ABSTRACT

Our objective was to develop a sole ulcer (SU) induction model that can be used to investigate new and more efficacious methods for the treatment and prevention of SU. Three iterations [phase (P)1, P2, and P3] of an SU induction model designed to mimic mechanical and presumed metabolic pathways for SU development were conducted. The results from P1 and P2 identified alterations for the subsequent phase. Each phase used cows with similar calving dates that were randomly assigned (n = 4) to treatments. Control cows (P1CON, P3CON) did not undergo any challenges to induce SU development. Treatment cows were challenged with a hoof block (B) applied to the right hind lateral hoof. Other treatments included restricted lying time (L), restricted feed intake (F), or systemic lipopolysaccharide (LPS) administration. Treatment comparisons were P1CON versus P1BL, P2B versus P2BL, and P3CON versus P3BLF and P3BLF+LPS for P1, P2, and P3, respectively. Pregnant nulliparous Holstein cows were used in P1 and P3, and the P1 cohort was used in P2 during mid-lactation [125.9 ± 7.20 d in milk (DIM)]. Challenges were applied during a set challenge period (P1: −14 to 14 DIM, P2: 126–168 DIM, P3: −14 to 28 DIM). The P1BL cows had a hoof block applied and lying time restricted for 5 h/d. The P2B and P2BL cows had a hoof block and P2BL cows also had their lying time restricted for 18 h/d for 2 d/wk. The P3BLF and P3BLF+LPS cows had a hoof block, 6 h/d of lying time restricted 2 d/wk, and had their DMI restricted by 30% for 2 d/wk. At weekly intervals during wk 1 to 3 postpartum, P3BLF+LPS cows received jugular administration of 0.031, 0.062, and 0.125 µg of LPS per kg of body weight, respectively. Primary response measurements included hoof lesion and locomotion scoring, lying time, hoof thermography, and weight distribution per hoof. No SU induction occurred but sole hemorrhages, a precursor to SU, occurred during the postchallenge period of all phases. Temperature of the blocked hoof at the end of the challenge period did not change for P3CON cows but increased by 5.5°C and 6.2°C for P3BLF and P3BLF+LPS, respectively. Notable increases in lameness and lack of weight-bearing on the blocked hind hoof occurred for challenge treatment cows during the challenge period of P2 and P3. These changes did not persist after the hoof blocks were removed, indicating that hoof blocks succeeded in altering cow gait mechanics, but not enough to induce long-term lameness or SU. Lying restriction challenged cows in P2 and P3, indicated by a compensatory increase in lying time on the day following lying restriction compared with that on the day before restriction. In P3, lying time had the greatest depression during restriction and compensation following restriction in P3BLF+LPS cows, with LPS challenges potentially increasing the other challenge’s effects. Future iterations of the SU induction model should include hoof block use, evaluate longer and more frequent standing and inclusion of forced walking bouts, and include DMI and LPS metabolic challenges.

Key words: dairy cow, induction model, lameness, sole ulcer
chronic (Newsome et al., 2017), and costly (Cha et al., 2010) hoof lesion types. The high prevalence and consequences of SU indicate a need for properly evaluated preventative and therapeutic treatment strategies. The evaluation of these strategies is hindered by the recurrent and multifactorial nature of SU and the lack of an experimental model that can be used to evaluate causative risk factors for new lesions (Randall et al., 2018a).

Researchers approaches to prevent and treat new SU development can be a challenge with observational and experimental research studies. Existing research from epidemiological studies investigating metabolic (Abuelo et al., 2016) and environmental (Cramer et al., 2009; Proudfoot et al., 2010) risk factors associated with hoof-health related lameness have failed to differentiate between new and recurrent lesions. Clinical trials that accounted for SU chronicity (i.e., new vs. chronic lesion cases) and occurred over a 6- to 16-mo period on a sample size of 3,300 cows found that only 60 new cases occurred, even in a population which horn lesion (SU, white line disease, hemorrhage) occurrence was 10 to 15% (Stoddard, 2018; Omontese et al., 2020). This is important to consider, because the risk of SU occurrence as well as methods for treatment and prevention of SU may differ when considering a first instance compared with chronic cases. As such, there is a need to extend beyond traditional clinical and epidemiological studies to identify alternative, novel approaches that can elucidate the pathogenesis of SU development to tackle the prominent economic, production, and welfare issues that arise from SU within the dairy industry.

Induction models are a potential tool for improving our ability to study SU development and pathology. The use of an induction model provides an experimental approach to evaluate research questions on the disorder without necessitating the use of large numbers of animals that would be required in field trials using naturally occurring cases. Successful induction models have been applied to the study of digital dermatitis (Kotschwar et al., 2009; Gomez et al., 2012; Coetzee et al., 2014). However, these models for an infectious lesion do not transfer to the induction of hoof horn lesions such as SU, which requires the development of a hoof horn-specific induction model.

Although the full scope of SU pathogenesis is not yet known, studies have identified potential biomechanical and metabolic disruptions that play a causal role in development. Alterations in normal biomechanics of weight-bearing result from accelerated horn growth of the lateral claws of the hind legs (particularly the heel) and place greater pressure on the sole corium (Shearer and van Amstel, 2017). The effects of these alterations can be compounded when animals are subjected to extensive periods of weight-bearing, such as extended bouts of standing (Eriksson et al., 2021). Prolonged periods of glucose deficit at the corium disrupt hoof horn growth and increase lesion risk (Lübbe, 2015). Recent evidence suggests that metabolic changes around calving, combined with the increased stress of reduced lying time, are also potential components to hoof horn lesion pathogenesis in dairy cows (Newsome et al., 2016; Randall et al., 2018a; Omontese et al., 2020).

