A field trial comparing four oral nonsteroidal anti-inflammatory drugs on controlling cautery dehorning pain and stress in calves

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ABSTRACT: The purpose of this study was to compare the analgesic effect of four nonsteroidal anti-inflammatory drugs (NSAIDs) administered as a single, standardized, oral dose in dairy calves at the time of cautery dehorning. The NSAIDs investigated have pharmacokinetic properties in cattle that produce persistent plasma concentrations that may provide prolonged analgesia with the added practicality of a simple administration regimen. One hundred and eighty-five Holstein calves aged approximately 50 d old were either sham dehorned (n = 31) or cautery dehorned following oral administration of carprofen (n = 31), firocoxib (n = 31), flunixin meglumine (n = 30), meloxicam (n = 31) or placebo (n = 31) in a randomized, controlled trial. A standard dose of 2.0 mg/kg was administered to all calves receiving an oral NSAID. 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. Administration of oral meloxicam, flunixin meglumine, and firocoxib at 2.0 mg/kg resulted in decreased cortisol concentrations compared to placebo-treated controls for the first 24 h postdehorning ($AUEC_{0–24}$) (P = 0.03). Moreover, firocoxib, flunixin meglumine, and meloxicam attenuated the maximum cortisol concentrations compared to placebo-treated calves (P = 0.04, P= 0.02). In calves treated with flunixin meglumine, cortisol concentrations was reduced at 4 h (P = 0.04) and 8 h (P = 0.02). In addition, analgesic administration was associated with changes in ocular and dehorning area temperature differences (P = 0.09). Carprofen and meloxicam reduced heart rates during the entire study period (P = 0.003). Although a treatment effect (P < 0.0001) was observed in the determination of mechanical nociception threshold among all treatment groups, meloxicam expressed marginally significant effects (P = 0.09) among NSAID treated groups dehorned. A single dose of oral meloxicam, flunixin meglumine, or firocoxib administered at 2.0 mg/kg 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 changes in pain and stress outcome variables assessed in this study, NSAIDs should be administered at the time of dehorning.

Key words: cattle, dehorn, nonsteroidal anti-inflammatory drug, pain

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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 (Coetzee et al., 2012a; Glynn et al., 2013). 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. Nonsteroidal 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; Stewart et al., 2009; Coetzee et al., 2012a; Allen et al., 2013; Glynn et al., 2013), flunixin meglumine (Stilwell et al., 2008, 2009; Glynn et al., 2013; Huber et al., 2013; Kleinhenz et al. 2017), carprofen (Stilwell et al., 2012), and firocoxib (Stock et al., 2015). All NSAIDs share a common mechanism of action by inhibiting the production of prostaglandins by cyclooxygenase-1 and -2 with varying affinities that differ by drug and species.

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 et al. (2009, 2010) demonstrated the clinical efficacy of IM meloxicam following cautery dehorning. As meloxicam has a prolonged half-life in cattle (~27 h), we hypothesized that NSAIDs with persistent concentrations would reduce the pain responses associated with dehorning following a one-time administration (Coetzee et al., 2009; Malreddy et al., 2013). As such, the objective of this study was 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. The NSAIDs evaluated in this study have demonstrated long plasma elimination half-lives in calves (Stock et al., 2014; Stock et al., 2016) and can be administered as a single oral dose, which is a practical method for drug delivery in the field. In addition, the prolonged physiological response to pain after cautery dehorning was compared.

MATERIALS AND METHODS

This study protocol was approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC Log # 4-14-7789-B).

Animals

One hundred and eighty-five female Holstein calves from a commercial dairy farm were utilized. Calves were approximately 50 d of age (range, 41–60 d) at dehorning were included in this study. The mean weight for the calves at disbudding was 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 following a physical examination by a veterinarian.

Calves were housed in individual three-sided pens (1.82 m × 1.22 m) with straw bedding. Calves were placed in these pens at birth and remained within the pens through for the duration 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,

Table 1. Treatment groups characteristics (mean; 95% confidence interval) at the time of dehorning and dose administered

