Lameness is a common cause of pain, distress, and debilitation in cattle, leading to welfare concerns and economic loss across all sectors of the cattle industry. In the dairy industry, lameness remains second only to mastitis in terms of herd productivity losses. Losses due to lameness include decreased milk yield, longer calving-to-conception intervals, and an increased risk of involuntary culling than in healthy cows. Although many risk factors have been identified and preventative measures initiated, the high prevalence of lameness in cattle continues to be problematic. This is a major animal welfare issue, as the prevalence of lameness may be considered an indicator of dairy cattle welfare.

Few options exist for effective alleviation of lameness-associated signs of pain in dairy cattle. This is in part attributable to a lack of FDA-approved analgesic drugs available for this purpose. Consequently, analgesics may not be routinely provided to large numbers of cattle with painful lameness or to cattle undergoing potentially painful treatments because of regulatory constraints. Cost, convenience, labor constraints, and difficulty recognizing signs of pain have also limited the use of analgesics in cattle. Concerns about the welfare of lame dairy cattle appear to conflict with concerns regarding food safety and drug residues with the use of pharmaceutical intervention. As food safety regulations become more stringent with regard to extralabel use of drugs in food-producing animals, it is imperative that action be taken on the part of the cattle industry to ensure pharmaceutical analgesic options are available, with importance placed particularly on maintaining appropriate welfare standards.

Currently, flunixin meglumine is the only NSAID approved by the FDA for use in adult dairy cattle; however, it is not approved for treatment of signs of pain. Label indications for the drug include pyrexia associated with bovine respiratory disease, endotoxemia, and acute mastitis as well as inflammation associated with these disorders. Procurement of approval for flunixin meglumine and other similar NSAIDs is difficult because of a lack of FDA-approved analgesic drugs available for this purpose. Consequently, analgesics may not be routinely provided to large numbers of cattle with painful lameness or to cattle undergoing potentially painful treatments because of regulatory constraints. Cost, convenience, labor constraints, and difficulty recognizing signs of pain have also limited the use of analgesics in cattle.

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Objective — To characterize amphotericin B–induced lameness in cattle and to ascertain the analgesic effects of flunixin meglumine by use of multimodal assessment.

Animals — 10 healthy Holstein steers free from musculoskeletal disease.

Procedures — Steers were randomly allocated to a treatment or negative control group. Amphotericin B was injected into the distal interphalangeal joint of the lateral claw of the left hind limb of all steers. Treatment steers received flunixin meglumine at the time of synovitis-arthritis induction and at 12 hours after induction. Control steers received no medication. Multimodal analysis included vital parameters, visual lameness score, behavioral monitoring with accelerometers, pressure mat analysis, and plasma cortisol determination before and after induction. Data were analyzed by use of linear mixed models with treatment and time designated as fixed effects, accounting for repeated measures on individual calves.

Results — Amphotericin B injection induced moderate, transient lameness. Control steers were more than twice as likely to be lame as treatment steers (mean ± SD lameness score, 92.2 ± 8.1% vs 40.7 ± 2.5%). Treatment steers placed significantly greater force and contact area on the affected foot and greater force, impulse, and contact area on the paired claw, compared with control steers. Furthermore, treatment steers spent considerably less time in recumbency than controls.

Conclusions and Clinical Relevance — Amphotericin B successfully induced synovitis-arthritis in dairy steers that was transient in nature. Flunixin meglumine was efficacious in providing analgesia for these steers. (Am J Vet Res 2011;72:1431–1438)
of objective data regarding efficacy. Therefore, objective documentation of the efficacy of flunixin meglumine for lameness-associated pain would provide a potentially viable treatment option for lameness.

