ABSTRACT

The use of local anesthesia and a nonsteroidal anti-inflammatory drug (NSAID) can reduce indicators of pain and inflammation and encourage self-rewarding behavior in calves following disbudding. Although the use of sedation may be recommended as a best practice for disbudding, there is little research in this area. The objective of this study was to evaluate the effects of xylazine sedation in conjunction with a local anesthetic and an NSAID in calves undergoing cautery disbudding. One hundred twenty-two group-housed female and male Holstein calves fed milk with automated feeders, aged 13 to 44 d, were enrolled over 9 replicates and randomly allocated to 1 of 2 treatments: (1) sedated: lidocaine cornual nerve block, 0.5 mg/kg meloxicam (administered subcutaneously) and 0.2 mg/kg xylazine (administered intramuscularly), or (2) non-sedated: lidocaine cornual nerve block and meloxicam. Outcomes collected consisted of feeding behavior (collected using automated milk feeders), latency to drink milk following disbudding, play behavior (induced by adding bedding), lying behavior, mechanical nociceptive threshold (MNT, measured using a pressure force algometer), struggling behavior during disbudding, length of time to administer the nerve block, length of time to disbud, and serum haptoglobin concentrations. Data were analyzed using mixed models with a fixed effect for baseline values and a random effect for trial replicate. Linear regression was used to assess continuous outcomes, logistic regression for binary outcomes, and Poisson and negative binomial models for count data with negative binomial models used if the overdispersion term was significant. There were no detected differences between the treatment groups in mean daily milk consumption in the 72-h following disbudding. Sedated calves had reduced average milk drinking speed from 0 to 24 h and 24 to 48 h following disbudding compared with non-sedated calves, but no difference was detected from 48 to 72 h. Sedated calves had reduced MNT at 0, 60, and 240 min after disbudding, but no differences were detected between groups at 24 h after disbudding. Non-sedated calves had 4.5 times the odds (95% CI: 1.5–13.2) of struggling more than twice during the disbudding procedure compared with sedated calves, and it took less time to administer a nerve block to sedated calves compared with non-sedated. At +3 h, non-sedated calves were 79 times (95% CI: 22.4 to 279.2) more likely to play compared with sedated calves, and 24 h after disbudding, sedated calves were 2 times more likely to play compared with non-sedated calves (95% CI: 0.93–4.3). The results indicate that calves sedated with xylazine for cautery disbudding responded less to painful stimuli (disbudding and MNT) both during and following the procedure and had a higher rate of play behavior 24 h following sedation compared with the non-sedated calves, but xylazine may also have a prolonged carryover effect that affects suckling behavior for 48 h following sedation.

Key words: welfare, analgesia, bull, heifer

INTRODUCTION

Disbudding is the removal of the horn-forming tissue from young calves and is a common procedure performed in the dairy industry to protect the safety of humans, as well as other animals (Stock et al., 2013). Of the various methods of disbudding, cautery (hot-iron disbudding), is the most common, with 86% of Canadian producers (Winder et al., 2018a) and 70% of American producers (USDA, 2018) using this method. A survey conducted by Winder et al. (2018a) across Canada reported that of the producers performing cautery disbudding, 67% disbudded their animals between the ages of 3 and 8 wk. Of these producers, 68% reported use of local anesthesia, 34% used sedation, and 28% provided a nonsteroidal anti-inflammatory drug (NSAID). In the United States, producers using caut-
tery disbudded their animals on average at 7.1 ± 0.4 wk of age (USDA, 2018). For any method of disbudding in the United States, 28% of producers reported the use of an anesthetic or analgesic, and of the producers using hot-iron disbudding, 30% reported the use of one or more of these drugs (USDA, 2018). In Canada, proAction (a national, industry-led quality assurance program that includes an animal care component) requires the use of both a local anesthetic and an NSAID given before disbudding for all methods (DFO, 2020). However, a combination of anesthetics, analgesics, and sedation is recommended as best practice for disbudding in both Canada and the United States by other groups (NFACC, 2009; AVMA, 2014). Little research has been done to examine the effects of sedation in calves for disbudding procedures.

Although cortisol is commonly used as a biomarker of stress in calves following painful procedures such as disbudding (Petrie et al., 1996; McMeekan et al., 1998; Sutherland et al., 2002), it was not used in the current study, as previous work has shown concern for reliability in sedated calves (Stilwell et al., 2010). The use of local anesthetics and NSAID reduces cortisol concentrations following both cautery (Heinrich et al., 2009; Winder et al., 2018b) and caustic paste disbudding (Reedman et al., 2020), but higher cortisol concentrations have been reported in sedated calves compared with nonsedated, potentially due to the effects of xylazine on the calf, which include decreased arterial pressure and reduced tissue oxygenation (Campbell et al., 1979; Hodgson et al., 2002). Xylazine also does not have an anesthetic effect but provides conscious sedation by causing muscle relaxation and limits the ability of the animal to move or react to stimuli; thus, it has been reported that sedation alone does not control the pain of disbudding (Grøndahl-Nielson et al., 1999; Stilwell et al., 2010).

Therefore, the objective of this study was to evaluate the effects of xylazine sedation in conjunction with a local anesthetic (lidocaine) and an NSAID (meloxicam) following cautery disbudding in 2- to 6-wk old dairy calves. The primary outcome in this study was total daily milk consumption. We predicted that calves receiving xylazine would have increased milk consumption, a shorter latency to drink, increased play behavior, increased time spent lying, decreased struggling behavior, decreased MNT, decreased haptoglobin concentrations, and shorter time required to administer a nerve block and disbudd compared with the nonsedated calves. Latency to drink milk, as well as feeding, struggling, and lying, were examined to assess the negative affective state of the animals following the procedure, and play was examined to assess positive affective state. Haptoglobin concentrations and mechanical nociceptive threshold (MNT) measurements were collected to assess inflammation and pain, and time to disbud and administer the nerve blocks were collected to assess potential benefits and convenience to producers.

**MATERIALS AND METHODS**

This manuscript is reported according to guidelines for randomized controlled trials in livestock and food safety (O’Connor, 2010). An a priori trial protocol was published to the University of Guelph Institutional Repository on November 5, 2018, and is available at http://hdl.handle.net/10214/14385.

**Animal Use**

This trial was conducted between December 2018 and June 2019 at the Elora Research Station – Dairy Facility in Elora, Ontario, Canada. Use of animals and all methods for this study were approved by the University of Guelph Animal Care Committee in compliance with animal use guidelines (AUP#4060) of the Canadian Council on Animal Care (2009).

