Translational Animal Science, 2025, **9**, txaf010
https://doi.org/10.1093/tas/txaf010
Advance access publication 25 January 2025
Animal Health and Well Being

Effects of administering local anesthesia immediately before surgical castration on indicators of pain and discomfort of beef calves

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ABSTRACT

Forty Hereford cross calves (mean and SD of 47.9 ± 10.43 d old) were used to assess the efficacy of lidocaine administered immediately before surgical castration on physiological and behavioral indicators of pain and discomfort. Calves were assigned by age to one of two treatments: surgical castration following subcutaneous injection of 2.5 mL/100 kg body weight of meloxicam (Metacam 20 mg/mL, Boehringer Ingelheim, Burlington, ON, Canada) per kg body weight (**MEX**, n = 19); or the same treatment preceded 90 s before by a nerve block of the spermatic cord using 8 mL of buffered (1 mL:10 mL of 8.4% sodium bicarbonate USP, Hospira Inc., Lake Forest, IL, USA) lidocaine (4 mL per cord; 20 mg/mL, LIDO-2 with epinephrine, Rafter Products, Calgary, AB, Canada) (**LID**, n = 21). During the castration procedure, two observers scored how much pain each animal was experiencing (visual analog score, VAS), number of body shakes and leg kicks for each calf. Blood samples and exit scores were collected the day of castration and on d 7, and 14 to assess the neutrophil-to-lymphocyte ratio (N:L) and calf temperament, respectively. Hair samples were collected the day of castration and on d 14 to determine cortisol levels. Scrotal circumference, swelling, and healing scores were determined on d 7 and 14 to assess healing. An accelerometer (HOBO Pendant® G Data Logger, Onset, Cape Cod, MA) was placed on the left hind leg to measure lying behavior until d 6 after castration. Calves were video recorded for 1 hour after the castration procedure to observe behaviors indicative of pain and discomfort. Surveillance cameras were also used to assess the mobility of the calves when cow-calf pairs were moved from the holding pen to the pasture. The VAS, number of tail flicks and foot stamps were greater ($P < 0.01$) in MEX than in LID calves. During the first hour after castration, LID calves spent more time lying ($P = 0.03$) and less time standing ($P = 0.01$) than MEX calves. When moved from the holding pen, LID calves moved faster ($P < 0.01$) and closer to their dams ($P < 0.05$) than MEX calves. Hair cortisol was greater ($P < 0.05$) in LID than MEX calves on d 14. Results suggest that using lidocaine for local anesthesia immediately before surgical castration reduced behaviors indicative of pain and improved mobility post-castration.

Lay Summary

Castration is a common husbandry practice in the beef industry to reduce aggressive behaviors, prevent unwanted breeding, and improve meat quality of male cattle. The development and adoption of strategies to mitigate the pain caused by this procedure is a main priority due to animal welfare concerns. The use of both a local anesthetic (e.g., lidocaine) and an analgesic (e.g., meloxicam) has been the most effective approach for minimizing pain experienced from castration. Local anesthetics, however, are not commonly used for castration in commercial beef operations because its delayed onset of effect (5 to 10 min) is seen as an impediment when processing large number of calves. The objective of this study was to evaluate the effectiveness of lidocaine in controlling acute and chronic pain caused by knife-castration when administered immediately (90 s) before the procedure. The results show that the administration of local anesthesia shortly before castration reduced behaviors indicative of pain and improved the mobility of calves after the procedure. This study provided evidence that lidocaine can be administered closer to the time of castration, with a time of onset of 90 seconds, and still provide effective analgesia.

Key words: analgesia, calf welfare, pain mitigation

INTRODUCTION

Castration of male calves is a common practice in the beef industry to reduce aggressive behaviors towards humans or other animals, improve meat quality, and to prevent unwanted breeding (Stafford and Mellor 2005). However, castration causes acute and chronic pain (Stafford et al. 2002; AVMA 2014), manifested as agitated behaviors, with animals kicking, stamping feet, swishing tails, or otherwise appearing restless (Fisher et al, 2001; Thüer et al., 2007). Elevated cortisol levels and inflammation can also be observed as a physiological response to the pain and stress (Fisher et al. 1996; Pang et al. 2006). Due to animal welfare concerns, there is a demand to develop and adopt new pain mitigation strategies that can be widely adopted by the industry (Phillips et al. 2009).

The combination of an anti-inflammatory drug and a local anesthetic has been the most effective approach for minimizing pain experienced from castration (Stafford and Mellor 2011; Meléndez et al., 2018b). Local anesthetics such as lidocaine, however, are only used by a small proportion of veterinarians and producers during surgical castration of beef calves (Hewson et al., 2007; Coetzee et al., 2010). Low

Received April 22, 2024 Accepted January 23, 2025.

adoption of lidocaine use in castration procedures is likely due to delayed onset of lidocaine effect (5 to 10 min, Neves et al. 2017) and increased handling time (Coetzee et al., 2010) beyond what commercial beef operations consider to be practical, especially when large numbers of calves need to be processed on 1 day (Johnstone et al., 2021). In the search for practical methods for pain relief that could improve the effects produced by anti-inflammatory drugs, the use of lidocaine administered immediately before castration has not been explored in the literature (Van der Saag et al., 2019). While the impact preventing acute pain during the procedure (e.g., observable behaviors indicative of pain, temperament upon release from restraint) may be limited, due to the aforementioned pharmacokinetics, such inclusion of lidocaine as part of a multimodal pain control strategy could still be effective at reducing post-operative pain and discomfort (e.g., calf mobility, lying behavior, inflammatory response, chronic stress levels) (Laurence et al., 2016).

The objective of this study was to evaluate the effectiveness of lidocaine in controlling pain during and after knife-castration when administered immediately before the procedure. We hypothesize that the administration of lidocaine 90 s before the castration procedure, along with a systemic anti-inflammatory drug, would be effective at reducing behavioral and physiological biomarkers of pain. This could help increasing the adoption of this pain mitigation strategy by the industry.

