Effect of plane of nutrition and analgesic drug treatment on wound healing and pain following cautery disbudding in preweaning dairy calves

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ABSTRACT

The objective of this study was to determine the effect of a biologically normal plane of nutrition compared with a limited plane on the primary outcome wound healing, and one dose of nonsteroidal anti-inflammatory drug (NSAID) compared with 2 on the secondary outcomes: lying behavior, haptoglobin concentrations, and mechanical nociceptive threshold (MNT) in calves disbudded via cautery iron. Eighty female Holstein calves were enrolled at birth, individually housed, and fed via a Calf Rail system (Förster Technik). A 2 × 2 factorial design was used to assess the effect of plane of nutrition and an additional NSAID. Calves were randomly assigned to a biologically normal plane of nutrition (BN; offered up to 15 L/d) or a limited plane (LP; offered up to 6 L/d) and to receive one or 2 doses of meloxicam. All calves received a lidocaine cornual nerve block and a subcutaneous injection of meloxicam 15 min before cautery disbudding at 18 to 25 d of age, and half the calves received an additional injection of meloxicam (0.5 mg/kg) 3 d after disbudding. Tissue type present, wound diameter, and wound depth were evaluated 2 times per week for 7 to 8 wk as measures of wound healing. Tissue type present, wound diameter, and wound depth were evaluated 2 times per week for 7 to 8 wk as measures of wound healing, and MNT was evaluated 2 times/wk for 3 wk. Survival analyses were analyzed using Cox regression models (wound healing) and continuous data were analyzed using mixed-effect linear regression models. Only 12% of horn buds were completely healed by 7 to 8 wk after disbudding and 54% had re-epithelized at this time. At any time, wounds from BN calves were more likely to have had re-epithelization occur compared with wounds from LP calves (hazard ratio: 1.93, 95% CI: 1.18–3.14). Wounds from calves that received only one dose of NSAID were more likely to have re-epithelization occur, compared with wounds from calves given 2 doses (hazard ratio: 1.87, 95% CI: 1.15–3.05). Wounds from BN calves had smaller diameters and depths over time beginning on wk 3 compared with LP calves. Wounds from calves that received an additional NSAID had larger diameters and depths over time beginning on wk 4 and 3 respectively, compared with calves that only received one dose of NSAID. Calves that received an extra NSAID had larger diameters and depths over time beginning on wk 4 and 3 respectively, compared with calves that only received one dose of NSAID. Calves on the BN milk program were more active compared with LP calves with lower lying times, fewer lying bouts per day, and longer average lying bouts. Our results indicate that a BN milk feeding program for calves can result in faster healing times and more activity, and that providing an extra NSAID may result in less pain experienced by the calf 1 to 2 wk after disbudding. Calves on the BN milk program were more active compared with LP calves with lower lying times, fewer lying bouts per day, and longer average lying bouts. Our results indicate that a BN milk feeding program for calves can result in faster healing times and more activity, and that providing an extra NSAID may result in less pain experienced by the calf 1 to 2 wk after the procedure. This study is also among the first to demonstrate that after the complete removal of the horn bud, wounds can take more than 8 weeks to re-epithelize and fully heal.

Key words: nonsteroidal anti-inflammatory drug, calf, dairy, analgesia

INTRODUCTION

Disbudding is a common procedure in the dairy industry (USDA, 2018; Winder et al., 2016). Although it is clear it is a painful procedure (Stock et al., 2013), especially when performed without anesthesia or analgesia (Calderón-Amor and Gallo, 2020), it is unclear how long this pain persists. Although many individuals perceive disbudding to be a painful procedure (Hoe and...
Ruegg, 2006; Hokkanen et al., 2015) and are in favor of the use of pain control (Kling-Eveillard et al., 2015) believing it to be effective (Robbins et al., 2015), full adoption of pain control use in the United States and Canada has not been reached. In the United States, only 28% of dairy operations reported use any form of pain control (anesthetics or analgesics) for disbudding procedures (USDA, 2018), whereas in Canada, 66 and 25% of caustic users report using local anesthetics and analgesics, respectively (Winder et al., 2018).

The provision of a local anesthetic and a nonsteroidal anti-inflammatory drug (NSAID) substantially reduces pain-related outcomes for both cautery (Faulkner and Weary, 2000; Heinrich et al., 2010; Winder et al., 2018) and caustic paste disbudding (Stilwell et al., 2009; Winder et al., 2017; Reedman et al., 2020). Haptoglobin is an acute phase protein and an indicator of inflammation in cattle that has been reported to increase in response to disbudding (Allen et al., 2013; Glynn et al., 2013). Calves provided with both a local anesthetic and an NSAID for disbudding procedures have been reported to have decreased haptoglobin concentrations compared with calves receiving less or no pain control (Ballou et al., 2013; Erdogan et al., 2019; Reedman et al., 2020). Stressful or painful events such as disbudding have also been reported to affect the lying behavior of calves (Molony and Kent, 1997; Black et al., 2017; Sutherland et al., 2018a), including (in the 4 h after disbudding) spending less time lying (Sutherland et al., 2019) and being more restless (moving from standing to lying frequently) (Sutherland et al., 2018b). Some of these negative responses can be mitigated with the use of a local anesthetic and NSAID (Sutherland et al., 2018a).

Most previous studies have only evaluated calves on the day of disbudding or up to 4 d or 3 wk after (Theurer et al., 2012; Huebner et al., 2017; Sutherland et al., 2019). More recently, researchers have reported that wound re-epithelialization after disbudding can range between animals from 42 to 91 d and 40 to 70 d in calves after disbudding (Adcock and Tucker, 2018; Adcock et al., 2019, respectively), and 35 to 63 d in goat kids after disbudding (Alvarez et al., 2019). These researchers (Adcock and Tucker, 2018; Adcock et al., 2019; Alvarez et al., 2019) evaluated the length of time for wounds to form new epithelium, rather than the time it took for wounds to completely contract. During this time, the tissue around the horn bud is more sensitive for up to 105 d after disbudding compared with control animals (Casoni et al., 2019) and is more sensitive [lower mechanical nociceptive threshold (MNT)] compared with new epithelium formed on the wound for this entire healing process (Adcock and Tucker, 2018). Calves administered lidocaine 11 d after cautery disbudding exhibit behavioral changes consistent with experiencing ongoing pain (Adcock et al., 2020). Additionally, although sham disbudded calves find the provision of lidocaine to be a painful experience and will avoid it, disbudded calves will choose to receive an injection of lidocaine at 20 d after disbudding, indicating that they are still in pain at this point and will trade off the short-term pain of the lidocaine with the longer-term pain relief (Adcock and Tucker, 2020).

Nutrition has been reported to be a crucial part of the wound healing process in humans as malnutrition (encompasses poor nutritional intake to overall metabolic equilibrium) has been well documented to impede wound healing (Williams and Barbul, 2003; Stechmiller, 2010; Wild et al., 2010); however, this has not been evaluated in disbudding wounds in calves. Although it is becoming more common to feed an increased nutritional plane to young dairy calves, 33% of Canadian producers are still feeding calves low levels of milk (<6 L/d; Winder et al., 2018). When calves are offered milk ad libitum, they will drink between 10 to 12 L/d on average (>20% of BW by volume; Khan et al., 2011), and researchers have reported that calves fed 6 L of milk daily display signs of hunger (Rosenberger et al., 2017). There are many benefits to feeding an increased nutritional plane to young dairy calves including improving calf health and performance (Todd et al., 2017) and improved first lactation milk production (Gelsinger et al., 2016). Taken together, it is known that the plane of nutrition has long-term biological effects in calves, specifically, and in the context of wound healing in other species. It seems plausible that plane of nutrition, therefore, is worth exploring in the context of the large amount of variation in time taken for disbudding wounds to re-epithelialize (Adcock and Tucker, 2018; Adcock et al., 2019; Alvarez et al., 2019).