**Housing and Management**

Calf navels were dipped in 2.5% iodine solution immediately following calving, and once during the next day of life. All calves were administered one oral 3-mL dose of a Rota-Coronavirus vaccine (Calf-Guard, Zoetis, Kirkland, Quebec, Canada) as soon as possible after birth, before receiving colostrum and 1.5 mL of vitamin E and selenium s.c. within the first day of life (Dystosel; Zoetis; Selon-E; Vetoquinol). All calves were administered halofuginone lactate (Halocur, Merck Animal Health; 2 mL/10 kg) orally after the first colostrum feeding and once every 24 h for the first 7 d of life to prevent Cryptosporidium parvum. Calves were housed in individual pens for the first 4 to 5 d of life, then they were then moved to a group pen. For the purposes of this trial, all calves were housed in group pens (9 × 5 m) with a maximum of 15 calves/pen (1 pen/room). Both treatment groups were represented and comingling in each group pen. Bedding in these pens consisted of wood shavings, with new bedding added daily and entirely replaced every 4 d.

**Nutrition**

Calves were fed 3 L of colostrum within the first 2 h of life (and 30 min after administration of the oral vaccine) offered first in a bottle, but tube fed if necessary. Colostrum quality was measured using a refractometer and was required to be at a minimum Brix value of 22%. Calves were moved from calving pens to the calf...
room into an individual pen within 2 h of birth and another 3 L of colostrum were offered 6 to 12 h after the first feeding. For at least the first 4 d of life, calves were fed 3 times daily from a bottle. For the second feeding and the feedings for the second day of life, calves were fed transition cow milk. On d 3 and 4 of life, calves were fed milk replacer mixed at 150 g/L, and if the calf was healthy and drinking well, on d 5 they were moved to the automatic milk feeder (AMF) in the group pen and brought to the feeder 3 times daily until consuming more than 6 L/d voluntarily. Each group pen had an AMF with one feeding station (DeLaval CF1000S, DeLaval Inc.) and an automatic concentrate feeder with one feeding station (KFA3-MA3; Förster Technik GmbH) accessible to, and able to accommodate, all calves. All calves were trained on both automatic feeders before they were enrolled in the trial. The feeders read each calves’ radio-frequency identification tag when they visited the feeder to monitor milk intake and visits to the feeder. The AMF measured the number of rewarded and unrewarded visits to the feeder by the calf, daily milk consumption, and average milk drinking speed. From d 1 on the feeder to d 28, calves were fed ad libitum, with an alarm if a calf drank less than 8 L/d, in which case they were brought to the feeder to drink. Step-down weaning began on d 28 to 32; calves’ maximum milk allotment was reduced to 12 L/d and further reduced by 0.5 L/d until they reached 9 L/d. On d 33 to 42; calves were allowed a maximum of 9 L/d, from d 43 to 56 calves were further reduced by 0.5 L/d, from 9 L/d to 2 L/d, and after d 57 on the feeder (around 61 d of age) calves were completely weaned from the AMF.

**Enrollment**

All calves in a pen were health scored by researchers using the Calf Health Scorer (McGuirk, 2013; McGuirk and Peek, 2014) to determine their eligibility for the trial. They were enrolled if their health score on the day before disbudding was ≤3 (for rectal temperature, ocular discharge, nasal discharge, cough score, and feces) or ≤2 (ear position, naval, and joint) and were not appreciably polled by palpation of the horn buds. Both female and male calves were eligible for this trial, and only calves between 2 and 6 wk of age at the time of disbudding were considered for enrollment.

A replicate was completed every 3 to 4 wk, or when a pen of calves was full (15 maximum), and all calves were within the eligible age bracket (2 to 6 wk old). Calves within each pen were randomly assigned to a treatment with approximately half the calves in each pen assigned to each group.

**Treatment Groups**

The 2 treatment groups consisted of a sedated group and a nonsedated group. The sedated calves received a lidocaine cornual nerve block (6 mL per side, lidocaine hydrochloride injection 20 mg/mL, Bimeda-MTC Animal Health Inc.), an s.c. injection (in the neck) of 0.5 mg/kg meloxicam (Metacam 20 mg/mL Solution for Injection, Boehringer Ingelheim), and an i.m. injection (in the neck) of 0.2 mg/kg xylazine (Rompun 2% 20 mg/mL Injectable, Bayer Inc.), and they were disbudded using a cautery iron (Express Pistol-Grip Dehorner, The Coburn Company, Inc.). The nonsedated group received a lidocaine cornual nerve block and an s.c. injection of meloxicam and were disbudded using a cautery iron as previously described. Individual calf weights were determined using weigh tape the day before disbudding to calculate the appropriate dosages for the meloxicam and xylazine. The day before disbudding, before any MNT measurements being taken, the hair around the horn buds was shaved using electric clippers. At 30 min before disbudding, depending on the treatment, meloxicam and xylazine were administered, and at 15 min before disbudding, lidocaine nerve blocks were administered. Cornual nerve block technique was performed by insertion of an 18-gauge, 1.5-inch needle caudal to the eye, ventral to the temporal ridge, injecting 6 mL per side fanned out in 3 directions as described in Winder et al. (2017; Figure 1). Although some animals that may have some innervation coming from the cervical nerve roots are more difficult to block (Valverde and Doherty, 2008), these animals were not identified in this study and would have been randomly allocated to either treatment group. Therefore, some animals may have been exposed to some pain during disbudding. Directly before disbudding, MNT was assessed to determine whether the calf was properly desensitized from the nerve block. Disbudding was performed by the same researcher for the entire trial. The iron was preheated for at least 3 min until it reached a temperature of approximately 650°C, it was then applied to each horn bud (always beginning with the left) until a copper ring was observed and horn buds were removed by maneuvering the iron in a circular motion in on itself until each horn bud was fully removed.

**Primary Outcome and Data Collection**

Experimental days occurred on a Tuesday, Wednesday, and Thursday when a pen of calves was full (15 maximum, 11 minimum) and of the eligible age (between 2 and 6 wk), which occurred approximately every 3 to 4 wk. Calves remained in group pens for the entirety of the experiment. Calves that were polled or
were deemed too unhealthy to be enrolled were moved to an individual pen until the experiment was completed. On the Tuesday (−24 h), baseline values were collected, and researchers arrived on farm at 0900 h to begin data collection. All calves were haltered and tied around the outside of the group pen. Every calf was then health scored and palpated for horn buds and were either deemed eligible or ineligible for trial. Disbudding occurred on the Wednesday; researchers arrived on farm at 0900 h to begin data collection, and disbudding occurred at the same time of day (1100 h) for every replicate. On the Thursday, researchers arrived on farm at 1000 h to begin follow-up data collection. A timeline for when measurements and samples for all outcomes were taken is described in Figure 2.

