4). Contrast statements revealed sham animals tended to have a decreased heart rate at 47.5 h (P=0.10) and 95.5 h (P=0.064) post-dehorning compared with calves dehorned.

Heart rate has been previously used to evaluate changes in the ANS following noxious events in cattle (Stewart et al., 2009; Heinrich et al., 2010; Coetzee et al. 2012). Increased heart rate variability has been reported in castrated or dehorned cattle as a result of an imbalance in the autonomic nervous system (Stewart et al., 2009; Stewart et al., 2010). Although previous reports have indicated a significant reduction in heart rate in calves administered meloxicam prior to dehorning, this effect was not observed in our study (Heinrich et al., 2010; Coetzee et al., 2012). Differences in analgesic potential between NSAIDs and method of data collection may be reasons for the reported differences. In previous investigations, heart rates were collected using an electrical heart rate monitor (Coetzee et al., 2012) or obtained following blood collection (Heinrich et al., 2009), which differs from our study. It is noteworthy that no differences were observed between calves actually or sham dehorned in the first 24 h

post-dehorning potentially indicating the influence of the repeated handling for data collection. The decreased heart rate observed at 47.5 h in sham dehorned calves coincides with the observed reduction in cortisol concentration in sham animals. Taken together, these responses may indicate a persistent stress response in calves following dehorning.

Respiratory Rate

Statistical analysis was performed with and without calves treated for respiratory disease. Using data from all calves, respiratory rate was not affected by treatment (P=0.20) or time (P=0.086) (Table 4). Moreover, treatment groups did not differ in response over time (P=0.25). Although carprofen calves tended to have an increased respiratory rate in comparison to placebo treated calves (P=0.094), this effect was not observed if calves treated for respiratory disease were removed from the statistical analysis (P=0.38). Interestingly, carprofen treated calves had an increased respiratory rate at 47.5 h (P=0.0040) post dehorning compared to placebo treated dehorned calves despite removing pneumonia treated calves from the analysis. A previous report indicated that respiratory rates increase in placebo treated calves in comparison with calves treated with meloxicam. (Heinrich et al. 2009). Although the lack of agreement between studies may be due to differences in analgesic potential between NSAIDs used, respiratory rates were not assessed past 24 h in the previous study (Heinrich et al., 2009). The increase in respiratory rate observed in our study at 47.5 h may correspond with the reduction of carprofen's effect.

Average Daily Gain

Calves treated with oral carprofen had an increased average daily gain compared with those treated with subcutaneous carprofen (0.92±0.10 kg/d vs. 0.48±0.10 kg/d) (P=0.0039). No other differences were observed between treatment groups (P>0.1). Changes in average daily gain are equivocal with previous studies reporting no changes or increases compared to placebo treated controls (Baldridge et al., 2011; Glynn et al. 2013; Coetzee et al., 2012; Stock, et al., 2014). Greater increases were observed in older study populations suggesting the potential that dehorning has a larger impact on weight gain in older calves (Baldridge et al., 2011; Glynn et al. 2013; Coetzee et al., 2012). Improved appetite and weight gain has been observed in calves administered meloxicam subcutaneously on the onset of diarrhea (Todd et al., 2010). NSAIDs may attenuate the production of proinflammatory cytokines associated with sickness behavior including inappetance as observed in sheep (Baile et al., 1981) and swine (Johnson and von Borell, 1994) endotoxin models. We do not completely understand the exact mechanism of the difference in ADG observed between carprofen treatments in our study. Parameters that contribute to changes in ADG such as grain consumption were not measured in our study. Potentially, oral carprofen administration may act locally on the gastrointestinal tract inhibiting proinflammatory cytokines, thereby improving appetite and grain consumption. Further investigations into changes in average daily gain following carprofen administration are warranted using an increased sample size.

Conclusion

Cautery dehorning in dairy calves resulted in increased nociception throughout the 96 h study period. Moreover, the stress response was increased immediately following dehorning, after the loss of local anesthesia, and 48 h following dehorning in comparison to sham dehorned calves. Carprofen administration reached concentrations that moderately inhibited ex-vivo prostaglandin and were decreased for 72 h (SQ) and 96 h (PO) after administration compared with placebo treated controls; however, analgesic responses between treatment groups subsequent to dehorning were minimal. The observed tendency to reduced nociception and stress associated with dehorning in addition to the prolonged prostaglandin inhibition potentially warrants future dose titration studies.

Acknowledgements

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Table 1. As fed (%) components of the grain diet provided to calves throughout the

study.

Component	As Fed (%)
Corn	50
Oats	5
Commercial Supplement* (38% protein)	30
Commercial Supplement (34% protein)	10
Liquid Molasses	5

*contains monensin

Table 2. Pharmacokinetic parameters following a non-compartmental analysis for carprofen (RS±) administered orally prior to cautery dehorning (mean dose, 1.4 mg/kg; range, 1.13 to 1.63 mg/kg).

Parameter, Units	R(-)					S(+)				
	Mean	SD	Min	Median	Max	Mean	SD	Min	Median	Max
AUCExtrapolatedto∞, (%)	37.1	10.6	21.0	35.6	57.2	31.0	9.5	19.2	31.6	49.6
AUC0→∞, (h*µg/mL)	518.2	143.4	321.1	519.5	786.8	394.5	114.6	239.8	371.0	565.5
AUC0→96h, (h*µg/mL)	314.1	50.5	242.8	318.1	382.9	265.6	60.8	193.7	257.9	358.6
Cl/F, (mL/kg/h)	1.45	0.41	0.89	1.36	2.18	1.92	0.57	1.24	1.89	2.92
Cmax, (µg/mL)	4.41	0.63	3.53	4.58	5.16	3.94	0.84	2.82	3.70	5.14
T ½ λz, (h)	62.8	18.5	39.3	58.1	97.0	52.9	14.5	38.4	49.7	79.2
λz, (1/h)	0.012	0.0032	0.0071	0.012	0.018	0.014	0.0032	0.0088	0.014	0.018
Tmax, (h)	26.4	12.4	12	24	48	22.8	14.4	12	18	48
Vz/F, (mL/kg)	124.0	23.7	93.2	117.7	161.2	140.8	36.5	90.3	138.1	210.1

AUC – area under the plasma drug concentration curve; Cmax – maximum plasma concentration; Tmax – time to maximum plasma concentration; $T_{1/2}\lambda z$ – elimination half-life; λz – elimination rate constant; V/F – volume of distribution per bioavailability; CL/F – plasma clearance per bioavailability

Table 3. Pharmacokinetic parameters following a non-compartmental analysis for carprofen (RS±) administered subcutaneously prior to cautery dehorning (1.4 mg/kg).

Parameter, Units	R(-)					S(+)				
	Mean	SD	Min	Median	Max	Mean	SD	Min	Median	Max
AUC Extrapolated to ∞, (%)	43.1	16.1	26.5	42.1	77.2	33.9	15.5	19.3	29.7	70.3
AUC0→∞, (h*µg/mL)	753.6	458.5	383.5	656.3	1969.6	557.7	368.6	266.2	433.1	1526.5
AUC0→96h, (h*µg/mL)	369.6	59.6	284.1	369.4	463.2	319.6	77.7	214.9	307.0	452.7
Cl/F, (mL/kg/h)	1.14	0.45	0.34	1.06	1.80	1.58	0.63	0.46	1.62	2.63
Cmax, (µg/mL)	5.74	0.57	4.76	5.66	6.74	5.27	0.85	4.24	5.10	6.61
T ½ λz, (h)	89.3	58.6	47.9	75.7	242.7	66.7	42.6	38.3	52.8	180.7
λz, (1/h)	0.0098	0.0040	0.0029	0.0092	0.014	0.013	0.0043	0.0038	0.013	0.018
Tmax, (h)			6	6	12			6	9	12
Vz/F, (mL/kg)	118.3	16.1	79.3	122.2	138.3	124.5	20.6	90.9	130.5	147.3

AUC – area under the plasma drug concentration curve; Cmax – maximum plasma concentration; Tmax – time to maximum plasma concentration; T1/2λz – elimination half-life; λz –elimination rate constant; V/F – volume of distribution per bioavailability; CL/F – plasma clearance per bioavailability

Table 4. Summary table of backtransformed response variables (geometric means (95% confidence interval)) of carprofen and placebo treated calves post-dehorning. N=10 unless otherwise noted. Within a row, means without a common superscript differ (P<0.05) **.**

Response, (Units)	Carprofen		Controls		Treatment (P value)	Time (P value)	Time x Treatment (P value)
	SQ Mean (95%CI)	PO Mean (95%CI)	PLCBO Mean (95%CI)	SHAM Mean (95%CI)			
MNT,[1] (kgf)	0.71[a] (0.56 to 0.88)	0.72[a] (0.57 to 0.90)	0.58[a] (0.46 to 0.73)	1.84[b] (1.47 to 2.32)	<0.0001	<0.0001	0.99
Ocular temperature, (°C)	38.4[a] (37.7 to 39.0)	38.5[a] (37.9 to 39.2)	38.1[a] (37.5 to 38.8)	38.2[a] (37.6 to 38.9)	0.65	<0.0001	0.26
Heart rate, (bpm)[2]	94.3[a] (87.3 to 101.9)	98.5[a] (91.3 to 106.2)	97.0[a] (89.9 to 104.6)	90.4[a] (83.7 to 97.8)	0.43	0.0092	0.96
Respiratory rate, (bpm)[2]	40.5[a] (37.1 to 44.1)	38.4[a] (35.2 to 41.8)	36.1[a] (33.1 to 39.3)	38.4[a] (35.2 to 41.8)	0.20	0.086	0.25
ADG, (kg/day)	0.48[a] (0.27 to 0.68)	0.92[b] (0.71 to 1.12)	0.69[ab] (0.48 to 0.90)	0.68[ab] (0.47 to 0.88)	0.0081		
Cortisol, (nmol/L)	10.5[a] (8.4 to 13.2)	11.0[a] (8.7 to 13.8)	11.9[a] (9.5 to 15.0)	9.4[a] (7.5 to 11.9)	0.48	<0.0001	0.22
Substance P, (pg/mL)	16.3[a] (14.2 to 18.7)	17.0[a] (14.8 to 19.5)	17.0[a] (14.8 to 19.5)	16.4[a] (14.3 to 18.9)	0.77	0.23	0.53
Prostaglandin E2, (%)	-65.6[3a] (-73.7 to -55.2)	-62.2[a] (-70.7 to -51.5)	-21.7[3b] (-39.8 to 1.6)	-8.3[b] (-28.5 to 17.5)	<0.0001	<0.0001	<0.0001

1: Mechanical Nociception Threshold; 2: Beats per minute; 3: n=9

Table 5. Area under the effect curve and maximum cortisol and substance P concentration (geometric means (95% confidence intervals)) of carprofen (SQ & PO) and placebo (PLCBO) treated calves post-dehorning. N=10 for all response variables evaluated. Within a row, means without a common superscript differ (P<0.05).

Response	Carprofen		Controls		P value
	SQ Mean (95%CI)	PO Mean (95%CI)	PLCBO Mean (95%CI)	SHAM Mean (95%CI)	
Cortisol, (nmol*h/L)					
AUEC[0-96]	1087.9[a] (814.1 to 1453.7)	1145.8[a] (850.9 to 1542.9)	1201.8[a] (896.1 to 1612.0)	898.4[a] (669.6 to 1205.3)	0.51
AUEC[0-2]	16.3[a] (12.1 to 21.8)	17.5[a] (13.0 to 23.7)	20.0[a] (14.8 to 26.9)	19.5[a] (14.5 to 26.3)	0.64
AUEC[2-8]	80.1[a] (55.7 to 115.2)	94.0[a] (64.8 to 136.5)	108.4[a] (75.1 to 156.6)	69.6[a] (48.2 to 100.6)	0.34
AUEC[8-96]	978.7[a] (715.4 to 1338.9)	1022.1[a] (740.9 to 1409.9)	1058.1[a] (770.3 to 1453.2)	789.6[a] (574.6 to 1084.9)	0.55
Cmax, (nmol/L)	30.5[a] (24.3 to 38.4)	29.4[a] (23.3 to 37.2)	35.7[a] (28.3 to 45.0)	28.1[a] (22.3 to 35.4)	0.41
Substance P, (pg*h/mL)					
AUEC[0-96]	1599.3[a] (1342.8 to 1904.7)	1625.5[a] (1364.9 to 1936.2)	1622.0[a] (1361.6 to 1932.4)	1586.5[a] (1332.0 to 1889.9)	0.96
AUEC[0-8]	124.6[a] (107.4 to 144.5)	129.7[a] (111.8 to 150.5)	134.8[a] (116.1 to 156.4)	131.0[a] (112.9 to 152.0)	0.70
AUEC[8-96]	1473.9[a] (1232.4 to 1762.9)	1494.4[a] (1249.5 to 1787.5)	1486.7[a] (1242.6 to 1778.5)	1454.5[a] (1215.9 to 1739.9)	0.96
Cmax, (pg/ml)	23.1[a] (18.9 to 28.2)	23.1[a] (18.9 to 28.3)	23.6[a] (19.3 to 28.8)	22.9[a] (18.7 to 27.9)	0.98