Therefore, the objectives of this study were to assess the effect of a biologically normal plane of nutrition (BN; up to 15 L/d) or limited plane (LP; up to 6 L/d) on wound healing after disbudding in dairy calves, as well as the effect of an additional dose of meloxicam 3 d after disbudding on pain and inflammation (haptoglobin). We predicted that BN calves would have improved wound healing, smaller wound diameters and depths over time compared with LP calves. We also predicted that calves receiving an additional dose of NSAID would have lower haptoglobin concentrations, decreased MNT, and improved lying behavior outcomes compared with calves only receiving one dose.

MATERIALS AND METHODS

This manuscript is reported according to guidelines for randomized controlled trials in livestock and food...
Colostrum quality was measured using a refractometer and was required to be at a minimum Brix value of 22% (Calf Lab refractometer; Golden Calf Company LLC). For the second feeding on the first day of life and the 3 feedings on the second day of life, calves were fed transition cow milk (milk from the second to sixth milkings from fresh cows) by bottle in 2-L quantities. Until calves were fully weaned at d 63, they were fed using a Calf Rail system (Förster Technik) that ran 5 times daily at 0500, 0900, 1300, 1700, and 2100 h. Beginning on their third day of life, calves were fed an acidified milk replacer at a concentration of 150 g/L (5.4 pH, 26% CP, 18% crude fat; BioForce Acidified Milk Replacer, Grand Valley Fortifiers) by the Calf Rail system. Depending on the calf’s milk treatment group, they were offered either 3 L (BN) or 1.2 L (LP) of milk replacer each time the Calf Rail ran. Calves were trained (assisted if needed) at every feeding until they could reliably get up on their own to drink their allotted milk. Barn staff checked daily at 1730 h for calves that had not consumed 4 L of milk since the first feeding of the day; these calves were bottle fed milk replacer until they had drunk at least 4 L for the entire day with the option to drink more milk at their last feeding at 2100 h as well. All additional bottle feedings were recorded and used to determine the total daily milk consumption of each individual calf. Further information on the Calf Rail system and calves’ nutrition protocol is reported in Parsons et al. (2022).

Calves had access to water in an 8 L bucket attached to the back of their pen 24/7 from birth and were offered a solid starter diet in an 8 L bucket attached to the back of their pen beginning at d 5 of life. Calves on this trial were a part of a nutrition trial being conducted in collaboration with another research group at the same time as the present trial, examining the effect of a novel milk by-product-based starter diet compared with a grain-based starter diet (AUP#3722; Parsons et al., 2022).

Both milk treatment groups were weaned using a gradual weaning program. The Calf Rail automatically gradually decreased the calves’ allotted milk in equal increments beginning on d 43 until d 63 and on d 64 all calves no longer received any milk. Calves remained on this trial after weaning in their individual pens until d 77 of life, and on d 78 they were moved to group heifer housing and were no longer followed by the researchers.

Enrollment

All heifer calves born were eligible and enrolled onto the trial. Calves were health scored by researchers using the Calf Health Scorer App (McGuirk, 2013; McGuirk...
and Peek, 2014) twice weekly beginning at d 2 of life. Any calf that was appreciably polled by palpation of the horn bud at the time of disbudding was excluded from the trial. Calves defined as ill (any one of: 3 fecal consistency and rectal temperature; ≥2 for all other health parameters) on the day of disbudding were not disbudded but could be included the following week if healthy at that time. Calves defined as ill both on their original disbudding date and one week later were excluded from the trial.

**Treatment Groups**

This was a 2 × 2 factorial design study that consisted of 2 separate variables of interest (milk feeding level and second dose of NSAID) with 2 treatment groups within each variable. Within each milk treatment group, there were an equal number of calves assigned to the 2 NSAID treatment groups to make up 4 treatment groups (BN and control; BN and additional NSAID; LP and control; LP and additional NSAID). Calves on the BN treatment were offered up to 15 L/d of milk, split into 5 feeding of 3 L each feeding from d 2 to 42, and LP calves were offered up to 6 L/d of milk split into 5 feedings of 1.2 L each feeding from d 2 to 42. All calves received one dose of meloxicam before disbudding, with half receiving a second dose 3 d after disbudding. Calves were disbudded when they were between 18 and 30 d of age. Treatment groups were all balanced to ensure an equal number of calves per room in both milk and NSAID treatments, while also accounting for the 2 additional starter feed treatments, which included in the collaborating nutrition trial feeding (1) only a mixture of 95% grain-based starter pellet (20% CP, Bionic Calf Grower Pellet, Grand Valley Fortifiers) and 5% wheat straw, and (2) 150 g/d (as-fed) of a whey-based starter (LifeLaunch 4C; Grand Valley Fortifiers), with the grain-straw mix also provided once calves consumed the entire 150 g/d on 2 out of 3 consecutive days. From the previously mentioned treatment groups, an equal number of calves within each of those 4 groups was assigned to each of the starter diets to balance for these as well.

Before all baseline MNT measurements being taken, the hair around the horn buds was clipped. Fifteen minutes before disbudding, all calves received a lidocaine cornual nerve block (6 mL per side, lidocaine hydrochloride injection 20 mg/mL, Bimeda-MTC Animal Health Inc.) and a subcutaneous injection (in the neck) of 0.5 mg/kg meloxicam (Metacam 20 mg/mL Solution for Injection, Boehringer Ingelheim). Cornual nerve block technique was performed as described in Winder et al. (2017) by insertion of an 18-gauge, 1.5-inch needle caut-

dal to the eye, ventral to the temporal ridge, injecting 6 mL per side fanned out in multiple directions. Calves in the extra NSAID group received their second dose 72 h after disbudding as described above.

To assess the efficacy of the nerve block, MNT was assessed immediately before disbudding, any calf that was not adequately desensitized (MNT value of 10 kilograms of force at all 8 locations) was administered an additional 2 mL of lidocaine on the appropriate side. Disbudding was performed by CNR (author) for the entirety of the trial. The horn bud was removed completely from the calf and will be referred to as a “bud-out” technique. The cautery iron (Express Pistol-Grip Dehorner, The Coburn Company, Inc.) was preheated for at least 3 min until it reached a temperature of approximately 650°C. The iron was then applied only once 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 all horn bud tissue was fully removed, as described in Reedman et al. (2021).

**Primary Outcome and Data Collection**

The research team attended farm on Tuesdays and Fridays to collect measurements for this trial. Disbudding always occurred on a Tuesday and all calves that were eligible (18 to 25 d of age and considered healthy as previously described upon initial assessment, but eligible up to 30 d of age if the calf was too sick on their original disbudding day) on that day were disbudded. After disbudding measurements for MNT and wound assessments were taken at the same time with MNT measurements collected first, followed by wound measurements, and finally photos of the wounds were taken last.

