Impact of oral meloxicam administered alone or in combination with gabapentin on experimentally induced lameness in beef calves

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Abstract
This study examined the pharmacokinetics and analgesic effect of oral meloxicam (MEL) administered alone or in combination with gabapentin (GABA) in an experimental bovine lameness model. Eighteen male British × Continental beef calves aged 4 to 6 mo and weighing 297 to 392 kg were randomly assigned to receive either 1) 0.5 mg/kg lactose monohydrate placebo (PLBO; n = 6), 2) 0.5 mg/kg MEL (n = 6), or 3) 0.5 mg/kg MEL combined with 15 mg/kg GABA (MEL-GABA; n = 6) once daily for 4 d. The first treatment was administered 4 h after a chemical synovitis/arthritis was induced with injection of 15 mg amphotericin B into the left hind lateral distal interphalangeal joint. Changes in activity were evaluated continuously with pedometers. Contact force, contact area, contact pressure, impulse, and stride length were recorded once daily with a pressure mat and visual lameness scores were determined by a masked observer using a 5-point scale. Cortisol and drug concentrations were determined daily by immunoassay and HPLC-mass spectrometry, respectively. Outcomes were compared statistically using a random effects mixed model and analysis of covariance. There was a positive association between lameness scores and serum cortisol concentrations (P = 0.02) and a negative association between lameness score and step count (P < 0.0001), total force (P = 0.001), force applied to the lateral claw (P= 0.02), contact pressure (P = 0.005), and impulse of the lateral claw (P = 0.01). Step count was greater in MEL calves compared with PLBO (P = 0.008) and MEL-GABA (P = 0.04) calves. Impulse was greater in the MEL-GABA calves compared with the PLBO calves (P = 0.03). There was an inverse relationship between plasma MEL concentrations and lameness score (P = 0.02) and a positive association between MEL concentrations and force applied to the lateral claw (P = 0.03), total contact pressure (P = 0.03), and impulse on the lateral claw (P = 0.02). There was a tendency towards a positive association between GABA concentrations, total impulse, and impulse on the lateral claw (P = 0.08) and a negative associate between GABA concentrations and step count (P = 0.08). The results of this study suggest that MEL administered alone or in combination with GABA reduced the severity of lameness in calves following induction of lameness with amphotericin B. These findings have implications for developing analgesic protocols in lame calves that address both production and welfare concerns.

Keywords
analgesia, cattle, gabapentin, lameness, meloxicam

Disciplines
Large or Food Animal and Equine Medicine | Statistical Methodology | Veterinary Anatomy | Veterinary Physiology

Comments
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ABSTRACT: This study examined the pharmacokinetics and analgesic effect of oral meloxicam (MEL) administered alone or in combination with gabapentin (GABA) in an experimental bovine lameness model. Eighteen male British × Continental beef calves aged 4 to 6 mo and weighing 297 to 392 kg were randomly assigned to receive either 1) 0.5 mg/kg lactose monohydrate placebo (PLBO; n = 6), 2) 0.5 mg/kg MEL (n = 6), or 3) 0.5 mg/kg MEL combined with 15 mg/kg GABA (MEL-GABA; n = 6) once daily for 4 d. The first treatment was administered 4 h after a chemical synovitis/arthritis was induced with injection of 15 mg amphotericin B into the left hind lateral distal interphalangeal joint. Changes in activity were evaluated continuously with pedometers. Contact force, contact area, contact pressure, impulse, and stride length were recorded once daily with a pressure mat and visual lameness scores were determined by a masked observer using a 5-point scale. Cortisol and drug concentrations were determined daily by immunoassay and HPLC-mass spectrometry, respectively. Outcomes were compared statistically using a random effects mixed model and analysis of covariance. There was a positive association between lameness score and step count (P < 0.0001), total force (P = 0.001), force applied to the lateral claw (P = 0.02), contact pressure (P = 0.005), and impulse of the lateral claw (P = 0.01). Step count was greater in MEL calves compared with PLBO (P = 0.008) and MEL-GABA (P = 0.04) calves. Impulse was greater in the MEL-GABA calves compared with the PLBO calves (P = 0.03). There was an inverse relationship between plasma MEL concentrations and lameness score (P = 0.02) and a positive association between MEL concentrations and force applied to the lateral claw (P = 0.03), total contact pressure (P = 0.03), and impulse on the lateral claw (P = 0.02). There was a tendency towards a positive association between GABA concentrations, total impulse, and impulse on the lateral claw (P = 0.08) and a negative association between GABA concentrations and step count (P = 0.08). The results of this study suggest that MEL administered alone or in combination with GABA reduced the severity of lameness in calves following induction of lameness with amphotericin B. These findings have implications for developing analgesic protocols in lame calves that address both production and welfare concerns.

Key words: analgesia, cattle, gabapentin, lameness, meloxicam


INTRODUCTION

Involuntary culling of lame cattle has a significant impact on both dairy and beef production. The prevalence of lameness in dairy herds has been reported as 33.7 and 36.8% in Wisconsin and the United Kingdom, respectively (Cook, 2003; Barker et al., 2010). Roeber
et al. (2001) found that 31.4% of cattle audited in the 1999 National Market Cow and Bull Beef Quality Audit were lame. Tibbetts et al. (2006) reported a 6.5% prevalence of foot rot in feedlot steers and observed that lame calves spent an average of 5 d longer on feed and gained 0.049 kg/d less during the finishing period than nonaffected cattle. As a consequence, the direct cost of lameness in the feedyard is estimated to be US$59.94/case.

Lameness is one of the most important welfare challenges in livestock production and is considered a chronic pain syndrome because hyperalgesia persists for at least 28 d after the causal lesion has resolved (Ley et al., 1996; Whay et al., 1998, 2003). Lameness pain has both an inflammatory and neuropathic component. Inflammatory pain responds modestly to treatment with nonsteroidal anti-inflammatory drugs (NSAID; Whay et al., 2005; Flower et al., 2008) but neuropathic pain is considered refractory to the effects of NSAID (Woolf and Mannion, 1999). Gabapentin (GABA) is a γ-aminobutyric acid analogue used extensively for the management of chronic pain in humans (Hurley et al., 2002; Cheng and Chiou, 2006). Meloxicam (MEL) is a NSAID that is approved outside the United States as an adjunctive therapy of acute respiratory disease, diarrhea, and acute mastitis. The pharmacokinetic profile of oral GABA and MEL supports clinical evaluation of these compounds for management of chronic pain in cattle (Coetzee et al., 2011). The present study was conducted to test the hypothesis that oral MEL administered alone or in combination with GABA would mitigate signs of experimentally induced lameness in beef calves.

MATERIALS AND METHODS

All experimental procedures in this study were approved by the Kansas State University (KSU) Institutional Animal Care and Use Committee under the supervision of the University Veterinarian (protocol number 2863).

