



Objective determination and quantification of pain and inflammation associated with digital dermatitis in feedlot cattle

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ABSTRACT

Digital dermatitis (DD) is an infectious skin disease in cattle that causes pain and discomfort, significantly impacting animal welfare. Although DD lesions are painful and prone to bleeding when touched, pain resulting from DD has not yet been objectively quantified. The aim of this study was to objectively quantify pain associated with DD and determine the association between pain and locomotion score (LS). A second objective was to determine the association between foot temperature (FT) and pain. In total 480 cattle (heifers and steers) from 3 feedlots were enrolled. Biweekly pen walks were performed to assess hind feet for DD and altered gait. Cattle presenting with clinical signs of DD during pen walks and at routine re-handling were selected for detailed foot examination. Cattle were assigned an LS (4-point; normal to severely lame) as they walked four strides down an alleyway. Next, while restrained in a chute cattle were clinically appraised for DD, mechanical nociceptive threshold (MNT) measured using pressure algometry, and FT captured using thermal images. Each hind foot was scored as DD absent or present. Further, DD lesions were classified as active or chronic. In total 116 of 510 feet had DD (61 active and 55 chronic) in 255 cattle. Feet with DD lesions withstood 6.37 N less pressure on average than feet without ($P < 0.001$). Active lesions were most sensitive, withstanding 8.11 N less pressure than feet without ($P < 0.001$) and 4.06 N less pressure than chronic lesions ($P = 0.004$). Feet with chronic lesions withstood 4.05 N less pressure than feet without lesions ($P = 0.001$). Seventy-eight cattle presented with DD of which 34 were lame. Moderate to severely lame cattle withstood 3.1 N less pressure than non-lame cattle ($P = 0.01$). An increase of 1 °C in maximum foot temperature (MFT) was associated with a 0.60 N decrease in MNT ($P < 0.001$). Feet with DD had higher MFT compared to feet without ($P < 0.001$). MFT was higher in active ($P = 0.011$), and chronic ($P = 0.001$) lesions compared to feet without lesions. No difference was observed between active and chronic lesions ($P = 0.79$). MFT was higher in lame cattle with DD compared to non-lame cattle with DD ($P = 0.005$). Our results demonstrated that all DD affected cattle experience pain as measured using MNT, especially lame cattle who also expressed higher MFT. These are important findings when developing strategies for pain mitigation and detection.

1. Introduction

Digital dermatitis (DD) is a serious infectious foot disease that negatively impacts cattle production, economics, and welfare (Kulow et al., 2017; Orsel et al., 2018). DD lesions are characterized as being ulcerative or necrotic (Cheli and Mortellaro, 1974; Döpfer et al., 1997), inflammatory (Watts et al., 2018), painful and prone to bleeding upon touch (Read and Walker, 1998), and a major cause of lameness (Terrell et al., 2017; van Huyssteen et al., 2020). Lesions are typically located above the heel bulbs and can be described macroscopically using the 6-point M-stages ('M' for Mortellaro) scoring system, which scores

lesions based on morphological changes in the lesion over time (Döpfer et al., 1997; Berry et al., 2012). Although DD is common in dairy cattle, few studies have examined DD in beef cattle. Furthermore, in Canada, DD is considered an emerging disease in feedlot cattle (Orsel et al., 2018).

Although pain cannot be directly measured in animals, it can be inferred by measuring production parameters, physiological responses, and behaviour (Prunier et al., 2013). Assessing and quantifying pain associated with DD is essential to understanding the welfare impact of DD on cattle, for the development of pain prevention protocols and to determine the need for pain mitigation. Previous studies have quantified

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DD-associated pain in dairy cattle by evaluating (1) behaviours such as limb withdrawal, kicking and falling (Stilwell et al., 2019), (2) changes in locomotion (Laven and Logue, 2006), (3) behaviour grouped into subjective pain scores (Britt et al., 1999; Shearer and Hernandez, 2000), (4) nociceptive threshold (Whay et al., 2005; Dyer et al., 2007), and (5) infrared thermography (IRT) as a proxy for inflammation (LokeshBabu et al., 2018). In this study we used a combination of indicators including locomotion scoring, mechanical nociceptive threshold (MNT), and IRT to investigate pain and inflammation associated with DD in beef cattle.