The objective of our study was to develop an SU induction model that can be used to evaluate treatment such as pain management and preventative treatment strategies. We anticipate that an effective SU induction model would help address gaps in knowledge regarding factors contributing to SU development and provide an additional tool for future hoof horn lesion research. To achieve this objective, we used an iterative process similar to that of Krull et al. (2016). In this process, different combinations of challenges were evaluated for their potential associated roles in hoof horn lesion development in each iteration, with the results of each iteration informing the modifications made to the challenges applied in the subsequent iteration. Additionally, the study was conducted as a proof-of-concept study, an approach in which a smaller population of animals is used to determine the feasibility of the use of an SU induction model to elicit the desired outcome of SU development in dairy cows.

MATERIALS AND METHODS

The research was conducted as a proof-of-concept study toward the creation of a successful SU induction model. The study was done in 3 study phases, herein referred to as phase 1 (P1), phase 2 (P2), and phase 3 (P3). The results of each phase informed the induction model challenges applied to the subsequent phase. All research in this study was conducted at the University of Minnesota St. Paul Dairy Cattle Teaching and Research Center and approved by the University of Minnesota Institutional Animal Care and Use Committee (protocol #1905-37075A). Cows were housed in tiestalls (P1 and P3) or in a compost-bedded pack (P2). All cows were milked in a double-5 herringbone parlor twice daily and fed once daily between 4 and 6 h after the a.m. milking. Amounts of feed delivered and refused were determined using a portable TMR mixer (Jaylor International) and Feed Supervisor software (Supervisor Systems). All animals were hoof trimmed using an adaptation of the functional hoof trimming method (Stoddard, 2018) 0 to 4 wk before the start of the study period and at the end of the study period.
Animals and Induction Model Challenges

Each study phase included a challenge period and a postchallenge period (Figure 1). The challenge period was defined as the period during which challenges with an associated role in SU development were applied to the cows. During the postchallenge period, data were collected to assess longer-term effects of the challenges on hoof health and cow performance. We expected that, during the challenge period, damage would occur to horn-producing cells. Observations during the postchallenge period were intended to allow signs of this damage to become visibly apparent as horn growth occurred.

Two primary challenges were applied across all 3 iterations of the SU induction model. The first was the use of a hoof block, placed directly underneath the flexor tuberosity of the third phalanx (Figure 2) of the lateral hoof of the right hind (RH) leg (by G. C.). The intent of the block was to place direct pressure in the typical SU location and to create a foot angle (unmeasured) that would place more weight toward the heel. The RH was chosen as that is where SU occurrence is more common (Solano et al., 2016; Newsome et al., 2017). All cows underwent lying restriction challenges, in which access to a lying area was removed for a set period, with the duration and frequency of the restriction periods differing between phases. Lying restriction occurred in a section of the parlor holding area (5.5 m wide × 7.6 m long) that cows entered twice daily for milking for P1 and P3. The area had rubber flooring and cows had access to water but not feed. In P2, lying restriction occurred on a concrete section of the feed alley (4.9 m wide × 6.7 m long) in the compost pack facility, and cows had access to both feed and water during the restriction period. Cows were able to move freely within the area. Cows were monitored visually at least hourly, and any cow that attempted to lie down was encouraged to stand by the observing staff. To avoid undue stress, cows that attempted to lie down more than twice during a restricted lying session were returned to their normal housing location for the remainder of that session. The frequency of this occurring was noted by research staff, but individual cow numbers were not always recorded. Cows that developed illness or lameness during the restriction period were excluded from any remaining lying restriction periods and treated.

Phase 1. An overview of the treatments and associated challenges applied during each phase are provided in Table 1. Eight purchased, pregnant nulliparous Holstein cows with similar calving dates were housed in the tiestall facility. Phase 1 of the research study was conducted from July to November 2019. The study period was from −14 to 84 DIM, with the challenge period occurring from −14 to 14 DIM. Cows were enrolled and randomly assigned using the “randomizr” package in R (R 3.6.0; https://www.r-project.org/) to a control group (P1CON; n = 4), with no challenges applied during the study period, or a treatment group (P1BL; n = 4). Treatment cows had a hoof block (details below) applied to the RH lateral hoof from −14 d relative to their expected calving date to 14 DIM. The P1BL cows were removed from their stall to restrict their ability to lie down for 5 h/d throughout the 28-d challenge period. The 5-h time period was divided into two 2.5-h blocks starting at 0700 and 1200 h.

For the hoof block challenge, a therapeutic plastic hoof block (Accu-Bloc, 130 mm long × 62 mm wide × 18–22 mm high, with height presented from heel to toe; Comfort Hoof Care Inc.) was attached to the hoof using a hoof block adhesive (Bovi-Bond, Vettec Hoofcare, Royal Kerckhaert Horseshoe Factory). The block was placed on the hoof such that the side with the lower height was positioned under the expected flexor tuberosity location and the block length was adjusted to 90 to 100 mm based on hoof length.

Phase 2. To test the hoof horn lesion induction model efficacy on nontransition cows, the 8 cows from P1 were enrolled during mid-lactation (mean ± SD: 126 ± 7 DIM) from December 2019 to March 2020. The study period was a total of 100 d from an average of 126 to 226 DIM, with the challenge period occurring from an average of 126 to 168 DIM. Cows were housed in the compost-bedded pack facility and randomly assigned (n = 4 cows/treatment) to either the P2B or P2BL treatment. Randomization occurred within P1 treatments, with P1 lesion history being accounted for when assigning cows to P2 treatment groups. Cows in both treatments had hoof blocks applied at enrollment and removed 42 d after application. During the challenge period, cows in the P2BL treatment were also subjected to a period of lying restriction. The P2 lying restriction protocol restricted lying access on 2 nonconsecutive days per week over the course of the challenge period for 18 h/d, divided into two 9-h blocks (a.m. and p.m. following milking).