Experimental Cattle

Eighteen male British × Continental beef calves aged 4 to 6 mo and weighing 297 to 392 kg were acquired from a Kansas livestock producer. On arrival calves received florfenicol (Nuflor; Merck Animal Health, Summit, NJ; lot number 8668101) for control of bovine respiratory disease at 40 mg/kg, doramectin pour-on (Deco-max; Pfizer Animal Health, Madison, NJ; lot number OAWM7) as an anthelmintic at 500 μg/kg, permethrin pour-on (Ultraboss; Merck Animal Health; lot number 90414C) for fly control at 1 mL/15 kg, and a clostridial vaccine (Covexin 8; Merck Animal Health; lot number 1381C). All animals were examined on arrival and were found to be healthy and subjectively free from lameness with the exception of 4 animals that presented with signs of bovine keratoconjunctivitis. These cattle responded to therapy with florfenicol before study commencement.

Housing and Husbandry

Study calves were initially housed in a dry lot confinement facility at KSU Animal Resource Facility for approximately 90 d after arrival. Thereafter, calves were blocked by body weight and randomly assigned to 3 pens of 6 calves each, so that each pen contained 2 calves from each treatment group. Pens comprised a linear row of outdoor concrete pads (9.75 by 18.29 m), each with a partial roof over straw bedding. The diet consisted of water and grass hay ad libitum with a ration composed of cracked corn, alfalfa pellets, soybean meal, molasses, vitamins, and minerals delivered at 5 to 6 kg per calf per day, divided and offered twice daily in open bunks. Due to the nature of the diet and the housing arrangement, it was not possible to measure individual feed intake.

Jugular Catheterization

Approximately 24 h before study commencement, all calves were individually restrained in a squeeze chute using a rope halter and the attached head gate. Following restraint, the area over the right jugular vein in period 1 and the left jugular vein in period 2 was clipped and disinfected using povidone iodine and 70% isopropyl alcohol swabs. The catheter site was infiltrated with approximately 0.5 mL of 2% lidocaine injection (Hospitala Inc., Lake Forest, IL) and a small skin incision with a number 22 blade was made to facilitate placement of a 14 gauge (G) by 140 mm catheter (Abbo cath-T; Abbott Ireland, Sligo, Rep. of Ireland), which was sutured to the skin using 2–0 nylon suture (Burns Veterinary Supply, Inc., Westbury, NY). Catheter patency was maintained by twice daily flushing using 3 mL heparin saline containing 3 IU heparin sodium/mL 0.9% saline (Heparin Sodium Injection, USP; Baxter Healthcare Corporation, Deerfield, IL). The catheters were removed immediately after the last blood collection time point in each period.

Group Assignment, Randomization, and Study Procedures

The study was conducted using a parallel design with a period of baseline data collection (period 1) followed by a study period (period 2). Before period 1, study animals were blocked by body weights determined 24 h before study commencement and randomly assigned to treatment groups based using a computer-generated random number (Microsoft Excel 2007; Microsoft Corpora-
tion, Redmond, WA) to ensure that treatment groups had similar mean body weights (Fig. 1).

**Baseline Data Collection (Period 1)**

The study commenced at 17 h 25 min on d –7 with an acclimatization period, which included collection of baseline data (cortisol, pedometer, and pressure mat data) from nonarthritic calves (period 1) collected once daily for 5 d. Baseline data were used as a covariate to improve the fit of the statistical model that was used to compare treatment effects following lameness induction.

**Lameness Induction (Period 2)**

Period 2 commenced on d 0 with lameness induction. Before lameness induction, all calves were restrained in a chute with head gate as conducted throughout the acclimatization period, and the left hind leg was restrained with ropes at the fetlock and stifle. After restraint, the lateral digit pastern region was prepared with close clipping of hair (number 40 clipper blade) and aseptic skin preparation using povidone iodine scrub and 70% isopropyl alcohol swabs. Lameness was induced as previously described (Kotschwar et al., 2009; Schulz et al., 2011). Briefly, a sterile 18 G 1 1/2-inch (3.8-cm) needle was inserted 1 cm proximal to the coronary band and 1 cm abaxial to the tendon of the long digital extensor muscle and angling distally toward the sole. After the sterile needle was inserted, correct placement into the distal interphalangeal joint was verified by aspiration of synovial fluid back into the syringe. A dose of 15 mg amphotericin B (X-Gen Pharmaceuticals, Inc., Big Flats, NY) was injected using 3 mL of a 5 mg/mL solution. Continued position within the distal interphalangeal joint was verified periodically throughout the injection by ease of injection followed by back-flow of synovial fluid and amphotericin B into the syringe. This solution was fully injected into the distal interphalangeal joint to complete the procedure. All procedures occurred at approximately 5 min intervals and were performed by a single veterinarian (DEA) to avoid interoperator variation.

Rescue analgesic therapy options for unresolved lameness after final data collection included flunixin meglumine at 2.2 mg/kg intravenously (IV) once daily, butorphanol tartrate at 0.05 mg/kg subcutaneously (SC) once daily, morphine at 0.1 mg/kg SC once daily, and 100 mg of lidocaine 2% administered as a single intra-articular injection.

**Drug Administration**

At 4 h after lameness induction, 0.5 mg/kg MEL (Meloxicam tablets 15 mg [NDC 65862-098-01]; Aurobindo Pharma USA, Dayton, NJ; lot number MX1509019-A) was administered alone or in combination with 15 mg/kg GABA (Gabapentin capsules, USP 100 mg and 400 mg [NDC 0228-2667]; Actavis Elizabeth LLC, Parsippany, NJ; lot number 832J91). The oral dose was rounded to the nearest whole capsule or tablet. Calves in the placebo (PLBO)-treated group received an equivalent dose of D (+)-lactose monohydrate (Fluka Analytical, Buchs, Germany), a pharmacologically inactive excipient used in the manufacture of MEL tablets, by mouth (PO). Treatments were administered at 24 h (±1 h) intervals for 4 d. The contents of the capsules, whole tablets, and lactose powder was suspended in 50 mL of water in a 60 mL catheter-tip syringe (BD Luer-Lok Syringe; Becton-Dickinson, Franklin Lakes, NJ) and administered as an oral drench within 5 min of suspension.

**Blood Sample Collection**

Fifteen milliliters of whole blood for determining drug concentrations in treated calves was collected into syringes using the preplaced jugular catheter immediately before lameness induction and again at 12, 24, 48, 72, 84, 96, 108, 120, 132, and 144 h thereafter. Samples for assessing cortisol concentrations in all calves were collected into syringes using the preplaced jugular catheter immediately before lameness induction and again at 12, 24, 48, 72, and 96 h thereafter. Immediately after obtaining the blood sample, 3 mL of heparin saline flush, as described above, was used to maintain patency of the catheter. Blood was immediately transferred to a 7 mL sodium heparin vacutainer tube (BD Diagnostics, Franklin Lakes, NJ) and 7 mL vacutainer tube (BD Diagnostics) containing no additive. The vacutainer tubes were stored on ice for no more than 60 min pending sample
processing. Thereafter, blood samples were centrifuged at 1,600 × g for 15 min at 0°C. Serum and plasma were pipetted from their respective tubes and placed in cryovials identified with calf identification, date, time point, sample, and treatment group. The samples were stored at –80°C before sample analysis. All samples were analyzed within 60 d of sample collection.