AUEC: area under the effect curve; Cmax: maximum concentration
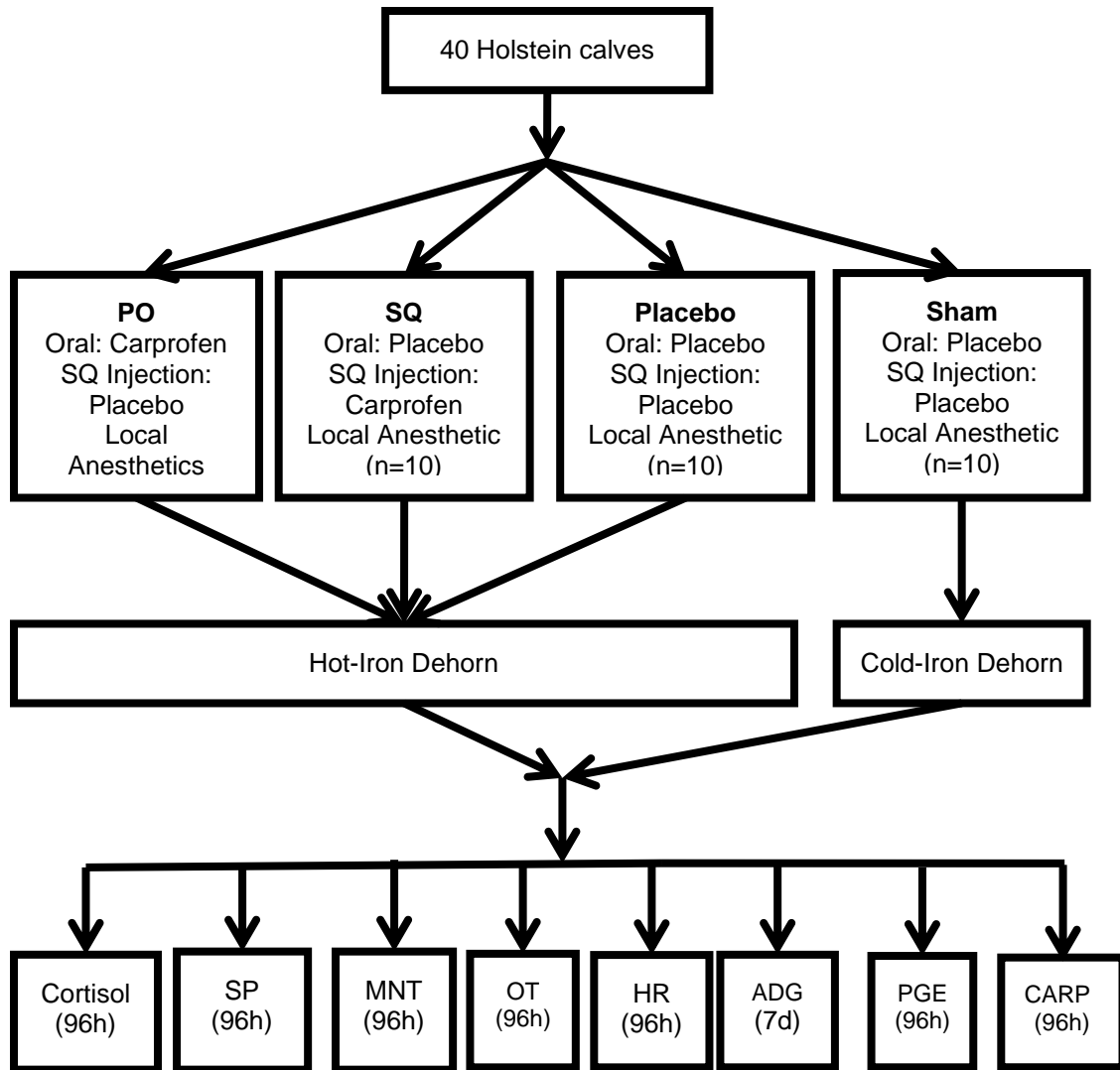
Figure 1. Flow chart outlining the timing of the study events. Calves were dehorned and monitored for cortisol, substance P (SP), mechanical nociception threshold (MNT), ocular temperature (OT), heart rate (HR), average daily gain (ADG), ex-vivo prostaglandin E_2 (PGE), and carprofen (CARP) concentration. The times in parentheses represent the duration of data collection for each variable.
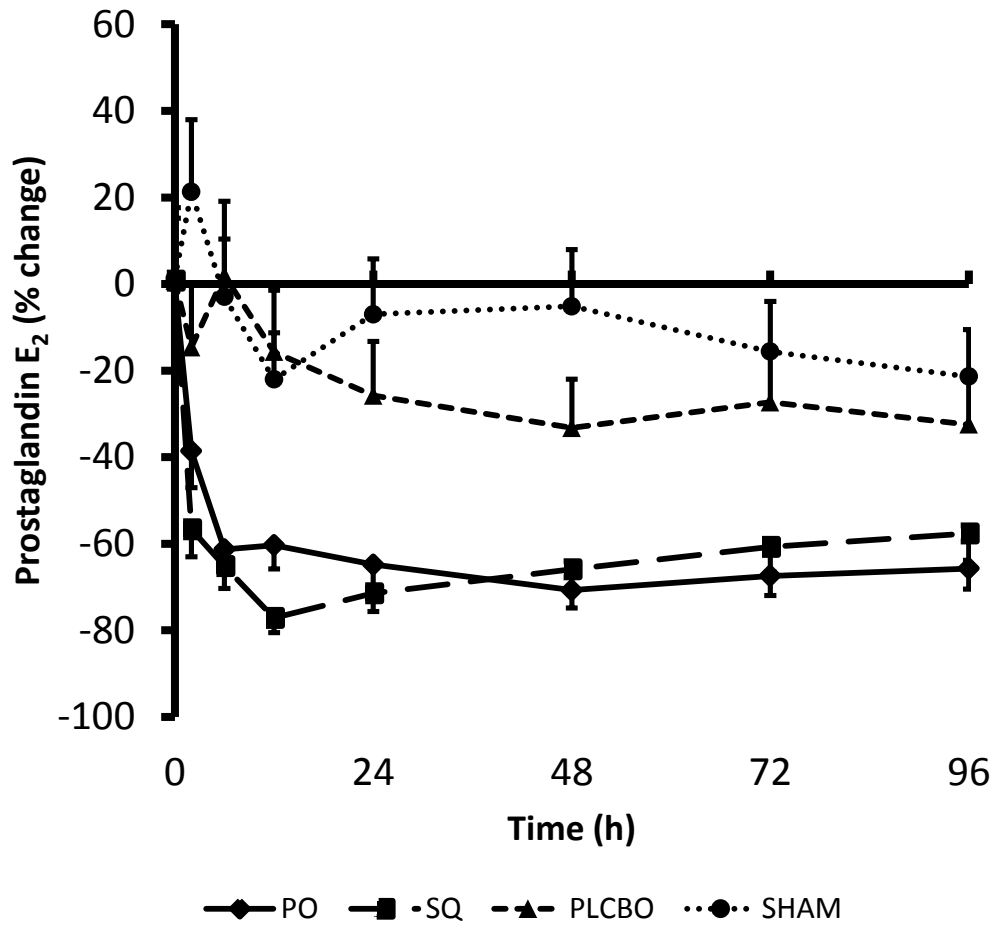
Figure 2. Back transformed geometric mean (±SE) ex-vivo prostaglandin E_2 concentrations over 96 h in carprofen and placebo treated calves post-dehorning.