One of the most frequently used behavioural indicators of pain associated with foot disorders in cattle is locomotion scoring (Gigliuto et al., 2014), which has been shown to be correlated with the severity of foot lesions (Whay et al., 1997; Winckler and Willen, 2001; O'Callaghan et al., 2003). Using locomotion scores solely to quantify pain is challenging, as these scores are based on subjective scales, and training and intra- and inter-observer agreement over time are essential for proper usage and interpretation (Engel et al., 2003; Channon et al., 2009; Flower and Weary, 2009). Also, multiple scoring systems are available for dairy (Whay, 2002) and beef (Larson et al., 2014; Edwards-Callaway et al., 2017) cattle preventing direct comparison of results between studies.

Changes in pain sensitivity can be measured objectively by using pressure algometry, an objective technique that applies continuing pressure from a noxious stimulus to the area where pain is being assessed; the pressure at which withdrawal response is observed is identified as the MNT (Coetzee et al., 2017). Pressure algometry has been shown to have good inter-observer reliability when used to assess MNT at several anatomic locations on dairy cows (Raundal et al., 2014). Inter-observer reliability was determined using the Bland-Altman plot (Bland and Altman, 1986) and the difference between observers (95% limits) was 0.2 kgf (2.8) which is considered small. Additionally, the agreement index for the algometer was 0.58 which is considered good (Kampen et al., 2004). IRT is a non-invasive technique used to measure surface temperatures (radiated heat) of animals and variation in temperature signaling inflammation or fluxes in metabolic activity of underlying tissues (LokeshBabu et al., 2018). Inflammation, an immune response to damage or pathogens can be painful due to swollen tissue pushing against nerve endings.

Unlike many dairy DD studies, which have taken the approach to evaluate the efficacy of treatment strategies (Whay et al., 1998; Laven and Logue, 2006; Cutler et al., 2013; Capion et al., 2018; Kasiora et al., 2021) the aim of this study was to objectively quantify pain associated with DD M-stages and determine the association between pain and locomotion score. A second objective was to determine the association between foot temperature and pain. We hypothesized that pain and inflammation resulting from DD could be objectively determined using pressure algometry and infrared thermography and that these methods could improve the current methods for assessing pain due to DD.

2. Material and methods

This study was conducted between November 2018 and November 2019. In accordance with the ethical principles of the Canadian Council on Animal Care, this study was approved by the University of Calgary Veterinary Services Animal Care Committee (AC17-0224). Written informed consent was obtained from participating feedlot owners for the use of their cattle.

2.1. Feedlot and cattle selection

Three feedlots located in southern Alberta Canada were enrolled in this study. These feedlots were a good representation of the feedlot industry in Western Canada with total capacity between 5000 and 10,000 cattle on outdoor dirt pens protected with wind breakers. Feedlot inclusion criteria included: commercial feedlot with a history of DD within the last three years, a working squeeze chute for detailed hind foot

examination, cattle procured from auction, and a readiness to participate. Using a stratified systematic sampling method, 480 of 3406 Angus type cattle (heifers and steers) from 13 pens were enrolled in the study. Initial mean body weight (BW) \pm SD for 320 heifers was 279 ± 67 kg, and 340 ± 77 kg, for 160 steers. Both calves (8–10 months of age) and yearlings (11–13 months of age) were enrolled. On arrival at the feedlot, cattle were assigned a unique feedlot ID (colour coded, numbered plastic ear tag), processed (vaccination, parasiticide, growth-promoting agents) and housed in outdoor hay-bedded dirt pens with their contemporary group. Each pen housed between 200 and 280 cattle. Across feedlots cattle transitioned between five to eight different rations (high-energy grain based) depending on their age at arrival at the feedlot, days on feed (DOF), sex, grain type and market endpoint. General management was similar across feedlots.

2.2. Digital dermatitis classification

DD was scored visually using the M-stage lesion scoring system, which is currently the most widely used system in research. Developed by Döpfer et al. (1997) and modified by Berry et al. (2012) the M-stage lesion scoring system classifies DD lesions into six stages: M0 - digital skin is normal with no lesions; M1 - small focal active state < 2 cm across; M2 - larger ulcerative active stage > 2 cm across; M3 - healing stage covered by a scab; M4 - chronic stage proliferative hyperkeratotic growth; and M4.1 - chronic stage with a small active M1 focus.