**Milk Consumption and Feeding Behavior**

Milk consumption and feeding behavior data were collected using the AMF (DeLaval Calf Feeder CF1000+, DeLaval Canada Inc.). The AMF collected continuous data for every day including when a calf approached the feeder, whether they drank or not, how much they drank, and what their average drinking speed was for each visit, as well as the specific time of day that the calf exhibited these behaviors or consumed milk. These data were imported into Microsoft Excel (version 16.45, Microsoft Corp.) and were cleaned/organized by a blind member of the research team into the various outcomes to capture the 24 h increments beginning at 1200 h on each of the days. Calves were released from their halters at 1200 h following disbudding; therefore, by organizing the data to begin and end at 1200 h on each of the experimental days, we were able to accurately capture the time before and following the disbudding procedure. These data were categorized into 4 outcomes: total daily milk consumption (mL), average drinking speed (mL/min), number of rewarded visits to the feeder, and number of unrewarded visits to the feeder. The 24 h before disbudding served as the baseline values for these outcomes.

**Secondary Outcomes and Data Collection**

**Mechanical Nociceptive Threshold.** Mechanical nociceptive threshold was measured using a pressure force algometer (Force Ten FDX Compact Digital Force Gauge, Wagner Instruments) following the protocol described by Reedman et al. (2020). One researcher collected all MNT measurements for the entire trial. The algometer was equipped with a rubber tip (~1 cm in diameter) and measurements were taken at 4 locations around each horn bud in the same order, always beginning with the left horn bud (Figure 3). Calves were restrained using a halter; the algometer was placed lightly on the site at first before force was fully applied. Force was applied until there was a withdrawal response from the calf. The sensitivity of the area was measured in kilograms of force (kgf) applied to each location and was referred to as the MNT. The MNT values at the 4 locations on each horn bud (8 sites total) were averaged to calculate one value for each calf at each time point. Minimum values were recorded at 0.5 kgf and maximum values at 10 kgf. These responses included, but were not limited to, the calf jerking or shaking their head, pulling back sharply or jumping up or forward. Directly before disbudding (15 min following nerve block administration), MNT measurements were taken to determine whether the nerve block was successful at desensitizing the animal (the calf showed no response to the maximum value of 10 kgf at all 8 locations).

**Serum Haptoglobin.** Blood samples for haptoglobin were taken using red top Vacutainer tubes via jugular venipuncture. Blood samples were centrifuged for 15 min at 2,000 × g at 4°C. Serum from each sample was separated and stored at −20°C until time of testing, as one batch at the end of the trial. Haptoglobin samples were run at the Animal Health Laboratory at the Ontario Veterinary College (Guelph, Ontario, Canada) as a single batch on a Roche Cobas 6000 c501

---

*Figure 1.* Needle position and location used to administer lidocaine cornual nerve blocks, caudal to the eye and ventral to the temporal ridge. Illustration created by Shelby Nielson.
biochemistry analyzer (Hoffmann-La Roche Limited) using a methemoglobin stock reagent with formulas and operating conditions developed by J.G. Skinner Laboratory, Veterinary Investigation Centre (Aberdeen, Scotland; Makimura and Suzuki, 1982; Skinner et al., 1991). Methemoglobin combines with haptoglobin to form a stable methemoglobin-haptoglobin complex, measurement of haptoglobin is based on the peroxidase activity of this complex in acidic conditions, a hydrogen peroxide/guaiacol solution served as the substrate for the reaction and color development was read at 480 nm wavelength. Standards and controls were used to calibrate the instrument before running the samples. The inter assay coefficient of variation was 5.4%.

Play Behavior. Play behavior was induced by adding fresh bedding to the group pen and video recording the calves for 15 min immediately following addition of the bedding. Wood shavings were brought into the center of the pen in a large bin and were distributed among the entire pen, rousing any calves that were sleeping or lying down. On experiment days, the bedding that was added to induce play replaced the usual daily provision of bedding (i.e., the pens were bedded only once a day) and the pens were not cleaned out on experiment days. Videos were recorded using an iPad mini (Apple Inc.) that was mounted on a 1.5-m stand with a view of the entire group pen the calves were housed in. While the video recording was taking place there were no people in the room. Behavior that was recorded included running, bucking, and head-to-head contact (Table 1) as described by Mintline et al. (2013). The videos that were recorded for the −24 h and the +24 h time points were recorded at the same time of day on each of the days (1100 h), but on the day of disbudding, the +3 h video was recorded at 1400 h because it had to be taken long enough after sedation that the calves were mobile again. Once the trial was complete, a third-party observer, blind to treatment groups and study design (i.e., were unaware of which videos represented −24 h, +3 h, and +24 h, and unaware of the research question), was trained to score the behavior using training videos created by a member of the research team. Calves were easily identifiable in the videos by their radio-frequency identification tags and unique markings. Each calf’s scoring sheet for recording behavioral observations included a photo of the calf to help the observer identify the specific animal in each video. For scoring, we used

![Figure 3](image_url) Locations around horn buds measured by algometry numbered in the order in which they were measured. Algometry measurements were always taken beginning with the left side of every calf. Illustration created by Shelby Nielson.
continuous all-occurrences observation to record the events of each behavior (Martin and Bateson, 1993) for all calves over the 15-min time period. An interobserver reliability score was calculated for each specific behavior based on behavioral scoring of 10 calves. A cut-off of 0.70 was used for assessing the kappa statistic, because this represents moderate agreement and there was room for variation in the scoring of behavioral events based on the ethogram. Once the third-party observer was trained, they watched and scored all of the videos and recorded the data into Microsoft Excel (version 16.45, Microsoft Corp.).

**Standing and Lying Behavior.** The HOBO Pendant G Data Loggers (Onset Computer Corp.) were used to measure standing and lying bouts. Loggers were set to record every 60 s and were attached horizontally to the rear right leg of each calf as described and validated by Bonk et al. (2013), using a cohesive bandage. Data were downloaded using HOBOware Pro Software (Onset Computer Corp.), imported into Microsoft Excel and categorized into 3 outcomes; average time spent lying, average lying bout length, and number of lying bouts. These data were then further sectioned into 6-h increments, with each segment from the day before disbudding used as the baseline value for the day following disbudding in analysis to account for differences in time of day (daylight vs. night time; e.g., 0–6 h; 6–12 h: 1600–2200 h, 12–18 h: 2200–0400 h, 18–24 h: 0400–1000 h).

**Struggle Behavior.** Struggling, or pain- or escape-related behavior patterns, were recorded during the disbudding procedure. While the calves were being disbudded, a researcher video recorded the procedure using an iPad mini (Apple Inc.), so the entire body of the calf was in full view. The video recording began immediately before the application of the cautery iron and stopped once both horn buds had been removed. Once the trial was complete, a third-party observer was trained to score the behavior using training videos created by a member of the research team. For scoring this behavior, we used continuous all-occurrences observation to record the events of each behavior (Martin and Bateson, 1993) for all calves over the period of time it took to disbudd them. An interobserver reliability score was calculated for each behavior based on behavioral scoring of 10 calves. A cut-off of 0.70 was used for assessing the kappa statistic (as described above), and once the third-party observer was trained, they watched the videos and scored them based on whether the calf was lying down or standing up at the beginning of the video (Table 2) and recorded the data into Microsoft Excel.