MATERIALS AND METHODS

This work was approved by the University of Saskatchewan's Animal Research Ethics Board (Protocol 20190066) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Experimental Design

Forty Hereford crossed bull calves (mean and SD of 47.9 ± 10.43 d of age) and their dams were housed at the Livestock and Forage Centre of Excellence (University of Saskatchewan, Saskatoon, SK, Canada). Cow-calf pairs were together on pasture throughout the 14-d study, except during the castration procedure on castration day, and during sample collection (on d 7 and 14 after castration), when they were processed through the handling facilities at the farm. Due to logistic and time constraints, the 40 calves were ranked by age and split into two groups that were castrated on two consecutive days each (d 0 and 1). Briefly, all 40 calves were sorted by date of birth. Following that list, from youngest to oldest, the first two calves were assigned to group 1, the next two calves to Group 2, continuing in that fashion until the end of the list.

Similarly, on each castration day, calves were ranked by age and randomly assigned to one of two surgical castration protocols: 1) with a subcutaneous injection of meloxicam in the neck (0.5 mg/kg body weight, Metacam 20 mg/mL, Boehringer Ingelheim, Burlington, ON, Canada) (**MEX**, n = 19); or 2) the same treatment preceded 90 s before (as counted with a timer) by a nerve block of the spermatic cord using 8 mL of buffered (1 mL:10 mL of 8.4% sodium bicarbonate USP, Hospira Inc., Lake Forest, IL, USA) lidocaine (4 mL per cord; 20 mg/mL, LIDO-2 with epinephrine, Rafter Products, Calgary, AB, Canada) (**LID**, n = 21). For the castration procedure in both treatment groups, calves were moved into a tipping table (Calf Tipping Table, 2W Livestock Equipment, Nanton, AB) where the hind legs were tied with a rope to expose the scrotal region. An experienced veterinarian then cut the skin of the scrotum with a Newberry knife, and the testicles were manually exposed so an emasculator could be used to cut and seal the spermatic cord.

While restrained, calves were also administered three commercial vaccines including Covexin® Plus(Merck Animal Health, Kirkland, QC), Bovi-Shield GOLD® 5 (Zoetis Canada Kirkland QC), and Anthrax Spore Vaccine (Colorado Serum Company, Denver, CO), the vaccines were administered as indicated of their labels.

Temperament and Behavior Assessments

Before the castration procedure, and in each sampling day (d 7 and 14), calves were processed through a chute and a score from 1 to 4 (modified from Vetters et al., 2013; and Parham et al., 2019) was assigned based on whether calves exited the chute 1) calmly, 2) promptly, 3) briskly, or 4) frantically.

Visual analogue scores were determined using the method described by Moya et al. (2014). Briefly, two people blind to the treatments were located around three meters behind the veterinarian's back. They could not see and they were not informed about the use of lidocaine during the castration procedure. They were only notified when the castration procedure started (cutting of the scrotum) and ended (with the release of the emasculator). Based on this, they placed a mark along a 10-cm horizontal line, with the far left indicating no pain response and the far right representing an extreme pain response from the calf considering the number of body shakes, vocalizations, kicks, urination, and defecation. The distance from the start point to the mark was measured to the nearest mm, and the values from each observer were averaged and used as an indicator of the calf's pain response to castration. The interobserver reliability was deemed acceptable, with a spearman correlation of 0.84.

Immediately after the castration procedure, while calves were on the tipping table, an accelerometer (HOBO Pendant® G Data Logger, Onset, Cape Cod, MA) was placed using self-adherent bandaging tape (VetrapTM, 3M Animal Care Products, St. Paul, MN) on the left hind leg of each calf, as described by Meléndez et al. (2021). The accelerometers were set to record data at 1-min interval during the first 6 d after castration to determine the proportion of time lying and the daily number of lying bouts according to the University of British Columbia's Animal Welfare Protocol (UBC AWP 2013).

Pen Behavior

While cow-calf pairs were processed through the chute immediately before castration, a heavy-duty paper label (Livestock Identification, W.J. Ruscoe Co., Akron, OH) was glued to their back to identify each pair with a random combination of a color (blue, green, red, or white) on a one-digit number (0 to 9). Following castration, calves were held with the cows in an observation pen (21 × 21 m) equipped with a surveillance camera (super HD wired security system, Lorex, Markham, ON) to record the animals for up to one hour after the last calf entered the pen. Cows and calves were provided with straw bedding and ad libitum access to water during the recording period. A 1-h of footage for each calf, starting the moment they entered the observational pen, was later analyzed by one observer blind to the treatments (intra-observer correlation of 0.88) with

Table 1. Ethogram of behaviors recorded in calves during the 1-h observation period after castration. Adapted from Molony et al. (1995) and Meléndez et al. (2017b)

Behaviour	Data output	Definition
Eating	Total duration, number of bouts	Suckling from the udder or ingesting hay or straw from the ground or the feeder
Lying	Total duration, number of bouts	Either lateral (lying with the hip and shoulder on the ground with at least 3 limbs extended) or ventral (lying in sternal recumbency with legs folded under the body or one hind or front leg extended) lying
Walking	Total duration, number of bouts	Walking forward more than 2 steps
Standing	Total duration, number of bouts	Standing on all 4 legs
Not in sight	Total duration	Calf was out of the observer's sight because it was behind a cow or calf or there was no visibility of the hindquarters
Foot stamping	Frequency	Hind legs are lifted and forcefully placed on the ground or kicked outward while standing
Head turning	Frequency	Head turned and touching the side of the calf's body when standing, including head turning to groom
Tail flicking	Frequency	Forceful tail movement beyond the widest part of the rump; movement to one side is counted as one action
Lesion licking	Frequency	Head turning to lick the lesion caused by castration while standing

The Observer® XT (Noldus, Wageningen, the Netherlands). The ethogram described in Melendez et al. (2017b), which was adapted from Molony et al. (1995), was used to identify behaviors indicative of pain and discomfort (Table 1), including foot stamping, head turning, tail flicking and lesion licking.