**Wound Assessments**

Wound diameter and depth were measured by the same researcher for the entire trial. These measurements were collected using a digital caliper, in mm (Mastercraft Digital Caliper, 6 in). The scoring system for tissue type during wound healing is described in Figure 1. The diameter measurements were taken at the widest part of each wound and were collected until there was no longer any crust or granulation tissue present, only new epithelium. Depth measurements were collected using the depth rod function on the caliper. Depth was measured at the deepest part of each wound and was collected until the necrotic tissue had fully detached from the skull of the calf and there was no longer depth measurable on the wound. If there was pu-
rulent discharge in the wound, the depth of the wound was not measured. Wound measurements were collected directly after disbudding and every Tuesday and Friday until the calves left the calf room (when they were 78 d of age). Therefore, depending on the age of the calf on the day of disbudding, measurements were collected at +3, +7, +10, +14, +17, +21, +24, +28, +31, +35, +38, +42, +45, +49, and +52 d relative to disbudding with some calves being followed up to +56 and +59 d after disbudding if they were disbudded earlier in the eligible window.

**Wound Healing**

Photos of the disbudding wounds were taken by the same researcher for the entirety of the trial. Following the protocol described by Adcock and Tucker (2018), photos were taken using an iPhone XR (Apple Inc.) to meet the following criteria for the wound: centered in the frame, in full view (taken straight on, not at an angle), clear, sharp and in focus, lighting was uniform (always taken in the calf nursery, no shadows), only the immediate area surrounding the wound was in

<table>
<thead>
<tr>
<th>Example</th>
<th>Tissue</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Wound characterization on day of disbudding</strong></td>
<td>Bone or fat</td>
<td>Smooth, white surface, may have pink tinge, and/or yellow ridged tissue</td>
</tr>
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| **Healing process (scored d 1 or later)** | Attached necrotic tissue | Yellow or white centre (likely sequestrum or fat) surrounded with dried black exudate that is below the plane of undamaged tissue; dried exudate may obscure yellow centre; none of the wound edge has lifted away from scalp |
| | Detaching necrotic tissue | The outside edge of the ring around the wound has started to lift away from scalp |
| | Pus/purulent discharge | Thick, fresh, exudate that is cloudy with a pink or gray tinge |
| | Granulation | Light red/dark pink, opaque, bumpy tissue; only scored once necrotic tissue begins to detach |
| | Crust | Rough tan, dark red or black dried exudate that is at or above the plane of undamaged tissue |
| | Epithelium | Layer of translucent skin is present; other tissue types or blood are absent |
| | Healed/fully contracted | Wound is fully contracted; scar line may be visible; other tissue types or exudate are absent |

*Figure 1.* The scoring system used for evaluating tissue types in disbudding wounds.
frame (no open space, ear, or eye), and skin was not folded around the wound. Photos of each wound were taken directly after disbudding and following the same schedule as the wound measurements. Photos were taken directly after measurements. Once the trial was complete, a third-party observer, blind to treatment groups, was trained to score the wound photos based on the presence or absence of the tissue types described in Figure 1. This chart was developed based on the scoring system described in Adcock and Tucker (2018), but modified by SJJA and CBT for bud-out disbudding. An interobserver reliability score was calculated for each tissue type based on this observer’s scoring of 300 photos (9 calves from the day of disbudding to the end of their follow-up) from the training module by SJJA and CBT. A cut-off of 0.8 was used for assessing the kappa statistic (considered very good agreement).

Once the third-party observer was trained, they scored all of the photos (2,534) and recorded the data into Microsoft Excel (Version 16.5, Microsoft Corp.).

Secondary Outcomes and Data Collection

**Standing and Lying Behavior.** Standing and lying behavior were measured using HOBO Pendant G Data Loggers (Onset Computer Corp.) attached horizontally to the rear right leg of each calf using a cohesive bandage and set to record the tilt of the x-, y-, and z-axis every 60 s, as described by Bonk et al. (2013). The y-tilt was used to evaluate when the calves were lying down versus standing. Data were downloaded using HOBOware Pro Software (Onset Computer Corp.), imported into Microsoft Excel (version 16.6, Microsoft Corp.), and categorized into 3 outcomes: average daily lying time, average daily lying bout length, and number of lying bouts in a day. Each day that the loggers collected data for began at 0000 h and ended at 1159 h. The HOBO sensors were attached to the calves when they were 11 d of age (1–2 wk before disbudding) and collected data until the calves completed the trial (77 d of age). The data collected in the 7 d before disbudding were used as baseline values for average daily lying time, average daily lying bout length, and number of lying bouts in a day. Data from the loggers were downloaded every week after attachment with new loggers attached each week. The exact time that HOBO loggers were switched for each individual calf was recorded in Microsoft Excel (Microsoft Corp., Redmond, WA) and was used to splice together all 8 to 9 wk of lying behavior data into one file per calf. If data collected by the loggers was incorrect upon visual inspection and was able to be corrected (such as the logger flipping upside down while collecting data), this was accomplished by adjusting the data however necessary (eg., for an upside down logger the data were adjusted by 180°).

**Serum Haptoglobin.** Haptoglobin was collected to evaluate inflammation after the disbudding procedure. Blood samples were collected using red top vacutainer tubes by venipuncture of the jugular vein. Baseline values for this outcome were collected at −60 min relative to disbudding. Further samples for this outcome were collected at +4 h and daily for 7 d after the disbudding procedure. The haptoglobin sample on d 3 was collected in the afternoon 4 h after the second dose of the NSAID was administered to calves assigned to that treatment group. As soon as possible after collection and once samples had clotted, samples were centrifuged for 15 min at 2,000 × g on farm. Serum from each sample was separated, collected, and stored at −20°C until the time of testing. All samples from trial were run as one batch at the Animal Health Laboratory at the Ontario Veterinary College (Guelph, Ontario, Canada) on a Roche Cobas 6000 c501 biochemistry analyzer (Hoffmann-La Roche Ltd.) 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 binds with haptoglobin to form a stable methemoglobin-haptoglobin complex. Measurement of haptoglobin was based on the peroxidase activity of this complex in acidic conditions. A hydrogen peroxide and guaiacol solution served as the substrate for the reaction and color development was read at 480 nm wavelength. The instrument was calibrated using standards and controls before running the samples. The interassay coefficient of variation was 5.2%.

**Mechanical Nociceptive Threshold.** The MNT was measured using a pressure force algometer (Force Ten FDX Compact Digital Force Gage, Wagner Instruments), following the protocol described by Reedman et al. (2021). One researcher collected all MNT measurements for the entire trial, for further information on MNT training and inter- and intra-observer reliability (see Reedman et al., 2020). The algometer was equipped with a rubber tip (approximately 1 cm in diameter) and measurements were taken at 4 locations around each horn bud in the same order (based on the numerical order in Figure 2) always beginning with the left horn bud (Figure 2). Calves were restrained using a halter tied to the side of their pen; the algometer was initially placed lightly on the site until the calf was motionless and then force was applied slowly until there was a withdrawal or pain response from the calf; note that rate of pressure application was not formally measured or controlled. These responses included the
calf jerking or shaking their head, pulling back sharply, or jumping up or forward. 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 locations in 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. The first MNT measurements were collected 60 min before disbudding to acclimate the calf to the test, and then baseline values were collected from each calf 30 min before the procedure. Directly before disbudding (15 min after 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). Follow-up measurements were collected 4 h after disbudding and then on the same days as the wound measurements and photos for 3 wk after disbudding. These measurements were the first collected every day to attempt to eliminate any type of annoyance response to this test by the calf due to over handling.