**Time to Administer Nerve Block.** Directly before the administration of the cornual nerve block, a

---

**Table 1. Definitions of play behavior used to evaluate the effects of sedation after disbudding in dairy calves (Mintline et al., 2013)**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run (duration and count)</td>
<td>Any gait faster than a walk, including trot and gallop, that lasts longer than 1 s, stopped when calf resumes walking or halts</td>
</tr>
<tr>
<td>Head-to-head (duration and count)</td>
<td>Heads or necks of 2 calves touch for 1 s or longer, stopped when calves break away from contact</td>
</tr>
<tr>
<td>Buck (count)</td>
<td>The body ascends from front to back, top of the head is level with or lower than the shoulders, and both hind hooves are lifted off the ground; legs may be kicked outward</td>
</tr>
</tbody>
</table>

**Table 2. Definitions of struggling or pain- or escape-related behavior used to evaluate the effects of sedation during disbudding in dairy calves**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves that began the procedure standing</td>
<td>Sharp backward or sideways extension of the hind right or left leg</td>
</tr>
<tr>
<td>Kicking</td>
<td>Any sharp or abrupt movements or behavior, including lunging forward, backward, or sideways while standing, or rapid head movements in any direction</td>
</tr>
<tr>
<td>Escape attempts</td>
<td>A controlled downward movement involving the animal dropping onto the front knees first and hips second</td>
</tr>
<tr>
<td>Lying down</td>
<td>A rapid or abrupt downward movement of the body involving the animal collapsing where one or both of its hips meet the ground first</td>
</tr>
<tr>
<td>Calves that began the procedure lying down</td>
<td>Rapid outward extensions of one or more of the right or left hind or front legs while the animal is lying down</td>
</tr>
<tr>
<td>Falling</td>
<td>Movement of the calf from laying down to standing, abrupt or at a normal speed, once disbudding has begun</td>
</tr>
<tr>
<td>Kicking while lying down</td>
<td>Any sharp or abrupt movements or behavior, including lunging forward, backward, or sideways while standing, or rapid head movements in any direction</td>
</tr>
<tr>
<td>Standing up</td>
<td>A rapid or abrupt downward movement of the body involving the animal collapsing where one or both of its hips meet the ground first</td>
</tr>
</tbody>
</table>

---
member of the research team began video recording the blocking procedure using an iPad mini and continually recorded the procedure until the lidocaine had been fully administered. Once the trial was complete, a third-party observer watched the videos and recorded the length of time it took to fully administer the nerve block beginning from the insertion of the needle to the first side (the left) until the needle had been removed from the second side (the right). This length of time was recorded in seconds.

**Time to Disbud.** Directly before the application of the cautery iron, a member of the research team began video recording the disbudding procedure using an iPad mini, and continually recorded the procedure until both horn buds had been completely removed. Once the trial was complete, a third-party observer watched the videos and recorded the length of time it took to fully remove the horn buds from the calf beginning from the application of the iron to the left side (disbudding always began on the left) and continually recording until the horn bud had been completely removed from the right side. This length of time was recorded in seconds.

**Latency to Drink.** Before the disbudding procedure, all calves were haltered and tied around the group pen with enough room to lay down if necessary. All calves in one pen were disbudded on the same day in the order they were tied up (starting from one side of the pen and working around to the other side) and the exact time that disbudding occurred for each individual calf, as well as the exact time when calves were released from their halters, were recorded on a calf specific worksheet. Once all disbudding had been completed for a replicate, all calves were released from their halters at the same time. Latency to drink was measured using data from the AMF to assess the length of time in minutes from being released from the halters to their first drink of milk at the AMF after disbudding.

**Sample Size.** The sample size was calculated a priori considering mean daily milk consumption as the primary outcome based on an expected difference of 2 L between treatment groups (8 vs. 6 L) with a standard deviation of 3 L, with 95% confidence and 80% power and was adjusted for clustering by day (average n sampled per cluster = 15, ICC = 0.045). The calculated sample size was 60 calves/treatment group for a total of 120 calves.

**Treatment Allocation and Blinding.** Treatments in this study were blocked by group; each day that trial was conducted, a full pen of calves (maximum 15) were enrolled with treatments randomly assigned among all calves; half of the pen assigned to one group and half assigned to the other. Treatments were assigned by use of a premade list numbered 1 to 15 with the letters A and B in a different randomized order every day by use of a random number generator. Calf numbers were written numerically from the youngest calf to the oldest on each list every trial day with calves assigned to the treatment (A or B) corresponding with their number on the list. The research team was blinded to the xylazine treatment with the exception of one member who knew which treatment group received xylazine and administered the sedative while the other members were not present in the pen; this person was not involved in any further treatment administration, evaluation of outcomes, or analysis. However, based on the clinical effects of the xylazine, it was apparent to the researcher performing the disbudding which calves were sedated. Caretakers on the farm were not present while calves were clinically sedated and remained blind to treatment groups throughout the trial, as well as outcome assessors measuring play behavior as by this time following disbudding, calves did not show any clinical signs of sedation. Once statistical analysis was complete, treatment allocation was revealed to the blinded researchers for interpretation of results.

**Statistical Analysis**

All recorded data were entered into Microsoft Excel and imported into STATA15 (Stata/IC Version 15.1 for Mac, StataCorp). Descriptive statistics were reviewed for all variables. The experimental unit for analysis in this study was the calf. Continuous variables (MNT, serum haptoglobin, average drinking speed, total daily milk consumption, time to block, time to disbud, latency to drink, time spent lying, and average lying bout length) were also assessed for normality, linearity, and variation through assessment of outliers and residuals. Model fit was assessed for Poisson and negative binomial models using normality of Anscombe residuals. Results were considered statistically significant if the P-value was <0.05 and were considered to have a tendency to be statistically significant if the P-value was >0.05 but <0.1.

Struggling behavior patterns (kicking, escape attempts, falling, lying down, kicking while lying down, and standing up) were examined individually for the most common behavior patterns observed (escape attempts, falling, and standing up), and the behavior patterns with few (<5%) or no observed counts were discarded from further analysis (kicking, lying down, kicking while lying down). Falling behavior ranged between 0 and 1 counts during the disbudding procedure, standing ranged between 0 and 2, and escape attempts ranged most commonly from 0 to 3 counts. A similar pattern of treatment effect on behavior was observed between all 3 behaviors and, therefore, they were
summed to generate a total of struggling behaviors. Zero inflated negative binomial models either did not converge or were unstable so the outcome was analyzed as binary data in a mixed logistic regression model. Because the duration of disbudding was variable for the individual calf and a longer disbudding time could allow for more instances of struggling behaviors from the calf, disbudding time was offered to the model to control for this. Disbudding time was assessed for collinearity and confounding in this model. The variance inflation factor was less than 5 for this variable, and it was not a statistical confounder; therefore, disbudding time was kept in the model to control for the individual calf variation. Data were analyzed first as 1 = calves showed any struggling behavior, 0 = no struggling behavior displayed, then as 1 = calves struggled more than once, 0 = calves struggled once or not at all, and finally as 1 = calves struggled more than twice, 0 = calves struggled twice or less. The majority of calves (82%) enrolled on the trial displayed 2 or fewer incidences of struggling during the disbudding procedure. Because there were no baseline data collected on calves’ struggling behavior due to handling, this outcome was analyzed as binary data where 1 = calves struggled more than twice and 0 = calves struggled twice or less.