The same therapeutic plastic hoof block applied in P1 was used in the P2 hoof block challenge in addition to a secondary plastic hoof block (Accu-Soel therapeutic sole, 122 mm long × 60 mm wide × 6–10.5 mm high; Comfort Hoof Care Inc.), graded from axial to abaxial hoof side, which was glued to the initial hoof block before placement on the hoof using a hoof adhesive (Accu-Bond, Comfort Hoof Care Inc.). The combined blocks were positioned with the thicker side placed abaxially and the thinner side axially to place more pressure on the axial part of the hoof.

Phase 3. Following the P2BL treatment, 8 cows were enrolled during mid-lactation (mean ± SD: 126 ± 7 DIM) from December 2019 to March 2020. The study period was a total of 100 d from an average of 126 to 226 DIM, with the challenge period occurring from an average of 126 to 168 DIM. Cows were housed in the compost-bedded pack facility and randomly assigned (n = 4 cows/treatment) to either the P3B or P3BL treatment. Randomization occurred within P3 treatments, with P3 lesion history being accounted for when assigning cows to P3 treatment groups. Cows in both treatments had hoof blocks applied at enrollment and removed 42 d after application. During the challenge period, cows in the P3BL treatment were also subjected to a period of lying restriction. The P3 lying restriction period involved lying access on 2 nonconsecutive days per week over the course of the challenge period for 18 h/d, divided into two 9-h blocks (a.m. and p.m. following milking).

The same therapeutic plastic hoof block applied in P1 was used in the P3 hoof block challenge in addition to a secondary plastic hoof block (Accu-Soel therapeutic sole, 122 mm long × 60 mm wide × 6–10.5 mm high; Comfort Hoof Care Inc.), graded from axial to abaxial hoof side, which was glued to the initial hoof block before placement on the hoof using a hoof adhesive (Accu-Bond, Comfort Hoof Care Inc.). The combined blocks were positioned with the thicker side placed abaxially and the thinner side axially to place more pressure on the axial part of the hoof.
Phase 3. Phase 3 was conducted from October 2020 to January 2021. Cows were enrolled from \(-28\) to \(84\) DIM, with the challenge period occurring from \(-14\) to \(28\) DIM. Twelve nulliparous cows from the University of Minnesota St. Paul campus dairy were enrolled and randomly assigned to 1 of 3 treatment groups (\(n = 4\) cows/treatment). Control heifers (P3CON) had no challenges applied during the challenge period. Animals in the P3BLF and P3BLF+LPS treatment groups had a hoof block applied at \(-14\) d relative to anticipated calving date until +14 DIM, and lying access was restricted on 2 consecutive days/week (d 3 and 4 of each week of the challenge period). During lying restriction periods, access to a lying area was restricted for...
6 h/d, divided in two 3-h blocks starting at 0700 and 1200 h (Tucker et al., 2021). Due to a high number of cows attempting to lie down repeatedly by the second week of P3 (2 P3BLF, 3 P3BLF+LPS), wooden boxes (Creutzinger et al., 2021) were placed in the holding area during the restriction intervals for the rest of the challenge period. These boxes were designed to reduce the floor space on which the cow could lie down while (...
leaving standing room unaltered. The size of the holding area was also reduced to 4 m wide and 5.3 long for this group.

For the hoof block challenge, a wood hoof block (UniBloc, 120 mm long × 57 mm wide × 22 mm high; Comfort Hoof Care Inc.) was adhered (Accu-Bond) to a plastic therapeutic sole (Accu-Sole). As in P2, the therapeutic sole was graded from the axial to the abaxial hoof side before placement on the hoof using a hoof adhesive (Accu-Bond), with the thicker side of the block placed abaxially and the thinner side axially.

Metabolic stressors were added as additional challenges in P3. Two methods intended to induce a glucose deficit in treatment cows were added to the challenge period: (1) 2 consecutive days of restricted DMI (d 2 and d 3), and (2) once-weekly systemic LPS administration. During DMI restriction, feed offered to P3BLF and P3BLF+LPS cows was restricted to 70% of the individual cow’s normal DMI, as per Gross et al. (2011), calculated from the day before each 2-d restriction period. Weekly LPS challenges were administered intravenously via jugular vein to P3BLF and P3BLF+LPS cows before the start of the second lying restriction day in wk 1, 2, and 3 postcalving. Cows only received a LPS challenge if they were not systemically ill (temperature <39°C and eating). The LPS (Escherichia coli serotype O111:B4; MilliporeSigma) was diluted with 10 mL of 0.9% saline (Teknova Inc.) to achieve doses of 0.03125, 0.0625, and 0.125 µg/kg of BW for wk 1, 2, and 3, respectively. Cows were observed for 2 min following the LPS injection for any immediate adverse physiological responses to the injection and then released to the lying restriction area.

Outcome Measures

Sampling intervals for all outcomes measured across the 3 study phases are depicted in Figure 1.

Lesion Scoring. Hooves on both the left and right hind feet were scored by a single observer (G. C.) while cows were in a hoof trimming chute, and the type and number of lesions were recorded. Hooves were evaluated for presence of sole hemorrhages (SH), SU, toe ulcers, white line disease, digital dermatitis, root rot, and other nonspecific limb conditions, referred to as “injury” as per Cramer et al. (2008). Due to the presence of hoof blocks, the observer was not fully blinded to treatment. Digital photographs were also taken of the hind hooves while cows were in the chute. The pain withdrawal response of each hind hoof was evaluated using a hoof tester (Jorgensen Labs) when SH were present to evaluate a withdrawal response by placing pressure on the hemorrhage site by a single observer (G. C.). Pain was considered to be present when a withdrawal response was elicited.