Mobility Assessment

Following the recording of pen behavior, calf mobility was assessed by moving the cow-calf pairs in a 2-leg journey (93 and 82 m long, respectively) between pens at the farm. Two surveillance cameras (super HD wired security system, Lorex, Markham, ON) located at the end of each trip were used to record videos with a time stamp to determine the time it took for each animal to reach the goal line, and the time between the dam and calf crossing the goal line (cow-calf latency).

Wound Healing and Swelling

On d 7 and 14, calves were rounded up from the pasture, separated from the cows, and then processed through the chute where one observer blind to treatments conducted a wound healing and swelling assessment. Wound healing was scored from 0 to 4 based on Mintline et al. (2014), where: 0) completely healed and the incision no longer visible, 1) an incision less than 25% of the length of the scrotum and a small scab or discoloration is present, 2) an incision less than 75% of the length of the scrotum and scabbing with many wrinkles present, 3) an incision greater than 75% of the length of the scrotum with scabbing and some wrinkles, and 4) an incision runs the length of the scrotum with few wrinkles and scabbing may be present.

Swelling was scored from 0 to 4 based on Molony et al. (1995), where: 0) no swelling, inflammation or infection visible or palpable; 1) certain degree of swelling without obvious erythema; 2) increasing degree of swelling with obvious erythema but without pus; 3) presence of pus with increased inflammatory response; and 4) presence of pus with inflammatory response that needs intervention. Scrotal circumference with a flexible measuring tape, and calves sensitivity to palpation (yes or no) were also determined. On d 14, two calves from the MEX group had an abscess on the scrotum that had to be drained and treated with antibiotic (Liquamycin® LA-200®, Zoetis Canada Inc., Kirkland, QC).

Neutrophil to Lymphocyte Ratios and Hair Cortisol

Blood samples were collected from the jugular vein into 6-mL blood tubes with an anticoagulant (Vacutainer® EDTA, BD vacutainer, Franklin Lakes, NJ) immediately before castration, and on d 7 and 14. Blood smears from each sample were prepared on glass slides, and then stained using a staining kit consisting of a fixative, stain solution, and counterstain (Jorvet® DipQuick Stain, Jorgensen Laboratories Inc., Loveland, CO). Each slide was then examined under the microscope at 100 × magnification to count 100 cells and then calculate the proportion of neutrophils, lymphocytes, and the neutrophil-to-lymphocyte ratio.

Hair samples were collected on the day of castration and on d 14 by shaving a 20 × 20 cm area from the hip of the calves. The hair was stored in paper envelopes at room temperature until further analysis in the lab. Hair cortisol extraction was conducted as described by Moya et al. (2013). Once extracted, samples were reconstituted with the assay diluent, and analyzed using a commercial ELISA kit (Salimetrics Elisa, State College, PA, USA) following the manufacturer's specification. A plate reader was used to detect the absorbance value of each well at 450 nm with a second correction of 490 nm. Using a 4-parameter logistic curve fit, the concentration of cortisol was obtained. The intra- and inter-plate assay CV were 3.63 and 2.21%, respectively

Statistical Analysis

All data analyses were conducted using SAS OnDemand for Academics® (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). All parameters were checked for normal distribution using the Proc Univariate of SAS. Normality of the data was assessed based on the histogram for data distribution, the Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests for normality, the curve of normal

Table 2. The visual analogue scores, pen behaviors, and mobility of calves given either meloxicam alone (MEX, n = 19) or meloxicam in combination with lidocaine (LID, n = 21) 90 s before castration

	Treatment			
	MEX	LID	SEM	*P*-value
VAS, cm	3.71	2.14	0.350	<0.01
Pen behaviors				
Event behaviors				
Tail flicks, no.	585.6	93.9	71.07	<0.01
Foot stamps, no.	9.21	1.95	1.344	<0.01
Head turning, no.	0.68	0.90	0.273	0.58
Lesion licking, no.	-[1]	-	-	-
State behaviors[2]				
Lying, min	4.47	8.15	1.681	0.03
Standing, min	17.6	13.8	1.78	0.01
Walking, min	4.31	3.36	0.721	0.11
Eating, min	4.03	3.15	0.596	0.31
Not in sight, min	31.0	31.4	1.28	0.82
Mobility				
Time to goal line, s	13.44	2.89	4.612	0.01
Latency between dam and calf, s	173.4	165.7	6.17	0.01

[1]Not enough events detected to perform a statistical analysis, as only one LID and one MEX calf performed this behavior.
[2]Number of minutes performing each given behavior within the 60-min observation period.

quantiles, and plot of residuals. Variables that were not normally distributed were log transformed. The mixed model analyses for repeated measures over two sampling times (i.e., mobility assessment, wound healing and swelling, scrotal circumference, and hair cortisol) was the following:

$$Y_{ijkl} = \mu + T_i + D_j + TD_{ij} + G_k + A_l + e_{ijkl},$$

where Y_{ijkl} is the dependent variable; μ is the overall mean; T_i is the fixed effect of the ith treatment (i = 1, 2); D_j is the fixed effect of the jth sampling time (j = 1, 2); TD_{ij} is the fixed effect of the interaction between treatment and sampling time; G_k is the random effect of the kth castration group (k = 1, 2); A_l is the random effect of the lth animal (l = 1, 2,…, 40); and e_{ijkl} is the residual error of the model. The error degrees of freedom (DFe) of such model were 39. Those parameters measured over three (i.e., exit scores and blood samples; j = 1, 2, 3) or 6 (i.e., accelerometer data; j = 1, 2,…, 6) sampling times, had DFe of 78 and 195, respectively. Those parameters measured one time (i.e., VAS and pen behavior), had a DFe of 39.

For all the models, the covariance structure was selected based on the best fit according to Schwarz's Bayesian information criterion. The Tukey's test was used to compare the adjusted means, and p-value between two variables. All data presented in tables and figures are least squares means along with the average standard error of the mean (SEM) from each effect in the corresponding model. Main effects were considered significantly different when $P < 0.05$, and a trend if $0.05 \leq P < 0.10$.

