ABSTRACT

Caustic paste disbudding is increasing in popularity on commercial dairy farms in the US, but little research has explored the pain and welfare implications beyond the acute period of this procedure. Researchers have reported it takes 7 to 9 wk, on average, for hot-iron disbudding wounds to re-epithelialize in dairy calves. Our objective was to describe wound healing and sensitivity following caustic paste disbudding. Jersey and Holstein female calves were disbudded using Dr. Naylor’s caustic paste at 3 d of age (n = 18) while control calves received a sham procedure (n = 15). Before disbudding, calves received a local block and an NSAID. Calves ≥34 kg and <34 kg at birth had 0.3 or 0.25 mL of paste applied per unshaved horn bud, respectively. Following disbudding, wounds were scored 2x/wk for the presence/absence of 8 tissue categories including the final stages: new epithelium and fully healed. Control calves were removed from the experiment after 6 wk to be hot-iron disbudded and avoid more invasive procedures. Mechanical nociceptive threshold (MNT) measures were collected weekly to evaluate wound sensitivity until calves were removed from the study or wounds were healed. Wounds were slow to re-epithelialize (16.2 ± 5.7 wk, mean ± SD; range: 6.2–32.5 wk) and contract to be considered fully healed (18.8 ± 6 wk, mean ± SD; range: 8.7–34.1 wk). Compared with non-disbudded controls, paste calves exhibited lower MNT values for all 6 wk (mean ± SE, Control: 1.46 ± 0.16; Paste: 1.18 ± 0.12 N). These data indicate that wounds from caustic paste disbudding are more sensitive than intact horn buds for at least 6 wk. Future work should examine if aspects of paste application (e.g., amount, time rubbed in, age, pain mitigation), could improve healing time and sensitivity.

Key words: disbudding, dehorning, pain, burn

INTRODUCTION

Disbudding is a common procedure performed on 94% of US and 80% of EU dairy farms (Cozzi et al., 2015; USDA, 2018). Disbudding permanently damages the horn bud tissue either with a hot iron (heat) or caustic paste (chemical). Paste has become more popular, but we know relatively little about it. We evaluated the wound healing process and sensitivity following caustic paste disbudding. Wounds took an average of 18.8 wk to heal and were more sensitive than intact horn buds for at least 6 wk. Future work should examine if aspects of paste application (e.g., amount, time rubbed in, age, pain mitigation), could improve healing time and sensitivity.
A growing body of work has also shown that hot-iron disbudding is painful for calves throughout the 7 to 9 wk healing period (Adcock and Tucker, 2018; Adcock et al., 2019; Reedman et al., 2022). Disbudded calves experience decreased mechanical nociceptive thresholds (MNT), a form of stimulus evoked pain, throughout the healing period (Adcock and Tucker, 2018; Casoni et al., 2019). Evidence for ongoing pain during the healing period includes a preference for stimuli paired with pain relief (lidocaine) 3 wk after the procedure (Adcock and Tucker, 2020). Additional behavioral responses in the weeks following hot-iron disbudding include a decrease in rumination for up to 11 d, less time spent active, increased sleep and sucking for at least 21 d after disbudding compared with controls (Adcock et al., In press). Taken together, the existing body of literature demonstrates that calves experience ongoing and evoked pain from hot-iron disbudding both in the acute phase and throughout the multi-week healing process.

To address the pain associated with disbudding, producers have increasingly adopted the use of pain mitigation and sought disbudding methods that are perceived as less painful (USDA, 2018), such as caustic paste (Saraceni et al., 2021). However, caustic paste creates a 3rd degree chemical burn, typically with a strong alkaline agent like sodium and calcium hydroxide (Stock et al., 2013). A limited amount of research has evaluated the welfare implications of caustic paste disbudding but, like hot-iron disbudding, acute pain is present (Vickers et al., 2005; Stafford and Mellor, 2011; Winder et al., 2017). Pain-related responses in the hours and days following caustic paste disbudding include increased cortisol (Stilwell et al., 2009), pain-related behaviors such as head shakes and rubs (Vickers et al., 2005), reduced play the day after the procedure (Rushen and de Passillé, 2012), and place aversion by spending less time in the pen where the procedure occurred (Ede et al., 2020). Currently no research is available looking at the weeks following caustic paste disbudding in dairy calves, including sensitivity, wound healing, or size. However, research in dairy goats has shown the chemical burns from caustic paste are present for at least 6 wk (Hempstead et al., 2018a).