Prior to data collection three observers completed a training program to identify and score DD lesions. Consistent with the program developed by Gibbons et al. (2012), each observer received digital coloured photographs with descriptors illustrating the M-stage scoring system along with 30 lesions to score. Three and seven days later each observer scored all 30 lesions again. Three additional training sessions were conducted throughout the study, using 30 photographs of different lesions each time. Kendall's coefficient of concordance was used to determine inter-observer agreement throughout the study. Observers remained in agreement throughout the study ($P < 0.001$).

2.3. Pen observations

To identify cattle for detailed foot examinations, pen walks were completed. Biweekly, the hind feet of cattle were observed from a distance, with a focus on the area above the coronary band and between the heel bulbs for the presence or absence of DD, and each hind foot was assigned an M-stage score. Observers stood directly behind cattle ensuring a direct line of sight to hind feet and when needed binoculars (Nikon PROSTAFF 3S, Nikon Canada Inc., Mississauga, Ontario) were used to zoom in on feet. Next, locomotion was observed, and a locomotion score (LS) assigned using ZINPRO's Step-Up Locomotion Scoring System (ZINPRO, 2013), where an LS of 0 (LS0) is normal, no apparent lameness or change in gait; an LS of 1 (LS1) indicates mild lameness, cattle exhibit short strides and dropping of the head slightly when walking; an LS of 2 (LS2) is considered moderate lameness when cattle have an obvious limp and slight head bob when walking; and an LS of 3 (LS3) is representative of severe lameness when cattle apply little or no weight to the affected limb with an obvious head bob and arched back when walking. A subset of six cattle per pen (representative of each M-stage), were selected for detailed examination (locomotion scoring in the alleyway and foot examination in the squeeze chute) two to four times throughout the feeding period. Criteria for selection for detailed examination included: DD lesion absent, DD lesion present or LS greater than 0 with signs of DD. Cattle were also selected for detailed examination during routine re-handling events which occurred every 60–240 DOF. Re-handling events were in accordance with the feedlot's management protocol and included processing activities such as vaccinations, weight sort, and administration of parasiticides and growth-promoting agents.

2.4. Alleyway observations

Prior to detailed foot examination in the squeeze chute, cattle were moved individually down a dirt alleyway a minimum of four strides for locomotion assessment. While walking, cattle were video recorded from the rear (Sony NEX-VG10, Sony, Tokyo, Japan) and sides (GoPro Hero 3+ (GoPro, San Mateo, CA, USA). Using these videos, an experienced observer (observer 1) assigned all cattle an LS corresponding to the time of detailed foot examination. A second observer (observer 2) scored a subset of the videos (20%), and inter-observer reliability was determined.

2.5. Chute observations

Two groups of cattle, those selected from pen walks ($n = 194$) and those selected at routine re-handling ($n = 116$), were moved to a squeeze chute where each hind foot was examined in detail (MNT, DD M-stage and IRT). One observer (observer 1) measured MNT twice on each hind foot, once while standing still and upright (SMNT) and again while a hind foot was lifted and secured with a rope attached to the squeeze chute (LMNT). MNT was measured at the lesion site (area between the heel bulbs) using a pressure algometer (ProdPlus, Topcat Metrology Ltd, Ely, Cambridgeshire, England). The ProdPlus algometers provide an objective measure of pain as they are designed with an air cushion which absorbs any movement from the animal without distorting the threshold force reading. Additionally, these algometers have a pressure rate onset which modulates applied pressure and retains the force reading once removed, allowing for consistency in data collection. The algometer tip, a 1 mm brass end with an edge radius of 10% of the diameter, was pressed against the lesion site at a rate of 2 N/sec until the withdrawal response (withdrawal of the foot). The algometer measurements were censored at 20 N, which was the maximum value that could be recorded. The minimum value of 0 N was possible when a withdrawal response occurred immediately after the algometer touched the lesion site. Next, with the hind foot still lifted, the lesion site was cleaned with water and a brush and then dried with paper towel to remove any manure or dirt. Two observers visually appraised each hind foot for DD and assigned an M-stage score. Finally, a thermal image camera (FLIR E60 Thermal Imaging Camera, FLIR Systems, Inc., USA) was used to capture IRT photos of the lesion site. The process was repeated for the second hind foot.