Sample Size. Our initial trial protocol included a sample size considering wound diameter as the primary outcome based on an expected difference of 2 mm with a standard deviation of 4 mm for the milk treatment groups, with 95% confidence and 80% power and was adjusted for mild clustering by day (average n sampled per cluster = 3, intraclass correlation coefficient = 0.02). The calculated sample size was 60 calves per milk treatment group for a total of 120 calves. In March 2020, the trial was halted due to the COVID-19 global pandemic and resumed in June 2020. During this time a blinded interim analysis was conducted using data from the calves that had completed the trial by March 2020 (n = 26 calves total). Based on this, the difference in wound diameter between treatment groups of one variable of interest (BN and LP calves; unknown by the researcher which treatment group was which at the time of analysis) was 5 mm (5.3 mm and 10.3 mm) with a standard deviation of 6 mm. As a result, we reduced our sample size to a total of 40 calves in each milk treatment group, for a total of 80 calves.

Treatment Allocation and Blinding. A random pattern of treatments was created using a random number generator in Microsoft Excel (Microsoft Corp.) and was repeated until 80 calves had been enrolled onto the trial. Treatments were assigned based on the next available pen that a new calf was to be placed into. The pattern of treatments was correlated with available pens, following the same flow of pens as a room filled up (a total of 11 individual pens per room). For example, treatments were A, B, C, D, and were allocated in the pattern D, B, A, C. Treatment D was assigned to the first pen in room 1, pen 2 was assigned to treatment B, pen 3 to treatment A, pen 4 to treatment C, pen 5 to treatment D, and so on. As a calf was born, she was placed in the next available pen and assigned to the corresponding treatment group associated with that pen. This randomization protocol controlled for the effect of the calves’ assigned pen location within a nursery room. CNR performed all data collection and disbudding was blinded to the treatments that calves were assigned to. This researcher administered the primary dose of the NSAID as well as the lidocaine cornual nerve block. A separate researcher (SDP), who was not involved in any evaluation of outcomes or analysis, was not blinded to the treatment groups and administered the second dose of the NSAID. This researcher also assigned calves to their appropriate treatment group and measured their milk consumption. Caretakers on the farm were blind to the NSAID groups but were aware of the milk treatment group for the calves. Once statistical analysis was completed by the blinded researcher, treatment allocation was revealed for interpretation of the results.

Statistical Analysis

All recorded data were entered into Microsoft Excel (Microsoft Corp.) and imported into STATA15 (Stata/
Wound healing outcomes were assessed at the level of the horn bud as well as at the calf (both wounds in one calf reaching the healed or epithelium stage). For all models built, univariable models were built first to assess the statistical significance of independent variables using a liberal \( P \)-value of 0.2. Variables were then offered to the multivariable model to further assess significance using a \( P \)-value of 0.05 and tendencies at >0.05 but <0.1. For all linear models (wound diameter, wound depth, MNT, haptoglobin, daily lying time, number of lying bouts, and average lying bout length), continuous independent variables (age on the day of disbudding and time since disbudding) were assessed for linearity with the dependent variable. If an independent variable was detected to have a nonlinear relationship with the dependent variable (time since disbudding) it was categorized. First order interaction terms were assessed between milk treatment, NSAID treatment, starter treatment, and time since disbudding for all models and were kept if statistically significant. For wound diameter and depth models, time was modeled in weeks since disbudding. Before the removal of any variable from the multivariable model for each outcome, each variable was assessed statistically for confounding effects on other variables in the model. If a variable was found to be a confounder (as previously described), it was kept in the model.

Wound healing outcomes were assessed at the level of the horn bud as well as at the calf (both wounds in one calf reaching the healed or epithelium stage). Regardless of the experimental unit, hazard ratios (HR)
for treatment groups were evaluated from Cox proportional hazard models. Kaplan-Meier failure curves were also constructed to visually compare differences in healing time between treatment groups while controlling for the other treatment not being assessed (i.e., if comparing milk treatments, the curve controlled for the effects of the NSAID treatment as well). When evaluated at the horn bud level, the length of time it took for individual wounds to reach the end points (epithelium or healed) was modeled based on the first day these tissue types were present for. For evaluation at the calf level, the length of time it took for both wounds in an individual calf to reach the end points (epithelium or healed) was modeled. The presence of pus or purulent discharge was evaluated and noted in the wound photos as well.

In the lying behavior models (daily lying time, number of lying bouts, and average lying bout length) an individual model was built for each outcome. Because milk treatment differences were detectable and present before disbudding, all the lying behavior models evaluating milk treatment were built using the entire time period that lying behavior was collected for (d 11 of age to d 77 of age). However, because NSAID treatment was only given 3 d after disbudding, differences were not detectable across the entire 66 d that data were collected for. Due to biological plausibility, lying behavior models (daily lying time, number of lying bouts, and average lying bout length) evaluating NSAID treatment were built to assess differences in the week after disbudding while controlling for baseline values the week before disbudding and controlling for differences attributable to the milk treatment.

RESULTS

In total, 95 calves were enrolled in this study, with 80 calves completing the trial. Two calves were excluded because they were polled, 2 were euthanized during the study due to severe respiratory disease and injury, 2 were excluded because they were disbudded with a different cautery iron, one was excluded because it was sick on the day of disbudding and a week later, and 8 were excluded because they were lost to follow-up due to the COVID-19 pandemic. Of the 80 calves, 39 were enrolled in the BN group and 41 were enrolled in the LP group. As well, 39 calves received one dose of meloxicam and 41 were given 2 doses. For both the milk and NSAID treatments, 20 calves were BN with 2 doses, 19 calves were BN with 1 dose, 21 calves were LP with 2 doses, and 20 calves were LP with 1 dose. All calves enrolled in the study received the intended treatment depending on their group and were followed for the entire study period from birth to d 77 of age. All samples and measurements collected were used in the analysis. Milk consumption (L/d) over time by milk treatment group is illustrated in Figure 3; treatment groups were statistically different in milk consumption beginning on d 7 and were different until weaning at d 63 (P < 0.01). Moreover, calves on the BN group had greater (P < 0.01) average ME intake (Mcal/d) from birth until weaning (d 0–63) compared with the LP calves (BN ME/d: 5.89 ± 0.04 Mcal/d, 95% CI: 5.81–5.96, LP ME/d: 5.16 ± 0.04 Mcal/d, 95% CI: 5.09–5.24). There were no deviations from the trial protocol for treatment allocation, randomization, blinding, data collection, or analysis. All data collected and analyzed from this trial that are not reported in this manuscript, are reported in Supplemental File S1 (https://doi.org/10.5683/SP3/CHTHRJ).

Baseline Characteristics

Calf age on the day of disbudding ranged from 18 to 30 d. The mean age was higher in the LP group compared with BN and higher in the control group compared with extra NSAID (BN: 20.9 ± 0.13 d, LP: 21.6 ± 0.13 d; P < 0.01, control: 21.5 ± 0.11 d, extra NSAID: 21.1 ± 0.13 d; P = 0.05). No difference was detected in baseline values between the different treatment groups for MNT (BN: 6.65 ± 0.09 kgf, LP: 6.83 ± 0.08 kgf; P = 0.18, control: 6.77 ± 0.09 kgf, extra NSAID: 6.72 ± 0.09 kgf; P = 0.68) and haptoglobin concentrations (BN: 0.15 ± 0.001 mg/mL, LP: 0.15 ± 0.0009 mg/mL; P = 0.25, control: 0.15 ± 0.001 mg/mL, extra NSAID: 0.15 ± 0.0009 mg/mL; P = 0.06) or between the NSAID treatment groups in daily lying time (BN: 20.9 ± 0.13 d; P < 0.01) average ME intake (Mcal/d) from birth until weaning (d 0–63) compared with the LP calves (BN ME/d: 5.89 ± 0.04 Mcal/d, 95% CI: 5.81–5.96, LP ME/d: 5.16 ± 0.04 Mcal/d, 95% CI: 5.09–5.24). There were no deviations from the trial protocol for treatment allocation, randomization, blinding, data collection, or analysis. All data collected and analyzed from this trial that are not reported in this manuscript, are reported in Supplemental File S1 (https://doi.org/10.5683/SP3/CHTHRJ).