Our objectives were (1) to evaluate how long caustic paste wounds were present in dairy calves when disbudded at 3 d of age; (2) measure the size and depth of wounds throughout the healing process; and (3) examine when and for how long the wounds are sensitive during healing. We predicted that (1) caustic paste wounds would persist for a similar amount of time as those from hot-iron disbudding (7 to 9 wk); (2) wound size would decrease in diameter and depth as they healed; (3) disbudded calves would be more sensitive to mechanical stimulation at the wound site than intact control calves; and (4) disbudded calves would have a lower growth rate.

**MATERIALS AND METHODS**

This study was conducted from July 2021 to March 2022 at the University of California, Davis Dairy Teaching and Research Facility. The University of California Davis Institutional Animal Care and Use Committee approved all procedures (protocol #21601). Based on a power analysis (assuming \( \alpha = 0.05, 2\)-tailed) with pressure algometry data in disbudded vs. intact horn buds, we found that at least 8 calves are needed for a between-subject design to achieve power \( \geq 0.8\). This calculation was based on an expected mean difference between treatments of 1 N force and SD = 0.7 N force.

**Animals and Housing**

On the day of birth, 24 Holstein and 9 Jersey female calves were individually housed in outdoor plastic hutches (2.0 × 1.5 × 1.4 m; length x width x height) with an attached wire-fenced pen (2.0 × 1.5 × 0.9 m; length x width x height) below a shade cloth structure. Two calves were housed in wire-fenced pens (4.0 × 1.5 × 0.9 m; length x width x height) under an open sided barn for 10 d due to an outdoor hutch shortage (1/treatment). Rice hulls were provided as bedding ~15–20 cm in depth. Fresh rice hulls were added on top of existing ones approximately once per week. Ear tags were applied on 2 d of life. Calves had visual and auditory interaction with conspecifics, but physical contact was limited by 0.5 m of space between hutches.

Calves were fed according to the farm’s standard operating procedure. From birth, access to grain (Starter Calf Feed 901033, Associated Feed and Supply Co.) and water was available ad libitum. Jerseys and Holsteins were fed 1.4 and 1.9 L of milk respectively twice daily (0900 and 1600). Milk provision increased by 0.5 L per feeding at 8, 18, and 32 d of age. From 0 to 5 d calves received colostrum, then re-hydrated milk replacer (26% CP, 16% fat, 15% total solids, mixed as indicated at a rate of 142 g/L of hot water; Calva Products Inc.) via a bottle until 35–38 d of age. Milk was fed via a bucket from this time through weaning. Weaning began at 50 d in a step-down manner with calves receiving milk 1x/d and added ad libitum access to TMR. At 60 d, calves were fully weaned from milk. Before weaning, electrolytes were fed to 14 calves evenly split across treatments for 1–3 feedings each for loose manure. Of those individuals, 8 calves were preconditionally treated with Sustian III (Bimeda Inc.) once if loose manure or lack of appetite persisted after 1 feeding of electrolytes.
One calf received 3 cc of Liquamycin La-200 (Zoetis Animal Health) for pink eye.

Weaned heifers were moved to an open-sided, covered group pen (5.7 \times 4.1 \text{ m}) bedded with rice hulls at 64–70 d of age (mean = 66 d). Group pens housed 8 similarly aged heifers. At 70–139 d of age (mean = 105 d), heifers moved to a larger group pen (43.2 \times 7.2 \text{ m}) with 30 animals. An open-sided barn and rice hull bedding was available in part of the pen (6.6 \times 7.2 \text{ m}). At 100–206 d of age (mean = 161 d), heifers moved to a new group pen similar in structure to the previous one. A TMR mix of grain and alfalfa was fed to heifers in all group pens (12% CP, 34% ADF, 48% NDF, 7% Ash, 61% TDN, 0.63 NEI, Mcal/Kg).

**Treatments**

We assigned all healthy female calves born on the farm between July 3 and October 2, 2021, to 1 of 2 treatments: caustic paste disbudding (n = 18; 13 Holsteins and 5 Jerseys) or control (n = 15; 11 Holsteins and 4 Jerseys). Calves were assigned to treatments using a random number generator. Treatments were balanced through date of enrollment and by breed. Each treatment had a similar birth weight (mean ± SD: disbudded 36.6 ± 9.2 kg; sham 37.2 ± 7.9 kg).