**Plasma Gabapentin and Meloxicam Analysis**

Plasma concentrations of GABA and MEL were determined with HPLC (Shimadzu Prominence; Shimadzu Scientific Instruments, Columbia, MD) and mass spectrometry (API 2000; Applied Biosystems, Foster City, CA) as previously described (Coetzee et al., 2011; Malreddy et al., 2013). Plasma samples or standards (100 μL) were added to 100 μL of internal standard (pregabalin 5 μg/mL in methanol) and 400 μL of methanol with 0.1% formic acid to precipitate the proteins. Quantitation was performed by calculating the ratios of GABA m/z 172.1 → 154.1 and MEL m/z 352.09 → 114.90 responses relative to the internal standard m/z 160.00 → 142.00 transition. The samples were vortexed for 5 s and centrifuged for 10 min at 15,000 × g at 4°C. The supernatant, 200 μL, was transferred to an injection vial with an injection volume of 25 μL. The mobile phase consisted of 100% B from 0 to 1 min with a linear gradient to 50% A and 50% B at 3 min, which was maintained until 6 min, followed by a linear gradient to 100% B at 6.5 min with a total run time of 8 min. The solvent “A” was acetonitrile and the solvent “B” was 0.1% formic acid at a flow rate of 0.4 mL/min. Separation was achieved with a phenyl column (Hypersil Gold, 150x2.1, 5 μM; Thermo Scientific, Waltham, MA) maintained at 40°C. The standard curve was linear from 0.05 to 10 μg/mL for GABA and 0.025 to 2.5 μg/mL for MEL and was accepted if the correlation coefficient exceeded 0.99 and predicted values were within 15% of the actual values. The accuracy of the GABA assay was 97 ± 10% and the coefficient of variation was 10% determined on replicates of 3 at 0.05, 0.1, 0.5, 5, and 10 μg/mL. The accuracy of the MEL assay was 100 ± 9% and the coefficient of variation was 6% determined on replicates of 3 at 0.025, 0.05, 0.25, 1.0, and 2.5 μg/mL. The limits of detection were 0.05 and 0.025 μg/mL for GABA and MEL, respectively, defined as the lowest concentration on the standard curve with predicted concentrations within 15% of the actual concentration.

**Pharmacokinetic Analysis**

Pharmacokinetic analyses were performed with computer software (WinNonlin 5.2; Pharsight Corporation, Cary, NC). The calculated variables included the area under the curve from time 0 to infinity using the linear trapezoidal rule and the terminal drug elimination half-life. The accumulation index was calculated as the ratio between the peak plasma drug concentration (Cmax) at steady state and the Cmax after the first dose. The maximum (Cmax), minimum, and average plasma concentration and time to maximum serum concentration (Tmax) were determined directly from the data.

**Clinical Lameness Scoring**

The degree of lameness was scored using a 0 to 4 scale adapted from Sprecher et al. (1997; Table 1) as previously described (Kotschwar et al., 2009). Lameness scores were determined once daily, after blood sample collection, to document presence of lameness and to visually score severity of lameness. To eliminate interobserver variation, all lameness scores were assigned by 1 blinded veterinarian (DEA) with training and expertise in bovine lameness assessment. Intra-observer variability was assessed periodically by randomly selecting calves for repeated assessment to ensure consistency of scoring. All lameness examinations were performed on even, nonsloped concrete floors free of obstructions and debris. Each lameness score was determined by watching the calf walk a minimum of 20 m in a straight line, turn, and walk 20 m back to the starting point.

**Cortisol Analysis**

Serum cortisol concentrations were determined as previously described and validated in bovine plasma (Coetzee et al., 2007) using a solid-phase competitive chemiluminescent enzyme immunoassay and an automated analyzer system (Immulite 1000 Cortisol; Siemens Medical Solutions Diagnostics, Los Angeles, CA). 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 analytical sensitivity of 5.5 nmol/L.
Pedometer

Pedometers (NL-800; New-Lifestyles Inc., Lees Summit, MO) were placed within a protective neoprene sleeve that was attached to the lateral aspect of the metatarsus immediately proximal to the fetlock as previously described (Hanzlíček et al., 2010). Pedometers contained an accelerometer inside of them that monitored the number of steps each calf took based on the up and down movement of the calf’s leg. The pedometer data were recorded during the acclimatization period and for 96 h after lameness induction at which time data were downloaded into a spreadsheet for analysis.

Pressure Mat Analysis

A commercially available floor mat-based pressure/force measurement system (MatScan; Tekscan, Inc., South Boston, MA) was used to record and analyze the affected feet of each calf as previously described (Kotschwar et al., 2009; Schulz et al., 2011). Data were collected once daily at the time of blood sample collection. The pressure mat was calibrated daily and each time the computer software was engaged using a known mass to ensure accuracy of the measurements at each time point. Video synchronization was used to ensure consistent gait between and within calves for each time point. Using research grade software (HUGEMAT Research 5.83; Tekscan, Inc.), contact pressure, contact area, impulse, and stance phase duration in the affected feet were measured. Surface area was calculated by area only of the loaded or “contact” sensing elements inside the measurement box. Contact pressure was calculated as force on the loaded sensing elements inside the measurement box divided by the contact area. Impulse was calculated as the area under the force vs. time curve. This reflects the association between force and the time the foot was on the ground. The stance phase duration was determined as the period of time when the foot was in contact with the ground.

Average Daily Weight Gain

Calves were individually weighed on d 0 and 4 using a commercial livestock scale (For-Most Livestock Equipment, Hawarden, IA). Food and water were not withheld before weighing. Average daily gain was calculated by subtracting the prelameness weight and the postlameness weight and dividing this by the number of days that passed between weigh dates.

Data Analysis and Statistics

Hypothesis tests were conducted using the GLIMMIX procedure of SAS (version 9.2; SAS Inst. Inc., Cary, NC). The mean ± SEM were calculated for each outcome variable at each time point. Mean baseline outcome values were used as covariates in regression models. Two structures of fixed effects were considered. One is ANOVA structure with treatment group, time, and their interaction; the other is an analysis of covariance type with drug concentrations (MEL or GABA; continuous), lameness score, and time (categorical) included in the model. Model assumptions were considered to be appropriately met based on diagnostics conducted on studentized residuals. Estimated least square means and corresponding standard errors are presented. A significant difference was considered to exist when \( P \leq 0.05 \), and a marginal difference was considered to exist if \( 0.05 < P \leq 0.10 \). Relevant pairwise comparisons were conducted when the significance of the interaction term was \( P \leq 0.10 \) using Tukey-Kramer or Bonferroni adjustments, as appropriate in each case, to avoid inflation of Type I error rate due to multiple comparisons. Analysis of variance was also used to evaluate differences in single measurement, normally distributed data (ADG).