RESULTS

VAS, Pen Behaviors, Calf Mobility and Exit Score

The visual analogue scores during the castration procedure were significantly lower (F(1, 39) = 72.60, $P < 0.01$) in LID compared to MEX calves (Table 2). After castration, while in the pen, LID calves had less frequent tail flicks (F(1,39) = 33.21, $P < 0.01$) and foot stamps (F(1, 39) = 14.57, $P < 0.01$), and spent more time lying (F(1, 39) = 5.05, $P = 0.03$) and less time standing (F(1, 39) = 7.04, $P = 0.01$) compared to MEX calves. Calves from the LID group moved faster between pens (F(1, 39) = 4.76, $P = 0.01$), and stayed closer to their dams (F(1, 39) = 5.71, $P = 0.01$) than MEX calves. There was a trend (F(2, 78) = 3.06, $P = 0.05$) for a treatment × day interaction, where MEX calves tended to have greater exit scores than LID calves on d 7 (Figure 1).

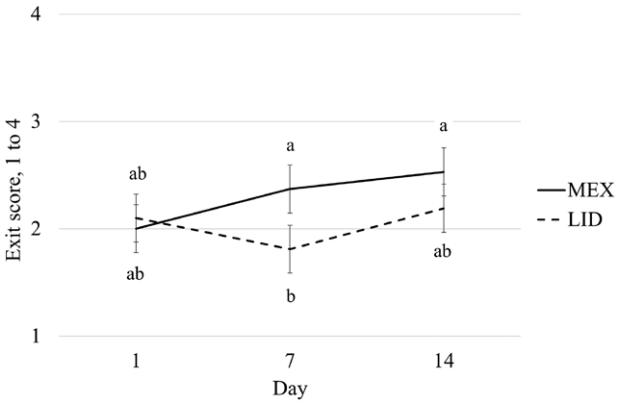

Figure 1. Average exit scores (SEM represented as error bars) of calves given meloxicam alone (MEX, n = 19) or meloxicam in combination with lidocaine (LID, n = 21) 90 s before castration. The statistical model showed no treatment (F(1, 78) = 0.97, $P = 0.33$) or time (F(2, 78) = 2.24, $P = 0.11$) effect, but there was a trend for a treatment × time interaction (F(2, 78) = 3.06, $P = 0.05$) identified with letters "a, b" in the figure.

Wound Healing and Swelling

There were no differences ($P > 0.10$) in wound healing (F(1, 39) = 0.01), swelling (F(1,39) = 0.01), or scrotal circumference

Table 3. Swelling scores, healing scores, and scrotal circumference of calves given either meloxicam alone (MEX, n = 19) or meloxicam in combination with lidocaine (LID, n = 21) 90 s before castration.

	Treatment		Day			*P*-value		
	MEX	LID	7	14	SEM	Treatment	Time	Treatment × Time
Healing score, 0 to 4[1]	3.61	3.6	3.75	3.45	0.095	0.94	0.03	0.79
Swelling score, 0 to 4[2]	1.89	1.88	1.73	2.05	0.123	0.94	0.07	0.96
Scrotal circumference, cm	15.29	15	16.16	15.13	0.421	0.63	0.95	0.17

[1]Based on Mintline et al. (2014), where 0 means completely healed and the incision no longer visible; and 4 means the incision runs the length of the scrotum with few wrinkles, and scabbing may be present.
[2]Based on Molony et al. (1995), where: 0 means no swelling, inflammation or infection visible or palpable; and 4 means presence of pus with inflammatory response that needs intervention.

Table 4. White blood cell count and chute exit scores of calves given either meloxicam alone (MEX, n = 19) or meloxicam in combination with lidocaine (LID, n = 21) 90 s before castration.

	Treatment		Day				*P*-value		
	MEX	LID	1	7	14	SEM	Treatment	Time	Treatment × Time
White blood cell count									
Neutrophils, %	36.9	38.6	48.6[a]	35.5[b]	29.3[c]	2.16	0.34	<0.01	0.36
Lymphocytes, %	58.6	56.6	46.3[c]	60.1[b]	66.4[a]	2.11	0.26	<0.01	0.31
Neutrophil-to-lymphocyte ratio	0.73	0.77	1.1[a]	0.68[b]	0.47[c]	0.08	0.61	<0.01	0.10

[a,b,c]Means with different superscript are different ($P < 0.05$).

between treatments (Table 3). Wound healing scores were lower on d 14 than on d 7 (F(1, 39) = 4.93, $P = 0.03$), while swelling scores tended (F(1, 39) = 3.48, $P = 0.07$) to be greater on d 14 than on d 7.

WBC Count and Hair Cortisol

The differential WBC counts showed no differences ($P > 0.10$) between treatments (Table 4). Between d 1 and 7, and d 7 and 14, the percentage of neutrophils and the neutrophil-to-lymphocyte ratio decreased (F(2, 78) = 41.66 and F(2, 78) = 33.08, respectively; $P < 0.01$), while the percentage of lymphocytes increased (F(2, 78) = 47.32, $P < 0.01$). The hair cortisol levels showed a treatment × interaction (F(1, 39) = 6.88, $P = 0.01$), where it was greater in LID than MEX calves only on d 14 after castration (Figure 2).

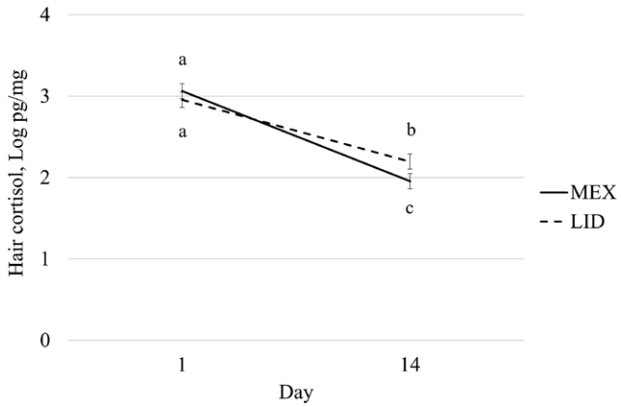

Figure 2. Hair cortisol levels (SEM represented as error bars) of calves given meloxicam alone (MEX, n = 19) or meloxicam in combination with lidocaine (LID, n = 21) 90 s before castration. The statistical model showed no treatment effect (F(1, 39) = 1.04, $P = 0.31$), but time (F(1, 39) = 198.1, $P < 0.01$) and treatment × time interaction (F(1, 39) = 6.88, $P = 0.01$) were significant, as identified with letters "a, b" in the figure.