2.6. Thermal image data extraction

Typically, three images of the lesion area (above the coronary band and between the heel bulbs) were taken and the sharpest image with the best focus was selected for analysis and data extraction. The FLIR E60 has a temperature range of $-20\text{ }^{\circ}\text{C}$ to $+650\text{ }^{\circ}\text{C}$, accuracy of $\pm 2\%$ and a resolution of 320×240 pixels. Emissivity was set to 0.98, reflective temperature at $20\text{ }^{\circ}\text{C}$, distance from foot was approximately 0.5 m, and images were amended with mean atmospheric temperature. The FLIR Tools software (v5.13.18031.2002) was used to extract the maximum temperature within the lesion area, which was outlined using the box measurement tool. Data from each image was organized in a Microsoft Excel spreadsheet (v16.0; Microsoft Corporation, Redmond, WA).

2.7. Data management

A dichotomous scoring system was created for each hind foot using the M-stages: DD absent (M0) and DD present (M1, M2, M3, M4 and M4.1 merged). Some M-stages had low frequencies: M0 ($n = 475$), M1 ($n = 9$), M2 ($n = 50$), M3 ($n = 5$), M4 ($n = 60$) and M4.1 ($n = 21$); therefore, we created another variable which combined M-stages into a simplified scoring system: DD absent (M0), active lesions (M1, M2 and M4.1 merged) and chronic lesions (M3 and M4 merged). Cattle with missing MNT measurements ($n = 55$) due to problems with the

algometer ($n = 43$) or cattle with only one hind foot measurement because of safety concerns (kicking; $n = 12$), were excluded from the analysis, resulting in 255 cattle included in the final analysis. Additionally, cattle with locomotion scores of LS2 and LS3 were merged as LS3 had low frequencies; LS0 ($n = 200$), LS1 ($n = 26$), LS2 ($n = 24$) and LS3 ($n = 5$). Data was organized in a Microsoft Excel spreadsheet.

2.8. Statistical analyses

For all analyses, a P-value < 0.05 was considered statistically significant and in cases of multiple comparisons, a Bonferroni correction was applied. Foot was the statistical unit, and all analyses were based on the DD M-stages (dichotomous or simplified scoring system). Based on a power calculation with the R package 'irr' (R for Windows, R Foundation for Statistical Computing) the sample size required to determine intra-observer agreement when assessing locomotion was calculated. Using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA) intra- and inter-observer reliability for locomotion scores were assessed using weighted kappa, κ_w (Cohen, 1968) with linear weights (Cicchetti and Allison, 1971). This measure of reliability is best suited for variables measured on an ordinal scale.

Stata/IC 15.1 for Windows (StataCorp LLC, College Station, TX, USA) was used to analyze MNT measurements. A multilevel mixed-effects tobit regression model (unstructured covariance matrix) was used to analyse MNT as this model is suitable for continuous responses where the outcome variable is censored. The tobit model estimates the linear effect on the uncensored latent variables. The Hausman Test was used as a proxy to determine if differences between point estimates caused by homoskedasticity, and normality are large enough relative to the standard errors to be statistically significant. The Hausman Test detects misspecifications that are large enough to cause point estimates to differ substantially. By fitting a multilevel model, we were able to account for the lack of independence of MNT measurements within animal. The following covariates: sex, DOF, BW, and LS were included in the model and through backward elimination, only significant covariates were considered for the final model. Feedlot was not included as a fixed effect in the model as sex was clustered within feedlot. The best model was assessed using Akaike Information Criterion (AIC) values. Only DD M-stage and LS were retained in the final multilevel tobit regression model.

Independence between MNT measurements within animal could not be assumed and using averages at the animal level would not be accurate as DD M-stage could differ between feet. Repeated measures correlation (rmcorr package, R for Windows, R Foundation for Statistical Computing) was used to determine the common within-individual association for paired measures assessed on multiple occasions for multiple animals.

IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA) was used to analyze maximum foot temperatures (MFT). Descriptive statistics were calculated for MFT by DD M-stage. As MFT were not normally distributed as assessed by the Shapiro-Wilk test ($P > 0.05$) and the data set was unbalanced, the Kruskal-Wallis H test was used to test the difference between M-stages and Dunn's post hoc tests were used on each pair of groups within the simplified scoring system. Locomotion scores were assigned on the animal level. There was no difference in MFT within animal as assessed by the Wilcoxon signed-rank test ($P = 0.83$), the Kruskal-Wallis H test was used to test the difference between locomotion scores.

3. Results

3.1. Locomotion scores (alleyway)

3.1.1. Intra- and inter-observer agreement

Observer 1 had very good intra-observer reliability (κ_w 0.876, 95% CI 0.742–1.010, $P < 0.001$; Landis and Koch (1977)). Inter-observer reliability was good between observers (κ_w 0.752, 95% CI

0.549–0.955, $P < 0.001$).

3.1.2. Locomotion and DD M-stage

In total 78 of 255 cattle presented with DD. Among cattle with DD, 44 were normal (LS0), 13 were mildly lame (LS1), 16 were moderately lame (LS2) and 5 were severely lame (LS3). Among cattle with active DD ($n = 49$), 30 presented as normal, 6 were mildly lame, 9 moderately lame and 4 severely lame. Among cattle with chronic DD ($n = 29$), 14 were normal, 7 were mildly lame, 7 were moderately lame and 1 was severely lame.

3.2. Mechanical nociceptive threshold

A total of 510 feet were included in the MNT analysis; 394 feet had no lesions and 116 had DD lesions (61 active and 55 chronic). Predicted LMNT was significantly different between feet with DD lesions and feet without lesions (Table 1). On average, DD lesions withstood 6.37 N less pressure than feet without lesions ($P < 0.001$). The predicted value of LMNT was 3.00 N less for cattle with an $LS \geq 2$ compared to non-lame cattle. Predicted LMNT was significantly different among M-stages (Table 2). On average, active lesions withstood the least amount of pressure when compared to feet with no lesions ($P = 0.001$) and chronic lesions ($P = 0.004$). The predicted SMNT value was not significantly different between feet with DD lesions and feet with no lesions ($P = 0.32$). Additionally, there was a weak positive linear relationship between SMNT and LMNT ($rrm = 0.24$, $P < 0.001$).

3.3. Infrared thermography

For every 1 °C increase in MFT there was a 0.60 N decrease in the MNT ($P < 0.001$). Median MFT was significantly higher in feet with DD compared to feet with no lesions; $\chi^2(1) = 12.778$, $P < 0.001$. Furthermore, median MFT were significantly different among M-stages; $\chi^2(2) = 14.032$, $P = 0.001$. Median MFT was higher in active, and chronic lesions compared to feet with no lesions (Fig. 1). However, no difference was observed between active and chronic lesions.

Lame cattle with DD had significantly higher median MFT compared to non-lame cattle with DD; $\chi^2(1) = 7.201$, $P = 0.007$. Median MFT was also different among LS; $\chi^2(2) = 7.319$, $P = 0.026$. Pairwise

Table 1

Multilevel mixed-effects tobit regression model for mechanical nociceptive threshold (MNT) in hind feet ($n = 510$) of cattle scored for digital dermatitis (DD; absence vs presence) at three feedlots in southern Alberta, Canada.

NT	Item	Coefficient	SE	95% CI	P-value	
LMNT ^a	Intercept	15.71	0.43	14.86	16.56	< 0.001
	M-stage ^b					
	Absence	Ref ^c				
	Presence	-6.37	0.81	-7.96	-4.79	< 0.001
	Lameness score ^d					
	LS0	Ref ^c				
	LS1	-0.38	1.21	-2.76	1.99	0.75
	LS ≥ 2	-3.00	1.19	-5.33	-0.68	0.01
SMNT ^e	Intercept	13.70	0.50	12.73	14.68	< 0.001
	M-stage ^b					
	Absence	Ref ^c				
	Presence	-0.97	0.98	-2.88	0.94	0.32
	Lameness score ^d					
	LS0	Ref ^c				
	LS1	0.56	1.40	-2.18	3.30	0.69
	LS ≥ 2	-0.33	1.36	-3.00	2.34	0.81

^a LMNT = Mechanical nociceptive threshold (foot lifted).