Primary Outcomes

Wound Diameter. The effect of milk treatment on wound diameter over time is illustrated in Figure 4 and the effect of NSAID treatment on wound diameter over time is illustrated in Figure 4. On the day of disbudding, the mean wound diameter was 18.8 ± 0.13 mm and was not different between the milk (P = 0.70) or NSAID (P = 0.16) treatment groups. On wk 3 after disbudding, BN calves had smaller wound diameters compared with LP calves (P = 0.05, F20, 1546 = 217.5) and from wk 4
to 8, BN calves had smaller wound diameters compared with LP calves \((P < 0.001, F_{20, 1546} = 217.5; \text{Figure 4})\). Beginning on wk 4 and until wk 8, calves that only received 1 dose of an NSAID had smaller wound diameters compared with calves that received 2 doses of an NSAID \((P < 0.01, F_{20, 1546} = 217.5; \text{Figure 4})\).

### Wound Depth

The effect of milk treatment on wound depth over time is illustrated in Figure 4 and the effect of NSAID treatment on wound depth over time is illustrated in Figure 4. On the day of disbudding, the mean wound depth was 5.2 ± 0.10 mm and was not different between the milk \((P = 0.53)\) or NSAID \((P = 0.93)\) treatment groups. On wk 2 after disbudding, BN calves tended to have smaller wound depths compared with LP calves \((P = 0.09, F_{20, 1539} = 71.5)\) and from wk 3 to 8, BN calves had smaller wound depths compared with LP calves \((P < 0.01, F_{20, 1539} = 71.5; \text{Figure 4})\). Beginning on wk 3 and until wk 8 calves that only received 1 dose of an NSAID had smaller wound depths compared with calves that received 2 doses of an NSAID \((P < 0.05, F_{20, 1539} = 71.5; \text{Figure 4})\).

### Full Wound Contraction

The time to the healed and fully contracted stage (Figure 1) was evaluated, but very few wounds or calves reached this stage by the end of the 7 to 8 wk of follow-up. Of the 160 horn buds evaluated in this trial, 19 of them healed completely by d 59 (median time to healing of 53.7 d), 10 were from BN calves and 9 from LP calves, and 8 from calves that received 1 dose of NSAID and 11 from calves that received 2 doses of NSAID. Out of all of the calves enrolled in this trial \((n = 80)\), only 3 calves had both of their wounds reach the healed stage by d 59 (median time to healing of 53.8 d; milk treatments: 1 in BN, 2 in LP; NSAID treatments: 1 in control group, 2 in extra NSAID group). Figure 5 depicts the time that wounds spend in each of the 8 stages of healing based on the scoring system in Figure 1 and the length of time since disbudding that these tissues were present for.

### Re-Epithelization: Horn Bud Level Analysis

Of the 160 wounds assessed for this study, 87 of them (from 56 calves) reached the epithelium stage of healing by the end of follow-up (59 d). For the 87 wounds which re-epithelialized, the time for individual wounds to reach this stage ranged from 28 to 59 d after disbudding with a median time of 49.2 d. The Kaplan-Meier failure curve for time to re-epithelization by milk treatment at the horn bud level is illustrated in Figure 6, and the Kaplan-Meier failure curve for time to re-epithelization by NSAID treatment at the horn bud level is illustrated in Figure 7. At any time during the healing process, wounds of BN calves were more likely to reach the epithelium stage compared with wounds of LP calves \((HR: 1.93; 95\% \text{ CI: } 1.18–3.14; P = 0.008)\), and wounds of calves on the control group that only received 1 dose of NSAID were more likely to reach the epithelium stage compared with wounds of calves on the extra NSAID group receiving 2 doses of NSAID \((HR: 1.87; 95\% \text{ CI: } 1.15–3.05; P = 0.01)\). Wounds from BN calves had 3.9 times the odds of re-epithelializing by d 59 after disbudding compared with wounds from LP calves \((95\% \text{ CI: } 1.33–11.2; P = 0.01)\), and wounds from calves on the control group receiving 1 NSAID dose had...
3.2 times the odds of having their wound re-epithelize by the end of follow-up compared with wounds from calves on the extra NSAID group (95% CI: 1.07 to 9.41, \( P = 0.04 \)).

**Re-Epithelization: Calf Level Analysis (Both Wounds).** Of the 80 calves enrolled on this trial, 31 had both of their disbudding wounds reach the epithelium stage by the end of follow-up. For the calves who had both wounds reach this stage, the time for both wounds in one calf to re-epithelize ranged from 38 to 59 d after disbudding with a median time of 51.7 d. At any time during the healing process, BN calves were 134% more likely to have both wounds reach the epithelium stage compared with LP calves (HR: 2.34; 95% CI: 1.1–4.9, \( P = 0.03 \)), and calves on the control NSAID group were 136% more likely to have both of their wounds reach the epithelium stage compared with calves on the extra NSAID group (HR: 2.36; 95% CI: 1.09–5.12; \( P = 0.03 \)). Calves enrolled on the BN program had 3.6 times the odds of having both of their wounds re-epithelize by the end of follow-up (d 59) compared with LP calves (95% CI: 1.18–11.1; \( P = 0.02 \)), and calves on the control NSAID group tended to have 2.8 times the odds of having both of their wounds re-epithelize by the end of follow-up compared with calves on the extra NSAID group (95% CI: 0.91–8.7; \( P = 0.07 \)).

**Presence of Purulent Discharge.** The presence of purulent discharge at any point during the study was noted in 18 out of 160 wounds (15 calves) during the scoring of the wound healing photos, with 12 of these being calves with one wound having discharge and 3 with both. Due to small sample size of wounds with purulent discharge present, this variable was not modeled. Of these calves, 11 were from BN calves and 4 were from LP calves, and 11 were from calves on the control NSAID group and 4 were from calves on the extra NSAID group. All 18 of these wounds reached the epithelium stage of healing by the end of follow-up (d 59).
Secondary Outcomes

**Haptoglobin Concentrations.** There were no differences in haptoglobin concentrations detected between NSAID treatment groups at any time point \( (P > 0.1 \text{ for all categorical time interaction coefficients}) \). There were no differences in haptoglobin concentrations detected between milk treatment groups from 60 min before disbudding (baseline) until 6 d after. However, there was a difference between milk treatment groups at d 7 after disbudding with BN calves having lesser haptoglobin concentrations compared with LP calves (BN adjusted mean: 0.083 mg/mL; LP adjusted mean: 0.113 ± 0.015 mg/mL; interaction coefficient: −0.03 mg/mL; 95% CI: −0.046 to −0.005; \( P = 0.02; F_{16, 509} = 3.23 \)). However, this difference was driven by the presence of large outliers in the data and when the data were analyzed with these outliers removed, there was no longer a detectable association between milk treatment and haptoglobin concentrations. These outliers were investigated and were not reporting errors or from calves who were sick or had received an intranasal vaccine in the past week, or had purulent discharge, therefore the removal of these outliers could not be explained or justified.