As a species in which overt displays of illness may lead to predation in the wild, cattle are often regarded as stoic. However, their physiologic and anatomic similarities to other mammals far outweigh the minor differences, making it likely that cattle are capable of feeling pain as other mammals do, even if cattle do not appear to respond similarly. The stoic demeanor of cattle can lead to difficulties in quantifying pain because few tools exist for assessment and quantification of pain in cattle. Pain is a complex, multidimensional phenomenon, and multimodal technologies may prove useful for validating the measurement and assessment of pain in cattle.

Models for pain research in cattle need to be validated, reliable, and reproducible; however, most existing models have serious limitations. Few studies have shown that the severity of lameness can be correlated with the severity of the lesion in cattle. Ideally, an agent used to experimentally induce lameness in cattle would allow development of a lameness scale that is reliable, sensitive, and valid. For pharmaceutical investigation, lameness induced with the agent would be transient in nature, would not cause permanent damage to musculoskeletal structures, would be moderate in severity, and would have a duration sufficient for evaluation of the chosen drug. Although no lameness-induction agent is ideal, the amphotericin B–induced synovitis-arthritis model embodies many of these characteristics.

Amphotericin B, a polyene antimicrobial, has been used since the late 1970s as part of an aseptic transient synovitis lameness model in horses. Intra-articular injection of polyene antimicrobials such as amphotericin B results in disruption of lysosomes and release of inflammatory mediators, leading to synovitis. We reported the use of an amphotericin B–induced transient synovitis-arthritis model to evaluate the effects of sodium salicylate on the resultant lameness in cattle. The objectives of the study reported here were to characterize lameness induced by an amphotericin B–induced synovitis-arthritis pain model and to determine the analgesic effects of flunixin meglumine administered IV in cattle by use of multimodal analysis. We hypothesized that flunixin meglumine would provide analgesia for lameness-associated pain in cattle.

**Materials and Methods**

**Animals**—Ten Holstein steers were obtained from a dairy steer facility. Inclusion criteria were absence of musculoskeletal abnormalities and unremarkable results of gait observation and physical examination. The steers were housed in individual stalls (3.7 m²) with free-choice access to water and brome hay. Additional feed included a balanced beef feedlot diet composed of cracked corn, whole oats, whole grain sorghum, dry distiller’s grain, and a protein, vitamin, and mineral supplement. At the time of procurement, the steers were allowed a minimum of 3 days of acclimatization prior to initiation of the trial. During this time, steers were walked for 15 minutes at a steady pace and bi-directionally back and forth through the pressure mat testing alleyway to establish a consistent gait and reduce the amount of variation during the trial. All experimental procedures in this study were approved by the Kansas State University Institutional Animal Care and Use Committee.

**Experimental design**—Steers were randomly allocated to a treatment (n = 5) or control (5) group on the basis of a Latin square design. This allocation method ensured balanced group sizes. The first calf was randomly chosen as the first calf to enter the chute for ear tag application. This calf was then assigned to a group by use of a coin flip. Each subsequent calf was assigned to alternating groups such that the random allocation proceeded progressively until all calves were assigned to a group. The investigators were blinded to the allocation of steers into treatment groups. Physical examination of the steers was performed every 12 hours starting immediately prior to induction of synovitis-arthritis (baseline) and continued until the end of the trial (60 hours). Vital parameters including heart rate, respiratory rate, and rectal temperature were recorded for analysis.

**Amphotericin B synovitis-arthritis induction**—Each of the 10 steers was directed into a cattle chute, and the distal aspect of the left hind limb was secured with a rope. The left hind lateral pastern region was clipped of hair with a No. 40 blade proximal to the dorsal aspect of the coronary band. The site was steriley prepared by use of povidone iodine scrub and 70% isopropyl alcohol. Sterile technique was used to place an 18-gauge, 3.8-cm needle 1 cm proximal to the coronary band and 1 cm abaxial to the tendon of the long digital extensor muscle. The needle was directed distally toward the sole at an angle of approximately 40° off the surface of the lateral aspect of the pastern joint into the distal interphalangeal joint of the lateral digit of each steer. Twenty milligrams of amphotericin B (2 mL of a 10 mg/mL solution) was administered. The intra-articular location of the injection was confirmed by aspiration of joint fluid, ease of injection of the amphotericin B solution, and positive pressure return of fluid into the syringe. The same investigator performed all injections.