For outcomes with repeated measures (MNT, serum haptoglobin, all play behavior, bucks, running, head-to-head contact, average drinking speed, total daily milk consumption, rewarded visits, unrewarded visits, number of lying bouts, total time spent lying, and average lying bout length), a mixed-effect model was built for each outcome with baseline measures as a covariate and calf nested within day and group as random effects. For continuous, normally distributed data (MNT, serum haptoglobin, average drinking speed, total daily milk consumption, time to block, time to disbud, latency to drink, time spent lying, and average lying bout length), linear models were used; for binary data (struggling behavior and maximum block score at disbudding), logistic models were used; and for count data (all play behavior, struggling behavior, and maximum block score at disbudding), Poisson and negative binomial models were used with the negative binomial model chosen if the overdispersion term was significant (P < 0.05). The effect of treatment on outcome was stratified by time, with single-level models built for each time point to assess the effect of the treatment at those specific times points.

**RESULTS**

In total, 122 calves were enrolled in this study with 62 calves in the sedated group and 60 calves in the non-sedated group. All calves enrolled in the study received the intended treatment depending on their group and were followed from 24 h before, to 24 h after disbudding (72 h after disbudding for feeder data). All samples and measurements collected were used in the analysis, all samples that were collected or were missing from analysis are reported in Table 3. All data collected and analyzed from this trial that are not reported in this manuscript, are reported in the Supplemental File S1 (https://doi.org/10.5683/SP2/AY0LRV, Reedman et al., 2021). There were no deviations from the trial protocol for treatment allocation, randomization, blinding, data collection, or analysis.

**Baseline Characteristics**

Calf age ranged from 13 to 44 d on the day of disbudding, the mean age did not differ between the treatment groups (sedated: 25.6 ± 0.9 d, nonsedated: 26.4 ± 0.9 d). Both males and females were enrolled into this study; 71 females and 51 males were randomly distributed among the 2 treatment groups with 37 females and 25 males in the sedated group, and 34 females and 26 males in the non-sedated group. Baseline values for each outcome are described in Tables 4, 5, and 6 by treatment group. No statistical difference was detected in baseline values between the treatment groups (P > 0.05).

**Primary Outcome**

**Milk Consumption and Feeding Behavior.** No differences were detected between the treatment groups in total amount of daily milk consumed on any of the days after disbudding (P > 0.5). No differences were detected between treatment groups in rewarded visits to the feeder on any days after disbudding (P > 0.2), and no differences were detected between groups in unrewarded visits to the feeder on any days after disbudding (P > 0.4). Data for milk consumption, rewarded and unrewarded visits to the AMF are reported in Supplemental File S1 (https://doi.org/10.5683/SP2/AY0LRV, Reedman et al., 2021). From 0 to 24 h after disbudding, nonsedated calves had faster average milk drinking speeds (mL/min) compared with sedated calves (43.7 mL/min; 95% CI: 5.7–81.7, P = 0.02).
Table 3. Number of samples included in analyses and missing data for each outcome by time point and treatment group

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Time relative to disbudding</th>
<th>No. analyzed (no. missing)</th>
<th>Sedated</th>
<th>Nonsedated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding data(^1)</td>
<td>−24 h</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+24 h</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+48 h</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+72 h</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td>Algometry(^2)</td>
<td>−24 h</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 min</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+60 min</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+120 min</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+24 h</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td>Haptoglobin(^3)</td>
<td>−24 h</td>
<td>62 (0)</td>
<td>0 (2)</td>
<td>60 (0)</td>
</tr>
<tr>
<td></td>
<td>−90 min</td>
<td>60 (2)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+60 min</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+240 min</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+24 h</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td>59 (1)</td>
</tr>
<tr>
<td>Standing or lying(^4)</td>
<td>−24 h</td>
<td>41 (21)</td>
<td>35 (25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−18 h</td>
<td>41 (21)</td>
<td>35 (25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−12 h</td>
<td>41 (21)</td>
<td>35 (25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−6 h</td>
<td>41 (21)</td>
<td>35 (25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+6 h</td>
<td>41 (21)</td>
<td>35 (25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+12 h</td>
<td>41 (21)</td>
<td>35 (25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+18 h</td>
<td>41 (21)</td>
<td>35 (25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+24 h</td>
<td>41 (21)</td>
<td>35 (25)</td>
<td></td>
</tr>
<tr>
<td>Play behavior(^5)</td>
<td>−24 h</td>
<td>61 (1)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+3 h</td>
<td>61 (1)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td>Struggle behavior(^6)</td>
<td>−24 h</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td>59 (1)</td>
</tr>
<tr>
<td>Blocking time(^7)</td>
<td>0 min</td>
<td>62 (0)</td>
<td>60 (0)</td>
<td></td>
</tr>
<tr>
<td>Disbudding time(^8)</td>
<td>−15 min</td>
<td>62 (0)</td>
<td>59 (1)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Data were collected using the automated milk feeders.
\(^2\)Test performed to determine the mechanical nociceptive threshold.
\(^3\)Blood sampling to determine haptoglobin concentrations.
\(^4\)Data collected using HOBO Pendant G Data Loggers (Onset Computer Corp.). Missing data due to technical failures of the loggers and improper attachment of some the loggers.
\(^5\)Fresh bedding added to the group pens to induce play behavior.
\(^6\)Behaviors observed during the disbudding procedure.
\(^7\)Length of time to administer the cornual nerve block to a calf.
\(^8\)Length of time to perform the disbudding procedure.