Parameter	Carprofen	Firocoxib	Flunixin	Meloxicam	Placebo	Sham	*P* value
N	31	31	30	31	31	31	
Age, d	51.2 (49.7–52.7)	50.5 (48.9–52.1)	50.6 (49.0–52.2)	50.7 (49.1–52.22)	50.42 (48.8–52.0)	50.7 (49.1–52.3)	0.98
Weight, kg	64.31 (61.45–67.17)	63.66 (60.71–66.62)	64.24 (61.28–67.19)	63.92 (60.96–66.87)	64.00 (61.05–66.96)	64.63 (61.68–67.59)	0.99
Breed: Holstein cross	4	3	4	3	2	5	0.87
Administered dose, mg/kg	1.97[a] (1.88–2.07)	2.02[a] (1.92–2.11)	2.21[b] (2.12–2.31)	1.99[a] (1.90–2.09)	0[c]	0[c]	<0.0001

[a, b, c] Indicates significant differences ($P \leq 0.05$) between treatments within rows.

consisting of corn, oats, molasses, protein/vitamin/mineral supplement, and monensin was added daily at approximately 0.45–1.36 kg. Calves were briefly handled in their pens daily for 2 d prior to baseline data collection to aid in the acclimatization of handling. The study was conducted from May through July.

During the study period, a veterinarian conducted observational examinations twice daily for health outcomes which included assessment of milk and grain consumption. 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 calf removal were diagnosed based on an elevated rectal temperature (>39.7 °C), spontaneous cough, ocular and/or nasal discharge, and inappetence. 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 design was used for this investigation (Figure 1). Study animals were randomly assigned to be dehorned and receive a target dose of 2.0 mg/kg orally of (Table 1):

- carprofen (Novox caplets, Vedco, Inc. St. Joseph, MO; ANADA 200–498) (actual dose 1.97 mg/kg; range 1.88–2.07 mg/kg) (n = 31)
- firocoxib (Previcox, Merial LLC, Duluth, GA; NADA 141–230) (actual dose 2.02 mg/kg; range 1.92–2.11 mg/kg) (n = 31)
- flunixin meglumine (Banamine Paste, Merck Sharp & Dohme Corp., Inc. Whitehouse Station, NJ; NADA #137–409) (actual dose 2.21 mg/kg; range 2.12–2.31 mg/kg) (n = 30)
- meloxicam (Meloxicam tablets, Carlsbad Technology, Inc., Carlsbad, CA; NDC: 61442-127-10) (actual dose 1.99 mg/kg; 1.90–2.09 mg/kg) (n = 31)
- lactose (NOW, Bloomingdale, IL) placebo with dehorning (PLCBO) (n = 31)
- lactose (NOW, Bloomingdale, IL) placebo without dehorning (SHAM) (n = 31)

SHAM animals were not dehorned prior to study enrollment. The NSAIDS were administered as either a paste (flunixin meglumine) using the commercially provided dosing syringe or in an oral gelatin bolus (Torpac Inc., Fairfield, NJ) using an oral balling gun. The calculated dose was rounded to the nearest tablet or dosing increment based on the weights determined 24 h prior to administration (Table 1). The actual dose for flunixin meglumine was based on the weight of the commercial tube obtained before and after administration. Calves treated with flunixin meglumine received an increased dose by approximately 0.2 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). Following randomization and treatment group assignment, calves were enrolled in one of six groups containing an equal number of animals from each treatment group (one animal/treatment group/replicate). In order to facilitate the number of calves enrolled in the study, animals