Steers in the treatment group received flunixin meglumine (1 mg/kg, IV) immediately after amphotericin B injection and at 12 hours after induction of synovitis-arthritis. The control steers (5) did not receive an NSAID at these times but were otherwise handled in a similar manner to the treatment steers at all measurement points.

**VLS**—Visual lameness scores were assigned by use of a previously described 5-point scale (Appendix). A VLS was assigned to each steer immediately prior to induction of synovitis-arthritis (baseline) as well as at 6, 12, 24, 30, 36, 48, 54, and 60 hours after induction. Each lameness score was determined by watching the steer walk 20 m in a straight line, turn, and walk 20 m back to the starting point. All lameness examinations were performed on even, nonsloped concrete floors free of obstructions and debris. Each steer was assigned a VLS by 2 separate investigators trained in lameness detection and blinded to the
study group allocation of each steer. The mean of these 2 scores for each measurement point for each steer was used for statistical analysis.

**Rescue analgesic protocol**—Given that an untreated control group was enrolled in the study, calves were assessed hourly for behavioral signs of excessive pain over a period of 10 hours following surgery. This was followed by twice-daily monitoring for 7 days. Calves with VLS of 4 or a VLS of 3 for > 48 hours, prolonged recumbency, anorexia, and signs of depression were to receive rescue analgesia with flunixin meglumine (2.2 mg/kg, IV, q 12 h) and intra-articular medication.

**Accelerometry**—At the time each calf was assigned to a treatment group, commercially manufactured remote sensor units were affixed to the lateral aspect of the left hind limb of the steers just proximal to the metatarsophalangeal joint by use of a protective plastic housing and canvas straps. Triaxial accelerometers were used to record the mean acceleration in x-, y-, and z-axes in addition to vector magnitude mean and maximum. Call behavior at each measurement point was categorized as walking, standing, or lying on the basis of a previously validated classification system. The categorized behavioral data were aggregated on a daily basis starting 1 day before induction of synovitis-arthritis (day 0) and extending through completion of the study (day 3). The final data represented the proportion of time cattle spent performing each behavior on each of the study days.

**Pressure mat analysis**—A pressure mat was placed in a designated alleyway with cattle panels placed parallel along each side of the mat, extending 3 m beyond both ends of the mat to allow for ease of directing cattle onto the mat. The pressure mat measured 0.9 X 2.4 m with 1.5 sensors/cm2. A 2-mm-thick rubber mat, extending the length of the alleyway, was placed over the pressure mat to ensure ambulation and traction were not impeded by hiding the mat from view and providing a consistent walking surface. Research software supporting the pressure mat system allowed for real-time recording of the stance phase of stride as well as the simultaneous recording of multiple foot falls on the pressure mat. Stance duration was calculated in a similar matter for the affected limb and contralateral limb. Video synchronization was used to assist in the determination of hoof placement on the mat. Characterization of weight bearing and force distribution of the hind limbs during locomotion was accomplished through calculation of stride duration, force, impulse, contact area, pressure, and integral measurements. Readings were obtained immediately prior to induction of synovitis-arthritis (baseline) as well as at 6, 12, 24, 30, 36, 48, 54, and 60 hours after induction.

**Cortisol measurement**—Blood samples were obtained from a jugular vein immediately prior to synovitis-arthritis (baseline) and at 6, 12, 24, 30, 36, 48, 54, and 60 hours after induction. Blood samples were collected in EDTA tubes and then stored on ice and centrifuged at 1,600 X g for 15 minutes at 0°C. Plasma was removed and frozen in cryovials at –40°C. Plasma cortisol concentrations were determined by use of a solid-phase competitive chemiluminescent enzyme immunoassay previously described and validated. A sample volume of 100 μL was used in each assay well. The reported calibration range for the assay is 28 to 1,380 nmol/L with an analytic sensitivity of 5.5 nmol/L.