(a)
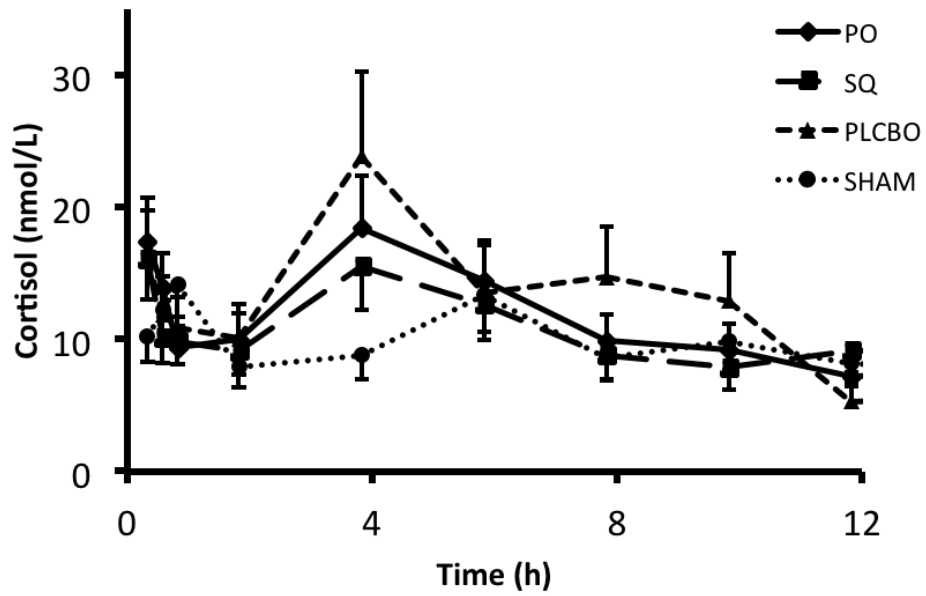
(b)
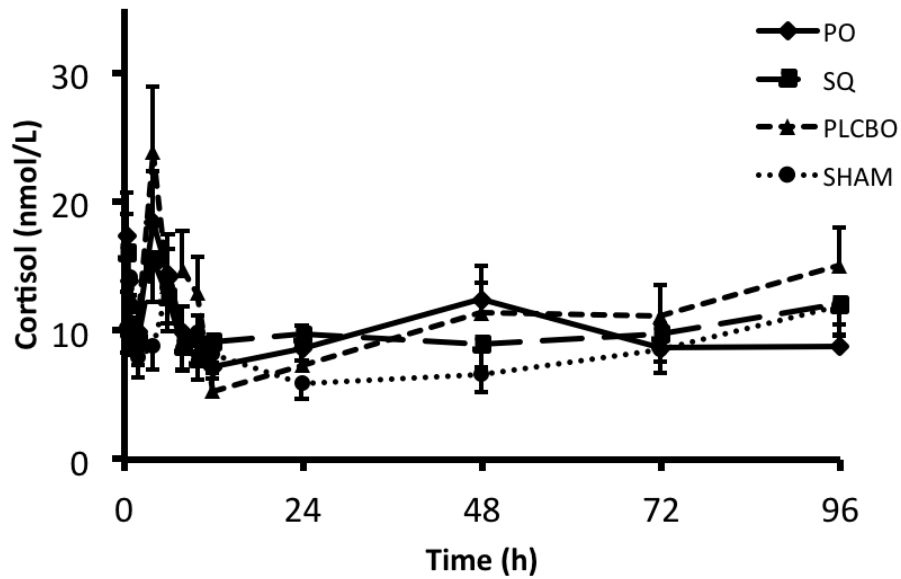
Figure 3. Back transformed geometric mean (±SE) cortisol concentrations over 12 h (a) and 96 h (b) for carprofen and placebo treated calves post cautery dehorning. Time 0 signifies the time of dehorning.
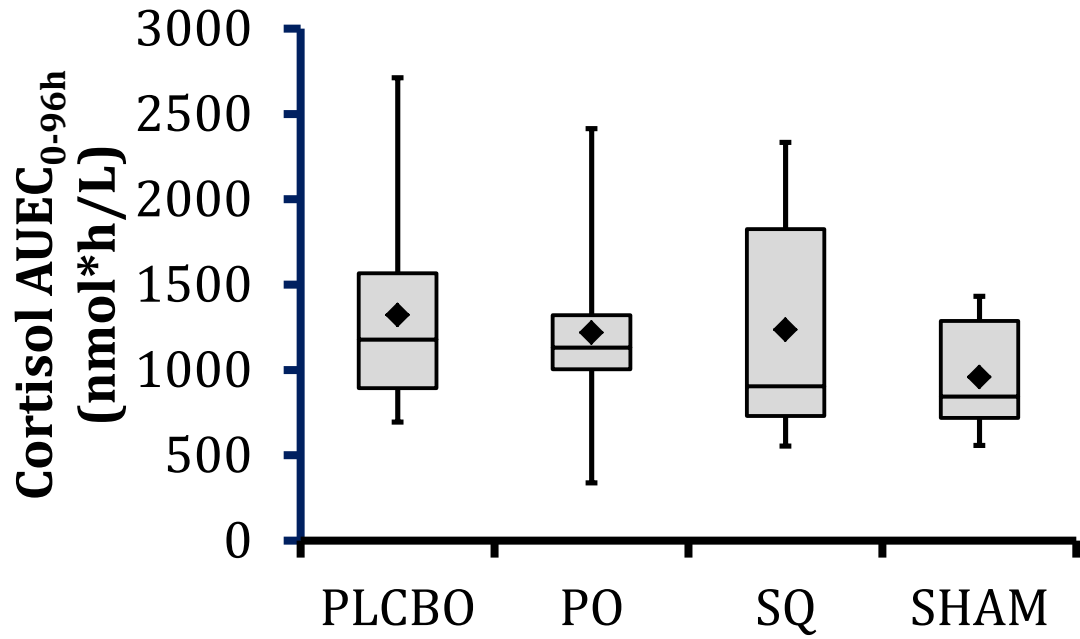
Figure 4. Box-and-whisker plots depict area under the effect curve (AUEC) for cortisol concentrations over the 96 h study period for carprofen and placebo-treated calves post-dehorning. Values were calculated using the linear trapezoid method.
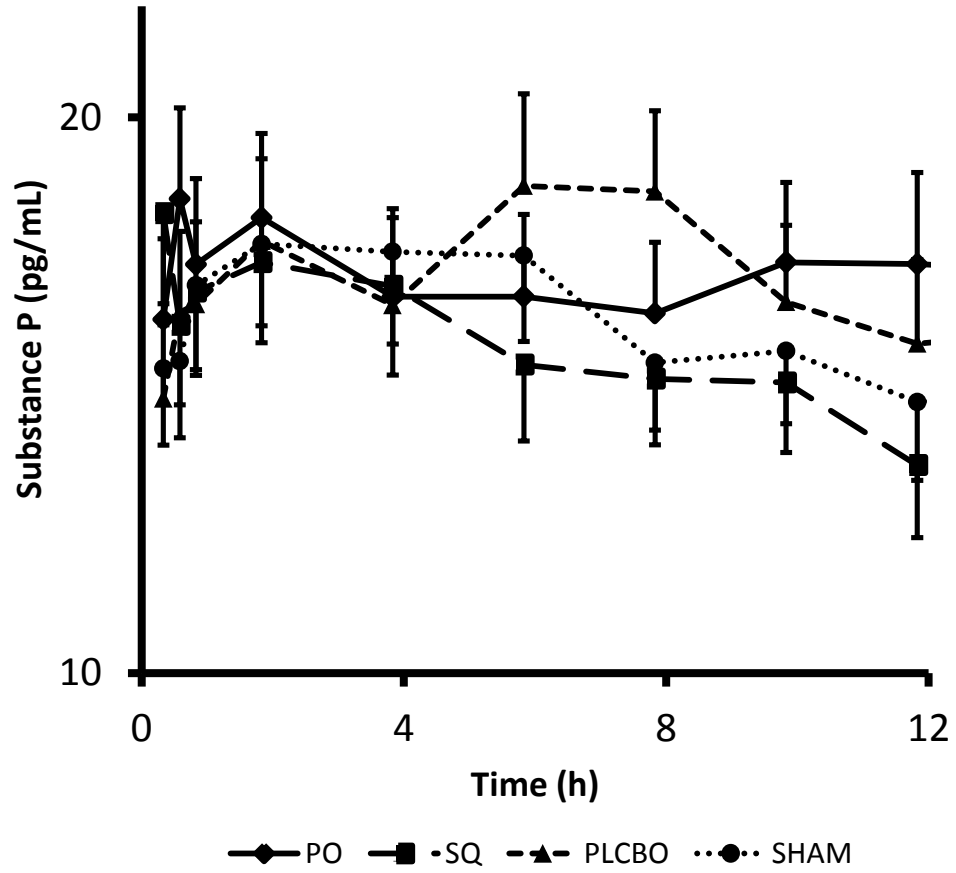
Figure 5. Backtransformed geometric mean (±SE) substance P concentrations over 12 h for carprofen and placebo treated calves post cautery dehorning. Time 0 signifies the time of dehorning.
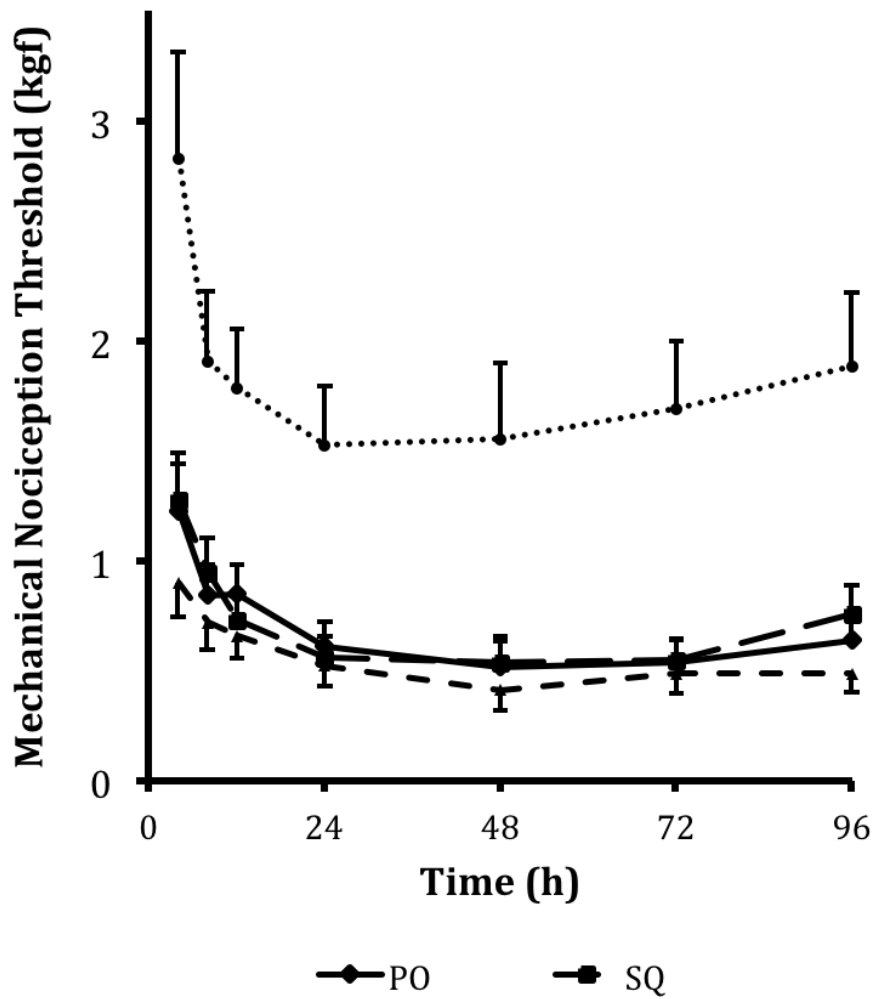
Figure 6. Backtransformed geometric mean (±SE) mechanical nociception threshold (MNT) as measured through pressure algometry over 96 h for carprofen and placebo treated calves. Time 0 signifies the time of dehorning. Pressure tolerance did not return to baseline values by the conclusion of MNT measurement for animals dehorned. Sham dehorned animals were significantly different at all time points post-dehorning (P<0.0001).

CHAPTER 6

A FIELD TRIAL COMPARING FOUR ORAL NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON CONTROLLING CAUTERY DEHORNING PAIN AND STRESS IN CALVES.

Modified from a manuscript to be submitted to the *Journal of Animal Science*

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Abstract

The purpose of this study was to compare a one-time oral administration of four non-steroidal anti-inflammatory drugs in their ability to control the pain and distress associated with cautery dehorning in dairy calves. The NSAIDs investigated have pharmacokinetic properties in cattle that produce a persistent plasma concentration, which may allow for prolonged analgesia with the added practicality of a simple administration regimen. One hundred and eighty five calves aged approximately 6 to 9 weeks old were either sham dehorned (n=31) or cautery dehorned following oral administration of carprofen (n=31), firocoxib (n=31), flunixin meglumine (n=30),

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meloxicam (n=31) or a placebo in randomized, controlled trial. A standard dose of 2.0 mg/kg was given to all calves receiving an oral NSAID. In addition, all calves received local anesthesia prior to actual or sham dehorning. Cortisol concentrations, heart rate, mechanical nociception thresholds, ocular and dehorning area temperatures and average daily gains were evaluated. A linear mixed effects model with repeated measures was used for statistical analysis. Planned contrasts evaluating analgesic treated calves and placebo treated controls were also analyzed. Analgesia administration was associated with changes in ocular and dehorning area temperature differences (P=0.020). In addition, administration of oral meloxicam, flunixin meglumine, and firocoxib resulted in decreased cortisol concentrations compared to placebo treated controls for the first 24 h post-dehorning ($AUEC_{0-24}$) (P=0.0499). Moreover, meloxicam attenuated the maximum cortisol released compared to placebo treated calves (P=0.018). Although carprofen administration reduced heart rates after 24 h (P=0.0466), it was unable to control the acute stress associated with cautery dehorning during the initial 24 h. Although a treatment effect (P<0.0001) was observed in the determination of mechanical nociception threshold among all treatment groups, no differences were observed among treatment groups dehorned. Average daily gain was not affected by treatment (P=0.91). A one-time dose of 2.0 mg/kg of oral meloxicam, flunixin meglumine, or firocoxib reduced the acute stress response associated with cautery dehorning; however, carprofen administration was associated with increased cortisol concentrations and dehorning area temperatures for the initial 24 h. Given the reduction in maximum cortisol concentrations, meloxicam appears to the most potent NSAID evaluated.

Introduction

Pain and distress associated with noxious husbandry procedures in cattle have resulted in changes in behavior, physiology, and the neuroendocrine response (Stafford and Mellor, 2011). Moreover, production losses including decreased average daily gain, and increased respiratory disease diagnosis and treatment can be associated with these painful procedures in cattle (Glynn et al., 2013; Coetzee et al., 2012a). Strategies to address the pain associated with dehorning have demonstrated the reduction of responses associated with this noxious stimulus.

Multimodal analgesia is currently recommended to address both the acute injury of horn removal performed by cautery or amputation as well as controlling the subsequent inflammatory pain (Stafford and Mellor, 2011; Stock et al., 2013). Local anesthesia such as lidocaine provides immediate pain relief lasting for approximately 2-3 h (Doherty et al., 2007; Stewart et al., 2008, 2009); however, the response following the loss of anesthetic activity indicates continued pain and distress. Non-steroidal anti-inflammatory drugs (NSAID) have been investigated to determine their analgesic effect during this prolonged response including sodium salicylate (Baldridge et al., 2011), ketoprofen (McMeekan et al., 1998; Faulkner and Weary, 2000; Duffield et al., 2010), meloxicam (Heinrich et al., 2009, 2010; Coetzee et al., 2012a; Allen et al., 2013; Glynn et al., 2013; Stewart et al., 2009), flunixin meglumine (Stilwell et al., 2008, 2009; Glynn et al., 2013; Huber et al., 2013), carprofen (Stilwell et al., 2012), and firocoxib (Stock et al., 2015).

Given the variation in pharmacokinetic properties of NSAIDs as well as the pharmacokinetic pharmacodynamic relationship, these investigated NSAIDs provided a

variety of analgesic responses. It is noteworthy that Heinrich and colleagues (2009, 2010)

demonstrated the clinical efficacy of IM meloxicam following cautery dehorning. As

meloxicam has a prolonged half-life in cattle (~27 hrs), we hypothesized that NSAIDs

with persistent concentrations would reduce the pain responses associated with dehorning

following a one-time administration (Coetzee et al., 2009). As such, the objective of this

study is to compare the analgesic properties of four NSAIDs administered at 2.0 mg/kg

once orally in calves following cautery dehorning under the same experimental field

conditions. These NSAIDs used in this study have demonstrated long half-lives in calves

and can be administered orally, a practical method for drug delivery in the field. In

addition, the prolonged physiology response post-cautery dehorning will be further

characterized.

Materials and Methods

Animals

One hundred and eighty-five female Holstein calves approximately between 6 and

9 weeks of age (mean, range; 50.6 d, (41 d – 60 d)) at dehorning were included in this

study. The mean weight (range) for the calves at disbudding was determined to be 64.1

kg (range: 45.5 – 88.6 kg). Weight and age at dehorning were not different between

treatment groups (Table 1). All calves were determined healthy by a physical

examination by a veterinarian. This study protocol was approved by the Institutional

Animal Care and Use Committee at Iowa State University (IACUC Log # 4-14-7789-B).

Calves were housed in individual three sided pens (1.82 m x 1.22 m) bedded with

straw, which was added as needed determined by the calf manager. Calves were placed in

these pens at birth and remained within the pens through the entirety of the study. Two liters of pasteurized waste milk were fed once daily in the morning for the length of the study. Water was provided twice daily and grain, consisting of corn, oats, molasses, protein/vitamin/mineral supplement, and monensin was added daily at approximately 0.45 – 1.36 kg. During the study period, a veterinarian conducted observational examinations which included assessment of milk and grain consumption. Calves were briefly handled in their pens daily for 2 days prior to baseline data collection to aid in the acclimatization of handling. The study was conducted May through July.