Visual Locomotion Scoring. Cows were recorded using a digital camera (Canon VIXIA HF R20, Canon Inc.) while walking another section of the holding area used for P1 and P3 lying restriction (i.e., parlor holding area). Videos for all phases were scored by a single trained observer blinded to the study treatments (E. S.). A 5-point numeric rating scale (Flower and Weary, 2006) was used for visual locomotion scoring (VLS), where a score ≥3 was considered lame. The total number of lame cows, by phase and group, were reported for each sampling period. No cow was lame at the start of any of the study phases.

Weight-Bearing Distribution. Weight-bearing by each leg was measured using a weighing platform (Pacific Industrial Scale Co.) as described by Chapinal et al. (2010). The platform contained 4 independent recording units with 4 load cells (3 mV Shear Beam Load cells, Anyload LLC) per unit and transmitted real-time data directly to a computer software program (CowWeight version 2.2., Pacific Industrial Scale Co. Ltd.) at a rate of 14 readings/s. Cows were recorded for a minimum of 5,000 readings. Weight-bearing by each leg was reported as a percentage of total BW.

Hoof Thermography. Hoof thermography was measured using an infrared thermal imaging camera (FLIR E8, Teledyne FLIR) following the image handling procedures outlined in Anagnostopoulos et al. (2021), with the area of interest being the SU location under the flexor tuberosity. To account for a lack of control for external ambient temperature, data were presented as the difference in hoof temperature between the lateral and medial hoof of the left (nonblocked) and the right (blocked) legs at each measurement.

Lying Time. Lying time was continuously measured using pedometers (IceQube pedometers, IceRobotics Ltd.) mounted on the RH leg of the cow, with activity data output reported in 15-min intervals and presented as total daily lying time (h/d). Pedometer data were collected and extracted weekly from 9-d pedometers during the P1 and P2 study periods. During P3, the data were collected using 200-d pedometers and extracted once at the end of the study.

Metabolic and Performance Indicators. Blood samples were collected from each P3 cow in the morning after milking during each sampling period from coccygeal vessels using a 20-gauge, 2.54-cm blood collection needle and a vacuum tube with lithium-heparin (Becton Dickinson Co.). Samples were centrifuged at 2,000 × g for 15 min for plasma separation and kept frozen at −20°C until analysis. Blood BHB, nonesterified fatty acids (NEFA), Ca, and glucose (Glu) were measured.
in duplicate using a small-scale biochemistry analyzer (CataChemWell-T; Catachem Inc.) with reagents and calibrators from the same manufacturer. The analyzer was calibrated every week. The BHB, NEFA, Ca, and Glu assays were based on β-hydroxybutyrate dehydrogenase, acyl-Co-A oxidase, arsenazo III, and UV-hexokinase methods, respectively. Intra- and interassay coefficients of variation for the BHB assays were <6.2 and 8.4%, respectively, <8.6 and 9.7% for the NEFA assays, <9.1 and 9.8% for the Ca assays, and <3.4 and 1.7% for Glu.

Daily milk yield (kg/d) was recorded for cows in P3 from calving until study end (84 DIM) and extracted at the end of the study from the dairy parlor software system (DelPro; DeLaval). Total daily rumination time was recorded continuously over the course of P3 using CowManager ear-tag accelerometers (Agis Automatisering BV). Data output was minutes per hour spent ruminating and reported as total rumination time per day (min/d).

Data Analysis

This study was designed as an iterative proof-of-concept approach to develop an SU induction model. The number of cows in each phase of the study represents a combination of ethical considerations, resource allocation, and other lameness induction models, in which sample sizes ranged from 4 to 10 (Kotschwar et al., 2009; Gomez et al., 2012). All results are presented descriptively with the intent of exploring outcomes for proof-of-concept and future adaptations of the SU induction model.

RESULTS

Lesion Scores and VLS

None of the challenges applied across the 3 phases induced SU development, but SH developed in all control and challenge treatments by the end of all phases (Table 2). A higher number of new SH developed in P2BL and P2B cows than in the other groups. Throughout the study, a greater number of SH were reported on the lateral hoof than the medial on both hind hooves. Interestingly, at the end of the study, challenge treatment cows in P2 and P3 developed the same number or more SH on the left hind (LH) hooves, contralateral to the foot on which the block was placed. Although P3CON cows saw no SH development on the RH hooves during the study period, these cows also experienced similar numbers of SH on the LH hooves as challenged cows. No cows in the P1BL treatment group had a VLS ≥3 by the end of the challenge period, compared with the same pool of cows after a 42-d challenge period at mid-lactation in P2, where 2/4 P2B and 3/4 P2BL cows ended the challenge period as lame (Table 2). Phase 3 saw similar levels of cows scored as lame over the course

Table 2. Total number of sole hemorrhages recorded on the medial (Med) and lateral (Lat) hooves of the left hind (LH) and right hind (RH) feet and total number of cows per group that were scored as clinically lame based on visual locomotion score (score ≥3 on a 5-point numeric rating scale)1

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<tr>
<th>Item</th>
<th>Phase 1</th>
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1Data are presented by treatment and study phase at the start of the study, end of the challenge period, and end of the study. Combined abbreviations represent the phase (P1, P2, P3 = phase 1, 2, and 3) and type of challenge applied during the challenge period (B, L, F, LPS), where CON = no challenges applied; B = hoof block; L = lying restriction; F = feed restriction; LPS = lipopolysaccharide challenge at wk 1, 2, and 3 post-partum.
of the challenge period for both P3BLF cows (3/4) and P3BLF+LPS cows (2/4) compared with P3CON cows (0/4). However, as with P2 results, little long-term gait change was achieved, with only 1/4 cows from each of the 3 P3 study groups scored as lame at the end of the study.