**Statistical analysis**—Visual lameness scores were evaluated with the aid of statistical software. The scores were transformed into binary variables, and the proportional odds of lameness were calculated by adding random effects for repeated measures (time, steer, and replicate group) to the logistic regression model. The binary breakpoint was set between a VLS of 0 and VLS > 0 to differentiate between nonlame and lame cattle.

Generalized linear models were developed by use of analytic software to evaluate accelerometer data, plasma cortisol concentrations, vital parameters data (heart rate, respiratory rate, and rectal temperature), and pressure mat variables (force, impulse, contact area, pressure, pressure integral, peak force, and peak pressure) for the affected limb, contralateral limb, affected claw, and paired claw. Values of P < 0.05 were considered significant. The models included fixed effects of treatment and time (after treatment) and random effects to account for lack of independence of individual measurements related to replicate group and repeated measures in individuals. In addition, cortisol measurements were analyzed with the baseline cortisol concentration at induction of synovitis-arthritis as a covariate in the model. With regard to time as an effect, although preinduction values were not directly compared with postinduction values, the pressure mat variables were evaluated over the entire study period, allowing for some inference regarding pre- and postinduction differences in values. The various effects were tested for interactions.

**Results**

**Animals**—The 10 steers enrolled in the study were a mean of 9 months old with a mean body weight of 400 kg. Environmental conditions during the study ranged from a mean temperature of 22°C to 26°C and humidity of 40% to 63.6%. Rescue analgesia was initiated for 1 steer (control group) because of lameness that did not decrease in severity or resolve by the end of the trial (VLS = 3 for > 48 hours). Lameness in that steer improved after lavage of the joint with 250 mL of lactated Ringer’s solution and intra-articular administration of 2 mL of 2% lidocaine HCl and flunixin meglumine (1 mg/kg, IV, q 12 h for 2 days). The steer subsequently recovered with no long-term deficits.

**Vital parameters**—The 2 research groups did not differ significantly in values of vital parameters, nor was there an interaction between treatment group and study day on those values. However, parameter val-
ues significantly changed as a function of time for the overall sample of steers (Figure 1). Changes in heart rate \((P < 0.01)\), respiratory rate \((P < 0.01)\), and rectal temperature \((P < 0.01)\) were associated with day of the study. Specifically, heart rate increased at 12 hours after synovitis-arthritis induction, then returned to near baseline at 48 hours after amphotericin B injection. Respiratory rate and rectal temperature increased slightly after injection and remained higher than baseline for the study period.

VLS—All steers \((n = 10)\) became visibly lame \((VLS > 0)\) within the first 6 to 12 hours after amphotericin B injection. Although a low degree of variation existed in VLSs between individual steers in each treatment group, there was a consistent peak in severity between the 6- and 12-hour marks, which subsequently dissipated over the course of the study, with most steers having a VLS of 0 by the end of the study (Figure 2). The interaction between treatment group and measurement time was not significant; however, there was an association between treatment group and VLS. Control steers had an increased probability of having a VLS > 0 at any measurement point during the study, compared with flunixin meglumine–treated steers \((92.2 \pm 8.1\% \text{ vs } 40.7 \pm 2.5\%, \text{ respectively}; P = 0.026)\).

Accelerometer analysis—Evaluation of the percentage of time calves spent in recumbency (lying) during the study period revealed a significant \((P = 0.001)\) interaction between treatment group and trial day. Flunixin-treated steers spent less time lying after lameness induction than did control calves in the initial postinjection period, but behavior was similar between the 2 groups by day 2 (Figure 3).