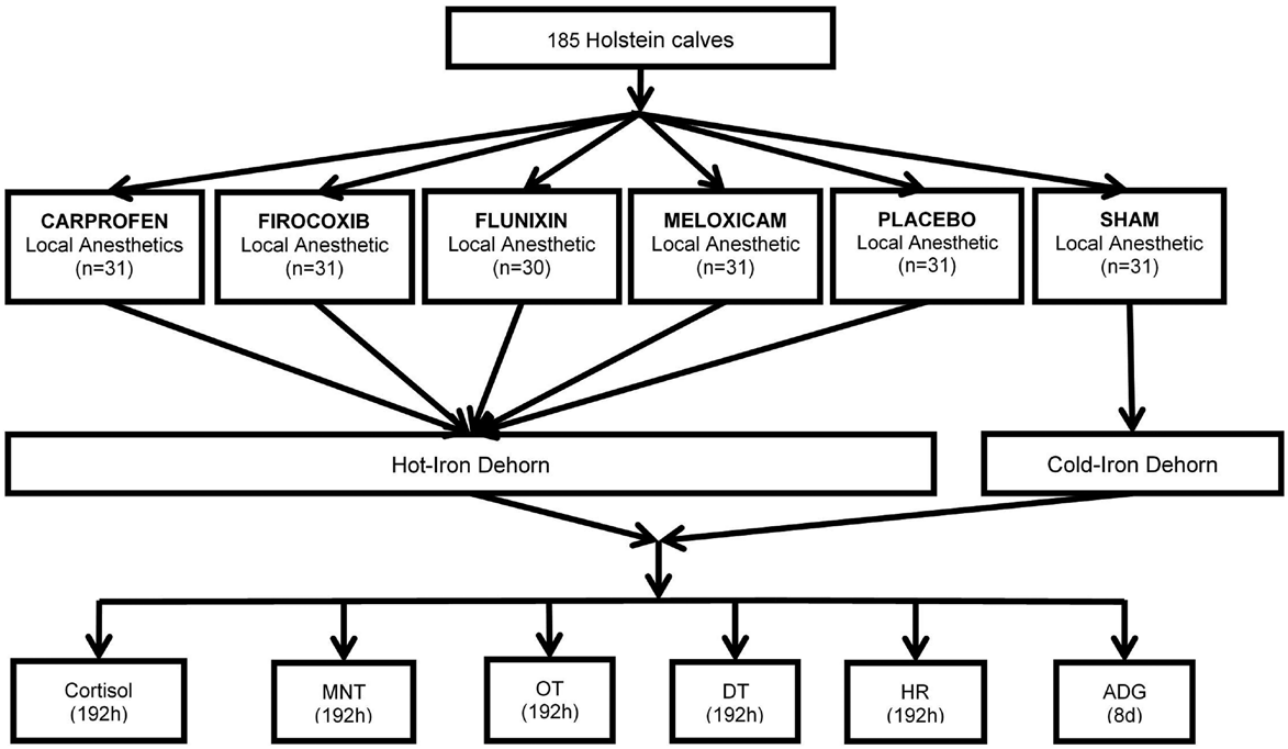

Figure 1. Flow chart outlining the timing of the study events. Calves were dehorned and monitored for cortisol, mechanical nociception threshold (MNT), ocular temperature (OT), dehorning area temperature (DT), heart rate (HR), and average daily gain (ADG). The times in parentheses represent the duration of data collection for each variable.

were enrolled over seven study periods with three to six treatment replicates per study period. Baseline data collection and dehorning were in the morning to account for diurnal variation.

Dehorning

Dehorning was performed in approximately 5 min intervals by a single dehorner and handler per study period adapting methods described by Stock et al., (2016). Calves were manually restrained by study personnel. Cautery dehorning was initiated approximately 5 min after 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 et al., (2013). Effective local anesthesia of the cornual tissue was confirmed using behavior responses (e.g., ear flicks, head shaking, strong escape behavior) to a needle prick approximately 3 min 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 an initial needle prick. Following confirmation of appropriate desensitization, calves were cautery dehorning by the placement of a preheated electric hot-iron (approx. 600 °C) (Dehorner X-50, Rhinehart Development Corporation, Spencerville, IN) on the horn tissue for approximately 15–20 s until a circumferential copper-colored ring surrounding the horn bud was formed. A duplicate, nonheated electric dehorner was used for SHAM animals.

Each calf was assessed for attitude, body posture, appetite, lying time, peri-operative swelling and signs of discharge or infection from the dehorning site. A rescue analgesia protocol of flunixin meglumine at 2.2 mg/kg, IV once daily for 3 d 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 blinded to the treatment groups.

Mechanical Nociception Threshold (MNT)

Mechanical Nociception Threshold (MNT), as defined by a maximum force that induces a withdrawal response, was determined at 4, 8, 24, 48, 96, 144, and 192 h postdehorning as previously described (Tapper et al., 2011 and Allen et al., 2013). Briefly, calves were restrained using a loose halter for mechanical nociception threshold determination. Using a handheld pressure algometer (Wagner Force Ten FDX 25 Compact Digital Force Gauge, Wagner Instruments, CT, USA), a force was applied perpendicular, at a rate of approximately 1 kg of force per second, at two locations (lateral and caudal) adjacent to the horn bud immediately adjacent to cauterized skin. Additionally, a third control located 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 postdehorning. 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 a 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 et 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 by Stock et al., (2016). Images were obtained from the left and right side of the calf, at an approximately 90° angle, and 0.5-m distance from the head. Ambient temperature (°C) and relative humidity (%) recorded using a weather meter (Acurite, Chaney Instrument Co., Lake Geneva, WI) were initialized for the cameras 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 24 h prior to dehorning and at 4, 8, 24, 48, 96, 144, and 192 h. The bell of a stethoscope (Littmann, St, Paul, MN, USA) was placed between the third and fifth intercostal space and beats were counted over a 15-s period. The value obtained was used to calculate beats per minute.