**Hoof Thermography and Weight-Bearing Distribution**

Average hoof temperature, represented as the difference between the lateral hoof and the medial hoof of each foot, was higher for the blocked leg of P3BLF (6.2°C; 95% CI: 2.34, 9.96) and P3BLF+LPS cows (5.5°C; 95% CI: 2.64, 8.25) by the end of the challenge period (Figure 3). The change in hoof temperature from study start to the end of the challenge period was greatest for P3BLF+LPS cows, with an increase of 4.4°C. The average difference in lateral and medial hoof temperatures remained close to zero for P3CON cows (−0.4°C; 95% CI: −4.43, 3.63). The lateral hoof temperature of the blocked foot of challenge treatment cows decreased to levels similar to or lower than those of the study start by the end of the study, whereas the contralateral foot’s lateral hoof temperature increased.

Weight-bearing across the hind limbs followed a similar pattern to the hoof thermography results (Figure 4). Due to scale malfunctions during P1, only P2 and P3 data could be processed. For challenge treatment cows in P2 and P3, the proportion of weight distributed to the blocked, RH limb of the cow (mean % of total BW across a 5-min period) was markedly lower (13.1 and 13.9% for P3BLF+LPS and P3BLF, respectively, and 13.3 and 17.7% for P2B and P2BL) by the end of the challenge period than the proportion of weight distributed to the unblocked, LH limb. However, as with lameness results, weight-bearing equalized across the hind limbs for all treatment groups with hoof blocks following block removal. Weight-bearing was lower for the unblocked limb by the end of the study period than for the blocked limb. Weight-bearing remained constant across each limb over the course of the study for P3CON cows.

**Lying Time**

The average duration of the period of lying restriction (mean h/d ± SD) for P1 was 4.6 ± 0.90 h/d (range: 1.5–5.3) over the course of the 28-d challenge period. In P2, the protocol dictated up to 2 d/wk of lying restriction at 18 h/d; however, the total number of restriction days carried out over the 42-d challenge period was 6 d (interval between challenge days: 3–17 d) at an average duration of restriction per lying restriction day of 12.8 ± 3.35 h (range: 7.5–16.3). Weeks 4 and 6 of restriction in P2 were skipped because most cows attempted to lie down during milking in the pre-

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Figure 3. Hoof thermography data for the left hind (not blocked) and right hind (blocked) leg of cows in each phase 3 (P3) treatment group, presented for the study start (wk −8), end of the challenge period (wk 0), and study end (wk 8). Hoof temperature for each leg is presented as the difference between the lateral and medial hoof temperatures on each foot. Crossbars represent the mean temperature and standard deviation across each time point. Abbreviations indicate the type of challenge applied during the challenge period (B, L, F, LPS) where CON = no challenges applied; B = hoof block; L = lying restriction; F = feed restriction; LPS = lipopolysaccharide challenge.
ceeding week. Twice-weekly consecutive lying restriction periods were carried out in P3, with lying restricted periods lasting an average of 5.5 ± 1.19 h/d (range: 3.0–6.3 h). One P3BLF+LPS cow skipped a p.m. lying restriction period due to being listed as systemically ill (i.e., reduced feed intake, temperature >39°C) in the week after first LPS administration.

Lying restriction protocols implemented under the induction model of each of the 3 phases were successful in reducing lying time during the challenge period (Figure 5). In P1, wherein lying was restricted daily over the course of the 28-d challenge period, lying time was consistently lower on each day of the challenge week for P1BL cows compared with P1CON counterparts,
with lying times between the 2 treatments being similar outside of the challenge period (Table 3). Lying time on the day following lying restriction periods was an average of 1.6 h/d and 1.0 h/d more than lying times reported on the day before restriction in P2 and P3 restricted cows, respectively. The largest reduction in lying time occurred on lying restriction days in P2, which can be attributed to the largest restricted lying time of all the phases. This variation in daily lying time over the course of the challenge period was, in turn, absent in the P3CON and P2B cows where no lying restriction occurred.

**Metabolic and Performance Indicators**

Only 2 cows in the P3BLF+LPS challenge received the 3 doses of LPS, with the 2 other cows only receiving 1 and 2 doses due to being systemically ill. Average DMI in P3 during nonrestriction days was 12.2, 10.2, and 12.4 kg/d for P3BLF, P3BLF+LPS, and P3CON, respectively. During DMI restriction days in the challenge period, P3BLF and P3BLF+LPS feed was successfully reduced to 70% of the nonrestriction day feed quantity. Refusals on challenge days averaged 3.7, 7.6, and 13.1% for P3BLF, P3BLF+LPS, and P3CON, respectively, and P3BLF and P3BLF+LPS had 5.6% (7.2% total refusal) and 7% (12.1% total refusal) lower refusals on the day following restriction compared with the day before restriction.