Animals were monitored twice daily for health observations. Animals appearing depressed or lethargic were examined by a veterinarian and monitored or treated accordingly. Removal of animals due to disease was determined following an examination by a veterinarian. Disease and study removal were diagnosed based on an elevated rectal temperature (>103.5°C), spontaneous cough, ocular and/or nasal discharge and inappetance. Three animals were removed from the study and the data collected prior to removal were not included in the analysis. Due to a recent history of respiratory disease with confirmed cases of *Mycoplasma bovis*, all animals received prophylactic tulathromycin (2.5 mg/kg) (Draxxin®, Pfizer Inc., New York, NY) according to the manufacturer's instructions during the initial weight determination at -24 h.

Study Design

A randomized controlled clinical trial was used for this investigation (Figure 1). Study animals that were dehorned were randomly assigned to receive an oral target dose of 2.0 mg/kg carprofen (Novox® caplets, Vedco, Inc. St. Joesph, MO; ANADA 200-498)

(actual dose 2.05 mg/kg; range 1.71 – 2.50 mg/kg) (n=31), firocoxib (Previcox®, Merial LLC, Duluth, GA; NADA 141-230) (actual dose 2.02 mg/kg; range 1.66 – 2.51 mg/kg) (n=31), flunixin meglumine (Banamine® Paste, Merck & Co., Inc. Whitehouse Station, NJ; NADA #137-409) (actual dose 2.30 mg/kg; range 1.61 – 3.73 mg/kg) (n=30), meloxicam (Meloxicam tablets, Carlsbad Technology, Inc., Carlsbad, CA; NDC: 61442-127-10) (actual dose 1.99 mg/kg; 1.72 – 2.11 mg/kg) (n=31) or a lactose (NOW®, Bloomingdale, IL) placebo with dehorning (PLCBO) (n=31) or without dehorning (SHAM) (n=31) (Table 1). SHAM animals were not dehorned prior to study enrollment. The test articles were administered as either a paste (flunixin meglumine) using the commercially provided dosing syringe or in an oral gelatin bolus using an oral balling gun. The calculated dose was rounded to the nearest tablet or dosing syringe notch based on the weights determined 24 h prior to administration (Table 1). The actual dose for flunixin meglumine was based on weights of the commercial tube obtained before and after administration. Calves treated with flunixin meglumine received an increased dose by approximately 0.3 mg/kg compared to the other treatment groups due to difficulties controlling the administered amount using the provided dosing syringe. Animals not receiving oral NSAIDs (PLCBO and SHAM) received a placebo (lactose powder) encapsulated in a gelatin capsule administered identically.

Randomization of group assignment was based on age and mediated by a computer generated random number (Microsoft Excel 2011, Redmond, WA, USA) as described in Theurer et al, 2012. Following randomization and treatment group assignment, calves were enrolled in one of 6 groups containing equal number of animals from each treatment group (1 animal / treatment group / replicate). In order to facilitate

the number of calves enrolled in the study, animals were in enrolled in 7 phases with 3 to 6 replicates of the treatment groups.

Dehorning

Dehorning was performed in approximately 5 min intervals by a single dehorner and handler per phase similarly described in Chapter 5. Calves were manually restrained. Cautery dehorning was initiated approximately 5 minutes following administration of the oral bolus and local anesthetic. A cornual nerve block using 2% lidocaine was provided to all calves prior to actual or sham cautery dehorning (VetOne®, Boise, ID) (5 ml / site) as described by Stock and others (2013). Effective local anesthesia of the cornual tissue was confirmed using behavior reactions (e.g. ear flicks, head shaking, strong escape behavior) to a needle prick approximately 3 minutes after administration of the cornual nerve block. Administration of lidocaine (1-2 ml) was repeated in the same manner described above if a response was observed to the initial needle prick. Following confirmation of appropriate desensitization, calves were cautery dehorning by placement of a pre-heated electrical hot-iron (approx. 600 °C) (Dehorner X-50, Rhinehart Development Corporation, Spencerville, IN) on the horn tissue for approximately 15-20 seconds until a circumferential copper-colored ring surrounding the horn bud was formed. A duplicate, non-heated electric dehorner (Dehorner X-50, Rhinehart Development Corporation, Spencerville, IN) was used identically for SHAM animals.

The dehorning sites were monitored daily for signs of discharge or infection, in addition to an assessment of attitude, posture, appetite, lying time, and peri-operative swelling. A rescue analgesia protocol of flunixin meglumine at 2.2 mg/kg, IV once

daily for 3 days was devised if obvert pain or distress was evident, such as increased

lying time, head pressing, inflammation with major drainage of the dehorning site,

dehydration or inappetence.

Data Collection

Variables of interest were evaluated in order from least to most invasive as

determined by the investigators to limit the effect of the assessment on the subsequently

tested variable. As such, the order was determined as follows: thermography images,

heart rate, pressure algometry, and blood collection via jugular puncture. Calves were

briefly manually restrained for infrared thermography, heart rate and blood collection.

Investigators collecting data were masked to the treatment groups.

Mechanical Nociception Threshold (MNT)

MNT, as defined by a maximum force which induces a withdrawal response, was

determined at 4, 8, 24, 48, 96, 144, and 192 h post-dehorning as described in Tapper et al.

2012 and Allen et al., 2013. Briefly, calves were restrained using a loose halter for

mechanical nociception threshold determination. Using a hand held pressure algometer

(Wagner Force Ten™ FDX 25 Compact Digital Force Gage, Wagner Instruments, CT,

USA), a force was applied perpendicular at a rate of approximately 1 kg of force per

second at 2 locations (lateral and caudal) adjacent to the horn bud. The 1 cm^2 rubber tip

of the algometer was placed immediately adjacent to cauterized skin. Additionally, a third

control location between the eyes on the frontal bone was used to evaluate MNT of an

area that was not adjacent to cauterized skin. A withdrawal response was indicated by an

overt movement away from the applied pressure algometer. The obtained pressure value was recorded by a second researcher prior to observation from the investigator applying the pressure. A maximum value of 10 kgf was determined *a priori*. Prior to placement of the pressure algometer, the investigator placed a hand on the posterior aspect of the poll and removed it immediately prior to placement of the pressure algometer. This method was employed to avoid provoking withdrawal associated with a startle response. Calves were blindfolded prior to MNT to avoid a response associated with visual cues. Both the order of locations tested and the side of the calf the researcher stood on was randomized. Locations were tested three times in sequential order and the value was averaged for statistical analysis.

Infrared Thermography

Using infrared thermography, eye and horn temperatures were recorded at 4, 8, 24, 48, 96, 144, 192 h post-dehorning. Baseline samples for the eye were determined at -24 h whereas the baseline sample for the horn was determined at -1 h. Horns were not imaged at -24 h as this was also when the hair around the horn buds was removed using electrical clippers, and the investigators were concerned about confounding effects of response to the recent hair removal. The maximum temperature (°C) within a circumferential area of each eye was recorded using an infrared thermography camera (FLIR SC 660, FLIR Systems AB, Boston, MA) as described by Stewart el al., 2008. In addition, a maximum temperature was recorded for a standard area surrounding each cauterized horn bud. A standardized method was used to collect images and record maximum ocular and dehorned area temperatures as previously described in Chapter 5.

Images were obtained from the left and right side of the calf, at an approximately 90°

angle, and 0.5 meter distance from the head. Ambient temperature (°C) and relative

humidity (%) recorded using a weather meter (Acurite®, Chaney Instrument Co., Lake

Geneva, WI) were entered into the camera in addition to the automatic calibration.

Images were analyzed using FLIR Tools (v. 4.1; FLIR Systems Inc, Boston, MA)

following collection. At each time point, two images were obtained and averaged for

statistical analysis.

Heart Rate

Heart rate was evaluated via auscultation at 4, 8, 24, 48, 96, 144, and 192 h. The

bell of a stethoscope (Littmann®, St, Paul, MN, USA) was placed between the 3rd and

5th intercostal space and beats were counted over a 15 second period. The value obtained

was used to calculate beats per minute. Baseline values were obtained at -24 h.

Average Daily Gain

Animals were weighed using a digital scale (Brecknell 200E, Avery Weigh-

Tronix, LLC, Fairmont, MN) 24 hours prior to the dehorning (d -1) and 8 days following

dehorning (d8). The scale was calibrated with weights of a known mass immediately

prior to obtaining the weight of a group of calves. Average daily gains were determined

using this data.

Blood sample collection

Trained handlers manually restrained animals during blood collection. Baseline samples were obtained at -24 h and immediately prior to drug administration. Blood samples were collected for animals receiving NSAID or placebo via jugular puncture at approximately 4, 8, 24, 48, 96, 144, and 192 h. Samples were immediately transferred to a blood collection tube with either sodium heparin or ethylenediaminetetraacetic (EDTA) with benzamidine (Vacutainer®, BD Diagnostics) and stored in a cooler with ice before processing. Blood samples were centrifuged for 15 minutes at 1,500g. Collected plasma was placed in cryovials and frozen at -70 °C until analysis.

Cortisol

Plasma cortisol samples were determined using a commercial radioimmune assay kit (Corti-Cote™, MP Biomedicals, LLC, Soton, OH) previously used and validated for bovine plasma (Fisher et al, 1996). Samples were assayed in duplicate with the reported concentration equaling the average cortisol concentration between duplicates. Samples were reanalyzed if there were subjectively large discrepancies between the duplicates. The average intra- and inter-assay coefficients of variation were 13.8% and 14.5%, respectively. Area under the effect curve (AUEC) was calculated using the linear trapezoidal rule as previously described (Glynn et al., 2013). Baseline values were obtained immediately prior to the administration of local anesthesia and drug administration.

Statistical analysis

Analyses were performed in SAS 9.3 (SAS Institute, Cary NC) using a linear mixed effects model with repeated measures. Data obtained from response variables was converted into a percent change from baseline and used for statistical analysis. Additionally, cortisol Cmax and AUEC were log transformed for normality and baseline values were used as covariates. The fixed effects were treatment (CARP, FIRO, FLU, MEL, PLCBO, SHAM), time, the interaction between treatment and time. Phase was a random effect and calf was the subject of repeated measures. F-tests were used to test the significance of main effects and interactions. If significant overall differences were identified among levels of a factor, pairwise comparisons were performed using Tukey's t-tests. The statistical analysis was performed for 3 time periods: 0 – 192 h, 0 – 24 h, and 24 h – 192 h. Planned contrasts were performed evaluating the responses of analgesia (CARP, FIRO, FLU, MEL) versus PLCBO treated calves following dehorning.

Results & Discussion

Calves treated with an NSAID at the time of dehorning resulted in reduced physiological responses compared with placebo treated controls. Evidence suggests that meloxicam, flunixin meglumine, and firocoxib may decrease the associated stress in the acute period (<24 h) following dehorning, with meloxicam reducing the maximum cortisol concentration compared to placebo treated controls. Interestingly, carprofen did not attenuate the stress response in the immediate period following dehorning suggesting it is unable to reduce pain acutely. Although not investigated in this current study, the

timing of maximum pain relief as well as persistence may reflect the pharmacokinetics of the NSAID administered.

Currently, there is a lack of pharmacokinetic-pharmacodynamic (PK/PD) models evaluating NSAIDs as analgesics in cattle. As such, accurate dose regimens demonstrating pain control are missing from the literature especially when compared to other veterinary species. For the drugs used within the study, a species dependent range of doses has been approved for use. For example, firocoxib has an approved dosing regimen of 0.1 mg/kg daily in horses while in dogs the approved dosing regimen is 5.0 mg/kg administered once daily. Although a multitude of factors affect dose determination, this dose range for the same NSAID in different species supports the necessity to determine an effective dose regimen in the target species through development of PK/PD models.

Given the uncertainty of an analgesic dose of NSAIDs in cattle, in order to control for the potential variation of drug concentrations resulting in analgesia, we choose to administer the same dose and route for all NSAIDs used in this study. Despite the pharmacokinetic and pharmacodynamic properties that determine plasma drug concentrations and potentially the analgesic effect, the use of the same dose and route provides conditions where the response observed is not influenced by the dose or route. It should be noted that although a dose of 2.0 mg/kg was targeted, flunixin meglumine was administered as a paste using a commercially available dosing syringe. As the dosing syringe used is based on 250 lbs increments for horses, the accurate actual dose administered was difficult to obtain in comparison with our calculated dose. Consequently, calves treated with flunixin meglumine received an actual mean dose of

2.3 mg/kg (range: 1.61 – 3.73 mg/kg). Due to the difficulty in controlling the actual dose administered, the authors refrain from recommending its use.

Cortisol

There was no treatment effect throughout the study in the percent change from baseline of cortisol concentrations (0.427); however a time effect (P<0.0001) and a time by treatment interaction (P=0.021) were observed (Figure 2). The percent change of cortisol at 4 h was reduced in flunixin meglumine treated calves (P=0.037) and tended to be reduced in meloxicam treated calves (P=0.056) compared to placebo treated controls. Moreover, the percent change of cortisol in carprofen treated calves was increased at both 4h (P<0.01) and 8h (P<0.05) compared to calves treated with firocoxib, flunixin meglumine, and meloxicam. Although no treatment effect was observed when evaluating the $AUEC_{0-192}$ (P=0.485), $AUEC_{0-24}$ indicates an overall treatment effect (P=0.0499) with placebo treated calves producing approximately 25% greater cortisol concentrations over the first 24 h compared to flunixin meglumine, meloxicam, and firocoxib treated calves (Table 2). In addition, maximum cortisol concentrations were attenuated in meloxicam treated calves compared to placebo treated controls (P=0.018) (Table 2).