Average Daily Gain

Animals were weighed using a digital scale (Brecknell 200E, Avery Weigh-Tronix, LLC, Fairmont, MN) 24 h prior to the dehorning (d –1) and 8 d following dehorning (d 8). 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 with a rope halter 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 1,500 × *g*. 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 interassay 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 generalized linear mixed-effects model with repeated measures. Data obtained from response variables were converted into a percent change from baseline, except for cortisol, 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, and their interaction (treatment × time). Phase was a random effect and calf was the subject of repeated measures. *F*-tests were used to test the significance of the main effects and interactions. The statistical analysis was performed for three time periods: 0–192 h, 0–24 h, and 24–192 h. Planned contrasts were performed evaluating the responses of analgesia (CARP, FIRO, FLU, MEL) vs. PLCBO treated calves following dehorning. Statistical significance was set at $P \leq 0.05$ and tendency towards significance was set at $P > 0.05$ to $P \leq 0.10$ a priori.

RESULTS AND DISCUSSION

Administration of an NSAID at the time of dehorning resulted in reduced physiological responses in medicated calves compared with placebo-treated controls. Evidence suggests that meloxicam, flunixin meglumine, and firocoxib may decrease stress in the acute period (<24 h) following dehorning, with firocoxib and meloxicam reducing the maximum cortisol concentration compared to placebo-treated controls. Interestingly, carprofen did not attenuate the stress response in the 24 h period after dehorning. This finding suggests that carprofen did reduce acute, inflammatory pain in calves and is less effective when administered at the same dose as the other drugs evaluated. Although not investigated in this current study, the timing of maximum pain relief, as well as persistence, may reflect the pharmacokinetics and/or pharmacodynamics of the NSAIDs administered.

Currently, there is a lack of pharmacokinetic-pharmacodynamic (PK/PD) models evaluating NSAIDs as analgesics in cattle. As such, effective dose regimens demonstrating pain control after oral administration are deficient in the literature especially when compared to other veterinary species. Additionally, there are no NSAIDs approved in the United States for analgesia at the time of dehorning. Thus all treatments listed in this study would be considered extra-label drug use and would need prescription by a licensed veterinarian.

For the NSAIDs evaluated in this study, a species-dependent range of doses has been approved. 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. Our group previously described the effect of oral firocoxib administered at 0.5 mg/kg on pain biomarkers after dehorning (Stock et al., 2015). Similarly, for carprofen injection, a dose of 4.4 mg/kg once daily is approved in dogs while a dose of 1.4 mg/kg is approved in cattle. Stock and others (2016) evaluated the effect of a single oral dose of carprofen at 1.4 mg/kg on the pain response of calves at dehorning. For meloxicam injection, a loading dose of 0.2 mg/kg is approved in dogs compared with 0.5 mg/kg in cattle and 0.6 mg/kg in horses. A dose of 1 mg/kg oral meloxicam has been shown to mitigate pain biomarkers in calves after dehorning (Allen et al., 2013). In horses and cattle, the dose of flunixin injection ranges from 1.1 to 2.2 mg/kg. Although there are many factors that may affect dose determination, this dose range for the same NSAID, administered by the same route, in different species, supports the necessity to determine an effective dose regimen in the target species through the development of PK/PD models. Taken together, these studies support the selection of 2 mg/kg as a single, standardized, oral dose in calves to compare the analgesic effects of the NSAIDs evaluated in this study.

Given the uncertainty of an analgesic dose of oral NSAIDs in cattle, in order to control for the potential variation of drug concentrations resulting in pain relief, 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 2.2 mg/kg (range: 2.12–2.31 mg/kg). Due to the difficulty in controlling the dose of flunixin paste administered, and the approval of a formulation of flunixin for topical administration, the authors do not currently recommend the extralabel use of oral flunixin paste in cattle.

Cortisol

There was no treatment effect throughout the study in the cortisol concentrations ($P = 0.71$) in addition to no treatment by time interaction ($P = 0.24$). However, a time effect ($P < 0.0001$) was observed (Figure 2). Cortisol at 4h and 8h was reduced in flunixin meglumine treated calves ($P = 0.04$, $P = 0.02$) and tended to be reduced in firocoxib treated calves ($P = 0.03$, $P = 0.07$) compared to placebo treated controls. Moreover, cortisol in carprofen treated calves was increased at both 4h and 8h compared to calves treated with flunixin meglumine ($P = 0.07$, $P = 0.008$) and firocoxib ($P = 0.06$, $P = 0.04$). Although no treatment effect was observed when evaluating $AUEC_{0-192}$ ($P = 0.74$), the $AUEC_{0-24}$ suggests an overall treatment effect ($P = 0.09$) 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 ($P = 0.03$), firocoxib ($P = 0.01$), and flunixin meglumine ($P = 0.02$) treated calves compared to placebo treated controls (Table 2).