In P3, BHB concentrations were relatively constant and uniform across the groups during the 2 wk before calving and from 28 DIM onward. Blood BHB levels only exceeded 1.2 mmol/mL, the threshold at and above which a cow is considered to be hyperketonemic (McArt et al., 2012), from 7 to 21 DIM: 3 P3CON cows at 7 DIM and 1 at 14 DIM; 1 P3BLF cow at 7, 14, and 21 DIM; and 2 P3BLF+LPS cows at 14 and 21 DIM, one of which skipped the second LPS dose due to systemic illness. Blood glucose decreased in the first week postpartum for all cows, and was lowest in P3CON at 7 and 14 DIM when BHB was highest in these cows (Figure 6). Only 1 P3CON cow was considered hypoglycemic (glucose ≤2.2 mmol/L; Gordon et al., 2013) at 14 DIM, with no other cows in P3 falling below this threshold. Calcium increased in all P3 cows in the first 21 DIM before declining to similar levels for the remainder of the study period, with no cows considered hypocalcemic (plasma Ca <2.0 mmol/L; Goff, 2008; Reinhardt et al., 2011). As with BHB, NEFA increased at 7 DIM for P3CON cows, with less change seen for the challenge treatments. Cows in all 3 treatments had similar blood concentrations for all 4 measures by the end of the study period.

Average daily milk yield across the challenge period was lowest in P3BLF+LPS (23.1 kg/d, 95% CI: 21.56, 24.72) cows, followed by P3CON (25.0 kg/d, 95% CI: 21.56, 24.72) and P3BLF (26.9 kg/d, 95% CI: 25.91, 27.79) cows. Although milk yield was relatively consistent for P3CON and P3BLF cows, the P3BLF+LPS cows had less consistency across the challenge period, with the lowest yields corresponding to LPS challenge days (d 4, Figure 7).

In P3, rumination decreased steadily across the DMI and lying restriction days, with the lowest average rumination times for P3BLF and P3BLF+LPS cows recorded on d 3, the final day of lying restriction (Figure 8). As with milk yield, P3BLF+LPS cows had the lowest mean rumination time over the course of the challenge period (448.3 min/d, 95% CI: 420.55, 476.04), followed by P3BLF (488.8 min/d, 95% CI: 468.24, 509.31) and P3CON (523.6 min/d, 95% CI: 500.95, 546.23).

### Table 3. Mean daily lying time (h/d) and 95% CI for treatments in each phase across the study period

<table>
<thead>
<tr>
<th>Period and treatment</th>
<th>Baseline</th>
<th>Challenge</th>
<th>Postchallenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1CON</td>
<td>14.3 (13.42, 15.27)</td>
<td>12.5 (12.18, 12.80)</td>
<td>12.7 (12.37, 12.93)</td>
</tr>
<tr>
<td>P1BL</td>
<td>14.8 (13.88, 15.66)</td>
<td>11.6 (11.16, 12.02)</td>
<td>12.9 (12.60, 13.16)</td>
</tr>
<tr>
<td>Phase 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2B</td>
<td>11.2 (10.81, 11.58)</td>
<td>13.0 (12.65, 13.36)</td>
<td>12.0 (11.63, 12.34)</td>
</tr>
<tr>
<td>P2BL</td>
<td>12.2 (11.80, 12.62)</td>
<td>12.3 (11.85, 12.83)</td>
<td>12.1 (11.68, 12.40)</td>
</tr>
<tr>
<td>Phase 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3CON</td>
<td>13.3 (12.67, 14.01)</td>
<td>11.0 (10.68, 11.39)</td>
<td>12.6 (12.44, 12.84)</td>
</tr>
<tr>
<td>P3BLF</td>
<td>11.0 (9.34, 12.71)</td>
<td>11.8 (11.32, 12.22)</td>
<td>11.1 (10.81, 11.34)</td>
</tr>
<tr>
<td>P3BLF+LPS</td>
<td>11.9 (10.09, 13.67)</td>
<td>11.9 (11.50, 12.30)</td>
<td>12.1 (11.93, 12.23)</td>
</tr>
</tbody>
</table>

1Baseline refers to measures of lying time before block application and start of the challenge period; challenge period is the 28-d (P1) to 42-d (P2 and P3) period during which induction model challenges were applied to study animal; postchallenge refers to the period following the challenge period (marked by hoof block removal)

2Combined abbreviations represent the phase (P1, P2, P3 = phase 1, 2, and 3) and type of challenge applied during the challenge period (B, L, F, LPS) where CON = no challenges applied; B = hoof block; L = lying restriction; F = feed restriction; LPS = lipopolysaccharide challenge at wk 1, 2, and 3 postpartum.
Figure 5. Average lying time (h/d) during the challenge period of phase 1 (P1; a), phase 2 (P2; b), and phase 3 (P3; c). P1 and P3 are presented as the entire challenge week, wherein lying restriction occurred every day in P1 and on d 3 and 4 of P3. Lipopolysaccharide challenges occurred on d 4 for wk 1 to 3 postpartum in the P3BLF+LPS treatment group. Abbreviations indicate the type of challenge applied during the challenge period (B, L, F, LPS) where CON = no challenges applied; B = hoof block; L = lying restriction; F = feed restriction; LPS = lipopolysaccharide challenge. Due to the interval of lying restriction periods in P2, lying time is presented for the day before each challenge day (d 1), the challenge day (d 2), and the day after each challenge day (d 3). Cross bars represent the mean lying time for each treatment across time points.
DISCUSSION

Like that of most hoof lesions, SU development is multifactorial in nature and can be influenced by several potential factors, including biomechanical injury, metabolic stress, parity, and DIM (Randall et al., 2018b). The challenges included in the SU induction models were selected to replicate these risk factors and presumed primary causes of SU in dairy cattle. However, even our most involved iteration, which incorporated both mechanical and metabolic challenges, did not induce SU. Although no single challenge or combination of challenges yielded a successful SU induction model, our more challenging treatments in P2 and P3 induction models did increase development of SH, which are considered precursors to SU (Newsome et al., 2016). Indeed, SH and SU are presumed to be different stages of the same disease process (Newsome et al., 2017), with SU being the later, more severe stage of the process. As a proof-of-concept study, the ability for the SU induction models in P2 and P3 to induce SH suggests that aspects of these models do have potential for activating presumed causal pathways for SU development. Thus, our results provide information that can be used to further refine the SU induction model with the expectation that improving the challenges applied based on these previous iterations will improve the potential for SU development. Moreover, it is important to note that, although P2 and P3 induction model challenges led to SH development, recovery rates for gait, lying behavior, and feed intake suggest that other aspects of animal health and wellbeing are not negatively affected beyond the challenge periods. This is valuable information from an animal health and welfare perspective when considering the use of an SU induction model.