The cortisol response following dehorning has been well characterized (Stafford and Mellor 2011). Moreover, attenuation of the cortisol response using NSAIDs following dehorning has been reported in previous studies for meloxicam (Heinrich et al., 2009; Allen et al., 2013), flunixin meglumine (Huber et al., 2013), carprofen (Stilwell et al., 2012) and firocoxib (Stock et al., 2015). As such these data are consistent with other published effects of NSAIDs reducing the cortisol response. Reduction in cortisol for the

first 24 h was similar between the treatment groups except for carprofen compared with placebo treated dehorned calves. In addition, meloxicam decreased cortisol Cmax compared to placebo treated controls, an effect not observed in the other treatment groups.

Carprofen treatment resulted in increased cortisol percent change for the first 8 h compared to the other NSAID treatment groups demonstrating the inability to control acute cortisol release and the stress response. This differs from previous reports using intravenous carprofen administration at the time of dehorning (Stillwell et al., 2012), but consistent with results presented by our group (unpublished data, presented in Chapter 5). The incongruity is most likely due to the route of administration. A prolonged time to reach maximum concentrations (median; range: 18 h; 12 h to 48 h) following oral administration may be responsible for the lack of pain relief during the acute period following dehorning (unpublished data, presented in Chapter 5). Although not significantly different, an 11% reduction in cortisol $AUEC_{24-192}$ was observed in carprofen treated calves compared to placebo treated suggesting a possible delay in effect of carprofen reducing stress.

Heart Rate

A treatment effect was observed throughout the study (P=0.024). A decreased percent change in heart rate observed in SHAM treated calves was greater than placebo treated calves over the study period (P=0.041). Moreover, analgesia administration tended to decrease percent change in heart rate for the duration of the study compared to placebo treated controls (P=0.085). Following the statistical analysis of the percent

change in heart rate in two distinct periods, 0-24 h and 24-192 h, the reported treatment

effect in SHAM treated calves was observed during the first 24 h. In addition, percent

change in heart rate was reduced in carprofen treated calves compared to placebo treated

calves between 24 h to 192 h (P=0.0466). During this same time period SHAM treated

calves tended to have a decreased percent change in heart rate compared to placebo

treated calves (P=0.0667).

The percent change of heart rate for all treatment groups was decreased

throughout the study (Figure 3). Most likely this was a result of an increased baseline

heart rate collected. Although calves remained in their home pens during their study and

were briefly handled daily for 2 days prior to the start of the study, multiple novel stimuli

including multiple unfamiliar people in the calf area were introduced during the

collection of baseline data. This may have resulted in stimulation of an increased heart

rate during the initial collection.

Multiple studies have reported the reductions in heart rate following dehorning

with the use of meloxicam (Heinrich et al., 2009; Stewart et al., 2009; Coetzee et al.,

2012a). While reductions in heart rates were observed in meloxicam treated calves, these

were not significantly different compared with placebo treated dehorned calves in our

study. Different methodology of heart rate collection may account for the differences

between studies. Interestingly, heart rates were significantly decreased in carprofen

treated calves compared to placebo treated calves from 24 h to 192 h indicating the

possible attenuation of the autonomic nervous system (ANS) response once drug

concentrations reached effective concentrations. This reduction in heart rate corresponds

with the decrease in $AUEC_{24-192}$ observed in carprofen treated calves compared to placebo treated controls.

Infrared Thermography: Dehorn area

SHAM dehorned calves had a greater increase in horn bud temperature throughout the duration of the study compared to all treatment groups (P<0.0001). A time effect (P<0.0001) and a time by treatment interaction (P=0.0005) were also observed. Among the dehorned treatment groups, carprofen treated calves had an increased percent change in dehorned area temperature at 4 h compared to firocoxib treated calves (P=0.025). Furthermore, this percent change in carprofen treated calves tended to be increased at 8 h (P=0.075) and 24 h (P=0.057) compared to firocoxib treated calves and at 8 h (P=0.075) for flunixin meglumine treated calves. An analysis of the initial 24 h indicates carprofen was associated with 1.4% greater increase in dehorned area temperature compared to firocoxib treated calves (P=0.0010) and 1.1% greater than flunixin treated calves (P=0.030). There were no treatment differences among dehorned treatment groups during the period 24 h to 192 h.

Infrared thermography has been used previously to demonstrate the effects of lameness on the surface temperature of the coronary band in cattle (Alsaaod et al., 2012). Changes in inflammatory patterns as observed in lame conditions result in differences between thermal profiles. Thermal imaging has not been used previously to determine the effect of cautery dehorning on the dehorning site. Skin temperature decreases with full or partially burned skin most likely due to disruption of peripheral blood perfusion (Hardwicke et al., 2013). As such, the increased temperature of non-dehorned SHAM

calves observed in this study was consistent with these findings most likely due to the
maintenance of blood flow in non-cauterized skin.

In this study, we report differences in the temperature of dehorned skin among
treatment groups following cautery dehorning. Calves treated with carprofen resulted in a
greater increase in temperature during the first 24 h post-dehorning in comparison to both
flunixin and firocoxib treated groups. If this effect correlates to increase inflammation as
demonstrated in lame cattle, this change in temperature parallels the increase in cortisol
percent change observed in carprofen treated calves. Although dehorning site temperature
in carprofen treated calves resulted in a greater numerical increase during the first 24 h
compared to placebo treated calves, it was not statistically different. Additional research
would be necessary to determine the contribution of carprofen administration to an acute
inflammatory response. Inflammation associated with burns is promoted by pro-
inflammatory cytokines such as IL-6 (Summer et al., 2008). In cattle, carprofen is
reported to affect both IL-6 (Pang et al., 2008) and IL-6 receptor (Vailati et al., 2015)
gene expression potentially leading to increased local concentrations of IL-6 in tissues.
An upregulation of proinflammatory cytokines resulting in a greater inflammatory
response may support the larger stress response observed.

Infrared Thermography: Ocular

Although the percent change from baseline of ocular temperatures changed over
time through the study (P<0.0001), no treatment effect (P=0.532) or time by treatment
interaction (P=0.712) were observed among treatment groups. These findings were
consistent when evaluating the response during the first 24 h as well as 24 h to 192 post

dehorning. However, comparing all analgesic treated calves to placebo treated controls indicated a decreased percent change in ocular temperature during the first 24 h in those animals receiving an NSAID (P=0.026). In addition, a treatment effect was observed when evaluating the difference between the eye and dehorn area temperatures over the study period (P<0.001). SHAM treated calves had a more narrow temperature difference compared to the other treatment groups (Figure 4). Moreover, a greater difference in temperature was observed between the dehorn area and the eye in calves treated with an NSAID compared to placebo (P=0.029).

Ocular temperature has been reported to decrease due to stimulation of the autonomic nervous system resulting in peripheral vasoconstriction (Stewart et al., 2010). In studies reporting this effect, ocular temperature was measured every 20 to 40 seconds and cattle were acclimated and restrained using a cattle chute with a head gate. The differences in methodology may be the reason for the incongruities in our studies evaluating ocular temperature over the course of the study period. In addition to collecting data at only specific time points, animals were manually restrained briefly to obtain a consistent image. Using our study methodology, no difference was observed between SHAM treated calves and placebo treated calves indicating a poor sensitivity in detecting differences associated with a noxious event. Despite obtaining the image before other data were collected, to avoid confounding factors of handling, the change in ocular temperature was increased in placebo treated controls in comparison to those treated with an NSAID during the initial 24 h period. This effect may be a result of the activity of an NSAID to inhibit prostaglandin production potentially resulting in alterations of peripheral blood flow (Przygodzki et al., 2015).

Mechanical Nociception Threshold (MNT)

A treatment effect was observed in the percent change of mechanical nociception threshold with SHAM treated animals tolerating increased pressure throughout the length of the study ($P<0.0001$). Moreover, a time effect ($P<0.001$) and a time by treatment interaction ($P<0.0001$) was observed with SHAM treated calves having increased MNT at all study time points post-dehorning (Figure 5). There was no treatment difference observed among treatment groups dehorned throughout the study.

The use of MNT to measure pain sensitivity following dehorning remains equivocal. Attenuation of the observed decrease in MNT post-disbudding has been previously reported following administration of IM meloxicam (Heinrich et al., 2010) and ethanol local anesthesia (Tapper et al., 2010); however other studies do not report treatment differences (Glynn et al., 2013; Allen et al., 2013; Stock et al., 2015). Methodology may remain a primary determinant of the ability to detect differences between treatment groups. In our study, MNT was useful in determining differences between animals dehorned compared to animals sham dehorned. However, MNT was insensitive in determining a treatment effect.

Heinrich and colleagues (2010) reported increased MNT at 4 h following cautery dehorning in calves treated with IM meloxicam relative to calves that were dehorned with a placebo injection. MNT was assessed only at this one time point. Although capable of determining differences between dehorned and not dehorned in our study, the evaluation of MNT at multiple time points may not be useful in characterizing subtle differences in nociception thresholds resulting from NSAID administration. This may be due to an

increase in learned avoidance behaviors in calves throughout the course of the study. As such, the timing of the initial data collection would be critical in determining the possibility of a treatment effect and should correspond with the time the analgesic drug has reached maximum concentrations.

Average Daily Gain (ADG)

Mean weight gain of 0.84 to 0.92 kg per day was observed during the study period. No treatment effect was observed in average daily gain among treatment groups including animals dehorned compared to those sham dehorned (P = 0.91). Equivocal results concerning ADG have been previously reported with multiple studies indicating increases in ADG (Glynn et al., 2013; Faulkner and Weary, 2000; Coetzee et al., 2012a) as well as those investigations resulting in no observed differences in weight gain (Grøndahl-Nielsen et al., 1999; Baldridge et al., 2011; Stock et al., 2015). The relationship between pain, stress, the neuroendocrine system and the immune system is complex. Investigations into castrations in cattle have indicated the benefits of using an NSAID at the time of castration resulting in a 50% reduction of bovine respiratory disease treatment (Coetzee et al., 2012b). All calves enrolled in this trial received metaphylaxic antibiotics to control respiratory disease 1 day prior to dehorning. This treatment may have masked the effect of using analgesia to control the pain and stress associated with cautery dehorning on weight gain. Moreover, individual housing used in this study may decrease competition for resources potentially influencing weight gain.

Conclusion

Cautery dehorning resulted in physiological, neuroendocrine, and nociception changes most dramatically during the first 24 h post-procedure. Perioperative oral NSAID administration resulted in the attenuation of the cortisol and heart rate response. Furthermore, NSAID use was associated with changes in temperature differences between the ocular and dehorning area. Based on cortisol response carprofen administration was not effective at controlling the associated stress in the first 24 h following cautery dehorning; however, meloxicam, firocoxib, and flunixin meglumine administration help to reduce cortisol release during the acute period post-dehorning. Moreover, meloxicam administration resulted in an attenuation of the maximum cortisol release. Despite the reduction in heart rate after 24 h, carprofen administration is not recommended based on its inability to control acute distress. Although not evaluated in this study, the variation in analgesic affect may be due to the variation in pharmacokinetic properties. Given the difficulties of controlling for an accurate dose using the oral flunixin paste in this dosing regimen, the authors would recommend meloxicam to control stress and pain associated with cautery dehorning.

Acknowledgments

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Table 1. Treatment groups characteristics at the time of dehorning and dose administered. Significant differences (P<0.05) between time points are indicated by different letters (a, b).

	Carprofen	Firocoxib	Flunixin	Meloxicam	Placebo	Sham	P value
N	31	31	30	31	31	31	
Age (d)	51.0 (41-60)	50.5 (41-60)	50.6 (42-60)	50.6 (42-59)	50.4 (42-59)	50.7 (41-60)	0.996
Weight	64.2 (49.1 – 77.3)	63.7 (45.5 – 80.5)	64.1 (54.5 – 80.0)	63.9 (47.3 – 88.6)	64.0 (47.7 – 80.5)	64.6 (49.1 – 81.8)	0.999
Breed: Holstein cross	3	3	4	3	2	5	0.871
Actual dose (mg/kg)	2.05^a (1.71 – 2.50)	2.01^a (1.66 – 2.51)	2.30^b (1.61 – 3.73)	1.99^a (1.73 – 2.11)	0	0	<0.0001

Table 2. Summary table of back-transformed cortisol concentrations of treatment groups post-dehorning. Significant differences (P<0.05) between time points are indicated by different letters (a, b).