Treatments occurred on d 3 of life to minimize the number of stressful events experienced on a single day (e.g., birth, processing, ear tagging, disbudding). On d 3 of life, calves were placed in a restraint in the home pen with their head in a relaxed, upright position, and an additional handler holding near the rump for support (Jimenez et al., 2019). For paste disbudded calves, a cornual nerve block of 5 mL of 2% unbuffered lidocaine hydrochloride (Vet One) was administered subcutaneously on each side of the head using a 20-gauge x 25-mm needle. A total of 10 mL of lidocaine was drawn up into a 12 mL luer-lock syringe. A clean needle was placed into the divot below the boney ridge of the calf’s head at a 45-degree angle pointing toward the horn bud. The needle was inserted subcutaneously up to the hub. Approximately 2.5 mL of lidocaine was injected as the syringe was moved in a fanning motion. The needle was withdrawn to approximately the halfway point and the remaining 2.5 mL was administered. Anesthesia was checked 10 min after the injection using a needle to gently prick around the base of each horn bud. If a behavioral response occurred, an additional 2 mL of lidocaine was administered on that side and checked after 5 min (1 horn bud received an additional injection). During the 10-min waiting period, calves were released from the head restraint then orally administered meloxicam at a dose of 1 mg/kg of birth weight with a balling gun. Before administration, meloxicam tablets were placed into a gelatin capsule (Torpac, size #13). Calves were placed back in the head restraint when anesthesia was checked and during paste application. Dr. Naylor’s paste (calcium hydroxide 37.8%, sodium hydroxide 24.9%, H. W. Naylor Company Inc.) was rubbed into unshaved horn buds at 0.25 or 0.30 mL/ per horn bud when calves had a birthweight of <34 kg or ≥34 kg, respectively. A 3-mL syringe was used to measure the paste. A gloved finger rubbed the paste in until a consistent layer was present over the buds (mean duration of rubbing/bud ± SD: 45 ± 11, 51 ± 20 s left and right bud, respectively). All paste was applied by AMD.

Control calves were handled for the same length of time as paste disbudded calves, received a sham injection with an air filled, needle-less syringe placed against the cornual nerve, were orally administered an empty gelatin capsule, and had their horn buds rubbed in a similar fashion to paste disbudded calves (mean duration of rubbing/bud ± SD: 38 ± 8, 38 ± 8 s left and right bud, respectively). To avoid more invasive procedures later, at 48 ± 2 d of age (46 - 51 d) control calves were removed from the study and hot-iron disbudded by AMD. Pain relief (lidocaine and meloxicam) was provided in the same manner as previously described for paste disbudded calves. An electric cautery iron (Rhinehart X50, Rhinehart Development Corp.) was heated (416 ± 10°C) and applied to each horn bud until a uniform copper ring formed (mean duration of application/bud ± SD: 14 ± 2, 13 ± 2 s left and right bud, respectively).

**Data Collection**

All calves were assigned to a weekly data collection day where all variables were measured (e.g., Tuesday), with the first observation occurring within 7 d of disbudding. A second weekly observation day where only wound healing was observed occurred 3 d later. During data collection, calves were placed in the previously described head restraint unless live wound tissue scoring was the only measure being collected. The head restraint was adjusted to the height of the calf so that her head could rest in an upright but natural posture. Before weaning, this restraint occurred in the home pen. After weaning, to avoid interference from conspecifics, calves were removed from their group pen and walked down an alley to a small enclosure ~3 m away where the head restraint was placed.

**Wound Healing.**

**Tissue type** Wounds were monitored for the presence/absence of 8 tissue types: fresh paste, attached
necrotic, detaching necrotic, exudate, granulation, crust, new epithelium, and fully healed (Figure 1). Live scoring occurred 2x/wk, once on the day of other measurements and 3 d later until all wounds were fully healed. Live wound scoring was completed by AMD and 2 trained observers. Reliability of identifying the 8 tissue types was determined using an 80-photo test and compared with AMD (Cohen’s κ > 0.80 for each type). The test contained a minimum of 10 photos per tissue type, but some had a combination of crust, granulation, and exudate which were not mutually exclusive. Wounds were considered re-epithelized when initially scored with that tissue and fully healed when they were scored as such for 2 consecutive measurements. To help with wound visibility, the hair around the wound was clipped the day after paste application and weekly until fully healed with a size 40 blade.