Lying Behavior

The lying behavior measured with accelerometers showed no significant differences ($P > 0.10$) between treatments (Table 5). The percentage of time lying per day tended (F(5, 195) = 2.06, $P = 0.07$) to be lower on d 2 compared to d 4 and 6; while the number of lying bouts per hour was greater (F(5, 195) = 0.60, $P < 0.01$) on d 1 compared to the rest of the days.

DISCUSSION

Lidocaine has been demonstrated effective at minimizing signs of pain due to castration for up to 2 hours after the procedure (Clarke and Trim 2014), particularly in combination with the use of NSAIDs such as meloxicam (Meléndez et al., 2018b). Lidocaine use in the field, however, is regarded as impractical due to the time required to numb the spermatic cords and/or scrotum (Egger et al., 2013; Johnstone et al., 2021). The shortest time allowed for the onset of effects in previous literature was 5 min (Lehmann et al., 2017; Neves et al., 2017; Van der Saag et al., 2019). To allow 5 min for lidocaine to be fully effective, calves would either need to be restrained for 5 min, or be released and restrained again later for the castration procedure, with the consequent stress for the animal and cost of labor for the producers (Coetzee et al., 2010; Moggy et al., 2017). However, based on the results of this study, the reduced VAS observed in LID compared to MEX calves suggests that lidocaine reduced the pain experienced during the procedure when administered only 90 s before castration. Although visual analogue scoring is a subjective measure of calf discomfort, in this study it was performed

Table 5. The time lying and number of lying bouts from the accelerometers placed on calves given either meloxicam alone (MEX, n = 19) or meloxicam in combination with lidocaine (LID, n = 21) 90 s before castration.

	Treatment		Day						SEM	*P*-Value		
	MEX	LID	1	2	3	4	5	6		Treatment	Time	Treatment × Time
Time lying, %	34.02	35.34	34.27	32.30	35.55	35.62	34.64	35.67	1.267	0.34	0.07	0.50
Lying bouts, no./hour	1.06	1.08	1.41[a]	0.93[b]	1.03[b]	1.00[b]	0.99[b]	1.05[b]	0.062	0.52	<0.01	0.70

[a,b]Means with different superscript are different ($P < 0.05$).

by two independent observers familiar with behaviors indicative of pain, as to mitigate possible biases. Lidocaine has been shown to reduce VAS, leg and head movements of calves during surgical castration (Meléndez et al., 2018b), while Van der Saag et al. (2019) showed that 2- to 4-mo old calves with a nerve block of the spermatic cord with lidocaine were more likely to display less severe responses during the procedure compared to calves given a vapocoolant spray.

During the first hour after castration, the frequency of tail flicks and foot stamps were also 6- and almost 5-fold lower, respectively, in LID calves compared to MEX calves. The frequency of tail flicks has been demonstrated to increase after surgical castration in previous studies (Meléndez et al., 2018a, 2018b). The increase in tail flicks and foot stamps is considered a response to pain and discomfort resulting from an increased nociceptive sensitivity in the scrotum and spermatic cords as inflammatory substances are produced and accumulated in the region (Tranquilli et al., 2013). The reduced tail flicking and foot stamping among the LID calves was likely the result of the lidocaine ring block being effective during that first hour after castration when the animals were observed. Moreover, calf mobility was also improved in LID compared to MEX calves. Both the time it took for each calf to arrive at a goal line, and how much time passed between the arrival of a cow and its calf, was shorter in the LID group. Especially the former, where MEX calves took over 4-fold the amount of time than LID calves (13.4 vs 2.9 s) to move between pens. These are novel methods of assessing mobility developed to capture what producers notice when using pain mitigation strategies, as they indicate calves would have better mobility when released to the pastures, and a quicker return to suckling behaviors with their dams. Previous studies used a different approach to assess mobility by using stride length, which was shown to decrease after surgical castration due to pain (Currah et al., 2009; Meléndez et al., 2017a). In this study, therefore, LID calves would be expected to have a longer stride length than MEX calves, allowing them to move faster and to better keep up with their dam's rhythm.

Chute exit score or flight speed are commonly used as a means to assess temperament in cattle (Vetters et al., 2013; Parham et al., 2019). When animals exit the chute at a faster or more frantic pace, they are considered to be more fearful or agitated (Parham et al., 2019). Upon first being restrained in the chute, the calves used in this experiment had similar exit scores (2.0 and 2.1 ± 0.223 for MEX and LID calves, respectively. Figure 1). An animal's response to handling is influenced by both an inherited temperament and the animal's previous experience (Grandin and Shivley 2015). Animals handled in a calm manner acclimatized to the handling experience over time, showing lower chute exit speeds in consecutive assessments (Parham et al., 2019). In this study, MEX calves tended to have greater exit scores than LID on d 7 (2.37 vs 1.81 ± 0.223, respectively), which suggests that MEX calves may have had a more adverse response to the handling experience on d 1 than LID calves. This along the reduced pain behaviors and improved mobility, would indicate that LID calves had a less traumatic or painful experience during castration, likely due to an effective numbing effect of lidocaine.

A reduced lying behavior has been observed in surgically castrated calves compared to sham castration (Meléndez et al., 2017b), as calves stand for longer in response to pain and discomfort in the scrotal area. In this study, the use of

accelerometers showed no differences between treatments in their lying behavior, although it did show that the day after castration all calves had the greatest amount of lying bouts compared to the test of the days. On the one hand, this suggests that calves were more restless, lying down more frequently, but not remaining in that position for long, which indicates discomfort (Molony et al., 1995). On the other hand, the results from the video analysis of the first hour post-castration showed that LID calves spent more time lying down and less time standing than MEX calves. The consideration of the results from both methods used to assess standing and lying time suggest that lidocaine reduced standing time only immediately after castration; and, that as the local anesthetic wore off, likely around 1 to 2 h later, standing and lying time returned to levels similar to calves given meloxicam alone. This agreed with previous studies (Stafford et al., 2002; Neves et al., 2017; Lehmann et al., 2017; Meléndez et al., 2018b) that showed administration of lidocaine reduced pain associated with castration, especially during or shortly after the procedure.