RESULTS AND DISCUSSION

Meloxicam and Gabapentin Plasma Concentrations

Following oral administration, plasma MEL concentrations declined slowly over the course of the study (Table 2; Fig. 2 and 3). In contrast, GABA was more rapidly eliminated from the plasma resulting in a shorter elimination half-life compared with MEL (Table 2; Fig. 3). There was no difference in plasma MEL concentrations in calves that received MEL alone compared with calves that received MEL combined with GABA (\( P > 0.5 \)).

Meloxicam is an NSAID of the oxicam class that is approved in the European Union and Canada for adjunctive therapy of acute respiratory disease, diarrhea, and acute mastitis and the alleviation of pain associated with disbudding in calves when administered at 0.5 mg/kg IV or SC (European Agency for the Evaluation of Medicinal Products [EMEA], 1999). Gabapentin is a \( \gamma \)-aminobutyric acid analogue originally developed for the treatment of spastic disorders and epilepsy in humans (Cheng and Chiou, 2006). Studies have established that GABA is also effective for the management of chronic pain of inflammatory or neuropathic origin (Hurley et al., 2002). Although the mechanism of action of GABA is poorly understood, it is thought to bind to the \( \alpha_2-\delta \) subunit of voltage gated calcium channels acting presynaptically to reduce the release of excitatory neurotransmitters (Taylor, 2009). The justification for co-administering GABA with MEL in the present study was the reported synergistic interaction with NSAID to
produce antihyperalgesic effects (Hurley et al., 2002; Picazo et al., 2006).

In a recent study we reported that the mean (±SD) Cmax, Tmax, and half-life for GABA (15 mg/kg) co-administered as a single oral dose with MEL (1 mg/kg) was 3.57 ± 1.04 μg/mL, 7.33 ± 1.63 h, and 8.12 ± 2.11 h, respectively (Coetzee et al., 2011). The mean (±SD) Cmax, Tmax, and half-life for MEL in the same study was 2.11 ± 0.19 μg/mL, 11.67 ± 3.44 h, and 20.47 ± 9.22 h, respectively. These concentrations are comparable to the steady state plasma concentrations maintained for the duration of the present study following once daily administration for 4 d.

The pharmacokinetic–pharmacodynamic relationship and dose response to MEL in horses with induced carpal arthritis has been previously reported (Toutain and Cester, 2004). Based on this work, the reported half maximal effective concentration (EC50) for MEL in the plasma of lame horses is approximately 0.2 μg/mL. Plasma GABA concentrations >2 μg/mL in humans are associated with a lower frequency of seizures (Sivenius et al., 1991). Similar doses are used to treat epilepsy and neuropathic pain suggesting that these concentrations will also be effective for analgesia. Concentrations above these levels were maintained for both drugs throughout the present study indicating that this treatment regimen was appropriate to achieve the goals of the trial.

In the United States, use of MEL and GABA for alleviating pain associated with lameness constitutes extra-label drug use (ELDU; Coetzee et al., 2009; Smith and Modric, 2013). Under the Animal Medicinal Drug Use Clarification Act of 1994 (U.S. Food and Drug Administration, 1994), ELDU is permitted for relief of suffering in cattle provided specific conditions are met. These conditions include that 1) ELDU is permitted only by or under the supervision of a veterinarian, 2) ELDU is allowed only for Food and Drug Administration approved animal and human drugs, (3) ELDU is only permitted when the health of the animal is threatened and not production purposes, (4) ELDU in feed is prohibited, and (5) ELDU is not permitted if it results in a violative food residue. Therefore, use of MEL and GABA to

Table 2. Mean (± SD) meloxicam and gabapentin plasma pharmacokinetic parameters following lameness induction with amphotericin B and treatment with oral meloxicam (MEL) at 0.5 mg/kg once daily alone or in combination with oral gabapentin (MEL-GABA) at 15 mg/kg once daily for 4 d. The maximum (Cmax), minimum (Cmin), and average (Cavg) plasma concentration and time to maximum serum concentration (Tmax) were determined directly from the data. The calculated variables included the area under the curve from time 0 to infinity (AUC INF) using the linear trapezoidal rule and the terminal drug elimination half-life (HL_Lambda_z). The accumulation index was calculated as the ratio between the Cmax at steady state and the Cmax after the first dose.

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<th>Outcome</th>
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<th>Cmax, μg/mL</th>
<th>Cmin, μg/mL</th>
<th>Cavg, μg/mL</th>
<th>AUC INF, h × μg/mL</th>
<th>Accumulation index</th>
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Figure 2. Mean ± SE for plasma meloxicam (MEL) concentrations after oral treatment with 0.5 mg/kg MEL once daily for 4 d (arrows) following lameness induction with amphotericin B at 6 h before first treatment.

Figure 3. Mean ± SE for plasma meloxicam (MEL) and gabapentin (GABA) concentrations after oral treatment with 0.5 mg/kg MEL alone or in combination with 15 mg/kg GABA once daily for 4 d (arrows) following lameness induction with amphotericin B at 6 h before first treatment.
alleviate pain associated with lameness in cattle in the United States must by law comply with these regulations (Coetzee, 2013a). At the conclusion of the present study all study animals were humanely euthanized and incinerated because a tissue withhold period for GABA in cattle has not been established. It is acknowledged that further research to establish a meat withhold period for GABA is needed before widespread use in animals intended for human consumption can be recommended.

**Clinical Lameness Scoring**

There was evidence of a time effect on clinical lameness score ($P < 0.0001$) but only marginal evidence of a treatment effect ($P = 0.089$) and no evidence of a time × treatment interaction ($P = 0.76$; Table 3; Fig. 4). Lameness scores were higher at 24 ($P = 0.005$) and 48 h ($P = 0.02$) after lameness induction across treatment groups. Lameness score tended to be greater in PLBO calves compared with MEL calves ($P = 0.07$) throughout the study but this was especially evident at 72 h after treatment ($P = 0.02$). The relative distribution of lameness scores over time in each treatment group are presented in Fig. 5a, 5b, and 5c. At 96 h after lameness induction, 100% of clinical lameness had resolved in the MEL group compared with 83% in the 0.5 mg/kg MEL combined with 15 mg/kg GABA (MEL-GABA) group and only 50% in the PLBO group. Plasma MEL concentrations were found to be inversely proportional to lameness scores ($P = 0.03$; Table 4). No animals required rescue analgesia during the course of the study.

Intra-articular injection of amphotericin B in the distal interphalangeal joint causes a chemical synovitis-arthritis resulting in a transient lameness in cattle, pigs, and horses (Fahmy et al., 1994; Kotschwar et al., 2009; Schulz et al., 2011; Karriker et al., 2013). Amphotericin B is a polyene antimicrobial that is approved for use as an antifungal agent. Following intra-articular injection, amphotericin B causes an aseptic synovitis as a result of disrupting lysosomes and release of inflammatory mediators within the synovial tissue. The results of the present study confirm the appropriateness of this model for demonstrating analgesic drug efficacy because lameness was reliably induced in all animals for at least 96 h after injection of amphotericin B.