Calves were monitored for horn regrowth using visual inspection and palpation throughout the experiment and approximately every other month after healing until 6 mo of age (Supplemental Figure S1). Scurs or regrowth occurred in 30% of paste disbudded horn buds. All regrowth was deemed too small to be of concern by the farm manager.

**Wound Measurement** Caustic paste disbudding wounds were measured 1x/wk until they were considered fully healed or December 9, 2021. At that point, due to time and labor constraints, only wound tissue scoring was continued until all paste disbudded calves were fully healed (March 2022). On December 9, 2021, 17 out of 36 horn buds were fully healed, and all control calves had aged out of the experiment. Wound measurements were taken with a 150 mm MC1630EWRI Digital Caliper (Mahr GmbH). A total of 4 measures per wound were collected: diameter of the wound in 2 directions (sagittal and frontal length), and depth at the cranial and caudal positions (Figure 2). Depth was measured at the inner edge of the wound from the bottom of the damaged tissue to the surrounding undamaged area. No formal reliability was conducted, but operators were trained for consistency on pilot calves by measuring wounds consecutively with AMD.

**Sensitivity** Tissue sensitivity was assessed on the same day as wound size through MNT with a digital algometer (ProdPlus; TopCat Metrology Ltd.) fitted with a 4-mm diameter round rubber tip placed ~3 mm from the edge of the wound or horn bud. Control calves were tested weekly for 6 wk. Paste calves were tested weekly until fully healed or December 9, 2021.

Tests occurred at 4 locations per wound or horn bud: caudal, lateral, cranial, and medial. We began with the caudal position and followed a clockwise pattern. An additional measurement was collected on a shaved patch of the left flank for each calf to determine whether disbudding increased sensitivity at a site distal to the injury. Testing order (left flank, left horn bud/wound then right) remained consistent across the experiment as previous work found no effect of testing order on MNT (Adcock and Tucker, 2018). Before data collection, the operator (AMD) met a set of criteria described in Adcock and Tucker (2018). Researchers and assistants were not blind to treatment.

During the tests, the calf was placed in the previously described head restraint and blindfolded to help keep her calm. The operator placed their fingers outside of the testing location and positioned the algometer tip on the calf’s skin using < 0.01 N of force. Once the calf had ceased any movement, force was applied at a constant rate (mean ± SD: 0.28 ± 0.11 N/s) until the calf reacted. For testing locations on the head, ear flicks or head movements stopped the test. On the flank, tail swishes or leg movement completed the test. Our approach, of first touching the calf and then applying the algometer, was designed to target c-fiber nociceptors, which have a delayed response compared with a-fibers that respond to touch alone (Ringkamp et al., 2013). As such, we are moving beyond the initial reaction of the calf, into responses that are processed by the central nervous system. No more than 10 N of force was applied. Videos (Panasonic HC-V550) were used to quantify the rate of force applied in each test (0.5% of videos missing overall). If force was applied at a rate < 0.1 or > 0.6 N/s these data were excluded (8% of tests). Tests were repeated if interruptions occurred (loud noises, animals urinating or defecation, fly landing on the animal). If a test was interrupted 3 times, it was abandoned (0.6% of tests).

**Weight** Calves were weighed on their DOB and 1x/wk (on the day of MNT and wound size measurement) until weaning began at 50 d of age (VS-660 scale, 300 kg limit/0.1 readability, A and A scales LLC.). Weights were taken 1 d before weaning began and 1 d before weaning ended. Average daily gain was calculated for each calf using the difference between the most recent weight and the previous measurement, divided by the number of d between them for wk 1–6. One ADG was calculated for the weaning period (wk 8).

**Statistical Analysis**

All data, RMarkdown files for analyses and figures, and a supplemental table containing means, SE, CI, test statistics, df, and P-values for analyses are available in the Dryad repository: https://datadryad.org/stash/share/wO6rgJ8-pC_xar0oV7fCj3oPjIrHTjE_P7fGmsKIdC8. Sample sizes will be given throughout
the results section to account for MNT and wound measurement data that were not collected after December 9, 2021. Two ADG measurements are missing from one calf in the Paste treatment group. Four negative ADG values were dropped, 2 from each treatment (3 in wk 1 or 2, and 1 in the weaning period).