**Mechanical Nociceptive Threshold.** There was no detectable treatment by time interaction for milk treatment and MNT values \( (P > 0.1 \text{ for all categorical time interaction coefficients}) \). Regardless of time point, calves on the LP program had greater MNT values compared with BN calves (BN adjusted mean: 5.29 kgf; LP adjusted mean: 5.49 kgf; model coefficient: 0.19 kgf; 95% CI: 0.032–0.35; \( P = 0.03; F_{19, 552} = 245.6 \)). Based on the interaction in the model, there were no detectable differences between the NSAID treatment groups 30 min before disbudding, directly before disbudding, or 4 h and 3 d after disbudding \( (P > 0.1, F_{19, 552} = 245.6) \). However, at 7 and 10 d after disbudding, calves that received an additional dose of NSAID tended to have greater MNT values compared with calves that only received 1 dose (control predicted adjusted mean 7 d: 1.39 kgf; extra NSAID predicted adjusted mean 7 d: 1.57 kgf; interaction coefficient at 7 d: 0.53 kgf; 95% CI: −0.015 to 1.08; \( P = 0.06; F_{19, 552} = 245.6 \)). However, at 10 d after disbudding, calves that received an additional dose of NSAID tended to have greater MNT values compared with calves that only received 1 dose (control predicted adjusted mean 10 d: 1.33 kgf; extra NSAID predicted adjusted mean 10 d: 1.47 kgf; interaction coefficient at 10 d: 0.5 kgf).

![Figure 5](image_url) **Figure 5.** Days relative to cautery disbudding that each tissue type was observed. The box shows the interquartile range with the bottom of the box representing the 25th percentile and the top representing the 75th percentile. The line in the middle of each box represents the median, and the whiskers are the upper and lower limit (1.5× interquartile range). The black circles on the plot represent the outside values or outliers, which are any values outside of the range of the box and whiskers.
kgf; 95% CI: −0.055 to 1.05; \( P = 0.08; F_{19, 552} = 245.6 \), and at 17 d after disbudding, calves that received an additional NSAID had greater MNT values compared with calves that only received 1 dose (control adjusted mean: 1.37 kgf; extra NSAID adjusted mean: 1.61 kgf; interaction coefficient at 17 d: 0.59 kgf; 95% CI: 0.044–1.14; \( P = 0.04; F_{19, 552} = 245.6 \)).

**Lying Behavior.** There was a milk treatment by time interaction for daily lying time (BN: 1,092.8 ± 1.15 min/d; LP: 1,094.4 ± 1.21 min/d), number of lying bouts (BN: 19.3 ± 0.08 bouts/d; LP: 18.6 ± 0.07 bouts/d), and average lying bout length (BN: 58.9 ± 0.26 min/bout; LP: 61.4 ± 0.25 min/bout) for the entire period that data were collected for (d 11 of age to d 77 of age; \( P < 0.05 \)). Based on the interaction from the model, calves on the BN program had a decrease in their amount of daily lying time from d 11 to d 77 of age, whereas calves on the LP program had a slight increase in their amount of daily lying time from d 11 to d 77 of age, whereas calves on the LP program had a slight increase in their amount of daily lying time from d 11 to d 77 of age (interaction coefficient: −0.30 min/d; 95% CI: −0.45 to −0.16; \( P < 0.001; F_{8, 209} = 7.6 \)). As well, calves on the BN program had fewer lying bouts in a d from d 11 to 77 of age and calves on the LP program had a slight increase in the number of lying bouts in a d from d 11 to 77 of age (interaction coefficient: −0.088 bouts/d; 95% CI: −0.097 to −0.080; \( P < 0.001; F_{8, 161} = 95.0 \)). Last, from d 11 to 77 of age, calves on the BN program had longer average lying bout lengths and calves on the LP program had shorter average lying bout lengths (interaction coefficient: −11.0 min/bout; 95% CI: −14.0 to −7.95; \( P < 0.001; F_{7, 156} = 64.5 \)).

The effect of NSAID treatment on daily lying time and average lying bout length in the wk after disbudding are described in Tables 1 and 2 respectively. Calves that received an extra NSAID spent less time lying in a day compared with control NSAID calves over the week after disbudding (Table 1; control predicted adjusted mean 6 d after disbudding: 810 min/d; 95% CI: 697–922, extra NSAID predicted adjusted mean 6 d after disbudding: 798 min/d; 95% CI: 687–910; control predicted adjusted mean 7 d after disbudding: 814 min/d; 95% CI: 702–927; extra NSAID predicted adjusted mean 7 d after disbudding: 800 min/d; 95% CI: 689–911; \( F_{6, 164} = 6.5 \)). No significant interaction was detected between NSAID treatment and the number of lying bouts in a day over the week after disbudding (\( P = 0.11 \)). Last, calves in the extra NSAID group also had shorter average lying bout lengths in the week after disbudding, whereas while calves in the control NSAID group had longer average lying bout lengths in this week (\( P = 0.013, F_{8, 150} = 9.9; \) Table 2). Interactions between milk and day and starter treatment and NSAID treatment were significant (\( P < 0.05, F_{8, 150} = 9.9 \)) in the average lying bout length model as well (Table 2), therefore these were kept in the model to control for the effect of these treatments.
DISCUSSION

Wound Healing

Although not every calf in the current study had new epithelium form by the end of their follow-up (56/80 calves), of the ones that did, we noted that it took between 4 and 8 wk for this new epithelium to form. This was the first study to quantify full healing (fully contracted wounds) and only 12% of wounds reached this stage by the end of follow-up (7–8 wk after disbudding). However, based on past studies which have reported an average of 7 to 9 wk for wound re-epithelialization to occur (Adcock and Tucker, 2018; Adcock et al., 2019), we did not expect wounds to fully contract faster than this.

Effect of Milk Treatment

We found wounds of BN calves (15 L/d) healed faster (tissue type during healing, wound diameter, and wound depth) and formed new epithelium earlier compared with LP calves (6 L/d), due to the higher plane of nutrition associated with the increased milk. The higher milk treatment may have provided the necessary nutrients for faster wound healing.

Figure 7. Kaplan-Meier failure curve of re-epithelialization at the horn bud level based on nonsteroidal anti-inflammatory drug (NSAID) treatment group [control (1 dose of NSAID 15 min before cautery disbudding) and additional NSAID (1 dose of NSAID 15 min before cautery disbudding and a second dose of NSAID 3 d after disbudding)] over time relative to cautery disbudding (d). Values in this graph controlled for the effect of milk treatment.

Table 1. Output from final mixed-effect linear regression model for daily lying time (min/d) in the week after the disbudding procedure with random effects for calf nested within calf room nested within disbudding date

<table>
<thead>
<tr>
<th>Item</th>
<th>Coefficient¹</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.16</td>
<td>0.084 to 0.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control²</td>
<td>Ref.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Extra NSAID³</td>
<td>6.96</td>
<td>−13.4 to 27.3</td>
<td>0.501</td>
</tr>
<tr>
<td>Limited plane⁴</td>
<td>Ref.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Biologically normal⁵</td>
<td>3.63</td>
<td>−12.8 to 20.1</td>
<td>0.660</td>
</tr>
<tr>
<td>Day since disbudding</td>
<td>4.59</td>
<td>2.46 to 6.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age on day of disbudding (d)</td>
<td>4.63</td>
<td>0.52 to 8.76</td>
<td>0.028</td>
</tr>
<tr>
<td>NSAID × day</td>
<td>−3.01</td>
<td>−5.99 to −0.020</td>
<td>0.048</td>
</tr>
</tbody>
</table>