Table 4. Baseline characteristics (obtained 24 h before disbudding) for age, algometry, drinking speed, total daily milk consumption, rewarded visits, unrewarded visits, bucks, running counts, head-to-head contact counts, and haptoglobin stratified by treatment group (mean ± SE)

<table>
<thead>
<tr>
<th>Item</th>
<th>Sedated (n = 62)</th>
<th>Nonsedated (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (d)</td>
<td>25.6 (±1)</td>
<td>26.4 (±1)</td>
</tr>
<tr>
<td>Algometry (kgf(^1))</td>
<td>6.9 (±0.2)</td>
<td>7.0 (±0.3)</td>
</tr>
<tr>
<td>Drinking speed (mL/min)</td>
<td>587.6 (±22)</td>
<td>569.2 (±25)</td>
</tr>
<tr>
<td>Total daily milk consumption (mL)</td>
<td>9,070.8 (±306)</td>
<td>9,151.6 (±319)</td>
</tr>
<tr>
<td>Rewarded visits (count/d)</td>
<td>6.4 (±0.3)(^2)</td>
<td>7.0 (±0.3)(^2)</td>
</tr>
<tr>
<td>Unrewarded visits (count/d)</td>
<td>3.4 (±0.9)(^2)</td>
<td>2.1 (±0.5)(^2)</td>
</tr>
<tr>
<td>Haptoglobin (mg/mL)</td>
<td>0.14 (±0.005)</td>
<td>0.13 (±0.003)</td>
</tr>
</tbody>
</table>

\(^1\)Kilograms of force.
\(^2\)IRR = incidence rate ratio.
relationship was also observed from 24 to 48 h after disbudding (40.9 mL/min; 95% CI: 4.9–76.8,  \( P = 0.03 \)) but was no longer detected from 48 to 72 h (15.0 mL/min; 95% CI: −22.6 to 52.7,  \( P = 0.4 \)).

**Latency to Drink.** The average latency to drink for a calf in the sedated group was 133.2 ± 12.5 min and in the nonsedated group was 142.7 ± 12.7 min. There were no detected differences between treatment groups in latency to drink at the AMF following release from the halters after disbudding (\( P = 0.6 \), full data available in Supplemental File S1, [https://doi.org/10.5683/SP2/AY0LRV, Reedman et al., 2021]).

**Secondary Outcomes**

**Time to Administer Nerve Block.** The average time it took to administer a nerve block to a calf in the sedated group was 24.1 ± 0.9 s, and the average time in the nonsedated group was 29.3 ± 0.9 s. It took 5.2 s (95% CI: 3.1–7.3,  \( P < 0.01 \)) longer to administer cor-...
disbudding there were no differences detected between the 2 treatment groups (0.14 kgf; 95% CI: −0.20 to 0.47, \(P = 0.4\); Figure 4).

**Time to Disbud**

The average time it took to disbud a calf in the sedated group was 82.1 ± 13 s, and the average time for a nonsedated calf was 88.2 ± 13.2 s. No differences were detected between sedated and nonsedated calves in the length of time required to disbud them (\(P = 0.14\), full data available in Supplemental File S1, https://doi.org/10.5683/SP2/AY0LRV, Reedman et al., 2021). On average, it took 64.9 ± 12.8 s to disbud female calves and 85.3 ± 12.3 s to disbud male calves. Therefore, it took +20.3 s (95% CI: 12.4–28.3, \(P < 0.01\)) longer to disbud males compared with females and for every 1 d increase in age of the calf, it took +1.0 s longer to perform the disbudding procedure (95% CI: 0.46–1.6, \(P < 0.01\)).

**Struggle Behavior.** Nonsedated calves had 4.8 times the odds of struggling more than twice during the disbudding procedure compared with sedated calves [odds ratio (OR) = 4.8; OR\(_{\text{sedated}} = 0.01 ± 0.01, OR_{\text{nonsedated}} = 0.05 ± 0.06, 95\%\ CI: 1.6–14.9, P = 0.006]}

**Standing and Lying Behavior.** Baseline characteristics for total lying time, number of lying bouts and average bout length are described in Table 5. Missing data from the data loggers are reported in Table 3, data on lying behavior were missing from 46 calves due to technical failure of the loggers and improper attachment of some of the loggers. From 0 to 6 and 6 to 12 h following disbudding, sedated calves had longer lying bout lengths compared with nonsedated calves (0–6 h, 40.3 min; sedated: 73.1 ± 11.4 min, nonsedated: 32.8 ± 11.9 min; 95% CI: 24.7–55.9, \(P < 0.01\); 6–12 h, 20.3 min, sedated: 72.2 ± 7.0 min, nonsedated: 51.9 ± 16.8 min; 95% CI: 1.4–39.2, \(P = 0.04\)), but from 12 to 18 and 18 to 24 h after disbudding, there were no detected differences between the 2 treatment groups (\(P > 0.1\), full data reported in Supplemental File S1). From 0 to 6 and 18 to 24 h after disbudding, nonsedated calves had a greater rate of lying bouts compared with sedated calves [0–6 h: incidence rate ratio (IRR) = 1.5; IRR\(_{\text{sedated}} = 3.4 ± 0.7, IRR_{\text{nonsedated}} = 4.9 ± 0.9, 95\%\ CI: 1.2–1.8, P < 0.01; 18–24 h: IRR = 1.3; IRR\(_{\text{sedated}} = 3.1 ± 0.5, IRR_{\text{nonsedated}} = 3.9 ± 0.6, 95\%\ CI: 1.0–1.6, P = 0.02\], but from 6 to 12 and 12 to 18 h after disbudding, there were no detected differences between the treatment groups (\(P > 0.2\), full data reported in Supplemental File S1). Figure 5 describes the differences between the treatment groups for total lying time.

**Play Behavior.** Mean number of the different play behavior patterns (bucking, running, and head-to-head contact) and summed behavior patterns are described in Table 6. Figure 6 is an interaction plot illustrating
the effect of treatment over time of all play behavior patterns recorded. There were no differences detected in the number of play behavior patterns exhibited between sedated and nonsedated calves 24 h before disbudding ($P = 0.3$) (Table 6). Three hours after disbudding, sedated calves did not display any bucking or running behavior, and nonsedated calves had a rate of playing (all play behavior) compared with nonsedated calves ($\text{IRR} = 2.0$; $\text{IRR}_{\text{sedated}} = 3.1 \pm 0.9$, $\text{IRR}_{\text{non-sedated}} = 1.5 \pm 0.4$, $P = 0.07$, 95% CI: 0.93–4.3).

**Serum Haptoglobin.** No differences were detected in haptoglobin concentrations between treatment groups at any of the time points relative to disbudding. Full data for haptoglobin concentrations is reported in Supplemental File S1 (https://doi.org/10.5683/SP2/AY0LRV, Reedman et al., 2021).

**DISCUSSION**

In this study we investigated the effects of xylazine sedation as a method of pain control for cautery disbudding when used in combination with local anesthesia and an NSAID. Similar to past research, our results demonstrate that the use of sedation for disbudding reduces struggling during the procedure (Grondahl-Nielson et al., 1999; Stilwell et al., 2010) and resulted in the calves being less sensitive to the MNT test following the procedure (Cuttance et al., 2019). We also noted that 3 h after disbudding, nonsedated calves played much more compared with sedated calves and compared with their own baseline play behavior. However, compared with nonsedated calves, sedated calves played more 24 h following the disbudding procedure, suggesting that sedation potentially has an effect on the play behavior of the animals at this time point. Sedated calves also had reduced drinking speeds for 48 h after disbudding. The implications of reduced drinking speeds, however, are not clear as this metric has
been used as a reliable indicator to predict sickness in calves (Knauer et al., 2017), and may reflect a negative affective state at this time point.