<table>
<thead>
<tr>
<th>Examples (Holstein and Jersey)</th>
<th>Tissue</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh paste</td>
<td>No visible damaged tissue. The wound area is covered in red/orange paste (Dr. Naylor’s). This tissue type is mutually exclusive.</td>
<td></td>
</tr>
<tr>
<td>Attached necrotic</td>
<td>Edges are defined and are in contact with the surrounding tissue. This tissue type is mutually exclusive.</td>
<td></td>
</tr>
<tr>
<td>Detaching necrotic</td>
<td>Any part of the wound edge is cracked or lifting away from the surrounding tissue underneath. This tissue type is mutually exclusive.</td>
<td></td>
</tr>
<tr>
<td>Exudate</td>
<td>Wound is moist and either bright red, yellow, cream, or white in color.</td>
<td></td>
</tr>
<tr>
<td>Granulation</td>
<td>Bumpy light red or dark pink tissue.</td>
<td></td>
</tr>
<tr>
<td>Crust</td>
<td>Rough tan, dark red or dried exudate.</td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>A layer of translucent skin is present; other tissue types and exudate are absent. Wound is light pink or opaque gray depending on the skin color. This tissue type is mutually exclusive.</td>
<td></td>
</tr>
<tr>
<td>Fully healed</td>
<td>Wound is fully contracted into a thin scar line with no round spots, at the plane of undamaged tissue. Other tissue types or exudate are absent. This tissue type is mutually exclusive.</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** The system used to evaluate tissue types in caustic paste disbudding wounds. Examples from both Holstein and Jersey calves are presented throughout the healing process.
Statistical analysis and plotting was conducted in R (R Core Team, 2022). Wound size and healing progress are descriptive and presented in boxplots (ggplot2 package version 3.3.6). All fixed and random effects included in our models were based on a-priori predictions, therefore no backward model selection was conducted.

We tested the effects of caustic paste disbudding on MNT in the first 6 wk using a linear mixed model with an identity variance structure to account for heterogeneous variance across weeks (nlme package version 3.1–155). Treatment, location around the horn bud, side, week, and the interaction between week and treatment were included as fixed effects while calf was a random effect. For flank MNT sensitivity, a linear mixed effects model was used to compare treatments in the first 6 wk with treatment, week, and their interaction as fixed effects, and calf as a random effect. During preliminary visual assessment of data, there was no evidence that breed had an effect on MNT and was not included in the model.

The effect of tissue type on sensitivity was tested in disbudded animals, using comparisons among their unhealed tissue types and MNT measurements once fully healed. Data were only used from horn buds that reached the fully healed point before MNT measurements were stopped. A 2-tailed paired t-test (t.test function in base R) was used.

The effect of disbudding on ADG was tested for the pre-weaning period (wk 1–6, n = 33) and the weaning period (wk 8, n = 32). Pre-weaning ADG was calculated with a linear mixed model with an identity variance structure to account for heterogeneous variance across weeks (nlme package version 3.1–155). Treatment, breed, week, and their 3-way interaction were fixed effects while calf was a random effect. Weaning ADG was calculated as 1 measure (wk 8) and assessed using a 2-tailed unpaired t-test (t.test function in base R). Breed was included as a fixed effect for ADG as it has been shown to influence these data (Ballou et al., 2013).

All linear mixed models were fit using the REML method implemented in the “nlme” package (version 3.1–155). A type 3 ANOVA was used on all linear mixed models (car package version 3.0–12). All model fits were checked for normality and homogeneity of variance using QQ plots and plots of residual versus fitted values (plot, boxplot, resid functions in base R); unless stated otherwise, all model assumptions were met.

Figure 2. Locations around the wound or horn bud where sensitivity (MNT) measurements were collected. The MNT locations consisted of caudal, cranial, medial, and lateral spots on both caustic paste disbudded calves and controls for both sides of the head. Disbudded calves also had wound size measurements collected for each side of the head in 4 locations: frontal and sagittal length, cranial and caudal depth (marked with x). Sagittal length was evaluated between the depth x’s. Frontal length was measured perpendicular to the sagittal line.
**RESULTS**

*Wound Healing*

**Tissue type.** Wounds took 114 ± 40 d (mean ± SD; range: 44–228 d) to be scored as re-epithelialized for the first time and 132 ± 42 d (mean ± SD; range: 61–239 d) to fully heal (n = 36 horn buds). Figure 3 shows the days relative to disbudding that each tissue was present during the healing process. Detaching necrotic tissue was present for the longest amount of time (mean ± SD: 91 ± 31 d; range: 31–175 d from disbudding).