¹Ref. = referent.
²Calves were given one dose of nonsteroidal antiinflammatory drug (NSAID) 15 min before cautery disbudding.
³Calves were given one dose of NSAID 15 min before cautery disbudding as well as an additional dose of NSAID 3 d after disbudding.
⁴Calves were offered up to 6 L of milk/d.
⁵Calves were offered up to 15 L of milk/d.
of nutrition fed to the BN calves. This relationship between nutrition level and wound healing has also been noted in human literature (Williams and Barbul, 2003; Stechmiller, 2010; Wild et al., 2010). Researchers have reported that feeding higher than the conventional 10% BW milk allotment to calves and feeding milk more than once daily results in improved growth and reduced hunger (Jongman et al., 2020), as well as increased energy intake and weight gain (Jasper and Weary, 2002; Mirzaei et al., 2018). Although the calves in our study did not always consume the entire amount of milk that they were offered in a day, calves on the BN group drank significantly more milk daily beginning on d 7 of life until weaning and had greater ME intakes per kilogram of body weight than LP calves. Since milk intake has been shown to increase BW milk allotment to calves and feeding milk more than once daily results in improved growth and reduced hunger (Jongman et al., 2020), mice and rats (Huss et al., 2019), as well as calves (Winder et al., 2018; Gladden et al., 2019; Reedman et al., 2020), they appear to also have a suppressant effect on wound healing. This slower healing may also have been a result of the increased activity level noted in the 2-dose NSAID calves. Pain results in minimized behaviors that impede wound healing and maximizes protective behaviors (Molony and Kent, 1997). Therefore, by reducing pain, 2-dose NSAID calves may have been less protective of their wounds. Although both the LP and 2-dose NSAID treatments hindered the healing process, the lower plane of nutrition had a larger effect on this outcome compared with an additional NSAID dose. The effect size of milk treatment was larger compared with the effect size of the NSAID treatments when looking at the formation of new epithelium by the end of follow-up at both the wound and calf level. Therefore, although an additional dose of NSAID impeded the wound healing process, it did not affect it to the degree that a lower plane of nutrition did. As well, we also noted that wounds on calves receiving 2 doses of NSAID had much fewer instances of purulent discharge (22% of cases were from wounds on calves receiving 2 doses), which could be a result of decreased inflammation.

**Haptoglobin**

There is a lack of agreement across past disbudding research about the effect of pain control on haptoglobin concentrations in calves. Although some researchers have noted calves provided with analgesics alone or in combination with a local anesthetic have lower haptoglobin concentrations compared with calves with less or no pain control for both castration (Fisher et al., 1997; Earley and Crowe, 2002; Ballou et al., 2013) and disbudding procedures (Ballou et al., 2013; Erdogan et

**Table 2.** Output from final mixed-effect linear regression model for average lying bout length/d in the week after the disbudding procedure with random effects for calf nested within calf room nested within disbudding date

<table>
<thead>
<tr>
<th>Item</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.05</td>
<td>-0.03 to 0.13</td>
<td>0.23</td>
</tr>
<tr>
<td>Control¹</td>
<td>Ref.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra NSAID³</td>
<td>9.31</td>
<td>4.02 to 14.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Limited plane⁴</td>
<td>Ref.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biologically normal²</td>
<td>-7.03</td>
<td>-11.5 to -2.56</td>
<td>0.002</td>
</tr>
<tr>
<td>Whey-based starter</td>
<td>Ref.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain-based starter</td>
<td>5.04</td>
<td>0.78 to 9.30</td>
<td>0.021</td>
</tr>
<tr>
<td>Days since disbudding</td>
<td>1.26</td>
<td>0.60 to 1.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Milk × day</td>
<td>-0.91</td>
<td>-1.70 to -0.11</td>
<td>0.026</td>
</tr>
<tr>
<td>NSAID × day</td>
<td>-1.01</td>
<td>-1.70 to -0.22</td>
<td>0.013</td>
</tr>
<tr>
<td>Grain × NSAID</td>
<td>-9.96</td>
<td>-15.9 to -3.95</td>
<td>0.002</td>
</tr>
</tbody>
</table>

¹Ref. = referent.
²Calves were given one dose of nonsteroidal anti-inflammatory drug (NSAID) 15 min before cautery disbudding.
³Calves were given one dose of NSAID 15 min before cautery disbudding as well as an additional dose of NSAID 3 d after disbudding.
⁴Calves were offered up to 6 L of milk/d.
⁵Calves were offered up to 15 L of milk/d.

**Effect of NSAID Treatment**

Although an increased level of milk intake in calves resulted in improved healing times, we also detected that providing calves with an extra NSAID 3 d after disbudding slowed the healing process (tissue type during healing, wound diameter, and wound depth). This finding has also been reported in mice (Huss et al., 2019) and humans (Kausal et al., 2006; Anderson and Hamm, 2012) following wounds due to incisions. Tissue damage triggers the healing process which consists of coagulation, inflammation, and healing (Lisowska et al., 2018). There are many different intricate parts to all of these mechanisms that result in wound healing and the formation of new epithelium; however, NSAIDs have been shown to have a depressant effect on this process (Anderson and Hamm, 2012). Briefly, NSAIDs inhibit the production of inflammatory mediators prostanoids, such as PGE2, which reduces pain; however, NSAIDs also have an antiproliferative effect on skin and blood vessels which is what causes them to affect the healing process (Anderson and Hamm, 2012). Therefore, although NSAIDs are proven to reduce pain in humans (Kausal et al., 2006; Anderson and Hamm, 2012), mice and rats (Huss et al., 2019), as well as calves (Winder et al., 2018; Gladden et al., 2019; Reedman et al., 2020), they appear to also have a suppressant effect on wound healing. This slower healing may also have been a result of the increased activity level noted in the 2-dose NSAID calves. Pain results in minimized behaviors that impede wound healing and maximizes protective behaviors (Molony and Kent, 1997). Therefore, by reducing pain, 2-dose NSAID calves may have been less protective of their wounds. Although both the LP and 2-dose NSAID treatments hindered the healing process, the lower plane of nutrition had a larger effect on this outcome compared with an additional NSAID dose. The effect size of milk treatment was larger compared with the effect size of the NSAID treatments when looking at the formation of new epithelium by the end of follow-up at both the wound and calf level. Therefore, although an additional dose of NSAID impeded the wound healing process, it did not affect it to the degree that a lower plane of nutrition did. As well, we also noted that wounds on calves receiving 2 doses of NSAID had much fewer instances of purulent discharge (22% of cases were from wounds on calves receiving 2 doses), which could be a result of decreased inflammation.
al., 2019; Reedman et al., 2020), similar to the current study, other researchers have demonstrated no differences in haptoglobin concentrations when comparing pain control strategies for disbudding procedures (Allen et al., 2013; Mirra et al., 2018; Reedman et al., 2021). Although we did not detect a difference at any time between the NSAID treatment groups in the present study, we did detect that calves on the LP program had increased haptoglobin concentrations (indicator of inflammation) 7 d after disbudding compared with calves on the BN program. However, this detected difference was driven by the presence of large outliers in the data. Compared with haptoglobin concentrations in other studies (Reedman et al., 2020, 2021), the large values in our data were comparable to large values from these data sets as well, suggesting that haptoglobin concentrations could be quite variable from calf to calf (range in this study: 0.06–0.6 mg/mL). Perhaps haptoglobin concentration differences are more detectable with larger sample sizes and increased power due to this variation. These differences between studies suggest that haptoglobin concentrations may not be a reliable biomarker when evaluating and comparing pain control strategies for disbudding procedures in calves.