Wound healing improved (lower scores) with time after castration, as expected, although the swelling score tended to increase from d 7 to 14, suggesting that the inflammation response to the surgical castration was still active, or that some complications (e.g., secondary infections) could have been present. There is evidence that lidocaine might have an anti-inflammatory effect by inhibiting pro-inflammatory cytokines and stimulating anti-inflammatory cytokines (Lahav et al., 2002). In this study, the administration of lidocaine did not affect healing or swelling when compared to MEX, but others (Meléndez et al., 2018b) have shown that lidocaine administered with meloxicam reduced scrotal circumference.

The neutrophil-to-lymphocyte ratio showed no treatment effect, although it decreased over time for the duration of the study. This was opposite to what was expected to occur in response to castration, where the N:L ratio has been observed to increase after surgical castration (Meléndez et al., 2017a), due to release of endogenous steroid in response to stress (Jones and Allison 2007). The increase in the lymphocyte population observed in this study is likely due to the administration of vaccines at the time of castration. Lymphocytes are mostly B and T cells, and B lymphocytes are responsible for the production of antibodies. These lymphocytes are expected to increase in population with exposure to an antigen and induction of a humoral immune response (Jones and Allison 2007). Such response to the vaccines administered in this study likely masked the effects of castration. It could be concluded, however, that vaccines might have stimulated a successful immune response in calves after surgical castration with administration of meloxicam, whether or not calves were given lidocaine.

Contrary to expected, a treatment × time interaction showed LID calves having greater hair cortisol levels than MEX calves only on d 14 (Figure 2). Hair cortisol has been used to evaluate long-term stress or chronic pain in cattle (Moya et al., 2013) on the premise that, as hair grows, it incorporates hormones and other substances from the blood stream, and hence it reveals the animal's overall state over time (Meyer and Novak, 2012). However, the relationship between chronic stress and cortisol levels is inconsistent in the literature (Otovic, 2014), with some studies reporting increased cortisol levels during chronic stress (Davenport et al., 2006), while others describing a blunted response of the hypothalamic-pituitary-adrenal axis, causing no response or reduced cortisol levels (Mormède et al., 2007). With respect to cattle castration, in a study comparing different castration methods across various age groups, hair cortisol levels did not reveal significant differences in long-term pain or stress (Marti et al., 2017). These findings suggest that the acute pain felt by calves during castration might not be intense or prolonged enough as to significantly affect hair cortisol levels. This would be in agreement with the lack of differences in the lying behavior of calves the days following castration. Further investigation is needed to determine whether surgical castration is a good model for chronic stress, and how it relates to hair cortisol concentrations.

There are some limitations to be acknowledged in this study, starting with the subjectivity of some measurements, such as the VAS, or the pen behaviors. In those cases, either the inter- or intra-observer reliability was above 80%, indicating that despite its subjective nature, measurements were consistent and reliable across observers or over time, respectively. The same could not be applied, however, to the healing and swelling scores, which could only be measured once by the same observer. In this case, those two parameters did not show any significant treatment effect that could bias our results interpretation or conclusions from the study. For all these parameters, observers were always blind to treatments to minimize potential individual biases.

The conditions of this experiment were intentionally kept as close to industry practices as possible in order to evaluate the viability of this pain control strategy in a real scenario. This meant that animal sampling was purposedly kept to a minimum, and hence the characterization of treatment effects was limited to a few time points over the course of 2 weeks after castration. This also meant that animal monitorization was limited to daily observations while cow-calf pairs were on the pasture, where adverse events such as swelling and wound infection may potentially occur associated to surgical castration (Coetzee et al., 2010).

Our results may not be necessarily applicable to all breeds, as they may have different behavioral phenotypes. The animals in this study were not used to human handling and therefore some variables could have been altered by the stress of chute restraint or pen allocation. Similarly, the diversity of outcomes in the literature (Van der Saag et al., 2019; Small et al., 2021) on the efficacy of lidocaine for pain-relief during cattle castration suggests that the breed and age of cattle, castration method, volume and route of lidocaine administration, or a combination of all these factors may lead to different outcomes.

Finally, the number of calves used in this trial only allowed for the assessment of one treatment (LID) compared to a negative control (MEX). Including a positive control, such as administering lidocaine 5 or 10 min before the procedure, would have provided a better understanding of the magnitude of the LID effects. Nevertheless, the results from this trial can be considered a starting point for reconsidering the efficacy of lidocaine as part of a practical multimodal pain mitigation strategy. A more methodological characterization of the effects of lidocaine when administered at different times before castration is warranted, as it would allow for a better understanding of the pros and cons of this pain mitigation strategy.

CONCLUSIONS

Lidocaine administered to nerve block the spermatic cord 90 seconds before surgical castration reduced the pain experienced during castration and for up to one hour after castration. This was evidenced by the reduced VAS during the procedure, and the reduced number of tail flicks, foot stamping, increased lying time, and improved mobility after the procedure, in calves treated with lidocaine in addition to meloxicam prior to castration. This study provided evidence that lidocaine can be administered closer to the time of castration, with a time of onset of 90 seconds, and still provide effective analgesia.

Acknowledgments

Financial support for this project was provided by the Western College of Veterinary Medicine and the University of Saskatchewan (Saskatoon, SK, Canada). For their assistance in the completion of this study the authors thank, Sharlene April, Michelle Jang, Yolande Seddon, Karen Gesy, and Lucy Kapronczai, and all the staff members at the Livestock and Forage Centre of Excellence (Clavet, SK, Canada). We also gratefully acknowledge the in-kind contribution of Metacam from Boehringer Ingelheim.