**Wound Measurement.** Sagittal and frontal length decreased throughout the healing process (Figure 3). At the first measurement, both the frontal and sagittal length were 24.1 ± 3.8 mm (mean ± SD). Cranial depth and caudal depth initially measured 1.6 ± 0.7 and 1.2 ± 0.6 mm respectively. Both the cranial and caudal depth increased or sunk further into the head before decreasing in size (range: 0.0–6.0 and 0.0–6.2 mm respectively).

**Sensitivity** Paste disbudding wounds were more sensitive to mechanical stimulation than intact horn buds for at least 6 wk (P = 0.01; Figure 4). There was no evidence that side, location around the wound, week, or an interaction between week and treatment affected sensitivity (P ≥ 0.19). There was no evidence that flank sensitivity was affected by treatment, week, or their interaction (6 wk mean ± SD: 2.8 and 2.9 ± 1.4 N paste and control calves, respectively; P ≥ 0.41; Figure 5).

Sensitivity throughout the healing process was analyzed by comparing each tissue type to when they were fully healed. Detaching necrotic tissue, granulation and crust wounds were more sensitive (P ≤ 0.05; Figure 6), while there was no evidence that attached necrotic tissue, exudate, and epithelium differed from fully healed tissue (P ≥ 0.63).

**Weight.** There was no evidence that ADG differed between caustic paste disbudded and non-disbudded controls during the pre-weaning period (wk 1–6; 0.64 ± 0.2 and 0.64 ± 0.3 kg/d respectively; P = 0.89, Figure 7). Week was a significant variable during the pre-weaning period (P < 0.01), with all calves gaining weight over time. Breed, and the 3-way interactions between breed, treatment, and week showed no evidence of differences (P ≥ 0.26). During the weaning period there was no evidence that ADG differed between caustic paste disbudded calves and controls (mean ± SE, 0.61 ± 0.05 and 0.75 ± 0.07 kg/d respectively; P = 0.16, Figure 7).

**DISCUSSION**

Caustic paste wounds took an average of 16.2 wk to re-epithelialize and 18.8 wk to fully heal. While the borders of the wounds contracted over time, the damaged tissue initially sunk before filling in and becoming flush with the rest of the head. The caustic paste wounds were more sensitive to mechanical stimulation than non-disbudded horn buds for 6 wk after the procedure. There was no evidence that ADG differed between treatments, but indicates growth was not optimal for these calves.

Wound healing is potentially affected by the paste amount, type, and application technique. However, it is difficult to compare our approach to the existing literature due to the variability in how caustic paste disbudding is reported and what information is described. For example, some studies report applying paste with a gloved hand (Karlen et al., 2019) while others report using a spatula (Stilwell et al., 2009). Still Brooks et al. (2021) report wiping the paste off 1 h after application, but no others have described this approach. Shaving the hair around the horn bud is reported in varying degrees of detail, with some mentioning clipping (Stilwell et al., 2009) while others report clipping a 30-mm radius leaving 1 mm length of hair (Karlen et al., 2019), but do not mention the blade size used (e.g.10 vs 40). The use of petroleum jelly to create a barrier around the paste is reported in several studies, but size is not mentioned or varies from 16 to 18+ mm in diameter (Hempstead et al., 2018b; Ede et al., 2020; Still Brooks et al., 2021). Paste brand is commonly reported, while quantity is either not reported (Stilwell et al., 2009; Winder et al., 2017), described as spread in 16–18 mm in diameter with a 1 mm thickness (Karlen et al., 2019; Ede et al., 2020), or volume is used with 1.6 mL/bud (Hempstead et al., 2018a; b). Only 2 other studies report the length of time paste was rubbed into the bud (Hempstead et al., 2018a; b). The inconsistencies of how paste is used and reported makes it difficult to draw conclusions among studies.