^b M-stage: Absence (M0), Presence (M1 to M4.1 inclusive); Döpfer et al. (1997); Berry et al. (2012).

^c Reference group.

^d Locomotion score: LS0 (normal), LS1 (mild), LS ≥ 2 (LS 2 and 3, moderate to severe merged).

^e SMNT = Mechanical nociceptive threshold (cattle standing upright).

Table 2

Pairwise comparison of predicted marginal means for mechanical nociceptive threshold (MNT) in hind feet ($n = 510$) of cattle scored for digital dermatitis (DD; absence vs active vs chronic) at three feedlots in southern Alberta, Canada.

MNT	DD Stage ^a	Contrast	SE	95% CI ^b	P-value ^b	
LMNT ^c	Active vs absence	-8.11	0.97	-10.43	-5.79	< 0.001
	Chronic vs absence	-4.05	1.08	-6.63	-1.47	0.001
	Chronic vs active	4.06	1.27	1.03	7.09	0.004

^a M-stage simplified scoring system: absence (M0), active (M1, M2 and M4.1 merged), and chronic (M3 and M4 merged); M-stages according to Döpfer et al. (1997) and (Berry et al., 2012).

^b Bonferroni correction applied.

^c LMNT = Mechanical nociceptive threshold (foot lifted).

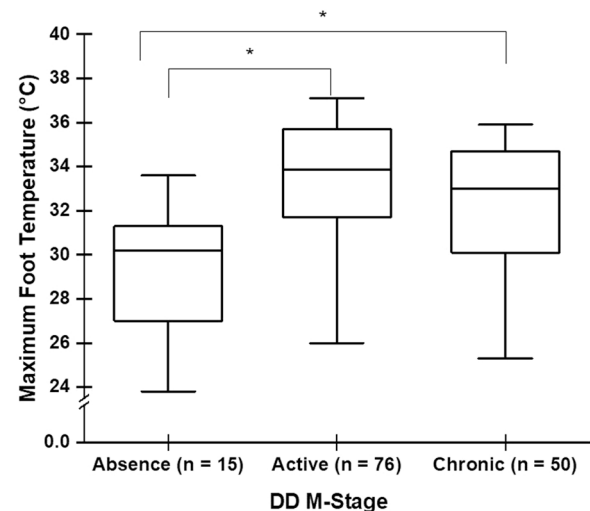


Fig. 1. Maximum foot temperature as measured by infrared thermography in hind feet ($n = 141$) of beef cattle scored for digital dermatitis (DD) using the M-stage scoring system (Döpfer et al., 1997; Berry et al., 2012). Absence represents feet without DD lesions (M0). Active represents ulcerative DD lesions (M1, M2 and M4.1). Chronic represents DD lesions with hyperkeratosis or surface proliferation (M3 and M4). Medians with an asterisk (*) are significantly different; $P < 0.01$.

comparisons between locomotion scores found no significant difference.

4. Discussion

The primary aim of the study was to objectively quantify MNT of DD lesions and determine the association between MNT and locomotion score. Feet with DD lesions had substantially lower MNT than feet without DD, providing evidence of hyperalgesia (enhanced sensitivity to pain) (Coetzee, 2011). Furthermore, lame cattle had significantly lower MNT compared to non-lame cattle. These findings are similar to those of Whay et al. (1998), who found that at treatment (initial inspection), lame dairy cattle had significantly lower nociceptive thresholds compared to non-lame cattle. Whay et al. (1998) reported mean MNT of $13.3 \text{ N} \pm 0.322 \text{ SEM}$ for control animals, $7.9 \text{ N} \pm 0.296 \text{ SEM}$ for unilaterally lame animals, and $11.3 \text{ N} \pm 0.787 \text{ SEM}$ for bilaterally lame animals. In our study, we estimated the linear effect on the uncensored latent variable and not the observed outcome as done in Whay et al. (1998) thus limiting our ability to make direct comparisons. Whay et al. (1998) also reported that mean thresholds for acute foot lesions (DD and foot rot; $7.6 \text{ N} \pm 0.639 \text{ SEM}$) were significantly lower when compared to control animals. Again, the predicted MNT values in our report would not be analogous to those reported by Whay et al. (1998) as they reported absolute values.