Author Contributions

Amanda Bernier (Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing—original draft, Writing—review & editing), Nathan Erickson (Methodology, Resources, Visualization, Writing—review & editing), John Campbell (Conceptualization, Methodology, Project administration, Resources, Supervision, Visualization, Writing—review & editing), and Diego Moya (Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing—original draft, Writing—review & editing)

Conflict of Interest Statement

The authors declare that they have no conflicts of interest related to this study.

REFERENCES

AVMA. 2014. Welfare Implications of Castration of Cattle; [accessed January 28, 2025] https://www.avma.org/sites/default/files/resources/castration-cattle-bgnd.pdf.

Clarke, K. W., and C. M. Trim. 2014. Veterinary anaesthesia. 11th Ed. Saunders, London: Elsevier Health Sciences.

Coetzee, J. F., A. L. Nutsch, L. A. Barbur, and R. M. Bradburn. 2010. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. BMC Vet. Res. 6:12. doi:10.1186/1746-6148-6-12

Currah, J. M., S. H. Hendrick, and J. M. Stookey. 2009. The behavioral assessment and alleviation of pain associated with castration in beef calves treated with flunixin meglumine and caudal lidocaine epidural anesthesia with epinephrine. Can. Vet. J. 50:375–382. PMID: 19436444; PMCID: PMC2657518.

Davenport, M. D., S. Tiefenbacher, C. K. Lutz, M. A. Novak, and J. S. Meyer. 2006. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. Gen. Comp. Endocrinol. 147:255–261. doi:10.1016/j.ygcen.2006.01.005

Egger, C. M., L. Love, and T. Doherty. 2013. Pain management in veterinary practice. Chichester, UK: John Wiley & Sons.

Fisher, A. D., M. A. Crowe, M. E. A. de la Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local Anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. J. Anim. Sci. 74:2336–2343. doi:10.2527/1996.74102336x

Fisher, A., T. Knight, G. Cosgrove, A. Death, C. Anderson, D. Duganzich, and L. Matthews. 2001. Effects of surgical or banding castration on stress responses and behavior of bulls Aust. Vet. J. 79:279–284. doi:10.1111/j.1751-0813.2001.tb11981.x

Grandin, T., and C. Shivley. 2015. How farm animals react and perceive stressful situations such as handling, restraint, and transport. Animals. 5:1233–1251. doi:10.3390/ani5040409

Hewson, C. J., I. R. Dohoo, K. A. Lemke, and H. W. Barkema. 2007. Canadian veterinarians' use of analgesics in cattle, pigs, and horses in 2004 and 2005. Can. Vet. J. 48:155–164. doi:10.4141/cjas68-021

Johnstone, E. C., J. F. Coetzee, P. J. Pinedo, and L. Edwards-Callaway. 2021. Current attitudes of veterinarians and producers regarding the use of local and systemic analgesia in beef and dairy cattle in the United States. J. Am. Vet. Med. 258:197–209. doi:10.2460/javma.258.2.197

Jones, M. L., and R. W. Allison. 2007. Evaluation of the ruminant complete blood cell count. Vet. Clin. North Am. Food Anim. Pract. 23:377–402, v. doi:10.1016/j.cvfa.2007.07.002

Lahav, M., M. Levite, L. Bassani, A. Lang, H. Fidder, R. Tal, S. Bar-Meir, L. Mayer, and Y. Chowers. 2002. Lidocaine inhibits secretion of IL-8 and IL-1β and stimulates secretion of IL-1 receptor antagonist by epithelial cells. Clin. Exp. Immunol. 127:226–233. doi:10.1046/j.1365-2249.2002.01747.x

Laurence, M., A. Barnes, T. Collins, T. Hyndman, and G. C. Musk. 2016. Assessing and mitigating post-operative castration pain in Bos indicus cattle. Anim. Prod. Sci. 58:909–919. doi:10.1071/an16210

Lehmann, H. S., G. C. Musk, M. Laurence, T. H. Hyndman, J. Tuke, T. Collins, K. B. Gleerup, and C. B. Johnson. 2017. Mitigation of electroencephalographic and cardiovascular responses to castration in Bos indicus bulls following the administration of either lidocaine or meloxicam. Vet. Anaesth. Analg. 44:1341–1352. doi:10.1016/j.vaa.2017.04.009

Marti, S., D. M. Meléndez, E. A. Pajor, D. Moya, C. E. M. Heuston, D. Gellatly, E. D. Janzen, and K. S. Schwartzkopf-Genswein. 2017. Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: II. Chronic pain. J. Anim. Sci. 95:4367–4380. doi:10.2527/jas2017.1763

Meléndez, D. M., S. Marti, E. A. Pajor, D. Moya, D. Gellatly, E. D. Janzen, and K. S. Schwartzkopf-Genswein. 2017a. Effect of timing of subcutaneous meloxicam administration on indicators of pain after knife castration of weaned calves. J. Anim. Sci. 95:5218–5229. doi:10.2527/jas2017.1978

Meléndez, D. M., S. Marti, E. A. Pajor, D. Moya, D. Gellatly, E. D. Janzen, and K. S. Schwartzkopf-Genswein. 2018a. Effect of a single dose of meloxicam prior to band or knife castration in 1-wk-old beef calves: I. Acute pain. J. Anim. Sci. 96:1268–1280. doi:10.1093/jas/sky034

Meléndez, D. M., S. Marti, E. A. Pajor, D. Moya, C. E. M. Heuston, D. Gellatly, E. D. Janzen, and K. S. Schwartzkopf-Genswein. 2017b. Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: I. Acute pain. J. Anim. Sci. 95:4352–4366. doi:10.2527/jas2017.1762

Meléndez, D. M., S. Marti, E. A. Pajor, P. K. Sidhu, D. Gellatly, D. Moya, E. D. Janzen, J. F. Coetzee, and K. S. Schwartzkopf-Genswein. 2018b. Effect of meloxicam and lidocaine administered alone or in combination on indicators of pain and distress during and after knife castration in weaned beef calves. PLoS One. 13:e0207289. doi:10.1371/journal.pone.0207289