Caustic paste wounds took an average of 16.2 wk to re-epithelialize and 18.8 wk to fully heal. While the borders of the wounds contracted over time, the damaged tissue initially sunk before filling in and becoming flush with the rest of the head. This is the first study to examine the entire wound healing process for caustic paste disbudding. In goat kids, caustic paste wounds have been shown to persist for at least 6 wk (Hempstead et al., 2018a). The caustic paste wounds in our study persisted for twice as long as those from hot-iron disbudding in dairy calves and goat kids, which have been reported to re-epithelialize in 7 to 9 wk (Adcock and Tucker, 2018; Adcock et al., 2019; Alvarez et al.,...
Figure 3. Days each tissue was observed relative to disbudding and measurement of wound size during that time. Whiskers indicate the 2nd and 98th percentiles and circles indicate arithmetic means. The top and bottom of the boxes indicate the 75th and 25th quartiles, respectively. The number of horn bud observations is displayed above each tissue type. Wounds were not measured once they were considered fully healed.
While these studies only measured wound healing to re-epithelialization rather than full contraction, the wounds in our study took an average of 16.2 wk to be covered in new tissue. Our study is the first to monitor all wounds until they were fully contracted. Reedman et al. (2022), also scored wound contraction after hot-iron disbudding, but only 19/160 wounds reached this point in their 7–8 wk observation period.

Necrotic tissue was present for an average of 13 wk after disbudding and represented the longest phase of the healing process. Following hot-iron disbudding, necrotic tissue has been reported to persist for only 2–4 wk (Adcock and Tucker, 2018; Adcock et al., 2019). In human burn patients, necrotic tissue is reported to inhibit wound healing and serve as a medium for bacterial growth (David and Chiu, 2018). It is possible the depth or sinking pattern of the caustic paste wounds may have influenced this tissue type. Indeed, in humans deeper burns has been shown to have longer healing times (e.g., Jackson, 2005; Merz et al., 2010). Additionally, once the necrotic tissue came off, calves moved quickly through the remainder of the healing period to re-epithelialized tissue in an average of 3.2 wk.

The borders of the wounds contracted over time, consistent with other studies that have measured wound size following hot-iron disbudding in goat kids and dairy calves (Huebner et al., 2017; Alvarez et al., 2019; Ridgway et al., 2022). Our wounds had an initial mean frontal and sagittal length of 24.1 mm which is larger than the 18–23 mm in diameter reported in hot-iron disbudding studies (Adcock et al., 2019; Alvarez et al., 2019; Reedman et al., 2022). While the absolute size of wounds differs among studies, a pattern of wound contraction throughout healing is seen, regardless of the method used. Reedman et al. (2022) also reported depth of the wound as they scooped the horn bud out with the hot iron creating a concave wound. On the day of disbudding, their wounds were 5.2 ± 0.1 mm deep which decreased over time (Reedman et al., 2022), again exhibiting a contracting pattern. In contrast, caustic paste wounds in our study had an initial cranial and caudal depth of 1.6 and 1.2 mm, but then sunk following the initial measurement before filling in and becoming flush with the rest of the head. This sinking pattern led to deep wounds that were unexpected and likely contributed to the long duration of the healing process. We expect the size of the wounds is influenced by the amount of paste used and application technique. Indeed, hot-iron disbudding wounds tended to differ in size for 2 wk after the procedure when 2 iron types were compared but the application technique was similar (Adcock et al., 2019).

Despite the increasing use within the US and unsupported perception that it is less invasive than cautery techniques, caustic paste disbudding is not a pain-free method (Winder et al., 2016; Saraceni et al., 2021). There is convincing evidence about acute responses (Stafford and Mellor, 2011; Stock et al., 2013), such as higher cortisol (Stilwell et al., 2009), more pain related behaviors (Vickers et al., 2005; Winder et al.,
higher heart rate (Winder et al., 2017) than non-disbudded controls. Ours is the first evidence that caustic paste wounds were more sensitive to mechanical stimulation than non-disbudded tissue for at least 6 wk. These findings are consistent with hot-iron results (Adcock and Tucker, 2018; Casoni et al., 2019), and demonstrate that calves experience stimulus-evoked pain following both methods of disbudding.