Treatment of hoof lesions commonly involves the application of a hoof block to shift the BW burden to the leg with an unaffected hoof, which relieves pressure on the lesion. In the current study, however, hoof blocks were the primary challenge used in our SU induction models and were applied to place continuous pressure directly underneath the flexor tuberosity of the third phalanx of the lateral right hoof. Our initial hypothesis was that block application during the transition period (P1, P3) when ligaments in the hoof are laxer (Tarlton
Figure 7. Milk yield (kg/d) of phase 3 (P3) cows, by treatment, averaged across days of the challenge period. Lying restriction occurred on d 3 and 4. Dry matter intake restriction to 70% of normal feed quantity occurred during d 2 and 3. The LPS challenges for the P3BLF+LPS treatment were administered on d 4 of wk 1 to 3 postcalving. Abbreviations indicate the type of challenge applied during the challenge period (B, L, F, LPS) where CON = no challenges applied; B = hoof block; L = lying restriction; F = feed restriction; LPS = lipopolysaccharide challenge. The boxplot box represents the interquartile range (IQR) and the line represents the median daily milk yield, with whiskers displaying the minimum and maximum IQR. Points represent individual cow daily milk yields.

Figure 8. Daily rumination time (min/d) of phase 3 (P3) cows, by treatment, averaged across days of the challenge period. Lying restriction occurred on d 3 and 4. Dry matter intake restriction to 70% of normal feed quantity occurred during d 2 and 3. LPS challenges for the P3BLF+LPS treatment were administered on d 4 of wk 1 to 3 postcalving. Abbreviations indicate the type of challenge applied during the challenge period (B, L, F, LPS) where CON = no challenges applied; B = hoof block; L = lying restriction; F = feed restriction; LPS = lipopolysaccharide challenge. The boxplot box represents the interquartile range (IQR) and the line represents the median total daily rumination time, with whiskers displaying the minimum and maximum IQR. Points represent individual cow daily rumination times.
blocks on both hooves in these initial phases. However, the ability of cows to move deterred us from implementing the SH in the contralateral limb. Concerns about the leg, but not on the RH, indicating that some unknown cause further evidenced by the appearance of SH on the un-blocked hoof. Indeed, our weight distribution data confirmed that higher temperatures (Eddy et al., 2001). Additionally, elevated temperatures on the hoof with lesions, particularly around the coronary band, have been reported for dairy cows with SH (Nikkhah et al., 2005). This is similar to the higher temperatures reported in our thermography results at the end of the challenge period for P3 blocked cows, suggesting underlying inflammation and possible lesion development. Although it is possible that the method of block application resulted in tendonitis, no excessive swelling of the tendon sheath was noted, and the presence of increased temperatures at the SU location suggests the changes in gait were due to pain as a result of inflammation in the hoof capsule, not tendonitis. In our study, we only used hoof testers when SH were visible; however, due to the suggested role of inflammation in SU development, future attempts to induce SU should use hoof testers at each hoof evaluation.

The effect of the block application in P2 and P3 is further evidenced by the appearance of SH on the un-blocked hind hoof. Although blocks were intended to put pressure on the blocked hoof, they likely also resulted in cows placing more weight on the unblocked hind leg to reduce pressure and discomfort of the blocked leg. Indeed, our weight distribution data confirmed that blocked cows placed 1.5- to 2.4-fold more weight on their unblocked LH leg when standing. This alteration in weight-bearing behavior corroborates that of Neveux et al. (2006), where cows standing on an uncomfortable surface (i.e., embedded with rocks vs. rubber) decreased weight-bearing on the foot positioned on the uncomfortable surface by around 5%. It is possible that this caused more sole hemorrhaging, not only on the blocked hoof but also on the unblocked hoof on which the cow placed more weight during the challenge period. However, P3CON cows also developed SH in the LH leg, but not on the RH, indicating that some unknown factors (e.g., cow, farm, management) might be causing SH in the contralateral limb. Concerns about the ability of cows to move deterred us from implementing blocks on both hooves in these initial phases. However, it is clear that this should be considered in the development of future induction models.

We included lying restriction challenges in the induction models to create prolonged periods of weight-bearing on the blocked hoof. Increased standing time has been shown to be a risk factor in the development of SH and SU (Singh et al., 1993). Eriksson et al. (2021) reported that increased total standing time and longer standing bouts increased the odds of SH or SU development by around 50%. However, despite reductions in lying time and, in P2 and P3, increases in compensatory lying after restriction periods, it is unclear how big a role lying restriction played in the current models compared with other challenges, as restricted cows had similar numbers of SH as nonrestricted cows in both P1 and P2. Although just 3 h of applied lying restriction daily is sufficient to negatively affect cow health and performance (Tucker et al., 2021), it is likely that the low frequency of restriction periods in P2 and P3 and the shorter restriction periods (5 h divided into 2.5-h bouts) allowed cows to increase lying time outside of restriction periods, decreasing the challenge’s efficacy. This is particularly evident in the day that followed a lying restriction period in P2 and P3, with challenge treatment cows increasing lying time. Physical limitations in the animals that led to the reduction of the number of lying restriction days in P2 and an adaptation of the restriction area in P3 to reduce attempts at lying down may also be a limitation to the use of lying restriction in SU induction models.