Pressure mat analysis—No significant treatment and time interactions with regard to pressure mat values were identified; therefore, only main effects (time and treatment) are reported. Stance duration (affected limb and contralateral limb) as well as force, peak force, impulse, contact area, pressure, peak pressure, and pressure integral were calculated for the affected limb, contralateral limb, affected claw, and paired claw, and significant findings or findings approaching significance were summarized (Table 1).

Steers that received flunixin meglumine exerted significantly greater maximum \((P = 0.032)\) and mean \((P = 0.049)\) force on the affected limb during the stance phase, compared with control steers. Flunixin-treated steers had a higher impulse on the affected limb during the stance phase than did control steers, but this difference was not significant \((P = 0.061)\). Flunixin-treated steers had significant \((P = 0.035)\) increases in mean contact area of the affected foot during the stance phase, compared with control steers. The maximum area for the contralateral foot during the stance phase was significantly \((P = 0.043)\) greater in flunixin-treated steers than in control steers. Steers that received flunixin meglumine exerted greater maximum \((P = 0.023)\) and mean \((P = 0.005)\) force on the paired claw (to the affected claw) during the stance phase, compared with control steers. The impulse exerted during the stance phase was significantly \((P = 0.023)\) greater for the paired claw (to the affected claw) in flunixin-treated steers, compared with control steers. The maximum \((P = 0.015)\) and mean \((P = 0.004)\) contact area of the paired claw (to the affected claw) during the stance phase...
phase were significantly greater in flunixin-treated steers, compared with values for the control steers. The mean peak force in the paired claw (to the affected claw) during the stance phase was greater in flunixin-treated steers than in control steers, but the difference was not significant ($P = 0.056$).

In all steers, a significant ($P = 0.036$) difference in the integral values exerted on the paired claw (to the affected claw) was evident over the course of the study. This was represented by a peak in the integral value at 6 hours after synovitis-arthritis induction, with values returning to baseline over the course of the study. All steers also had nonsignificant differences in maximum force ($P = 0.056$) and maximum peak force ($P = 0.06$) exerted on the paired claw (to the affected claw) as well as integral ($P = 0.075$) exerted on the contralateral foot over the course of the study. These maximum force and maximum peak force values varied over the course of the study with no apparent pattern. In all steers, the duration of the stance phase in the affected limb approached significance ($P = 0.067$) over time; that of the contralateral limb differed significantly ($P = 0.002$) over time.

Cortisol analysis—Plasma cortisol concentrations significantly ($P = 0.03$) increased during the study period in all steers (Figure 4). The initial postinduction cortisol concentration measured at 6 hours was increased, compared with concentrations at all other postinduction measurement points. No interactions were present between time and treatment. The control steers generally had greater plasma cortisol concentrations than flunixin-treated steers; however, the differences were not significant ($P = 0.13$).

Discussion

The present study involved evaluation of the magnitude and duration of amphotericin B–induced synovitis-arthritis in cattle combined with evaluation of the analgesic efficacy of a cyclooxygenase inhibitor in an induced lameness model. We previously reported that amphotericin B induced a predictable and moderate synovitis-arthritis that was transient in duration.26 In that study, sodium salicylate had minimal impact on the reduction of the induced lameness. In the present study, the amphotericin B–induced synovitis model provided a controllable and sustained but transient painful insult. Severity of lameness peaked between 6 and 12 hours after induction as judged by VLS assessment and resolved over 72 hours in most steers. The severity and duration of lameness in the control steers were considerably less than those described in the equine literature. For example, equine studies17–27 have shown a moderate to severe lameness for 3 days to 2 weeks in duration. In our steers, a consistent severity and duration of lameness were achieved. We chose to perform only 1 injection of amphotericin B, as opposed to multiple injections as performed in many of the aforementioned equine studies. The selected joint may also affect the severity and duration of lameness because amphotericin B was injected in the distal interphalangeal joint of the steers rather than the carpal or tarsal joints as in horses. Also, cattle are able to shift weight from one claw to the other, which likely decreases pain-associated lameness in the affected claw.26–28 These findings suggest the 2 species differ with regard to severity of synovitis and clinically apparent signs of lameness.