The analgesic efficacy of IV administered flunixin meglumine (1 mg/kg) has been previously evaluated using an amphotericin B induced lameness model (Schulz et al., 2011). Compared to untreated controls, animals receiving flunixin meglumine at the time of lameness induction and 12 h later were less likely to be lame as determined by clinical lameness score. In contrast, sodium salicylate (50 mg/kg) administered IV failed to significantly mitigate clinical signs following amphotericin B induced lameness in 4- to 6-mo-old steers (Kotschwar et al., 2009). The results of the present study provide evidence that MEL mitigated clinical lameness, thus providing support for our hypothesis.

Although the clinical response to treatment with MEL alone was similar to MEL-GABA in the present study, it should be noted that there were no calves with lameness scores of 3 or 4 at the time treatment was initiated. Given that more severe lameness scores are commonly

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PLBO</th>
<th>MEL</th>
<th>MEL-GABA</th>
<th>Treatment</th>
<th>Time</th>
<th>Treatment × Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sprecher</strong> lameness score, 0–4</td>
<td>1.33 (0.29)</td>
<td>0.38 (0.29)</td>
<td>0.79 (0.29)</td>
<td>0.09</td>
<td>&lt;0.0001</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Cortisol</strong>, nmol/L</td>
<td>15.78 (1.19)</td>
<td>15.18 (1.18)</td>
<td>15.79 (1.18)</td>
<td>0.98</td>
<td>&lt;0.0001</td>
<td>0.25</td>
</tr>
<tr>
<td>Step count</td>
<td>3,224.03a (163.70)</td>
<td>4,086.59b (176.98)</td>
<td>3,424.63a (161.36)</td>
<td>0.01</td>
<td>&lt;0.0001</td>
<td>0.92</td>
</tr>
<tr>
<td>Total force, kg force</td>
<td>788.75 (36.48)</td>
<td>863.81 (36.25)</td>
<td>910.20 (36.87)</td>
<td>0.10</td>
<td>0.30</td>
<td>0.59</td>
</tr>
<tr>
<td>Force (lateral claw), kg force</td>
<td>495.93 (47.38)</td>
<td>621.29 (47.86)</td>
<td>637.64 (48.28)</td>
<td>0.10</td>
<td>0.45</td>
<td>0.31</td>
</tr>
<tr>
<td>Force (medial claw), kg force</td>
<td>394.94 (23.04)</td>
<td>336.94 (23.09)</td>
<td>313.61 (24.25)</td>
<td>0.07</td>
<td>0.21</td>
<td>0.89</td>
</tr>
<tr>
<td>Total contact area, cm²</td>
<td>50.81 (1.59)</td>
<td>49.30 (1.57)</td>
<td>51.08 (1.60)</td>
<td>0.70</td>
<td>0.38</td>
<td>0.37</td>
</tr>
<tr>
<td>Contact area (lateral claw), cm²</td>
<td>28.96 (1.34)</td>
<td>30.25 (1.35)</td>
<td>31.67 (1.36)</td>
<td>0.38</td>
<td>0.20</td>
<td>0.13</td>
</tr>
<tr>
<td>Total contact pressure, kg/cm²</td>
<td>24.29 (1.10)</td>
<td>21.39 (1.07)</td>
<td>21.18 (1.12)</td>
<td>0.12</td>
<td>0.52</td>
<td>0.98</td>
</tr>
<tr>
<td>Contact area (medial claw), cm²</td>
<td>15.88 (0.90)</td>
<td>17.40 (0.90)</td>
<td>17.90 (0.91)</td>
<td>0.28</td>
<td>0.30</td>
<td>0.93</td>
</tr>
<tr>
<td>Total impulse, kg × s</td>
<td>384.33a (24.98)</td>
<td>409.77b (27.11)</td>
<td>488.43b (26.81)</td>
<td>0.03</td>
<td>0.12</td>
<td>0.80</td>
</tr>
<tr>
<td>Impulse (lateral claw), kg × s</td>
<td>235.06 (25.41)</td>
<td>282.97 (26.27)</td>
<td>320.73 (26.30)</td>
<td>0.09</td>
<td>0.33</td>
<td>0.68</td>
</tr>
<tr>
<td>Impulse (medial claw), kg × s</td>
<td>152.02 (17.76)</td>
<td>145.86 (19.00)</td>
<td>152.53 (19.16)</td>
<td>0.97</td>
<td>0.38</td>
<td>0.37</td>
</tr>
<tr>
<td>Stance phase duration, s</td>
<td>0.75 (0.03)</td>
<td>0.78 (0.04)</td>
<td>0.82 (0.03)</td>
<td>0.36</td>
<td>0.59</td>
<td>0.48</td>
</tr>
<tr>
<td>Stride length, cm</td>
<td>62.47 (1.46)</td>
<td>66.64 (1.42)</td>
<td>65.68 (1.43)</td>
<td>0.15</td>
<td>0.55</td>
<td>0.41</td>
</tr>
</tbody>
</table>

*a,bDifferent superscripts indicate significant differences between treatments.

1LS = least square.

2PLBO = placebo.
recorded at the start of analgesic therapy in field cases of lameness, an effect of GABA in cattle with established central sensitization cannot be excluded based on these results. However, these findings support the conclusion that administration of MEL alone at 0.5 mg/kg once daily would alleviate pain associated with early cases of mild (score 1 or 2) lameness. Further research is needed to assess the efficacy of MEL and GABA in alleviating pain in cattle with more severe, naturally acquired lameness.

**Serum Cortisol Concentrations**

There was a positive association between log-transformed serum cortisol concentrations and increasing clinical lameness scores \( (P = 0.02) \) but there was no evidence of a treatment effect or time \( \times \) treatment interaction on serum cortisol concentrations (Table 3). Nevertheless, there was evidence of a time effect on circulating cortisol concentrations (Fig. 6). Specifically, serum cortisol concentrations were elevated at 12 h after lameness induction in all treatment groups \( (P < 0.0001) \) but there was no difference in cortisol concentrations noted at any other time points. There was also no evidence of an association between log-transformed serum cortisol concentrations and circulating MEL \( (P = 0.13) \) or GABA \( (P = 0.54) \) concentrations (Table 4).

Cortisol secretion is a critical component of the physiologic stress response. 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 and castration, the relationship between stress and plasma cortisol concentra-

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**Figure 4.** Mean ± SE lameness scores after oral treatment with a placebo (PLBO; \( n = 6 \); panel a), 0.5 mg/kg of meloxicam (MEL; \( n = 6 \); panel b), or 0.5 mg/kg of meloxicam combined with 15 mg/kg gabapentin (MEL-GABA; \( n = 6 \); panel c) once daily for 4 d following lameness induction with amphotericin B at 6 h before first treatment. \( a,b \)Time points not connected by the same letter are significantly different \( (P < 0.05) \). Lameness Score (LS): 0 = normal: stands and walks normally, with all feet placed with purpose; LS 1 = mildly lame: stands with flat back but arches when walks; gait is slightly abnormal; LS 2 = moderately lame: stands and walks with an arched back and short strides with 1 or more legs; LS 3 = lame: arched back standing and walking, with 1 or more limbs favored but at least partially weight bearing; and LS 4 = severely lame: arched back, refuses to bear weight on 1 limb, or may refuse or have great difficulty moving from lying position.