The cortisol response following dehorning has been well characterized (Stafford and Mellor, 2011). Moreover, attenuation of the cortisol response by 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; Kleinhenz et al., 2017), 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.

Carprofen treatment did not attenuate cortisol concentration to the same extent over the first 8 h compared to the other NSAID treatment groups. This suggests that carprofen was less effective at controlling the acute release of cortisol associated with inflammatory pain after dehorning. This differs from previous reports that demonstrated a reduction in peak cortisol concentrations after intravenous carprofen administration at the time of dehorning (Stilwell et al., 2012). However, this is finding consistent with results presented by

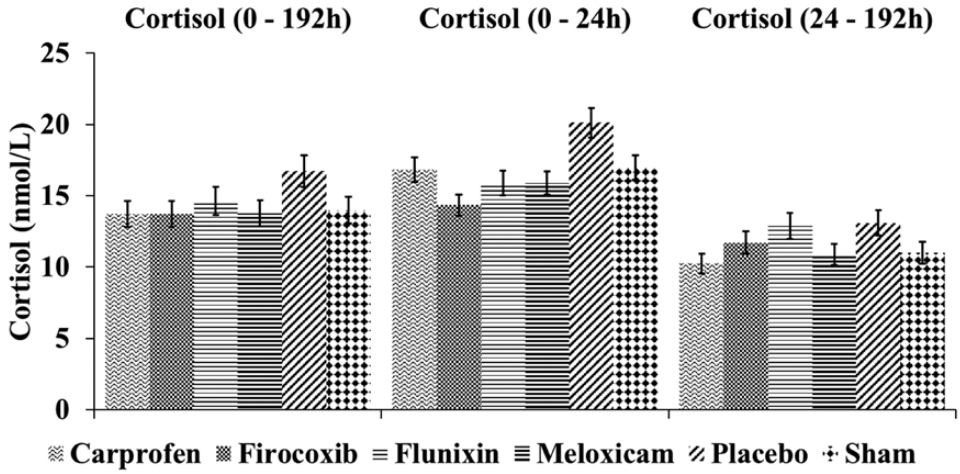

Figure 2. Mean cortisol concentration (±95% CI) from 0 to 192 h, 0 to 24 h, and 24 to 192 h for carprofen (2.0 mg/kg; $n = 31$), firocoxib (2.0 mg/kg; $n = 31$), flunixin (2.0 mg/kg; $n = 30$), meloxicam (2.0 mg/kg; $n = 31$), placebo ($n = 31$), and sham ($n = 31$) treated calves following actual or sham cautery dehorning.

Stock et al., (2016) after oral carprofen administration. The incongruity is most likely due to the different routes of administration used in these studies. Specifically, Stock and others reported a prolonged time to reach maximum concentrations (median; range: 18 h; 12 to 48 h) following oral administration of carprofen which may explain the lack of pain relief observed during the acute period after dehorning (Stock et al., 2016). Although not significantly different, a 21% reduction in cortisol $AUEC_{24–192}$ was observed in carprofen treated calves compared to placebo-treated suggesting a possible delay in the effect of carprofen reducing stress.

Heart Rate

A significant treatment effect ($P < 0.0001$; Table 3) and a significant time effect ($P = 0.006$) were observed throughout the study. A decreased percent change in heart rate observed in SHAM treated calves was greater than placebo-treated calves over the study period ($P < 0.0001$). Carprofen and meloxicam administration decreased the percent change in heart rate for the duration of the study compared to placebo-treated controls ($P = 0.003$). The heart rate percent change of carprofen treated calves was similar ($P = 0.38$) to SHAM calves between 0 and 192 h while a similar reduction was observed in meloxicam treated calves between 24–192 h, resulting in normal heart rate conditions despite the dehorning process.

The percent change in 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 the study and were briefly handled daily for 2 d prior to the start of the trial, multiple novel stimuli, including unfamiliar people in the calf area, were introduced during the collection of baseline data. This may have resulted in the stimulation of an increased heart rate during the initial collection.