Physiologic variables such as heart rate, respiratory rate, and rectal temperature are often used to assess pain

![Graph](image)

Figure 3—Least squares mean ± SD percentage time the steers in Figure 1 spent in recumbency after treatment with flunixin meglumine (1 mg/kg, IV; solid line) or nothing (dashed line).

Table 1—Pressure mat analysis data (least square means and SE) for force, area, and impulse of Holstein steers treated with flunixin meglumine (1 mg/kg, IV; flunixin; n = 5) or nothing (control; 5) after induction of synovitis-arthritis with amphotericin B injected into the distal interphalangeal joint of the left hind limb.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Flunixin</th>
<th>SE</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left hind limb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum force (kg-force)</td>
<td>82.73</td>
<td>103.03</td>
<td>14.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean force (kg-force)</td>
<td>52.32</td>
<td>67.17</td>
<td>11.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Mean area (cm²)</td>
<td>28.87</td>
<td>36.21</td>
<td>2.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Impulse (kg*s)</td>
<td>37.11</td>
<td>56.14</td>
<td>13.2</td>
<td>0.06</td>
</tr>
<tr>
<td>Left hind medial claw</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum force (kg-force)</td>
<td>38.87</td>
<td>49.08</td>
<td>10.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean force (kg-force)</td>
<td>20.49</td>
<td>30.43</td>
<td>6.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean area (cm²)</td>
<td>11.62</td>
<td>16.20</td>
<td>2.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Impulse (kg*s)</td>
<td>14.58</td>
<td>25.32</td>
<td>6.9</td>
<td>0.03</td>
</tr>
</tbody>
</table>

All models accounted for repeated measures on individual steers throughout the study period. Generalized linear models included fixed effects of treatment and time and random effects to account for lack of independence of individual measurements related to replicate (steer grouping) and repeated measures on individuals. Values of $P < 0.05$ were considered significant.
in research and clinical settings. Changes in heart rate occur when calves are castrated, suggesting an influence of pain on heart rate. In the present study, heart rate, respiratory rate, and rectal temperature of steers differed significantly over the study period. Given these findings, it is likely that synovitis-arthritis induction, pain, and perhaps distress caused by the procedure led to increased heart rates in the initial postinduction period. Further interpretation of these findings is difficult because of the potential effects of any distress caused by the procedure, the noxious nature of the induced synovitis-arthritis, and any anxiety caused by handling. Factors such as the ambient temperature and humidity can affect heart rate, respiratory rate, and rectal temperature; however, in the present study, these variables were similar throughout the study period. The chronicity of the tissue insult can also lead to less consistent changes in physiologic responses.

The duration of lameness induced via amphotericin B injection provided ample time to determine the efficacy of preemptive and postinduction administration of flunixin meglumine in the amelioration of lameness. In cattle, the half-life of flunixin meglumine is approximately 5.2 hours after IV administration. Other studies have been conducted to evaluate the effect of cyclooxygenase inhibitors such as ketoprofen in naturally acquired lameness. In a study, ketoprofen administration reportedly had a minimal effect on gait; however, it is unclear whether factors other than claw pain were responsible for the gait alterations observed. These other studies were conducted in cattle with lameness of various etiologies, thus limiting interpretation of initial degree of pain and response to drug treatment. Although the use of cattle with naturally acquired lesions lessens the ethical concerns of induction of pain in bovine research, it can be difficult to control the source, severity, and consistency of the lesions involved. A validated, reliable, and reproducible model of pain associated with lameness in cattle is useful to allow assessment of efficacy of various treatments under controlled conditions. Our model of amphotericin B–induced synovitis-arthritis offers a humane alternative with which to perform validation studies to develop treatments for painful, naturally occurring disease.