**Figure 5.** Distribution of lameness scores after oral treatment with a lactose monohydrate placebo (PLBO; \( n = 6 \); panel a), 0.5 mg/kg of meloxicam (MEL; \( n = 6 \); panel b), or 0.5 mg/kg of meloxicam combined with 15 mg/kg gabapentin (MEL-GABA; \( n = 6 \); panel c) once daily for 4 d following lameness induction with amphotericin B at 6 h before first treatment. Lameness scores (LS) were assigned according to the following system: LS0 = normal: stands and walks normally, with all feet placed with purpose; LS 1 = mildly lame: stands with flat back but arches when walks; gait is slightly abnormal; LS 2 = moderately lame: stands and walks with an arched back and short strides with 1 or more legs; LS 3 = lame: arched back standing and walking, with 1 or more limbs favored but at least partially weight bearing; and LS 4 = severely lame: arched back, refuses to bear weight on 1 limb, or may refuse or have great difficulty moving from lying position.
Kotschwar et al. (2009) reported that mean serum cortisol concentration was less in calves with a lameness score of 1 compared to that of calves with a lameness score of 3 ($P = 0.004$) after lameness induction with amphotericin B. It was also reported that cortisol concentrations tended to be elevated in control calves following lameness induction with amphotericin B compared with calves treated with 1 mg/kg flunixin meglumine (Schulz et al., 2011). Taken together these results combined with the findings of the present study support the assertion that pain associated with induced lameness causes activation of the hypothalamic–pituitary axis resulting in elevated serum cortisol concentrations.

Recently it was reported that plasma cortisol concentrations and lameness scores were significantly reduced in cows that received 0.5 mg/kg MEL IV once daily for 4 d following resection of the distal interphalangeal joint (Offinger et al., 2013). This finding contradicts the results of the present study that found no association between MEL administration and a reduction in cortisol concentrations in calves after induction of lameness. One explanation for these equivocal findings is that plasma cortisol concentrations were elevated for several days following digital resection compared with an increase only at 24 h after experimental lameness induction in the present study. This may have increased the likelihood of an analgesic drug mitigating serum cortisol concentration after digital resection because the overall magnitude and duration of the response was greater than in the present study.

### Step Count

Step count was inversely proportional to clinical lameness score ($P < 0.0001$; Table 5). An effect of treatment ($P = 0.01$) and time ($P < 0.0001$) on the number of steps taken after lameness induction was recorded using pedometers but no time × treatment interaction was observed ($P = 0.92$; Fig. 7; Table 3). Specifically, calves in the MEL group took more steps after lameness induction than calves in the PLBO group ($P = 0.008$) and the MEL-GABA group ($P = 0.04$). However, there was no difference in step count between the PLBO group and the MEL-GABA group ($P = 0.67$). There was also a positive association between increasing plasma MEL concentrations and the number of steps taken after lameness induction ($P = 0.0002$) but step count tended to be inversely proportional to plasma GABA concentrations ($P = 0.08$; Table 4).

### Table 4. Intercept, slope, and SEM for the correlation between plasma drug concentrations and the outcome measures after lameness induction with amphotericin B and treatment with oral meloxicam (MEL) at 0.5 mg/kg once daily alone or in combination with oral gabapentin (GABA; 15 mg/kg PO) once daily for 4 d

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intercept (±SEM)</th>
<th>Regression slope estimate (±SEM)</th>
<th>$P$-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEL</td>
<td>GABA</td>
<td></td>
</tr>
<tr>
<td>Sprecher lameness score, 0–4</td>
<td>7.37 (2.30)</td>
<td>–0.13 (0.06)</td>
<td>0.03</td>
</tr>
<tr>
<td>Log cortisol, nmol/L</td>
<td>1.75 (0.79)</td>
<td>–0.07 (0.04)</td>
<td>0.13</td>
</tr>
<tr>
<td>Step count</td>
<td>1,669.36 (599.44)</td>
<td>189.73 (46.70)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Total force, kg force</td>
<td>1,066.53 (188.46)</td>
<td>23.20 (12.43)</td>
<td>0.07</td>
</tr>
<tr>
<td>Force (lateral claw, kg force)</td>
<td>468.08 (180.47)</td>
<td>27.09 (11.98)</td>
<td>0.03</td>
</tr>
<tr>
<td>Force (medial claw, kg force)</td>
<td>289.34 (65.96)</td>
<td>–11.62 (7.11)</td>
<td>0.11</td>
</tr>
<tr>
<td>Total contact area, cm$^2$</td>
<td>37.61 (9.74)</td>
<td>–0.61 (0.51)</td>
<td>0.24</td>
</tr>
<tr>
<td>Contact area (lateral claw, cm$^2$)</td>
<td>28.02 (7.89)</td>
<td>0.35 (0.44)</td>
<td>0.43</td>
</tr>
<tr>
<td>Contact area (medial claw, cm$^2$)</td>
<td>15.27 (3.48)</td>
<td>–0.45 (0.33)</td>
<td>0.18</td>
</tr>
<tr>
<td>Total contact pressure, kg/cm$^2$</td>
<td>3.90 (5.31)</td>
<td>0.55 (0.25)</td>
<td>0.03</td>
</tr>
<tr>
<td>Total impulse, kg × s</td>
<td>404.55 (79.50)</td>
<td>9.49 (9.43)</td>
<td>0.32</td>
</tr>
<tr>
<td>Impulse (lateral claw, kg × s)</td>
<td>214.71 (48.95)</td>
<td>15.27 (6.42)</td>
<td>0.02</td>
</tr>
<tr>
<td>Impulse (medial claw, kg × s)</td>
<td>110.23 (33.57)</td>
<td>–1.43 (5.56)</td>
<td>0.80</td>
</tr>
<tr>
<td>Stance phase duration, s</td>
<td>0.49 (0.11)</td>
<td>0.01 (0.01)</td>
<td>0.41</td>
</tr>
<tr>
<td>Stride length, cm</td>
<td>46.49 (16.18)</td>
<td>0.57 (0.44)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

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Figure 6. Mean ± SE serum cortisol concentrations after oral treatment with a placebo (PLBO) or 0.5 mg/kg meloxicam (MEL) alone or in combination with 15 mg/kg gabapentin (MEL-GABA) once daily for 4 d following lameness induction with amphotericin B at 6 h before first treatment. 