Both active and chronic lesions had significantly lower MNT than

feet without lesions. MNT was lowest for active lesions indicating these lesions were most painful. The algometer tip used in our study was designed for cattle and other prey species (Topcat, 2022). This type of tip (1 mm brass end) may have elicited a quicker withdrawal response when applied to active DD lesions, as these lesions are characteristically ulcerative and erosive. The importance of algometer tip size, type and shape has been demonstrated in studies measuring MNT in humans, pigs, horses, and cattle. Duan et al. (2014) measured MNT in healthy male subjects using three different algometer tips, 1 cm², 0.1 cm² and 0.01 cm². They reported that the smaller algometer tips improved pain sensitivity detection and produced more accurate and consistent results. Fosse et al. (2011) compared three types of tips with the same maximum force and found that to provoke a response from the piglets they had to use the tip with the smallest diameter. Similarly, Janczak et al. (2012) found that the smallest commercially available tip did not elicit a withdrawal response in all piglets therefore they constructed a tip with a smaller diameter to evaluate stability and repeatability of MNT measures. To determine the appropriate algometer tip for horses, Taylor et al. (2016) compared four tips, 1.0 mm (sharp), 3.2 mm (blunt), 1.0 mm (spring-mounted) and 3 × 2.5 mm (3 tips) and reported that smaller tips are preferable when assessing MNT in horses as they produce less variable data. While assessing MNT on dairy cows kept in loose housing, Raundal et al. (2014) used two tip types, a 6.6 mm steel probe tip and a 0.8 mm plastic tip. They reported that the level of precision did not differ significantly between tips, however, a higher range of coefficient of variation was observed with the bigger tip suggesting a smaller tip may be preferable to decrease variation in MNT testing of dairy cows. Larger tip sizes require higher forces to elicit a response which results in more variable data when compared to smaller tips (Taylor and Dixon, 2012a,b). Additionally, with larger tips, care must be taken when applying force so as not to push the limb away from the algometer but rather have the animal withdraw the limb. We believe this algometer tip type was the most appropriate to obtain noticeable foot withdrawal responses.

Active lesions are likely more painful as they are susceptible to an exacerbated inflammation process found to be associated with increased local Cxcl-8, TLR4, and β -defensin gene expression whereas chronic lesions are shown to be associated with increased synthesis of anti-inflammatory IL-10 (Watts et al., 2018). There are two other studies, again in dairy cattle, that have looked at pain associated with different stages of DD. Cutler et al. (2013) examined the effectiveness of treatments to reduce pain associated with DD lesions characterized as active, healing and healed. The authors concluded that active lesions were most painful (Cutler et al., 2013). However, it is important to note that MNT values from their study and ours are not comparable for a number of reasons: they used a different DD scoring system, the algometer that they used had a 1 cm diameter flat rubber end, MNT values were aggregated (three time points) and their study was on dairy cattle rather than beef cattle which may differ in how or when they express pain due to the different ecological context in which artificial selection occurs for dairy and beef cattle (Hayes et al., 2009). Kasiara et al. (2021) examined the impact of adding a nonsteroidal anti-inflammatory drugs (NSAID) in addition to antibiotics in the treatment of active DD lesions. They observed a numerical difference in pain (assessed using mobility scores) on second evaluation between animals that received an NSAID and those that didn't. Although the difference between groups was not significant, their results show that active lesions are painful, which is consistent with the results of our study.