	Carprofen LSM (95% CI)	Firocoxib LSM (95% CI)	Flunixin LSM (95% CI)	Meloxicam LSM (95% CI)	Placebo LSM (95% CI)	Sham LSM (95% CI)	Treatment (P value)
Cortisol AUEC (0-192)	2034.9 (1645.0 – 2517.4)	2202.6 (1783.4 – 2720.3)	2217.4 (1791.5 – 2744.6)	1851.4 (1498.9 – 2286.6)	2198.7 (1780.1 – 2716.0)	1925.6 (1559.2 – 2378.0)	0.485
Cortisol AUEC (0 -24h)	376.8 (307.4 – 461.9)	290.4 (237.2 – 355.5)	291.6 (237.7 – 357.9)	285.8 (233.5 – 349.9)	384.1 (313.7 – 470.2)	324.9 (265.4 – 397.7)	0.0499
Cortisol AUEC (24-192h)	1568.9 (1214.4 - 2027.0)	1856.8 (1440.0 - 2394.4)	1810.8 (1400.8 - 2340.5)	1509.9 (1171.0 - 1947.1)	1758.6 (1363.6 - 2268.1)	1491.7 (1156.9 - 1923.5)	0.410
Cortisol Cmax	39.7^{ab} (35.0 – 44.9)	36.1^{ab} (31.8 – 40.8)	36.7^{ab} (32.4 – 41.7)	32.8^{b} (29.0 – 37.2)	43.8^{a} (38.6 – 49.6)	41.7^{ab} (36.8 – 47.2)	0.0187

AUEC: area under the effect curve; Cmax: maximum concentration
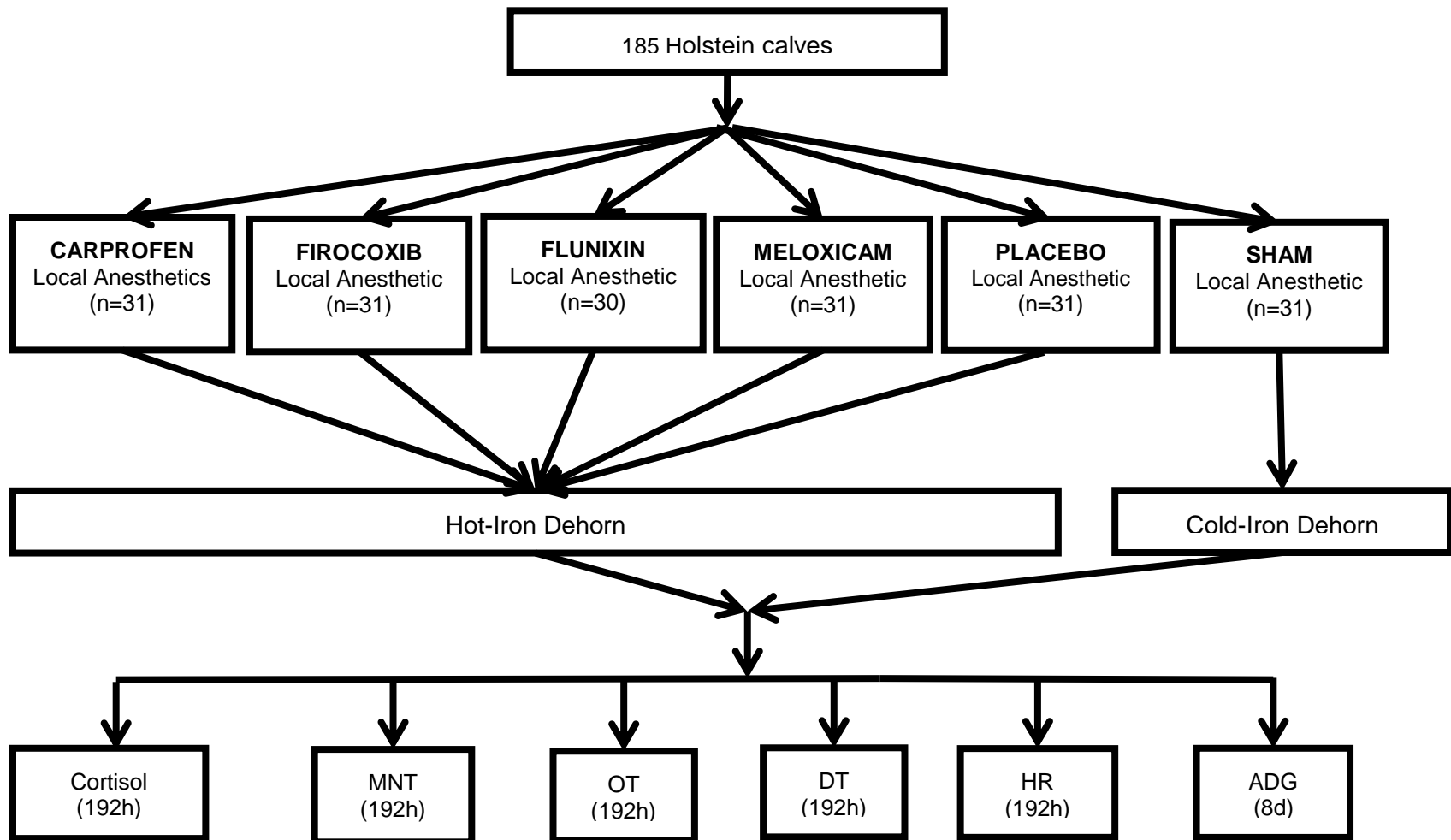
Figure 1. Flow chart outlining the timing of the study events. Calves were dehorned and monitored for cortisol, mechanical nociception threshold (MNT), ocular temperature (OT), dehorning area temperature (DT), heart rate (HR), and average daily gain (ADG). The times in parentheses represent the duration of data collection for each variable.
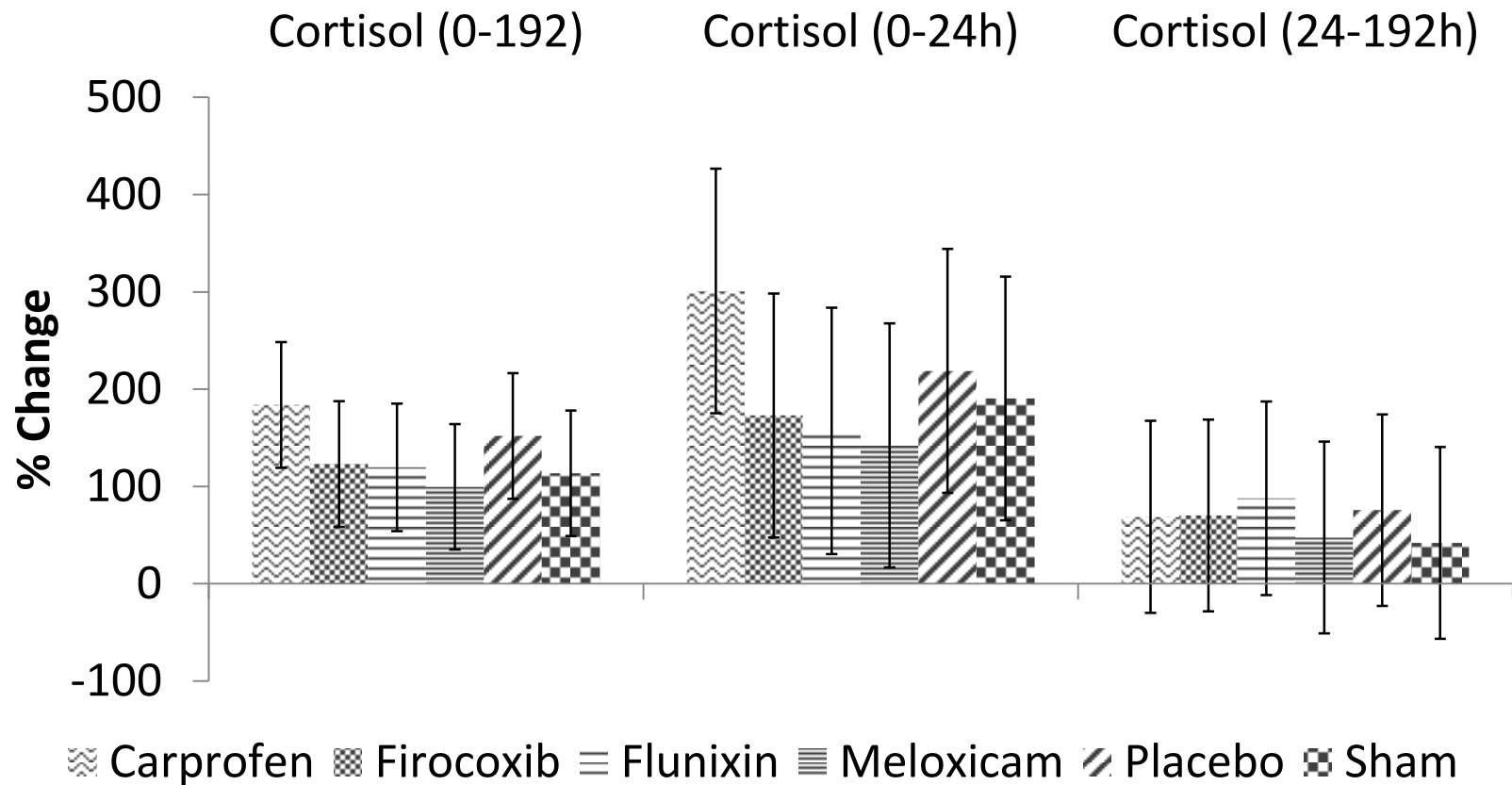
Figure 2. Mean percent change (±95% CI) in cortisol concentration from 0-192 h, 0-24h, and 24-192h for sham and carprofen, firocoxib, flunixin, meloxicam, placebo treated calves following actual or sham cautery dehorning.