In our study, gait evaluation by use of the VLS rating scale was useful in consistent characterization of gait. Various lameness scoring systems have been developed for adult cattle, but no VLS method has been standardized for calves. We chose to use the VLS system described by Anderson because of our familiarity with that scoring method. Control steers were more than twice as likely to be lame as flunixin-treated steers at any measurement point during study. The dramatic difference between the control steers and the flunixin-treated steers with regard to odds of lameness was likely influenced by the preemptive administration of flunixin meglumine in this study. After induction of synovitis-arthritis, the flunixin-treated steers ambulated less lamely than control steers as revealed by pressure mat analysis. This was indicated primarily through significantly larger force and contact area in the affected limb of flunixin-treated versus control steers and an apparent but non-significantly higher impulse. In a similar pattern, the paired claw (same limb as the affected claw) had increased force, impulse, and contact area as well as a trend of increased peak force in flunixin-treated versus control steers. These findings suggest that flunixin meglumine administration was successful in blunting inflammatory pain associated with the synovitis-arthritis through inhibition of prostaglandin production. Although flunixin-treated steers had fewer signs of pain in the affected limb than did their control counterparts, they shifted their axis of weight bearing toward the companion claw during the weight-bearing phase of stride. This suggests that although the flunixin-treated steers were more comfortable bearing weight on the affected limb, they did have some degree of discomfort. Administration of flunixin meglumine after the establishment of clinical pain may not have diminished the severity of lameness to the same degree as preemptive administration would have, but additional research is needed to explore this effect.

The driving forces of gait and force distribution in cattle are poorly understood. Weight shifting has been observed in cattle with sensitive or painful claws. Lame cattle can alter gait not only by the reduction of vertical ground forces in affected limbs but also through the modification of horizontal acceleratory and deceleratory forces. In an effort to shift weight away from a painful digit, cattle may alter force and associated limb acceleration in multiple planes. Force and contact area distribution in lame cattle are dynamic and differ among the multiple limbs. This creates unique challenges in studying lameness and response to treatment, compared with horses. Pressure mat analysis allows sensitive detection of alterations in weight distribution that are useful in assessment of subtle changes in limb pain. In our study, weight distribution could be assessed within the limb (companion claw) as well as between the limbs (contralateral limb).

As the study progressed, changes in integral values occurred in both groups as a function of time in the paired claw (the same limb as the affected claw). These changes were consistent with the time course of the synovitis-arthritis as evidenced by a significant increase in the integral of the paired claw at the peak of lameness 6 hours after synovitis-arthritis induction. Because the integral is a function of pressure over time to account for variation in acceleration, this increase was expected for steers when bearing a substantial amount of...
weight or force on a smaller surface area as when cattle have slight (ie, toe-touching) lameness. The maximum peak force increased, albeit nonsignificantly, toward the end of the study, indicating that the steers were likely placing increased amounts of force on the paired claw as they became more comfortable. These findings were true of both the control and treatment groups, suggesting that although flunixin administration appeared to mitigate pain associated with the synovitis-arthritis, it did not completely eliminate the pain.

The duration of the stance phase of both limbs varied over time in the study steers. In the affected limb, a nonsignificant increase in stance duration was noted, particularly at the time of peak lameness, compared with that at baseline. The duration of the stance phase nonsignificantly decreased over the course of the study, with the duration approaching baseline toward the study conclusion. In the same manner, the duration of the contralateral limb stance was significantly higher 12 hours after synovitis-arthritis induction, at which point the duration steadily decreased until it approached baseline values at the end of the study. These findings support the transient nature of the lameness achieved with the amphotericin B synovitis-arthritis model. The increased duration of stance was consistent with a decreased overall speed of locomotion as was seen after induction of synovitis-arthritis (data not reported), and slowing of locomotion is associated with lameness.40