In this study we measured MNT when cattle were restrained in the chute, standing on all four feet (SMNT) and when one hind foot was lifted and tied to the chute (LMNT). We compared the measurements obtained by both methods. To our knowledge, this is the first study in which SMNT are compared to LMNT. We found that the correlation between SMNT and LMNT was weak. We believe that SMNT measurements may not accurately represent pain threshold of DD lesions based on the challenges experienced during data collection. Challenges

included: (1) cattle stance which sometimes made it difficult to accurately press the algometer at the site of the lesion, (2) cattle with long or uneven hooves affect line of sight needed to identify location of lesions, (3) within the chute lighting on the ground can be poor, affecting lesion identification especially for cattle with black skin, (4) there was a high frequency of instant reactions which may be cattle reacting to activities within the surrounding environment and not the noxious stimulus, and (5) measurement collection could be unsafe as cattle could freely kick. Moreover, MNT measurements taken on limbs that are restrained versus limbs that allow for free movement may not be comparable; therefore, we recommend further research into pain sensitivity of DD lesions when feet are uninhibited but easily and safely accessible.

Feet with DD had significantly higher MFT than feet without DD. We used the maximum temperature detected in the area above the heel bulbs as an indicator of inflammation since maximum temperature has been shown to be the most accurate measure (Harris-Bridge et al., 2018) and other studies have used this measure to distinguish between healthy feet and feet with foot lesions in dairy cattle (Main et al., 2012; Stokes et al., 2012). In a field trial conducted by Alsaad et al. (2014), feet with DD had a significantly higher maximum surface temperature (coronary band and skin) than healthy feet. Similarly, Stokes et al. (2012) reported higher MFT in the plantar area of hind feet with DD when compared to feet with no lesions. In terms of absolute numbers, Alsaad and Büscher (2012) compared temperatures of feet with DD to those without DD and determined that temperature differences greater than 0.64 °C in the coronary band region are likely associated with DD, given a sensitivity of 85.7% and specificity of 55.9%. Our findings support the conclusion that foot lesions trigger an inflammatory response associated with increased blood flow and tissue metabolism leading to a localized increase in surface foot temperature (Nääs et al., 2014; Alsaad et al., 2015). When we looked specifically at the relationship between feet with DD lesions and locomotion score, we found that MFT is significantly higher in lame cattle with DD. Alsaad and Büscher (2012) also reported an increase in surface temperature of a lame limb when DD is present. Results presented by Harris-Bridge et al. (2018) suggest that temperatures of both feet of lame cattle are elevated even when only one foot has DD, and this may explain why lame cattle with DD had higher MFT compared to non-lame cattle with DD.

During data collection, cattle were restrained in a squeeze chute and each hind foot consecutively lifted and secured with a rope. It has been shown that pain can be suppressed when cattle are restrained or when human observers are present and these events can lead to stress-induced analgesia (Butler and Finn, 2009; Adcock and Tucker, 2018). Our results may not fully account for the pain resulting from DD lesions considering stress-induced analgesia would increase MNT. Multiple studies investigating stress in rodents and swine have shown that stress triggers stress-induced analgesia (Butler and Finn, 2009). However, the only study that has looked at stress-induced analgesia in beef cattle found no reduction in pain response after stress exposure at branding (Schwartzkopf-Genswein et al., 1997).

We used the DD M-stage scoring system to classify lesions in our study. Some stages had low frequencies and by grouping lesions we were not able to provide MNT information for each M-stage. Further studies would be recommended to look at each M-stage separately as this information could provide direction for pain management based on lesion severity. Also, cattle received an overall locomotion score, and further studies that assign a locomotion score for each limb might provide more information on the impact of lameness on MNT and would likely better account for foot temperature differences.

5. Conclusion

DD is associated with decreased locomotion, changes in behaviour, pain, and discomfort making it a welfare issue for feedlot cattle. With the use of a pressure algometer we were able to quantify pain associated with DD lesions. DD lesions are painful and active lesions are more

painful than chronic lesions. Lamé cattle with an obvious limp had lower pain thresholds compared to lamé cattle without an obvious limp or non-lamé cattle. Using thermal images, we were able to detect an increase in foot temperature associated with DD lesions and lameness. Using a combination of indicators: locomotion scoring, pressure algometry, and IRT we were able to more precisely describe pain and inflammation resulting from DD in beef cattle. These objective measures may be useful when refining current methodologies to diagnose DD related pain in beef cattle. Moreover, objective measures of pain and inflammation at different stages of DD will prove invaluable when determining the need for pain control, pain mitigation or pain intervention strategies that would be beneficial for cattle welfare and productivity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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