Several 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 and the degree of required animal restraint may account for the differences between studies.

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

Cortisol, ng × h/mL	Carprofen LSM[a] (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)
AUEC[b] (0–192 h)	1978.7 (1568.4 – 2496.3)	2199.0 (1789.1 – 2702.9)	2333.4 (1878.9 – 2897.8)	1977.7 (1909.6 – 3000.1)	2393.5 (1909.6 – 3000.1)	2045.5 (1684.4 – 2484.1)	0.74
AUEC (0–24 h)	448.0 (386.2 – 519.7)	361.6[a] (306.3 – 426.9)	370.7[a] (288.9 – 475.8)	377.0[a] (315.1 – 451.1)	505.2[b] (425.9 – 599.2)	422.0 (361.2 – 493.0)	0.09
AUEC (24–192 h)	1438.2 (1083.5–1909.2)	1780.7 (1406.9–2253.9)	1836.5 (1409.9–2392.1)	1544.8 (1192.0–2002.0)	1830.6 (1418.4–2362.6)	1512.8 (1175.9–1946.2)	0.66
Cmax[c], ng/mL	37.0 (32.8 – 41.8)	33.7[a] (29.4 – 38.7)	34.5[a] (29.7 – 40.1)	32.8[a] (28.8 – 37.4)	41.9[b] (35.7 – 49.3)	40.0 (34.3 – 46.8)	0.12

[a]LSM: Least square means
[b]AUEC: area under the effect curve;
[c]Cmax: maximum concentration

Table 3. Summary table of overall least square means values for percent change in heart rate, infrared thermography temperature of the dehorn area, ocular infrared thermography temperature, and mechanical nociception threshold measures; and average gaily gain of treatment groups postdehorning. Significant differences ($P < 0.05$) between time points are indicated by different letters (a, b)

	Carprofen LSM[a] (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)
% change Heart Rate	–8.5[a] (–14.2 to –2.8)	–2.8[b] (–8.5–2.9)	–2.6[b] (–8.4–3.1)	–4.3[a,b] (–10.0–1.4)	–1.5[b] (–7.2– 4.2)	–8.5[a] (–14.2 to –2.8)	<0.0001
% change ocular-dehorn temperature	0.45[a,b] (–0.27 – 1.17)	1.07 [a] (0.35 – 1.79)	0.27[a,b] (–0.49– 1.00)	0.60[a,b] (–0.12– 1.32)	0.32[a,b] (–0.40– 1.04)	–0.57[b] (–1.29 – 0.15)	<0.0001
% change mechanical nociception threshold	–58.8[a] (–68.0 to –49.6)	–58.0[a] (–67.2 to –48.9)	–56.2[a] (–65.4 to –47.0)	–60.1[a] (–69.3 to –50.9)	–58.0[a] (–67.1 to –48.8)	–5.1[b] (–14.2 – 4.1)	<0.0001
ADG, kg	0.88 (0.79 – 0.98)	0.87 (0.77 – 0.96)	0.90 (0.80 – 0.99)	0.84 (0.75 – 0.94)	0.92 (0.83 –1.02)	0.90 (.80- 0.99)	0.91

[a]LSM: least square means.