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($^a$)Time points not connected by the same letter are significantly different ($P < 0.05$). Lameness Score (LS): 0 = normal: stands and walks normally, with all feet placed with purpose; LS 1 = mildly lame: stands with flat back but arches when walks; gait is slightly abnormal; LS 2 = moderately lame: stands and walks with an arched back and short strides with 1 or more legs; LS 3 = lame: arched back standing and walking, with 1 or more limbs favored but at least partially weight bearing; and LS 4 = severely lame: arched back, refuses to bear weight on 1 limb, or may refuse or have great difficulty moving from lying position.
A reduction in the number of steps in lame cattle compared with sound cattle has been recorded using pedometers attached to a sound rear limb (O’Callaghan et al., 2003). This indicates that cattle tend to reduce their activity to avoid weight loading to the painful limb (Shearer et al., 2013). It was previously reported that calves treated with 1 mg/kg flunixin at the time of lameness induction with amphotericin B were more active than untreated controls (Schulz et al., 2011). In a recent study it was reported that cows that received 0.5 mg/kg MEL IV once daily for 4 d following resection of the distal interphalangeal joint took more steps per hour than control cattle (Offinger et al., 2013). The results of the present study support this observation. However, the reduction in the number of steps taken in the MEL-GABA-treated calves compared with the calves that were treated with MEL alone was unexpected. The tendency for step count to be inversely proportional with circulating GABA concentrations suggests that this may have been directly associated with the effects of the drug. It is noteworthy that GABA administration in humans is associated with sedation and dizziness (Mao and Chen, 2000). We therefore hypothesize that GABA may have a mildly sedative effect in cattle that could have contributed to the reduction in activity. To our knowledge, this is the first study to report a direct association between analgesic drug concentrations and changes in activity in lame cattle. These data may assist in the development of analgesic drug regimens in lame calves that address both production and welfare concerns.

**Pressure Mat Analysis**

**Force.** Clinical lameness score was inversely proportional to total force ($P = 0.001$) and force applied to the lateral claw ($P = 0.02$; Table 5). However, there was no effect of treatment ($P = 0.10$) or time ($P = 0.30$) or evidence of a time × treatment interaction ($P = 0.59$) on the total force applied to the claw after lameness induction (Table 3). There was evidence of a weak positive association between plasma MEL concentrations and the total force applied to the lame claw ($P = 0.07$; Table 4).

When each claw was examined individually, there was no effect of treatment ($P = 0.10$) or time ($P = 0.45$) on the force applied to the lateral claw. However, there was evidence of a positive association between increasing plasma MEL concentrations and force applied to the lateral claw ($P = 0.03$; Table 4). There was also marginal evidence of an effect of treatment on the force applied to the medial claw after lameness induction ($P = 0.07$).

Specifically, calves in the PLBO group tended to apply

### Table 5. Intercept, slope, and SE for the correlation between Sprecher lameness score and the outcome measures after lameness induction with amphotericin B and treatment with oral meloxicam at 0.5 mg/kg once daily alone or in combination with oral gabapentin (15 mg/kg PO) once daily for 4 d

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intercept (±SE)</th>
<th>Regression slope estimate (±SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log cortisol, nmol/L</td>
<td>1.26 (0.66)</td>
<td>0.20 (0.08)</td>
<td>0.02</td>
</tr>
<tr>
<td>Step count</td>
<td>2,468.92 (501.20)</td>
<td>-487.77 (84.81)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total force, kg force</td>
<td>1,146.92 (157.34)</td>
<td>-84.92 (24.65)</td>
<td>0.001</td>
</tr>
<tr>
<td>Force (lateral claw), kg force</td>
<td>562.47 (170.17)</td>
<td>-65.81 (26.37)</td>
<td>0.02</td>
</tr>
<tr>
<td>Force (medial claw), kg force</td>
<td>240.77 (68.90)</td>
<td>23.16 (16.55)</td>
<td>0.17</td>
</tr>
<tr>
<td>Total contact area, cm$^2$</td>
<td>38.75 (9.24)</td>
<td>-0.40 (1.13)</td>
<td>0.72</td>
</tr>
<tr>
<td>Contact area (lateral claw), cm$^2$</td>
<td>28.14 (7.01)</td>
<td>-1.46 (0.90)</td>
<td>0.11</td>
</tr>
<tr>
<td>Contact area (medial claw), cm$^2$</td>
<td>13.76 (3.51)</td>
<td>1.13 (0.71)</td>
<td>0.12</td>
</tr>
<tr>
<td>Total contact pressure, kg/cm$^2$</td>
<td>8.90 (4.42)</td>
<td>-1.56 (0.53)</td>
<td>0.005</td>
</tr>
<tr>
<td>Total impulse, kg × s</td>
<td>381.27 (77.09)</td>
<td>-37.08 (25.74)</td>
<td>0.16</td>
</tr>
<tr>
<td>Impulse (lateral claw), kg × s</td>
<td>219.71 (48.86)</td>
<td>-51.21 (18.69)</td>
<td>0.01</td>
</tr>
<tr>
<td>Impulse (medial claw), kg × s</td>
<td>105.09 (26.56)</td>
<td>6.41 (11.60)</td>
<td>0.58</td>
</tr>
<tr>
<td>Stance phase duration, s</td>
<td>0.53 (0.10)</td>
<td>-0.0007 (0.03)</td>
<td>0.98</td>
</tr>
<tr>
<td>Stride length, cm</td>
<td>44.23 (16.92)</td>
<td>-0.43 (0.95)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Figure 7. Mean ± SE step count assessed with pedometer after oral treatment with a placebo (PLBO) or 0.5 mg/kg meloxicam (MEL) alone or in combination with 15 mg/kg gabapentin (MEL-GABA) once daily for 4 d following lameness induction with amphotericin B at 6 h before first treatment. a,b,cTime points not connected by the same letter are significantly different ($P < 0.05$). Lameness Score (LS): 0 = normal: stands and walks normally; 1 = mildly lame: stands with flat back but arches when walks; gait is slightly abnormal; 2 = moderately lame: stands and walks with an arched back and short strides with 1 or more legs; 3 = lame: arched back standing and walking, with 1 or more limbs favored but at least partially weight bearing; and 4 = severely lame: arched back, refuses to bear weight on 1 limb, or may refuse or have great difficulty moving from lying position.
more force to the medial claw after lameness induction than calves in the MEL-GABA group ($P = 0.07$).

When the force applied to the lateral claw was expressed as a percentage of the total force applied to the claw, a treatment effect was observed ($P = 0.03$) but there was no effect of time ($P = 0.22$) or time × treatment interaction ($P = 0.66$; Fig. 8). Specifically, calves in the MEL group had a greater percentage of force distributed to the lateral claw than PLBO calves ($P = 0.03$). Calves in the MEL-GABA group also tended to have a greater force distribution to the lateral claw than calves in the PLBO group ($P = 0.08$). As expected, when the force applied to the medial claw was expressed as a percentage of the total force applied to the claw, a treatment effect was also observed ($P = 0.04$) but there was no effect of time ($P = 0.75$) or time × treatment interaction ($P = 0.29$; Fig. 9). Specifically, calves in the PLBO group tended to have a greater percentage of the total force distributed to the medial claw than calves in the MEL-GABA group ($P = 0.04$).