HR (0-192)   HR (0-24h)   HR (24-192h)
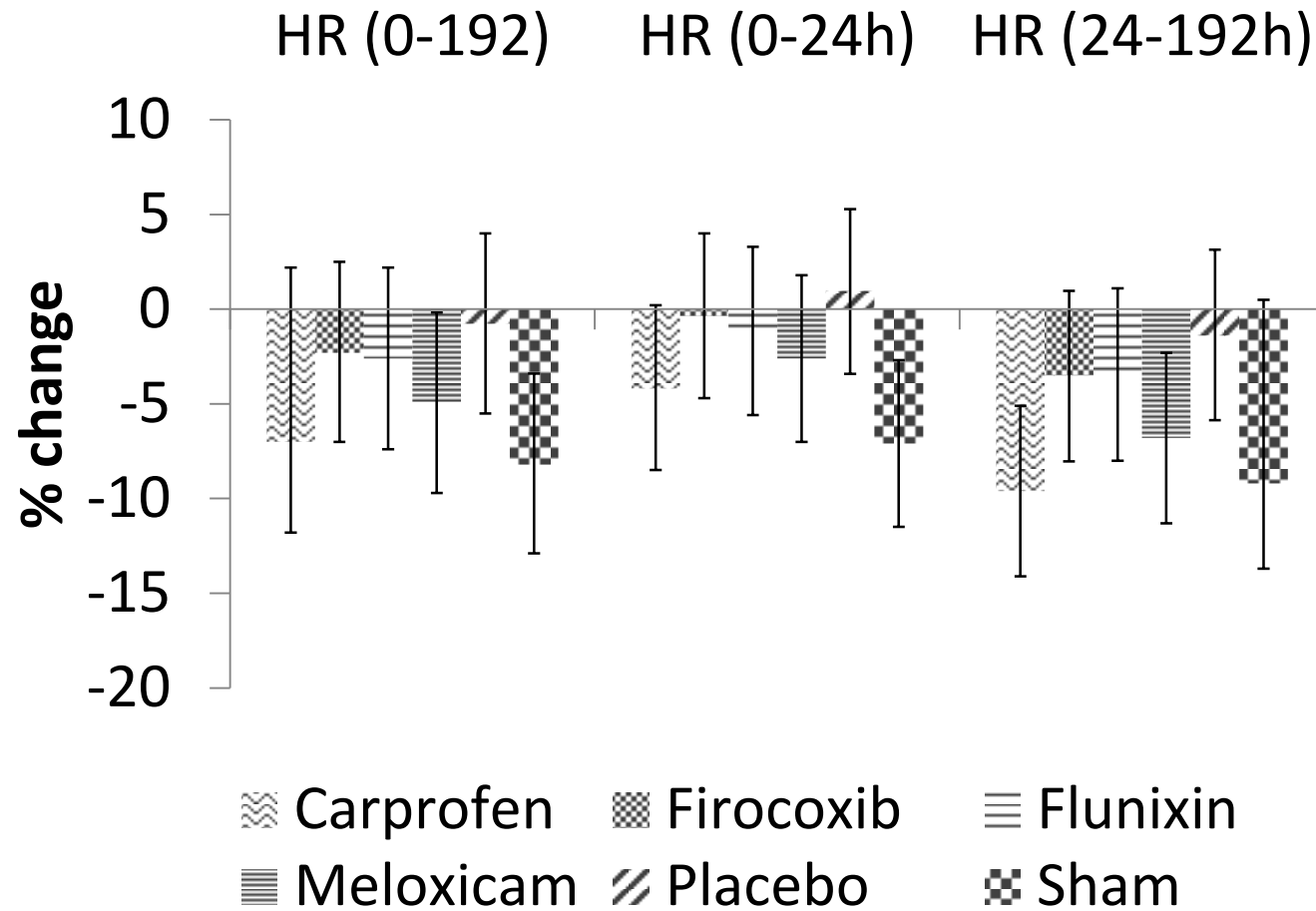
Figure 2. Mean percent change (±95% CI) in heart rate from 0-192 h, 0-24h, and 24-192h for sham and carprofen, firocoxib, flunixin, meloxicam, placebo treated calves following actual or sham cautery dehorning.
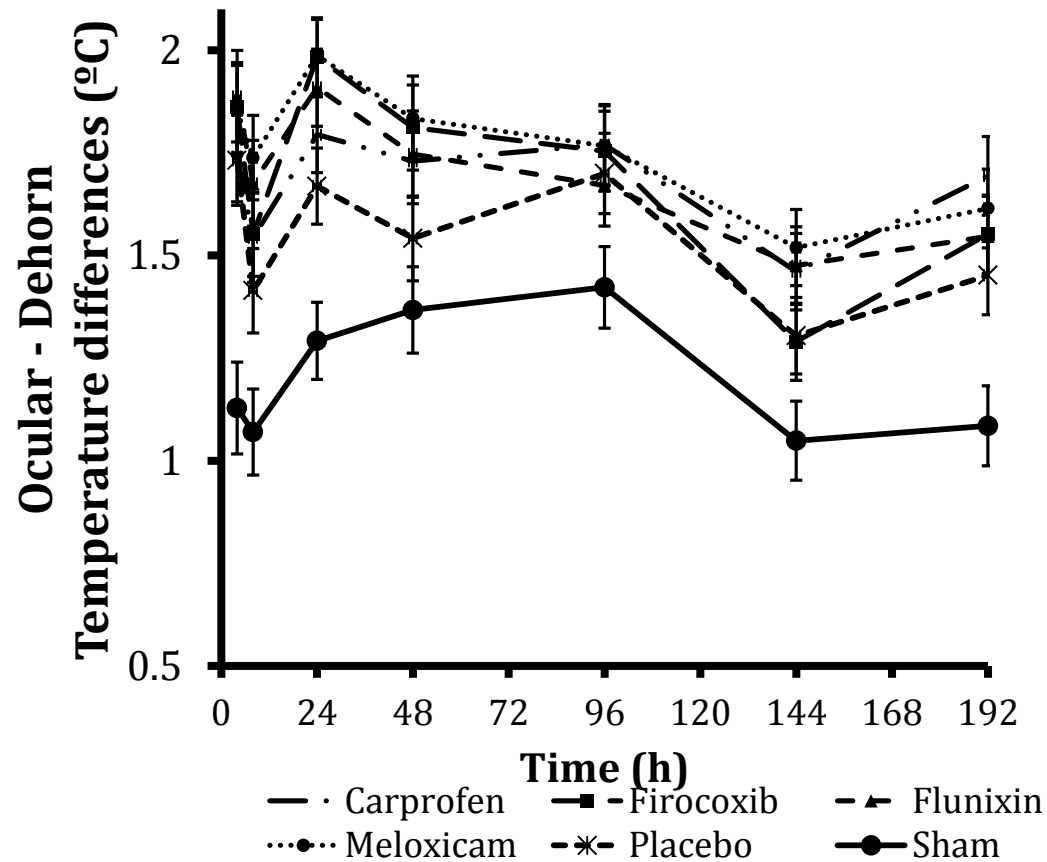
Figure 4. Mean (±SE) difference between ocular and dehorn are temperatures as measured using infrared thermography for sham and carprofen, firocoxib, flunixin, meloxicam and placebo treated calves following actual or sham cautery dehorning.
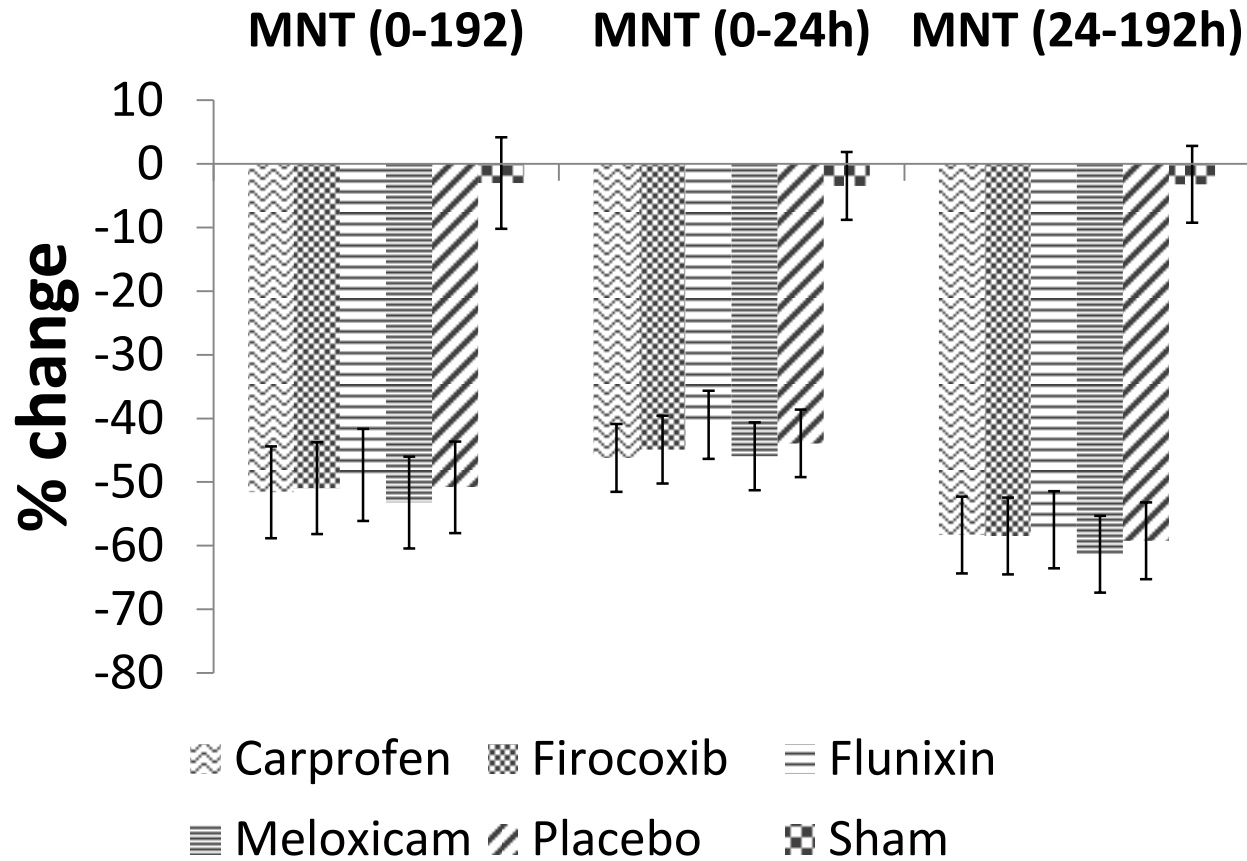
Figure 5. Mean percent change (±95% CI) in mechanical nociception threshold from 0-192 h, 0-24h, and 24-192h for sham and carprofen, firocoxib, flunixin, meloxicam, placebo treated calves following actual or sham cautery dehorning.

CHAPTER 7

DERIVATION OF A PAIN-STRESS INDEX

Introduction

The evaluation of pain is challenging. In disease states, using a single validated biomarker are advantageous; however, investigations validating biomarkers and responses in pain states continue to be elusive. As discussed by Molony and Kent (1997) the assessment of multiple indices of pain and stress are more likely to support the identification of a pain state. As such, we investigated the construction of a pain-stress index (PSI) where all response variables were standardized to derive a summary graph describing the response over time using one variable.

Methods

Response variable data was collected as described in Chapter 6 and used to generate the summary graph. In order to equally weight all variables in determination of the pain-stress index (PSI), responses were standardized on a scale of $0 - 10$ using the following formula:

$$\text{Pain-Stress Index (PSI)} = ((a + (X - Min) * (b - a)) / (Max - Min))$$

X: value of variable at given time point

a: 0; lowest value in normalized range

b: 10; highest value in normalized range

Max: maximum response value observed in all calves for the variable calculated

Min: maximum response value observed in all calves for the variable calculated

PSI was calculated for heart rate, mechanical nociception threshold (MNT), cortisol concentration, ocular temperature, and dehorned area temperature. As a decrease in MNT is associated with an increased nociception and pain, the inverse of the response was used in calculating the variables PSI. Additionally, due to increased dehorned area temperature observed in sham treated calves as a result of continued blood perfusion, this response was removed from sham PSI determination. As such, the average PSI for all response variables was used to determine the overall PSI at each calf at each time point in order to reduce the impact of removing a variable from a treatment group. The difference from baseline PSI was used for statistical analysis.

Analyses were performed in SAS 9.3 (SAS Institute, Cary NC) using a linear mixed effects model with repeated measures. Baseline PSI values were used as covariates. The fixed effects were treatment (CARP, FIRO, FLU, MEL, PLCBO, SHAM), time, and the interaction between treatment and time. Phase was a random effect and calf was the subject of repeated measures. F-tests were used to test the significance of main effects and interactions. If significant overall differences were identified among levels of a factor, pairwise comparisons were performed using Tukey's t-tests. The statistical analysis was performed for 3 time periods: $0 - 192$ h, $0 - 24$ h, and $24 - 192$ h.

Results & Discussion

There was an overall treatment effect ($P<0.0001$), time effect ($P<0.0001$) and time by treatment interaction ($P<0.001$) throughout the study period (Figure 1). SHAM treated animals had a decreased PSI difference compared to all other treatment groups ($P<0.0001$) at every time point ($P<0.01$). In addition, flunixin treated calves had a

decreased PSI at 8 h compared to carprofen (P=0.015) and placebo (P=0.052) treated calves. An analysis of the first 24 h indicates both sham (P<0.001) and meloxicam (P=0.044) treated calves had a decreased PSI compared to placebo treated controls (Figure 2). Other than a decreased PSI in SHAM animals compared to the other treatment groups, no treatment differences were observed in calves dehorned after 24 h.

The pain-stress index accurately detected differences between actual and sham dehorned calves. Moreover, placebo treated calves had an increased PSI for 24 h compared to meloxicam treated calves. This may provide further support for the analgesic superiority of meloxicam for a 24 h period of time at the current dosing regimen compared to other evaluated NSAIDs. Additionally, the calculated PSI indicated a reduction in pain and stress responses at 8 h for flunixin meglumine treated calves compared to placebo treated controls. This response may correlate to the plasma drug concentration profile and pharmacokinetic properties of oral flunixin meglumine (Odensvik, 1995). As previously reported in chapter 6, oral carprofen was not effective in comparison to other evaluated NSAIDs regarding the reduction of the pain-stress index for the initial 24 h. Although preliminary, the following model could be used to further investigate analgesic compounds providing a summary variable of multiple indices.