**Mechanical Nociceptive Threshold**

**Effect of Milk Treatment.** Based on our results, it appears that calves on the LP program were less sensitive to the MNT test compared with calves on the BN program, although there was no effect of milk treatment at any specific time during the 3 wk these measurements were collected for. The MNT is used to assess pain sensitivity in disbudding research, particularly with cautery disbudding (Heinrich et al., 2010; Stock et al., 2016; Adcock and Tucker, 2018). Past research has reported that during the healing process after disbudding, all tissue types are more sensitive compared with nondisbudded tissue and new epithelium (Adcock and Tucker, 2018; Alvarez et al., 2019). Although many researchers have reported differences in MNT values based on pain control method after disbudding (Heinrich et al., 2010; Espinoza et al., 2013; Stock et al., 2016), to our knowledge, very few if any researchers have reported differences in MNT values based on milk feeding level to young calves. There are several different reasons why calves on the LP program may have been less sensitive to the MNT test compared with calves on the BN program. This difference could be attributed to the improved healing time and shallower wounds in the BN calves compared with the LP calves during the time period when MNT measurements were being collected. Therefore, by decreasing healing time with a higher milk allotment to preweaning calves, their disbudding wounds might be in a stage of healing that is more sensitive compared with other stages. Although we are unsure whether this hypothesis has been investigated in human or animal literature, a study on wound healing in humans did report that opioid receptors affect mechanisms involved in wound healing and, thus, the pain from a wound helps to induce the healing process (Bigliardi et al., 2015).

Another potential reason for this difference in MNT between the milk treatment groups could be attributed to the calf’s ability to respond to the test. Although the MNT test is effective at determining the sensitivity of the horn bud area and pain experience of a calf after disbudding (Heinrich et al., 2010; Espinoza et al., 2013; Stock et al., 2016), calves must also have the ability to respond to the test for it to be effective. Previous research in younger calves (1–9 d old) suggest that calves in their first week of life may have less ability to respond adequately to the MNT test (Karlen et al., 2019; Reedman et al., 2020). Although our calves were much older than this, the results reported by Karlen et al. (2019) and Reedman et al. (2020) do indicate that the effectiveness of the MNT test depends on the individual calf’s ability to show a withdrawal response to the test. Therefore, it could be that the BN calves had more ability to respond earlier to the MNT test compared with calves on the LP program, due to their increased activity level. This raises the question whether the increased sensitivity noted in the BN calves is due to a potential improved ability to respond better to this pain, or if faster healing is associated with increased pain or sensitivity.

**Effect of NSAID Treatment.** The extra NSAID treatment in this study was administered to calves 3 d after disbudding, and no differences were detected between the NSAID treatment groups before this point in time. The half-life of meloxicam in calves is approximately 35 to 38 h, therefore this drug is typically effective and present in a calf’s system for up to 3 d (72 h) after administration (Allen et al., 2013). It also takes approximately 14 to 15 h for meloxicam to reach its maximum plasma concentration in calves (Allen et al., 2013), which may explain why there was no difference in MNT values 3 d after disbudding; MNT values on this day were collected 4 h after the additional dose of NSAID was administered. However, similar to past research, we detected that calves provided with an additional NSAID had decreased MNT around their horn buds compared with calves provided with less pain control (Allen et al., 2013; Glynn et al., 2013). We detected these differences up to 17 d after disbudding (no differences were detected on d 14 after disbudding), whereas other researchers have only reported these differences 6 h after disbudding between calves that received no
meloxicam compared with those that did (Winder et al., 2018). Although this effect was significant \((P = 0.03)\) 17 d after disbudding in our results, the difference in values between the treatment groups \((0.59 \text{ kgf})\) was not a large effect biologically, as our MNT values ranged from 0.5 to 10 kgf. We evaluated MNT for only 3 wk after disbudding; however, it would be interesting for future research to assess potential differences in MNT for a longer period of time.

Adcock and Tucker (2018) reported that calves disbudded at a younger age \((3 \text{ d compared with } 35 \text{ d})\) can be more sensitive to MNT tests on their rump later in life due to a systemic increase in pain sensitivity. Similar results have been reported in other species such as rodents where younger animals are more susceptible to long-term changes in pain sensitivity (Walker et al., 2009). Although our calves were not this young \((3 \text{ d of age})\) and we did not detect an effect of age at disbudding when evaluating MNT values between NSAID treatment groups, our results could suggest that by decreasing the pain experience of the calf through the provision of an extra NSAID for a couple of days after administration, calves were then less sensitive to the MNT test after the drug had worn off. Calves that were not provided with an additional NSAID could have remembered the test as being more painful without that additional pain relief and therefore reacted to the stimulation earlier compared with the calves that received the additional dose. However, it is unclear why this difference was not also observed at 14 d after disbudding. As well, we did not evaluate the concentration of this drug in the calf’s system, so we do not know how long it was present after the second dose was given. Further research evaluating the effects of an additional NSAID on the pain of calves after disbudding would be beneficial for understanding these results.

**Lying Behavior**

**Effect of Milk Treatment.** Calves on the BN program were more active compared with calves on the LP program. This was evident with decreased daily lying times, fewer lying bouts in a day, and longer lying bout duration across time in BN calves compared with LP calves. Although these differences were small biologically, this interaction between milk treatment and time was present when examining lying behavior across the entire 8 to 9 wk that data were collected but was not present when evaluating the time around disbudding specifically. Our statistical models controlled for the effect of baseline lying behavior values in the week before disbudding when evaluating the effect of milk treatment in the week after disbudding. When this was controlled for, there were no differences between the milk treatment groups in the week after disbudding suggesting that the difference observed across the entire data collection period \((d 11–77 \text{ of age})\) was due to an increase in activity in relation to greater milk intake rather than an effect of disbudding pain on lying behavior.

**Effect of NSAID Treatment.** Results from the present study indicate that in the week following disbudding, calves that received an extra NSAID were more active \((\text{decreased lying time, decreased average lying bout duration})\) compared with calves that only received one dose of NSAID. The effect of pain on long-term lying behavior has been evaluated in few studies. Sutherland et al. (2018b) followed calves for 48 h after cautery disbudding with and without a local anesthetic, and clove oil disbudding without a local anesthetic, and detected calves spent more time lying in a d 24 h after the procedure regardless of treatment group. Similarly, Heinrich et al. (2010) detected in the 5 h after cautery disbudding that calves given meloxicam in conjunction with a local anesthetic were less active compared with calves only provided with a local anesthetic. However, it has been suggested that when calves are in pain, they will display more protective behavior to minimize pain and assist in healing such as decreased activity (Mooney and Kent, 1997). This was reported by Sutherland et al. (2018b) as well, where 24 to 48 h after disbudding control handled calves were more active compared with all disbudded calves who spent more time lying in a day compared with their baseline values. Our results, in conjunction with our MNT results, suggest that providing an additional NSAID to calves 3 d after the procedure could result in decreased pain for the animals in the 1 to 2 wk after the procedure.

**CONCLUSIONS**

The results indicate that restricted milk feeding \((\text{up to } 6 \text{ L/d})\), as well as an additional dose of an NSAID 3 d after cautery disbudding, in preweaning heifer calves can slow the wound healing process; however, the latter has a lesser effect on this process comparatively. These results also demonstrate that the healing process after disbudding is quite long and variable between calves. Feeding a higher level of milk \((\text{up to } 15 \text{ L/d})\) also resulted in more active calves with more sensitive wounds, and providing calves with an additional NSAID 3 d after cautery disbudding can decrease behavioral indicators of pain in calves in the 1 to 2 wk after the procedure. In addition to the many reported benefits of feeding calves a biologically normal plane of nutrition, this study demonstrates an additional effect of improved wound healing in calves. Further research...
assessing the effects of an additional NSAID after disbudding would be beneficial for understanding the effect of this medication on pain and healing.

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