Meléndez, D. M., Marti, S., Haley, D. B., Schwinghamer, T. D., and Schwartzkopf-Genswein, K. S. 2021. Effects of conditioning,

source, and rest on indicators of stress in beef cattle transported by road. *PLoS One*. 16:e0244854. doi: 10.1371/journal.pone.0244854

Meyer, J. S., and M. A. Novak. 2012. Minireview: hair cortisol: a novel biomarker of hypothalamic-pituitary-adrenocortical activity. Endocr. J. 153:4120–4127. doi:10.1210/en.2012-1226

Mintline, E. M., A. Varga, J. Banuelos, K. A. Walker, B. Hoar, D. Drake, D. M. Weary, J. F. Coetzee, M. L. Stock, and C. B. Tucker. 2014. Healing of surgical castration wounds: a description and an evaluation of flunixin. J. Anim. Sci. 92:5659–5665. doi:10.2527/jas.2014-7885

Moggy, M. A., E. A. Pajor, W. E. Thurston, S. Parker, A. M. Greter, K. S. Schwartzkopf-Genswein, J. R. Campbell, and M. C. Windeyer. 2017. Management practices associated with pain in cattle on western Canadian cow–calf operations: A mixed methods study. J. Anim. Sci. 95:958–969. doi:10.2527/jas.2016.0949

Molony, V., J. E. Kent, and I. S. Robertson. 1995. Assessment of acute and chronic pain after different methods of castration of calves. Appl. Anim. Behav. Sci. 46:33–48. doi:10.1016/0168-1591(95)00635-4

Mormède, P., S. Andanson, B. Aupérin, B. Beerda, D. Guémené, J. Malmkvist, X. Manteca, G. Manteuffel, P. Prunet, C. G. van Reenen, et al. 2007. Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. Physiol. Behav. 92:317–339. doi:10.1016/j.physbeh.2006.12.003

Moya, D., L. A. González, E. Janzen, N. A. Caulkett, E. Fireheller, and K. S. Schwartzkopf-Genswein. 2014. Effects of castration method and frequency of intramuscular injections of ketoprofen on behavioral and physiological indicators of pain in beef cattle. J. Anim. Sci. 92:1686–1697. doi:10.2527/jas.2013-7298

Moya, D., K. S. Schwartzkopf-Genswein, and D. M. Veira. 2013. Standardization of a non-invasive methodology to measure cortisol in hair of beef cattle. Lives. Sci. 158:138–144. doi:10.1016/j.livsci.2013.10.007

Neves, A. C., J. C. B. Santos Júnior, R. L. Marucio, M. Midon, and S. P. L. Luna. 2017. Anesthetic effects of different volumes of lidocaine for spermatic cord block in cattle. Vet. Anaesth. Analg. 44:375–378. doi:10.1016/j.vaa.2016.05.002

Otovic, P., and E. Hutchinson. 2014. Limits to using HPA axis activity as an indication of animal welfare. Altex. 32:41–50. doi:10.14573/altex.1406161

Pang, W. Y., B. Earley, T. Sweeney, and M. A. Crowe. 2006. Effect of carprofen administration during banding or burdizzo castration of bulls on plasma cortisol, in vitro interferon-γ production, acute-phase proteins, feed intake, and growth. J. Anim. Sci. 84:351–359. doi:10.2527/2006.842351x

Parham, J. T., A. E. Tanner, M. L. Wahlberg, T. Grandin, and R. M. Lewis. 2019. Subjective methods to quantify temperament in beef cattle are insensitive to the number and biases of observers. Appl. Anim. Behav. Sci. 212:30–35. doi:10.1016/j.applanim.2019.01.005

Phillips, C. J. C., J. Wojciechowska, J. Meng, and N. Cross. 2009. Perceptions of the importance of different welfare issues in livestock production. Animal. 3:1152–1166. doi:10.1017/S1751731109004479

Small, A., A. D. Fisher, C. Lee, and I. Colditz. 2021. Analgesia for sheep in commercial production: where to next? Animals (Basel). 11:1127. doi:10.3390/ani11041127

Stafford, K. J., and D. J. Mellor. 2005. The welfare significance of the castration of cattle: A review. N Z Vet. J. 53:271–278. doi:10.1080/00480169.2005.36560

Stafford, K. J., and D. J. Mellor. 2011. Addressing the pain associated with disbudding and dehorning in cattle. Appl. Anim. Behav. Sci. 135:226–231. doi:10.1016/j.applanim.2011.10.018

Stafford, K. J., D. J. Mellor, S. E. Todd, R. A. Bruce, and R. N. Ward. 2002. Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. Res. Vet. Sci. 73:61–70. doi:10.1016/s0034-5288(02)00045-0

Thüer, S., S. Mellema, M. G. Doherr, B. Wechsler, K. Nuss, and A. Steiner. 2007. Effect of local anaesthesia on short- and long-term pain induced by two bloodless castration methods in calves. Vet J. 173:333–342. doi:10.1016/j.tvjl.2005.08.031

Tranquilli, W. J., Thurmon, J. C., and Grimm, K. A. 2013. Pain and its management. Page 36 In: Tranquilli, W. J., Thurmon, J. C. and K. A. Grimm, ed. Lumb and Jones' veterinary anesthesia and analgesia. 4th ed. Iowa, USA: Blackwell Publishing.

UBC, A. W. P. 2013. UBC Anima Welfare Program: SOP - HOBO Data Loggers. Vancouver (Canada): University of British Columbia.

Van der Saag, D., S. Lomax, P. A. Windsor, E. Hall, and P. J. White. 2019. Effect of lignocaine and a topical vapocoolant spray on pain response during surgical castration of beef calves. Animals. 9:126. doi:10.3390/ani9040126

Vetters, M. D. D., T. E. Engle, J. K. Ahola, and T. Grandin. 2013. Comparison of flight speed and exit score as measurements of temperament in beef cattle1,2. J. Anim. Sci. 91:374–381. doi:10.2527/jas.2012-5122