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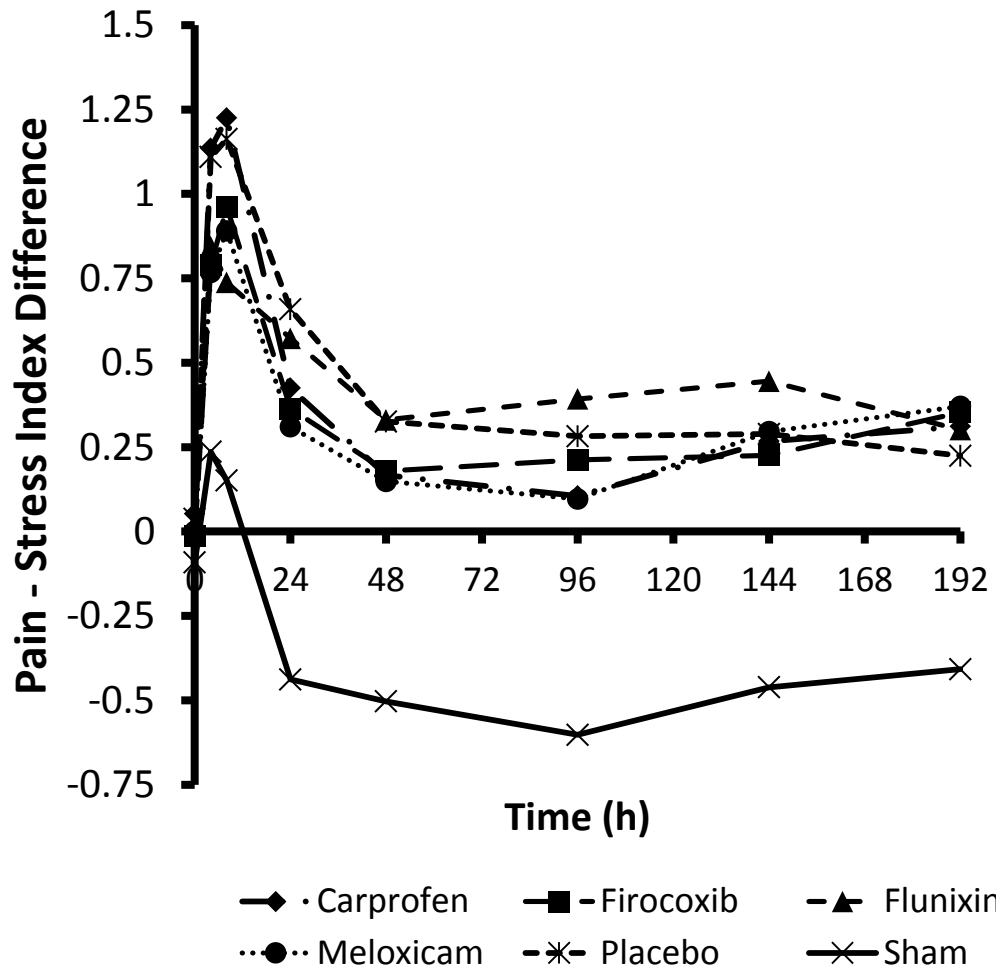
Figure 1. Pain-Stress Index difference from baseline as calculated using the average of values obtained from equally weighted response variables over the course of the 192 h study.
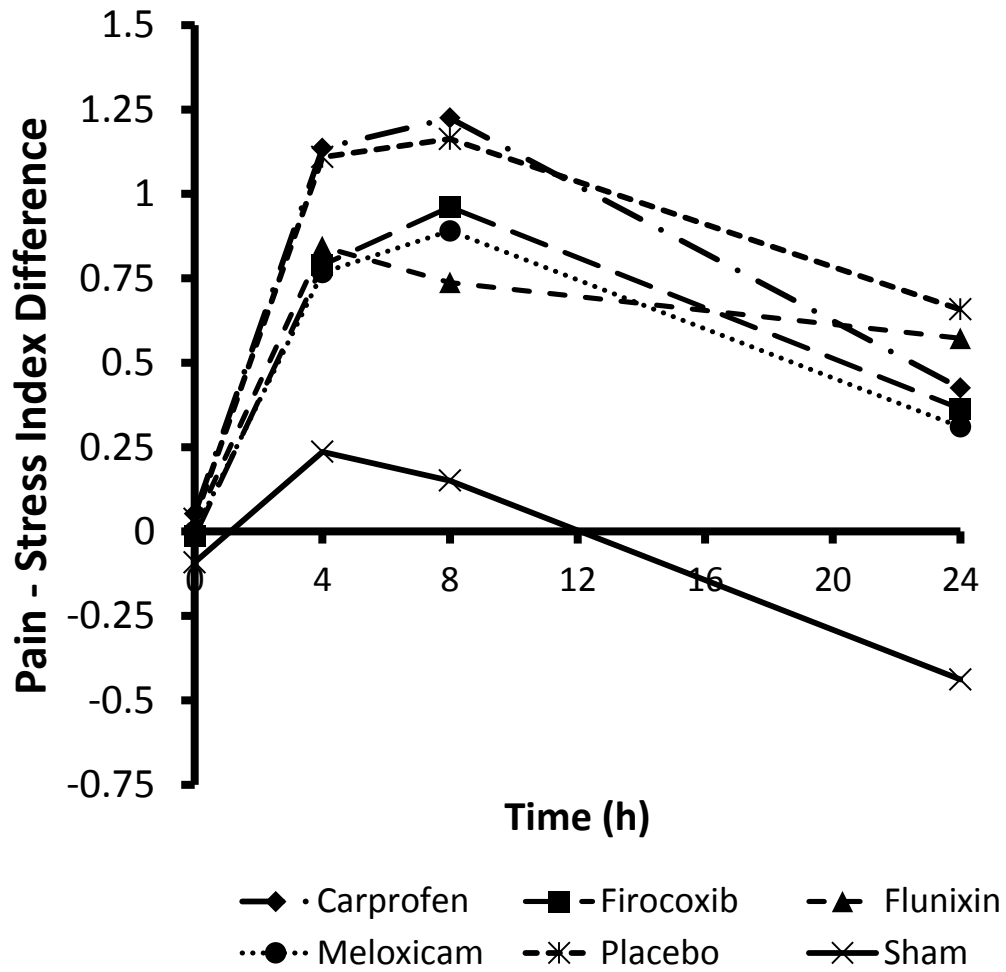
Figure 2. Pain-Stress Index difference from baseline as calculated using the average of values obtained from equally weighted response variables over the first 24 h.

CHAPTER 8
GENERAL CONCLUSIONS

The pain and distress resulting from cautery dehorning in cattle has been well documented and reviewed (Stafford and Mellor, 2005, 2011). Changes in cortisol concentrations (Heinrich et al., 2009, Doherty et al., 2007, Allen et al., 2013), heart rate (Heinrich et al., 2009; Coetzee et al., 2012), respiratory rate (Heinrich et al., 2009), ocular temperature (Stewart et al., 2009), heart rate variability (Stewart et al., 2009), mechanical nociception threshold (Heinrich et al., 2010; Allen et al., 2013), time budget (Theurer et al., 2012), and behavior indices (Faulkner and Weary, 2000) are responses that have been evaluated to determine the changes due to cautery dehorning. The importance of investigating multiple indices of pain has been stressed as a method to accurately assess the affect state (Molony and Kent, 1997). As such, I examined multiple response variables previously investigated to evaluate the analgesic potential of non-steroidal anti-inflammatory drugs to relieve pain associated with cautery dehorning.

Treating pain in cattle is challenging. In addition to the lack of overt responses observed in cattle following a noxious event, there is scant information publicly available such as pharmacokinetic/pharmacodynamic (PK/PD) models to evaluate appropriate analgesic dosing regimens. The goal of these investigations was to determine a simple analgesic regimen that would optimize pain relief following cautery dehorning. A literature review indicated a multimodal approach using both local anesthesia and an NSAID reduced the signs of pain and distress associated with cautery dehorning (Stafford and Mellor, 2005, 2011; Stock et al., 2013). Moreover, investigations supporting the analgesic properties of meloxicam, an NSAID with a prolonged half-life may optimally

treat pain with the added benefit of complying with a simple dosing regimen (Heinrich et al., 2009).

In the wake of studies indicating the pain relieving effects of meloxicam following cautery dehorning, I investigated firocoxib, an NSAID with a long half-life that produces analgesia in lame horses (Orsini et al., 2012). Chapter 3 describes the pharmacokinetics of firocoxib in calves, which demonstrated high oral bioavailability as well as a prolonged elimination half-life. Interestingly, firocoxib has a large volume of distribution, which is unique compared to other NSAIDs. As a result, firocoxib may accumulate in tissues throughout the body more so than most NSAIDs with a low volume of distribution. The pharmacokinetics of oral firocoxib detailed in our study would most likely support a once daily analgesic regimen for preweaned calves.

In the same study, I investigated the analgesic response of oral firocoxib (0.5 mg/kg) following cautery dehorning using a randomized controlled trial. The dosing regimen used resulted in plasma drug concentrations that inhibited ex-$vivo$ prostaglandin E_2 (PGE_2) production for up to 48 h compared to placebo treated calves; however, this finding did not translate to attenuated stress and nociception responses during the initial 24 h post dehorning. Although reductions in cortisol concentrations were observed in firocoxib treated calves at 48 h, responses evaluating physiology, neuroendocrine system, and nociception in the acute period did not differ from placebo treated controls. As such, further research would be required prior to making a conclusion on the analgesic effect of firocoxib in calves including dose titration studies following predictive PK/PD modeling.

Similar to firocoxib, carprofen is an NSAID often used in veterinary medicine primarily in dogs with pain associated with osteoarthritis. More recently, the oral

formulation has become available as a generic product significantly reducing its price. Carprofen has been previously investigated in calves, and is gaining approval as an anti-inflammatory for respiratory disease in Europe. PK/PD modeling of its use as an anti-inflammatory support, at most, a once daily administration supported by a prolonged terminal half-life in young calves (>44 hrs) (Brentnall et al., 2013; Delatour et al., 1996). Given the persistent anti-inflammatory activity observed, I hypothesized a one-time administration of carprofen would provide a prolonged analgesic effect in calves following cautery dehorning.

Using a similarly designed randomized controlled trial as previously employed for evaluating firocoxib, oral carprofen was investigated for its analgesic potential and compared to subcutaneously administered carprofen using a dose of 1.4 mg/kg. The pharmacokinetics of oral carprofen indicated a prolonged half-life (~50 h) with approximately 70% bioavailability relative to subcutaneously administered carprofen. In addition, the time to reach maximum concentration was prolonged at a median of 18 h (range: 12 – 48 h). Prostaglandin inhibition was similar between routes of administration with moderate inhibition observed over the 96 h study period. This moderate inhibition has been previously reported leading investigators to label carprofen as both a COX-dependent and COX-independent anti-inflammatory drug (Delatour et al., 1996; Lees et al., 1996).

Despite the persistent concentrations and prolonged reduction of prostaglandin E_2, responses suggestive of an attenuated pain and stress response were minimal in carprofen treated calves. Although carprofen treated calves tended to have an increased mean nociception threshold compared to placebo treated dehorned calves, other responses were

equivocal. As such, it was concluded that using the anti-inflammatory dose of 1.4 mg/kg administered orally at the time of dehorning was unsuccessful at controlling the associated pain. However, given the reduction in *ex-vivo* prostaglandin E_2 suggesting a prolonged activity, dose titration studies may be warranted.

Following studies evaluating the pharmacokinetics and analgesic responses of oral firocoxib and carprofen in calves at the time of dehorning, I addressed the question concerning the optimal NSAID to use to treat and control pain associated with cautery dehorning. My final study compared the analgesic effect of a one-time oral dose of carprofen, firocoxib, meloxicam, and flunixin meglumine in a randomized controlled field trial. In the absence of PK/PD analgesic modeling conducted in cattle using non-steroidal anti-inflammatory drugs, a single standard dose administered the same route was used to avoid the uncertainty of an observed response being related to the dose and route used.

Based on the cortisol concentrations, calves treated with meloxicam, flunixin, and firocoxib had reductions in stress during the first 24 h compared to placebo treated controls. Moreover, meloxicam and flunixin had a decreased percent change cortisol response at 4 h and meloxicam decreased the maximum cortisol response. In contrast, carprofen treated calves had a similar cortisol response during the acute period (<24h) as placebo treated controls. This may be due to an increase in inflammation around the dehorning site evidence by the observed increased temperature compared to other treatment groups. Overall, meloxicam, flunixin and firocoxib may reduce the stress associated with dehorning; however, given the reduction of maximum cortisol release in meloxicam treated calves, the results of this field trial support the use of oral meloxicam

for the treatment of dehorning pain. This conclusion is further reinforced through the derivation and statistical analysis of the pain-stress index, which compiled equally weighted responses post-dehorning into one summary variable.

Given the lack of PK/PD modeling for analgesia in cattle, future research and growth should be directed towards the development of these models. Using the plasma NSAID concentration data as well as the prostaglandin data generated from these studies, preliminary PK/PD models can be generated to calculate a preliminary analgesic dose. Previous literature using animal models have suggested targeting drug concentration that inhibit PGE_2 by 80% to 95% (IC_{80} or IC_{95}) to provide analgesia in rheumatoid arthritis in humans (Huntjens et al., 2005) or inflammatory conditions in cats (Giraudel et al., 2005a; Lees et al., 2004). Additionally, PK/PD models can be derived from already examined response variables including skin temperature or nociception thresholds in order to best elucidate the response most correlated with analgesia following cautery dehorning (Giraudel et al., 2005b).

In order to optimize a PK/PD model, it is necessary to further investigate and refine biomarkers and response variables. Concentrations of inflammatory mediators found in plasma or cerebral spinal fluid such as bradykinin, serotonin, prostaglandins, histamine, calcitonin gene related peptide, and substance P that are responsible for hyperalgesia and allodynia may provide a better measurement of pain in cattle. Determination of key pharmacokinetic parameters including maximum plasma drug concentration or area under the curve could potentially lead to generating accurate PK/PD models already present in antimicrobial research.

In the advent of determining a PK/PD model of NSAIDs in cattle, preliminary calculated doses can be examined *in-vivo* to determine the accuracy of the modeled dose. Moreover, the preliminary dosing regimens should be evaluated in both healthy animals as well as in painful animals to best elucidate the optimal pain relief in the given disease state. In other animal models, pharmacokinetics (Day et al., 2015) and pharmacodynamics (Zhang et al., 2012) have demonstrated altered parameters in disease states compared to healthy subjects.

Taken together, conclusions from this research continue to support the use of NSAIDs in cattle to control the pain associated with cautery dehorning. To best serve the welfare of the animal, it is important that the provision of analgesia has demonstrated effectiveness for the dosing regimen administered. Continued research both *in-vivo* and *in-silico* will be necessary to fully optimize pain relief in cattle.

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