**Feeding Behavior**

Milk consumption has been used in the past to assess performance of calves following disbudding procedures (Bates et al., 2015) as well as for identifying or predicting sickness in calves (Knauer et al., 2017; Cramer et al., 2020). Xylazine sedation has been reported to cause a decrease in core temperature in calves, particularly young calves, who do not thermoregulate as efficiently as older animals (Vasseur et al., 2014). Vasseur et al. (2014) reported that disbudding itself did not affect milk intake, but calves whose body temperature decreased less, or were in a warmer environment, drank more milk. We predicted in this study that xylazine-treated calves might have a greater milk consumption volume on the day of disbudding compared with nonsedated calves, due to a reduction in stress or pain, but we detected no differences between the treatment groups in the 72 h following disbudding.

Pain is a subjective state that results in suffering for the animal, similar to the effects of illness, both of which result in a negative affective state (Fraser et al., 1997). Illness in calves, whether it be from gastrointestinal or respiratory diseases, has been linked with reduced drinking speeds on AMF (Johnston et al., 2016; Knauer et al., 2018; Cramer et al., 2020). The connection between drinking speed and illness in calves has been used as a way to predict illness up to 4 d before farm personnel are able to identify a calf as sick and continues for 7 to 10 d after (Knauer et al., 2017). There is no previous research to our knowledge examining associations between reduced drinking speed with pain in calves. We were, however, able to detect differences between the treatment groups in average drinking speed for up to 48 h following the disbudding procedure. The majority of our results indicate that sedated calves responded less to painful stimuli and had reduced behavioral indicators of pain both during and following the disbudding procedure compared with nonsedated calves, but we did detect that sedated calves drank slower than nonsedated calves for 48 h after disbudding. Although these results might indicate that the sedated calves were potentially experiencing a more negative affective state compared with the nonsedated calves, this is in contrast with the majority of our results. However, it is important to note that a lack of responsiveness to stimuli could be due to a prolonged carryover effect of the xylazine as opposed to an analgesic effect. It is also possible that the xylazine may have caused a decrease in body temperature in the sedated calves, similar to what Vasseur et al. (2014) reported. Thus, rather than a decrease in milk intake by these calves, they may have reduced their drinking speeds. Vasseur et al. (2014) used a base value of 0.25 mL of xylazine for every calf rather than adjusting the dosage based on body weight of the calf, they did not assess drinking speed, and the calves were only fed milk in 2 meals/d. Those researchers reported that following sedation a decrease in body temperature was negatively associated with milk intake, but it is possible the reason for the reduced milk consumption stemmed from a reduction in drinking speed, although calves in this study were limited to a maximum of 4.5 or 9 L/d depending on treatment. In our study, calves were provided ad libitum access to the milk and were allowed to visit the feeder as often as they wanted, and even during the beginning of their step-down weaning were able to drink a maximum of 9 to 12 L/d. Additionally, the stocking density of the pen was amenable to all calves having adequate access to the AMF. Therefore, potentially due to the increased access to the milk in our study, or the differences in xylazine dosage, we were able to identify the reduced drinking speed in the sedated calves, but overall, those calves were able to drink as much milk as the nonsedated calves, although it may have taken them longer at each visit to the feeder.

We also predicted that sedated calves would have a shorter latency to drink compared with nonsedated calves, but we detected no differences between the treatment groups. As this was measured from the time that calves were released from the halters, and all calves were released at the same time (approximately 1 h after disbudding), at this point the sedation was not producing any clinical signs and sedated calves were mobile again. In a study by Miller-Cushon and DeVries (2016), the researchers reported that pair-housed calves consumed milk in smaller more frequent meals compared with individually housed calves. Perhaps a difference might have been detected in our study if the calves were individually housed and there was no social effect on milk consumption due to the calves being housed in a group pen.

**Play and Lying Behavior**

When the basic needs of animals are met, they play more, and during times of stress (such as weaning in calves), play behavior is reported to decrease (Krachun et al., 2010). Disbudding is a painful procedure and results in calves exhibiting negative judgment bias even with the provision of a local anesthetic and a sedative (Neave et al., 2013). Following disbudding procedures, calves play less (Rushen and de Passille, 2012; Winder et al., 2017), but the provision of pain control, namely
local anesthetics and NSAID, helps to encourage play after disbudding compared with providing calves with less or no pain control (Mintline et al., 2013). Until now, research has not specifically examined the effect of sedatives on the expression of play behavior after disbudding. In our study, on the day of disbudding (3 h after the procedure) little to no play behavior was exhibited by the sedated calves, even though at this point the calves had been mobile for at least 2 h, although this does not necessarily mean the xylazine did not continue to produce effects. Therefore, even though all sedated calves were mobile at this time, the lack of play seen on the day of disbudding could be due to an extended carryover effect from the sedative, especially because at this time, nonsedated calves played much more than the day before or the day after disbudding. On the day of disbudding, all calves were haltered and tied around the group pen for the majority of the morning while every calf was given pain control and disbudded. This lack of movement for a large part of the day could be a reason why the nonsedated calves played more later in the day once they were free to do so and were stimulated by the new bedding provided. It is also important to note that the play behavior observed on the day of disbudding (+3 h) was recorded at a different time of day compared with the −24 and +24 h videos. This could also be a confounding factor in the differences in play behavior recorded at this time point. However, on the day after disbudding, the sedated calves tended to play more than the nonsedated calves, suggesting that the similar increase in play that was observed in the nonsedated calves on the day of disbudding also occurred in the sedated calves 24 h later, potentially due to an extemated carryover effect. As well, housing the calves in group pens with both treatments represented in each pen could have biased our result toward the null. Assuming that one treatment group (nonsedated) is more active than the other (sedated), the more active group would most likely stimulate the other group to play more than they normally would, reducing the detectable differences between the treatment groups. Therefore, there may have been a greater difference in play behavior between the treatment groups than we were able to detect, due to the design of this study.