In healthy cattle, force is primarily exerted on the lateral claw at the point of heel strike after which the load becomes shifted to the medial claw (van der Tol et al., 2002). Weight shifting occurs in lame cattle resulting in a reduction of vertical ground forces in affected limbs and redistribution of weight from the lateral to the medial claw especially in the hind limbs (Schulz et al., 2011). Pressure mat analysis allows detection of subtle changes in weight distribution between claws that is useful in detecting analgesic drug efficacy (Shearer et al., 2013). In the present study, induction of lameness manifested as a redistribution of force from the lateral to the medial claw. Administration of analgesia resulted in increasing weight distribution to the lateral claw that was associated with increasing plasma MEL concentrations. This is similar to the findings reported by Schulz et al. (2011) that steers receiving flunixin meglumine applied a greater maximum and mean force on the affected limb during the stance phase compared with control steers. Taken together these findings support the hypothesis that MEL reduced the severity of lameness likely due to the analgesic and anti-inflammatory effects of the drug.

**Contact Area.** There was no relationship between clinical lameness score, total contact area, or the contact area of the individual claws (Table 5). Furthermore, there was no effect of treatment or time or evidence of a time × treatment interaction on the total or individual contact area of the claw after lameness induction (Table 3). There was also no association between plasma drug concentrations and the contact area (Table 4).

The results reported herein conflict with the findings reported by Kotschwar et al. (2009) and Schulz et al. (2011) that observed a significant decrease in contact area following lameness induction with amphotericin B. One explanation for this is that the lameness that was induced with 20 mg amphotericin B in the previous studies was more severe than the lameness induced with 15 mg amphotericin B in the present study. This would also explain why there was no treatment effect observed in the present study.

**Contact Pressure.** Total pressure applied to the claw after lameness induction was inversely proportional to clinical lameness score ($P = 0.005$; Table 5). However, there was no effect of treatment ($P = 0.28$) or time ($P = 0.30$) or evidence of a time × treatment interaction ($P = 0.93$) on the contact pressure applied to the claw after lameness induction (Table 3). Nevertheless, there was a positive association between an increase in total contact
pressure applied to the lame claw and plasma MEL concentrations ($P = 0.03$).

These findings contradict those reported by Kotschwar et al. (2009) who reported that contact pressure tended to be directly proportional to lameness score. This was likely because mean contact area decreased significantly in the previous study resulting in force being distributed over a smaller area, thus increasing the total contact pressure. Furthermore, in that study there was no effect of treatment with intravenous sodium salicylate on contact pressure following lameness induction with amphotericin B. The positive association between MEL concentrations and contact pressure reported herein suggests that analgesic drug administration maintained both the force applied to the affected limb and surface area on which this was applied.

**Impulse.** Although there was no association between clinical lameness score and total impulse ($P = 0.16$) and impulse of the medial claw ($P = 0.58$), there was an inverse relationship between impulse on the lateral claw and clinical lameness score ($P = 0.01$; Table 5). There was also evidence of a treatment effect on total impulse ($P = 0.03$) but no effect of time ($P = 0.12$) or time × treatment interaction ($P = 0.80$; Table 3). Specifically, total impulse was less in PLBO-treated calves compared with MEL-GABA-treated calves over the course of the study ($P = 0.0286$). Furthermore, there was marginal evidence of a treatment effect on impulse of the lateral claw ($P = 0.09$), which was also due to the tendency of PLBO-treated calves to have a lower impulse than MEL-GABA-treated calves ($P = 0.077$). There was also evidence of a weak positive association between plasma GABA concentrations and the total impulse ($P = 0.08$) and impulse on the lateral claw ($P = 0.07$; Table 4). Increasing plasma MEL concentrations was also positively associated with an increase in impulse on the lateral claw ($P = 0.02$). It was previously reported that flunixin-treated steers tended to have a higher impulse on the affected limb during the stance phase than did control steers ($P = 0.06$; Schulz et al., 2011). The results of the present study provide further support for field investigations that test the hypothesis that MEL combined with GABA will reduce the severity of lameness especially in advanced cases where central sensitization has become established.

**Stance Phase Duration and Stride Length.** There was no association between lameness score and stance phase duration ($P = 0.98$; Table 5). Furthermore, there was no effect of treatment ($P = 0.36$), time ($P = 0.59$), or time × treatment interaction ($P = 0.48$) observed in the study (Table 3). Changes in drug concentrations were also not associated with changes in stance phase (Table 4). There was also no association between lameness score and stride length ($P = 0.65$; Table 5). Furthermore, there was no effect of treatment ($P = 0.15$), time ($P = 0.55$), or time × treatment interaction ($P = 0.41$) observed in the study (Table 3). Changes in drug concentrations were also not associated with changes in stride length (Table 4). These findings indicate that lameness induction and subsequent drug administration did not significantly impact these outcomes.

**Average Daily Weight Gain**

There was no significant difference in ADG between treatment groups over the course of the study ($P = 0.15$; Fig. 10). The difference in ADG between calves in the MEL-GABA group and calves in the PLBO group and MEL group was $2.30 ± 1.10$ ($P = 0.13$) and $1.30 ± 1.10$ kg/d ($P = 0.49$), respectively. The numerical difference in ADG between the MEL-GABA group and the PLBO group was surprising because both groups were less active than calves within the MEL group based on the pedometer results. It was recently reported that calves treated with MEL-GABA had a greater ADG after dehorning compared to calves receiving either MEL ($P = 0.02$) or GABA ($P = 0.0006$) alone (Glynn et al., 2013). This finding suggests the potential for a synergistic effect of these compounds for improving the growth and performance of calves after a painful event but further research is needed before widespread use can be recommended. It is noteworthy that pedometer measurements are not useful for assessing the time budgets and the relative location of calves within a pen. Therefore, the reduced activity of the calves does not necessarily imply that they spent less time at the feed bunk. The recent validation of remote triangulation devices to assess cattle time budgets and relative location within a pen could further elucidate the effect of analgesic compounds on cattle behavior after lameness induction (Theurer et al., 2012).

The results of this study provide further support for the effectiveness of an intra-articular injection of amphotericin B as being a useful model for studying lameness in cattle. Oral MEL administered once daily for 4 d had a positive effect on step count after lameness induction and increasing drug concentrations produced lower lameness scores and was positively associated with increased total contact pressure, force, and impulse applied to the lateral claw. The combination of GABA and MEL had a positive effect on the impulse applied to the lame claw but appeared to produce a mild sedative effect in treated cattle. Although the results of this study assist in the development of analgesic protocols in lame cattle that will address both production and welfare concerns, further research in cattle with naturally acquired lameness is needed before widespread use of these compounds can be recommended.
Figure 10. Mean ± SE ADG in body weight (kg) after oral treatment with a placebo (PLBO) or 0.5 mg/kg meloxicam (MEL) alone or in combination with 15 mg/kg gabapentin (MEL-GABA) once daily for 4 d following lameness induction with amphotericin B at 6 h before first treatment. *a,b* Time points not connected by the same letter are significantly different (P < 0.05).

**LITERATURE CITED**


Meloxicam and gabapentin lameness