Our MNT results align with several studies, but also differ from others. The direction of our results (disbudded more sensitive than intact horn buds) is consistent with hot-iron (Adcock and Tucker, 2018) and caustic paste studies (Winder et al., 2017; Hempstead et al., 2018b). The magnitude of our thresholds is slightly lower than Adcock and Tucker (2018), 1.2 vs. 1.55 N, and Winder et al., (2019), once converted to comparable units (0.09 vs. 0.34 N/mm²). This could be in part due to the use of a more subtle response of ear flicking in the current study, compared with a calf moving her head away from the algometer described by Adcock and Tucker (2018) or to differences in brand or tip size of the algometry devices being used (TopCat ProdPlus 2017).

Figure 6. Disbudding wound sensitivity (measured as force, N) for each of the 7 tissue types present in the wound. Sensitivity varied significantly from fully healed tissue for detaching necrotic, granulation and crust (*; P ≤ 0.05; indicated by purple). There was no evidence that attached necrotic tissue, exudate, and epithelium were different from fully healed (P ≥ 0.35; indicated by light gray). Whiskers indicate the 2nd and 98th percentiles and circles indicate least squares means. The top and bottom of the boxes indicate the 75th and 25th quartiles, respectively. The number of horn buds in which each tissue was observed (indicated above the box) differed because some tissue types were present for longer periods than others and not all calves reached the fully healed stage when sensitivity measures were halted.

Figure 7. ADG for wk 1–6 (pre-weaning) and wk 8 (weaning) by treatment and breed. ADG was calculated by using the difference in weight from the most recent measurement divided by the number of days between them. There was no evidence that ADG differed between treatments during wk 1–6 (P = 0.48), or during the weaning period (P = 0.16).
with 12.5 mm² tip vs Wagner Force Ten with 79 mm² tip used by Winder et al., 2017). In contrast, 2 previous studies found no differences in MNT between paste and intact horn buds for calves 16 d old or less (Karlen et al., 2019; Reedman et al., 2020). These differences could be in part due to MNT technique. It is possible the use of the head restraint and subtle response of ear flicking in our study allowed us to detect differences during a time when the young calves are developing locomotor skills and easily fall over if not supported, particularly if pressure is applied to a body part.

While we observed no evidence of difference in flank MNT between paste and control calves (6 wk mean ± SD: 2.8 and 2.9 ± 1.4 N respectively, P ≥ 0.76), it is possible a longer period of observation could influence these results. Adcock and Tucker (2018) observed no difference in flank sensitivity between disbudded and intact calves for 3 wk following the procedure, but within disbudded calves an effect of age at the time of the procedure (3 vs 35 d of age) was present during the later stages of wound healing (3 d: 2.81 ± 0.29 N; 35 d: 3.93 ± 0.29 N). Disbudding occurs at an early developmental stage, which can potentially lead to lifelong negative welfare implications such as heightened sensitivity to future painful stimuli (Adcock, 2021). Previous work in rodents has shown a heightened sensitivity to mechanical stimulation later in life despite an initial similar or less sensitive response than control animals (Moriarty et al., 2019; Walker, 2019; van den Hoogen et al., 2020).

Sensitivity has also been shown to be heightened in hot-iron disbudded goat kids (Alvarez et al., 2019) and dairy calves (Adcock and Tucker, 2018) when wound tissue is present, compared with re-epithelialized skin. We found detaching necrotic tissue, granulation, and crust to be more sensitive compared with healed wounds. The lack of evidence of a difference between sensitivity of wounds with attached necrotic tissue and exudate could be, in part, due to our small sample size. Indeed, MNT values were collected on 10 and 6 horn buds for attached necrotic tissue present when data collection ended 6 wk after the procedure (Hempstead et al., 2018b). Feeding level may also influence MNT responses. Reedman et al., (2022) found that calves fed 6 L/d were consistently less sensitive during MNT testing than calves offered 15 L/d, indicating that, if anything, our results may underestimate how sensitive caustic paste disbudding wounds are. There are many benefits of feeding calves to a biologically-normal plane of nutrition (Jasper and Weary, 2002; Khan et al., 2011), but they are unlikely to reverse or resolve the serious concerns associated with disbudding wounds.

**CONCLUSIONS**

Caustic paste disbudding wounds took an average of 18.8 wk to fully heal, twice as long as those from hot irons (7 to 9 wk). These wounds were more sensitive than non-disbudded tissue for at least 6 wk. These results highlight the importance of understanding the long-term implications of routine management procedures used on dairy calves.

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