Excessive movement of the third phalanx has been suggested as a cause of SU (Lischer et al., 2002); this is influenced by, among other factors, walking distances and the use of harder flooring type with lower compressibility in the housing environment (Bicalho and Oikonomou, 2013). During our lying restriction periods, cows spent most of their time standing as opposed to walking. Given that our data show that the block applications led to weight shifting and that literature shows cows strike their lateral heel first when walking (Schmid et al., 2009), future induction models should likely incorporate increased walking on harder surfaces such as concrete to improve the efficacy of SU development. This is further supported by the higher number of SH reported in P2 cows, even while housed in a compost-bedded pack with characteristics that should be a protective factor against hoof horn lesions (Solano et al., 2016) and often has lower levels of SU development (Somers et al., 2003). One possible explanation for these outcomes is that the increased amount of time spent walking on the hoof blocks in this environment, despite more ideal lying surfaces, created the excessive movement needed to affect lesion development. Addi-
tionally, although flooring during the lying restriction period in P1 and P3 was covered in rubber mats, the extended period of standing on concrete in P2’s lying restriction environment may have increased the impact on the hoof.

Blood parameters selected for this study were those that are known to change around calving and have been found to be indicators of metabolic stress (Caixeta et al., 2015; Weber et al., 2015). Apart from mechanical injury, it is thought that glucose deficits that are substantial enough to disrupt hoof horn growth can lead to SU development (Lübbe, 2015). Low plasma concentrations of glucose and elevated NEFA and ketone bodies are common characteristics of cows in negative energy balance (van Dorland et al., 2009), which can be achieved by 70% feed restriction (Gross et al., 2011). Feed restriction of multiparous cows in early lactation to 65% of their ad libitum intake affected proxies of negative energy balance, including increased plasma NEFA and BHIB, decreased plasma glucose, and decreased milk production (Nielsen et al., 2003). Similarly, LPS challenges can elicit a robust immune response that can disrupt normal metabolic processes; induce hypoglycemia, hypocalcaemia, and hyperketonemia; and decrease overall performance (Horst et al., 2021). Feed restriction and LPS administration did not induce major alterations in plasma NEFA, glucose, or Ca concentrations in our P3 cows, but milk yield by P3BLF+LPS cows was reduced, particularly on the day (d 4) on which LPS challenges were administered. Reductions in milk yield of more than 3 kg/d in cows receiving low doses of LPS solution daily across a 1-wk period were also reported by Ning et al. (2018). This reduced milk yield indicates a reduction in nutrients and energy available for milk synthesis and for other biological functions. Thus, the combination of restricted DMI and LPS administration might have reduced glucose available for the hoof and contributed to the development of more SH that were apparent by the end of the study, but was not important enough to lead to disruption at the level of the corium needed for SU development.

We purposely selected first-lactation animals in an attempt to remove the potential effect of previous lameness and lesions (Newsome et al., 2016; Randall et al., 2018b). However, given their greater BW (Vaarst et al., 1998) and greater metabolic demands as added risk factors, it is possible that our SU induction model would have been more successful if we had used older cows without a history of SU or SH or any other lesions. Indeed, both the prevalence of SU and risk of SU increase with parity, particularly after the third lactation (Barker et al., 2009). Thus, comparing the effects of SU induction model challenges on multiple parities while accounting for previous lesion history should be considered in future iterations to better understand how this potential confounder influences performance of an SU induction model. Additionally, it is possible that the use of loose or freestall housing, where cows would have walked more and increased the force on the sole corium of the blocked hoof, would have increased our ability to induce SU.

In summary, although none of the induction models used in this study induced SU, our results do indicate the potential for modifications of certain challenges to be more successful. Hoof blocks used in P2 and P3 were graded toward the axial area of the medial hoof of the blocked hind leg and elicited changes in VLS and weight-bearing that increased pressure on the third phalanx and should lead to corium disruption and SU development. Although lying restriction increased standing time for a cow and increased pressure on the blocked hoof, increased walking during this period would be expected to amplify this effect further, particularly when paired with P2 or P3 hoof block methods. The LPS challenges we used were sufficient to affect physiological and performance metrics that should be explored in future iterations. In addition, cow-level factors associated with increased risk of SU development, particularly parity and foot angle, that were not explored in the current study could enhance the success of an SU induction model.

ACKNOWLEDGMENTS

The authors acknowledge the American Association of Bovine Practitioners (Ashland, OH) for their funding support. We extend our thanks to Zelmar Rodriguez (Michigan State University, East Lansing, MI) for his assistance with data collection during this study, and we acknowledge the assistance of the student research assistants throughout the many phases of this study. We also thank Laura Solano (Lactanet, Calgary, Alberta, Canada) for her input regarding the study development and Maddy Ellis-Cramer for the hoof illustrations. Finally, we extend our gratitude to the dairy managers, J. P. Salvador and Dani Johnson, and staff at the University of Minnesota St. Paul Dairy Cattle Teaching and Research Center (St. Paul). The authors have not stated any conflicts of interest.

REFERENCES


Lübke, K. 2015. Entwicklung und Einsatz eines In-vitro-Ishämienmodells zur Untersuchung zellulärer Pathomechanismen der Klaue-/Rehe des Rindes. DVM-PhD Diss. Leipzig University, Veterinary Anatomical Institute, Department of Veterinary Medicine, Leipzig, Germany.


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Stoddard, G. 2018. Evaluating the relationship between hoof trimming and dairy cattle well-being. PhD Diss. Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN.


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