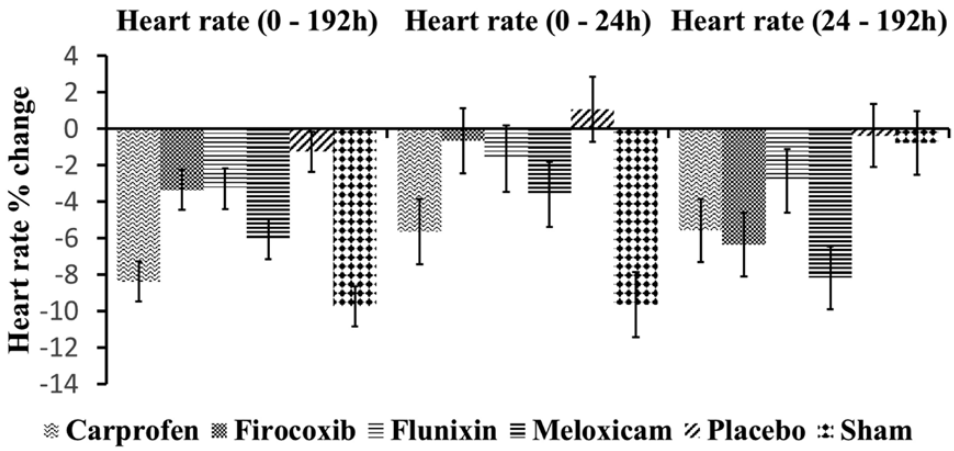

Figure 3. Mean percent change (±95% CI) in heart rate from 0 to 192 h, 0 to 24 h, and 24 to 192 h for carprofen (2.0 mg/kg; $n = 31$), firocoxib (2.0 mg/kg; $n = 31$), flunixin (2.0 mg/kg; $n = 30$), meloxicam (2.0 mg/kg; $n = 31$), placebo ($n = 31$), and sham ($n = 31$) treated calves following actual or sham cautery dehorning.

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 significant treatment and time effect ($P < 0.003$) was observed. Among the dehorned treatment groups, carprofen treated calves tended to have an increased percent change in dehorned area temperature at 8 h compared to flunixin ($P = 0.06$) treated calves. Furthermore, this percent change in carprofen treated calves tended to increase compared to firocoxib ($P = 0.03$), flunixin meglumine ($P = 0.06$), and meloxicam ($P = 0.08$) treated calves. The overall analysis indicates carprofen was associated with a 22% greater increase in dehorned area temperature change compared to firocoxib treated calves and 19% greater than flunixin treated calves.

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 nondehorned SHAM calves observed in this study was consistent with these findings, most likely due to the maintenance of blood flow in noncauterized skin.

In this study, we report differences in the temperature of dehorned skin among treatment groups after cautery dehorning. Calves treated with carprofen demonstrated a greater increase in temperature during the first 24 h postdehorning compared to all other treatment groups. Assuming this effect correlates to increased inflammation, as demonstrated in lame cattle, this change in temperature parallels the increase in cortisol 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 and meloxicam treated calves, this difference 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., 2006) and IL-6 receptor (Vailati et al., 2015) gene expression paradoxically leading to increased local concentrations of IL-6 in tissues. Upregulation of proinflammatory cytokines resulting in a greater inflammatory response may support the larger stress response observed.

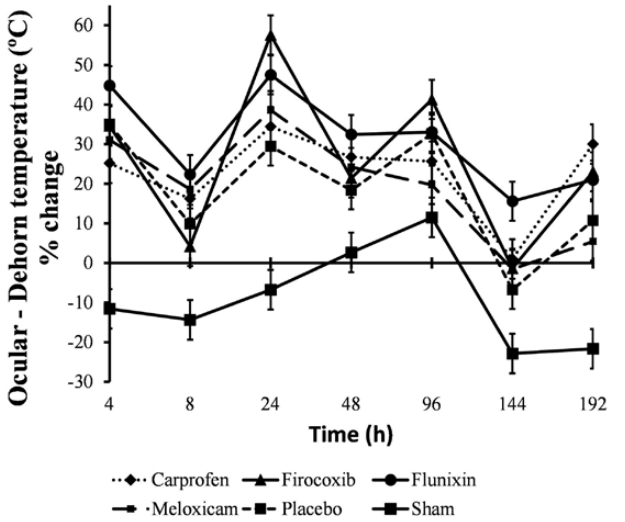

Figure 4. Mean (±SE) percent change difference between ocular and dehorn temperatures as measured using infrared thermography for carprofen (2.0 mg/kg; n = 31), firocoxib (2.0 mg/kg; n = 31), flunixin (2.0 mg/kg; n = 30), meloxicam (2.0 mg/kg; n = 31), placebo (n = 31), and sham (n = 31) treated calves following actual or sham cautery dehorning.

Infrared Thermography: Ocular

The percent change from baseline of ocular temperatures changed over time ($P < 0.0001$) and a treatment effect was observed ($P = 0.009$) throughout the study. However, compared with NSAID treated calves, SHAM treated controls tended to have a decreased percent change in ocular temperature during the study ($P = 0.09$). Calves that received carprofen ($P = 0.03$) and flunixin ($P = 0.05$) had the highest ocular temperature percent changes after dehorning compared to meloxicam-treated calves. In addition, a treatment effect was observed when evaluating the difference between the eye and dehorn area temperature changes over the study period ($P < 0.0001$) (Figure 4, Table 3).