Lying behavior and overall activity of calves have been reported to change in response to pain (Heinrich et al., 2010; Sutherland et al., 2018). As expected, the sedated calves in the current study had fewer lying bouts, a longer average length of lying bout, and longer total lying time in the first 6 h after disbudding compared with nonsedated calves because for the first h or so of this time, the calves were sedated and lying down. Little research has been conducted to examine the effect of disbudding on lying behavior, but of the research available, few have reported differences between treatment groups (Winder et al., 2017; Sutherland et al., 2018; Reedman et al., 2020). The time period in the current study from 12 to 24 h after disbudding is from 2200 to 1000 h, which is essentially overnight for the animals, and may make it less likely to observe differences between groups. Therefore, the lack of differences between the 2 groups in the length of lying bouts is reasonable. However, from 18 to 24 h after disbudding (0400 to 1000 h), the nonsedated calves had a greater rate of lying bouts compared with the sedated calves, indicating that the nonsedated calves were potentially more restless, needing to move more frequently. As well, from 12 to 18 and 18 to 24 h after disbudding (2200 to 1000 h), the nonsedated calves spent less time lying compared with the sedated calves. These results could indicate that the nonsedated calves were potentially more uncomfortable 12 to 24 h following the disbudding procedure compared with the sedated calves requiring them to move from standing to lying more frequently. Conversely, it could also indicate that the sedated calves were simply less responsive to stimuli up to 24 h due to a carryover effect, as opposed to being less painful.

**During the Procedure**

The amount of time it took to disbud and administer nerve blocks to the calves was evaluated to determine whether providing a sedative would reduce the length of time it took to perform either of these tasks, and potentially encourage producers to adopt these methods. We noted nonsedated calves reacted to handling during nerve block administration, as well as from the insertion of the needle. In a study by Jimenez et al. (2019), the researchers reported that cornual nerve blocks with lidocaine are painful and elicit escape behavior as well as increased heart rates in calves following insertion of the needle. The sedated calves did not have the same ability to react to these stimuli as the nonsedated calves and therefore it took less time to administer the nerve blocks to sedated calves compared with the nonsedated. Sedated calves were also less responsive to the MNT test performed immediately before disbudding and were more likely to achieve a maximum MNT score at this time. This may be due to their inability to respond to the test the same way the nonsedated calves were able to, or potentially because the sedated calves were more likely to have an effective nerve block due to their reduced ability to respond or try to escape during the administration of the nerve block. Regardless, there were no differences detected between the treatment groups in the length of time it took to perform the disbudding procedure. This could potentially be because...
the sedated calves were often in positions lying down that made it difficult to manipulate their heads into a position ideal for disbudding, whereas the nonsedated calves were standing and easily accessible.

**Pain and Inflammation**

Haptoglobin is an acute phase protein and can be used as an indicator of inflammation in cattle (Makimura and Suzuki, 1982). The use of haptoglobin for measuring pain and inflammation in calves following painful procedures has been increasing in the literature (Earley and Crowe, 2002; Erdogan et al., 2019; Reedman et al., 2020). Haptoglobin concentrations have been reported to increase from basal values at around 12 to 72 h following disbudding procedures in calves (Allen et al., 2013). In contrast to this, researchers assessing disbudding in goat kids have reported no changes in haptoglobin concentrations over time from −1 min to +24 h relative to disbudding when kids were not provided with any pain control (Hempstead et al., 2018).

The provision of a local anesthetic and an NSAID has been reported to prevent increased haptoglobin concentrations in calves 24 h following castration (Earley and Crowe, 2002), as well as dehorning (Ballou et al., 2013), compared with groups provided with no pain control. In the present study, we detected no differences between the treatment groups in haptoglobin concentrations at 1, 4, or 24 h after disbudding. To our knowledge, no research has examined haptoglobin concentrations in the context of sedation for disbudding, but the provision of a sedative does not appear to affect concentrations in the 24 h following cautery disbudding. Other researchers have also reported higher basal haptoglobin concentrations in young calves (3 to 9 d of age) (Mirra et al., 2018), but we detected no differences based on age, although all of our calves were at least 13 d of age, so this was not something that we anticipated detecting a difference in.

Mechanical nociceptive threshold is commonly used to assess pain and inflammation in relation to disbudding, particularly cautery disbudding (Heinrich et al., 2010; Stock et al., 2015, 2016) but also with caustic paste disbudding (Winder et al., 2017; Reedman et al., 2020). Following disbudding, calves are more sensitive when tested with a pressure force algometer than before the procedure (Heinrich et al., 2010), but with the provision of a local anesthetic and an NSAID this sensitivity can be greatly reduced (Heinrich et al., 2010; Stock et al., 2016; Reedman et al., 2020). There is little research on the effects of xylazine sedation on pain sensitivity and MNT in calves following cautery disbudding. In a recent study by Cuttance et al. (2019) the researchers reported higher MNT values in sedated calves compared with nonsedated calves for 24 h following the disbudding procedure, indicating that the sedated calves were less sensitive for this period of time. In our study, we did detect greater MNT values in sedated calves compared with nonsedated in the first 4 h after disbudding, but by the next day (24 h later) we no longer detected differences between the treatment groups. The contrast in results between our study and Cuttance et al. (2019) may be due to differences in methods, such as a smaller sample size for MNT measurements (total of 124 calves split into 6 treatment groups), only measuring MNT at locations 1 and 2 (Figure 3), and restraining calves using 2 people to restrain the animal while another took the measurements. Regardless of these differences, in both studies sedated calves were detected to be less sensitive to the MNT test in the first 4 h after disbudding. This indicates that for at least a short period of time following the procedure, the animals were either less sensitive and in less pain, or were unable to respond properly to the test due to the sedative or the muscle relaxation effects of the xylazine, but by 4 h after disbudding, in our study, all sedated calves no longer appeared to be under the effects of the sedative with respect to pain sensitivity.

**CONCLUSIONS**

The results of this study indicate that xylazine sedation, in conjunction with a local anesthetic and an NSAID, is potentially effective at reducing behavioral and physiological indicators of pain in calves during and following cautery disbudding, as well as encouraging play behavior 24 h after disbudding. However, in our study sedation negatively affect play behavior at 3 h after disbudding and reduced drinking speed for 48 h following sedation. Further examination of the relationship between feeding behaviors and sedation would be beneficial for understanding the full effect of providing this medication for disbudding, to determine if the effects on behavior stem from a lack of responsiveness as a direct result of the sedation, as opposed to an analgesic effect or decrease in stress.

**ACKNOWLEDGMENTS**

The authors thank the research facility, all of the farm staff, and research students for their contribution to this work. This project was funded by the Ontario Ministry of Agriculture, Food, and Rural Affairs–University of Guelph Ontario Agri-Food Innovation Alliance Research Program (Guelph, ON, Canada), Boeh-
REFERENCES


ORCIDS
Cassandra N. Reedman 🆓 https://orcid.org/0000-0003-3904-0993
Todd F. Duffield 🆓 https://orcid.org/0000-0001-6635-4669
Trevor J. DeVries 🆓 https://orcid.org/0000-0001-9364-2456
Kerry D. Lissemore 🆓 https://orcid.org/0000-0003-2979-4708
Ian J. Duncan 🆓 https://orcid.org/0000-0001-5787-2577
Charlotte B. Winder 🆓 https://orcid.org/0000-0002-7314-3657