Cortisol secretion is part of a complex physiologic stress response.41 Although plasma cortisol measurements have been the most extensively used assessment tool of pain-induced stress in models of acute pain in cattle such as dehorning, the relationship between stress and plasma cortisol concentration is not always clear.31,42,43 Confounding factors such as handling-induced distress must be differentiated from pain-induced stress. In the present study, plasma cortisol concentrations significantly differed over the study period. The increase in plasma cortisol concentration noted 6 hours after synovitis-arthritis induction may have corresponded to psychological distress, pain-induced stress, or a combination thereof. Although the control steers appeared to have a greater plasma cortisol concentration after induction of synovitis-arthritis, there was no significant difference between the treatment groups. This suggests that flunixin meglumine was ineffective at alleviating pain-induced increases in cortisol secretion or that plasma cortisol analysis was an insensitive tool for pain assessment in this model. Given the other findings of the study, it is likely that the initial spike in plasma cortisol concentrations was consistent with a pain response; however, additional research will be needed to ascertain the meaning of cortisol measurements in the amphotericin B–induced lameness model.

The accelerometer results in the present study characterized the behavioral manifestations of lameness associated with amphotericin B–induced synovitis-arthritis in steers. A significant interaction was detected between time and treatment with regard to time cattle spent in a recumbent position. In particular, the flunixin-treated steers spent significantly less time in recumbency than their control counterparts during the early postinduction phase of the trial. Lying behavior may be an indicator of cattle discomfort, and previous research has demonstrated changes in this behavior following castration.44 As the present trial progressed, the difference in degree of activity between the flunixin-treated group and the control steers slowly decreased. This finding was expected because the amphotericin B lameness model appeared to yield a peak in lameness severity 6 to 12 hours after synovitis-arthritis induction. As a nonspecific cyclooxygenase inhibitor, flunixin meglumine was expected to maintain effect for 6 to 12 hours after administration on the basis of an expected half-life in serum of 5.2 hours.

The pressure mat, gait, and accelerometer analysis findings in the study reported here showed the amphotericin B–induced synovitis-arthritis model yielded moderate but transient lameness in dairy steers. Flunixin meglumine administration was efficacious in providing analgesia in this model. Future research is needed to determine the dose-dependent analgesic effects of flunixin meglumine. Research is also needed to assess the efficacy of flunixin meglumine in ameliorating pain in cattle with naturally acquired lameness.

a. X-Gen Pharmaceuticals Inc, Big Flats, NY.

b. Motes, SENSIR, Elkader, Iowa.

c. Tekscan Hugemat 5400 XL, Tekscan, South Boston, Mass.

d. BD Diagnostics, Franklin Lakes, NJ.

e. Immitite 1000 Cortisol, DPS, Calif.

f. JMP, version 5.1.2, SAS Institute Inc, Cary, NC.

References

Appendix

Visual lameness scoring system used for a study of amphotericin B-induced synovitis-arthritis of the distal interphalangeal joint of the left hind limb in Holstein steers.

<table>
<thead>
<tr>
<th>Lameness score</th>
<th>Assessment criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal—no gait deficits</td>
</tr>
<tr>
<td>1</td>
<td>Mild lameness; bears weight on all 4 limbs when standing; walks readily and bears full weight on foot and limb; observable gait alteration; topline of back is normal</td>
</tr>
<tr>
<td>2</td>
<td>Moderate lameness; does not bear full weight on limb when standing; reluctant to walk but does use the limb to ambulate; shortened weight-bearing phase of stride; increased periods of recumbency; may arch topline of back</td>
</tr>
<tr>
<td>3</td>
<td>Severe lameness; prefers recumbency; reluctant to stand; non-weight bearing when standing; reluctant to walk unless forced; hops over limb when walking; topline of back arched with caudoventral tip to pelvis</td>
</tr>
<tr>
<td>4</td>
<td>Recumbent; unable to rise; euthanasia indicated</td>
</tr>
</tbody>
</table>