Ocular temperature has been reported to decrease due to the stimulation of the autonomic nervous system resulting in peripheral vasoconstriction (Stewart et al., 2010). This observation is confirmed by the results of this study. In studies reporting this effect, the ocular temperature was measured every 20 to 40 s and cattle were acclimated and restrained using a cattle chute with a head gate. In addition to collecting data at only specific time points, animals were manually restrained by study personnel briefly to obtain a consistent image. Using our study methodology, a difference was observed between SHAM treated calves and placebo in addition to the NSAID treated calves, except carprofen and meloxicam treatment groups. This indicates a good sensitivity in detecting differences associated with noxious events. The change in ocular temperature decreased in placebo and increased in SHAM 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 ($P < 0.0001$; Table 3). Moreover, a time effect ($P < 0.001$) was observed with SHAM treated calves having increased MNT at all study time points postdehorning (Figure 5). Despite the fact that there was no treatment difference observed among treatment groups, meloxicam treated calves tended to exhibit a greater percent change of pressure tolerance during the study when compared to flunixin ($P = 0.05$), firocoxib ($P = 0.08$), and carprofen ($P = 0.09$)

The use of MNT to measure pain sensitivity following dehorning remains equivocal. Attenuation of the observed decrease in MNT postdisbudding has been previously reported following administration of IM meloxicam (Heinrich et al., 2010) and ethanol local anesthesia (Tapper et al., 2011). However, other studies have not reported similar treatment differences (Allen et al., 2013; Glynn 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 only able to

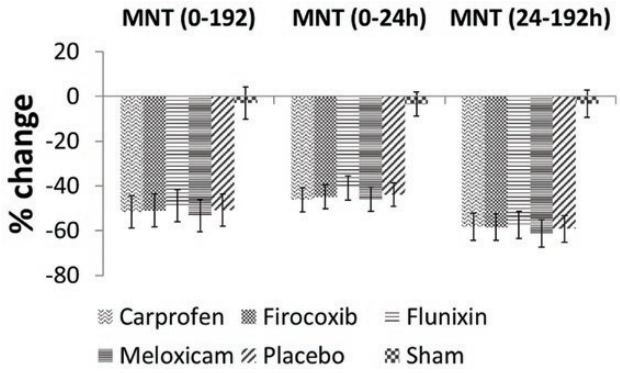

Figure 5. Mean percent change (±95% CI) in mechanical nociception threshold from 0 to 192 h, 0 to 24 h, and 24 to 192 h for carprofen (2.0 mg/kg; $n = 31$), firocoxib (2.0 mg/kg; $n = 31$), flunixin (2.0 mg/kg; $n = 30$), meloxicam (2.0 mg/kg; $n = 31$), placebo ($n = 31$), and sham ($n = 31$) treated calves following actual or sham cautery dehorning.

marginally discern an effect of meloxicam from other NSAID administrations.

Heinrich et al. (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 over time resulting from NSAID administration. A possible explanation is an increase in learned avoidance behaviors in calves throughout the course of this 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/d was observed during the study period (Table 3). 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 (Faulkner and Weary, 2000; Coetzee et al., 2012a; Glynn et al., 2013) 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. All calves enrolled in this trial received metaphylaxic antibiotics to control respiratory disease 1 d 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.

Cautery dehorning resulted in physiological, neuroendocrine, and nociception changes most dramatically during the first 24 h postprocedure. 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 stress associated with cautery dehorning in the first 24 h. However, meloxicam, firocoxib, and flunixin meglumine administration reduced stress as measured by assessing cortisol release during the acute period postdehorning. Moreover, firocoxib, flunixin meglumine, and meloxicam administration resulted in an attenuation of the maximum cortisol release between the time periods 0_192 h. 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 effect may be due to the variation in pharmacokinetic properties or affinity for the cyclooxygenase enzyme resulting in varying suppression of PGE synthesis.

As mentioned, differences in pharmacokinetic properties is a potential limitation for comparing different NSAIDs. These properties were not incorporated into the study design to keep drug administration at the time of dehorning as would be expected to occur on farms. Additionally, not sampling at an earlier time point may have resulted in unknown treatment differences. These differences would have had to be compared cautiously due to the use of local anesthesia, but may have highlighted the synergistic benefit gained by using NSAIDs with local anesthetics.

This study provides evidence that meloxicam, flunixin meglumine and firocoxib, administered as a single oral dose of 2 mg/kg at the time of dehorning, reduces pain and stress. Given the difficulties of controlling for an accurate dose, as well as newer alternative formulations approved for cattle, the use of oral flunixin paste in this dosing regimen is not recommended. These data support the recommendation that NSAIDs be administered in conjunction with lidocaine to control stress and pain associated with cautery dehorning.

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