Animal 15 (2021) 100415

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences

Embedded microcomputer-based force plate system validation when evaluating lameness severity differentiation under an induced synovitis model in lactating dairy cattle

R. Warner[a], B.C. Smith[b], K.J. Stalder[c], L.A. Karriker[a], S. Plaengkaeo[d], B.C. Ramirez[b], P.J. Gorden[a,*]

[a] Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[b] Department of Agricultural and Biosystems Engineering, College of Agriculture and Life Sciences, Iowa State University, Ames, IA, USA
[c] Department of Animal Science, College of Agriculture and Life Science, Iowa State University, Ames, IA, USA
[d] Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand

ARTICLE INFO

Article history:
Received 18 January 2021
Revised 2 November 2021
Accepted 5 November 2021

Keywords:
Amphotericin B
Bovine
Lameness Detection
Non-invasive
Welfare

ABSTRACT

Bovine lameness has relatively large negative economic and welfare implications on the U.S. dairy industry. Due to the ramifications, early lameness detection will aid in assisting dairy producers to mitigate downstream effects through early treatment. The objective of this study was to determine the minimum standing time required among 2-, 3-, 4-, 5-, and 10 min time intervals to obtain an accurate weight distribution estimate for each leg when attempting to detect lameness. An embedded microcomputer-based force plate system was developed to measure vertical forces from individual cow limb weight distribution to detect bovine lameness when utilizing an induced synovitis lameness model. The force plate has four quadrants, with each load cell quadrant measuring the force placed on it from a single limb. The force plate recorded weight (kg) every second from each load cell quadrant, after which, a 60 s moving average for weight distribution was calculated. A sequential study design was employed to evaluate non-lame and induced lameness to ensure time requirements were consistent. Prior to induction, the force plate system was used to measure weight distribution every second for 15 min. After lameness induction, additional 15 min increments were recorded every 24 h for seven days. Lameness was induced by injecting the left hind distal interphalangeal joint in three cows with amphotericin B, 12 h prior to the start of the study. Data were analyzed using a linear mixed effect that included the fixed effects of day relative to lameness induction, time period, foot and injected foot. Cow within replicate was included as a random effect. Cumulative minutes were assessed up to 15 min by comparing the least square rolling 60 s cumulative means expressed as a percentage of each animal's BW percentage placed on each leg for 2-, 3-, 4-, 5-, and 10 min intervals. Results indicate that the minimum time needed for accurate lameness detection in cows was 2 min.

Implications

Lameness remains an economic and welfare issue plaguing the dairy industry. To combat this, an embedded microcomputer-based force plate system shows great promise as a non-invasive, early lameness detection modality for research applications. The primary focus of this pilot study was validation of the embedded microcomputer force plate system through an induced synovitis model. Our results indicate that the minimum time needed to stand on the force plate system for accurate lameness detection in cows was 2 min. Early lameness detection is key to mitigate negative welfare concerns through prompt treatment and pain alleviation.

Introduction

In the U.S. dairy industry, there is no dispute that lameness has extensive economic repercussions. Lameness is a condition in which an animal displays dysfunction within the locomotion system and is therefore externalized as abnormalities in the gait or posture (Sprecher et al., 1997). On average, the estimated cost for a single lameness event is $412 (Greenough and Weaver, 1997). This value takes into consideration reduced fertility, decreased

* Corresponding author.
E-mail address: pgorden@iastate.edu (P.J. Gorden).

https://doi.org/10.1016/j.animal.2021.100415
1751-7311/

production, increased labor, culling or replacement costs, and veterinary services (Greenough and Weaver, 1997). In 2019, the U.S. Department of Agriculture reported that there are approximately 9.33 million dairy cows in the United States. Under the assumption of a 20% lameness prevalence, this would result in an annual financial loss of greater than $550 million for U.S. dairy producers. This is a conservative estimate, as Wisconsin has reported that the lameness incidence was 21% in the summer and 24% in the winter among their dairy operations (Cook, 2003). Lameness is considered the third most costly issue for U.S. dairy herds, behind mastitis and reduced fertility (Brand et al., 1996). Lameness is financially correlated to increased medical treatments (O'Callaghan, 2002), decreased milk production (O'Callaghan, 2002), decreased body condition, and reduced reproductive performance (Sprecher et al., 1997; O'Callaghan, 2002; Vermunt, 2005). Additionally, lameness has been shown to decreased social interaction (Galindo and Broom, 2000), increased time spent lying, reduced feed intake, and increased culling rates (Sprecher et al., 1997; O'Callaghan, 2002). The economic implications for lameness are far reaching; therefore, early lameness detection validation techniques that can be used on farm are crucial to mitigating the burden on the cow and producer.

Force plate systems have been used in cattle along with other species. They are often manufactured using individual load cells under each limb to evaluate vertical weight distribution in real time. As animals are standing stationary on the force plate, locomotion is not detectable in this approach. In a study evaluating lameness in swine, an embedded microcomputer-based force plate system was developed for measuring sow weight distribution to detect lameness. The force plate measured vertical forces exerted by each limb (i.e., weight from each leg) and was able to generate data that enabled individual sow lameness detection (Sun et al., 2011). Another study used a lameness synovitis model to validate the time required to detect induced sow lameness using the embedded microcomputer-based force plate system (McNeil et al., 2018). In cattle, a force plate scoring system was interfaced in a hoof trimming box to evaluate the sensitivity and specificity relative to visual lameness scoring (**VLS**) systems (Ghotoorlar et al., 2012). The VLS system ranks lameness on a scale of 1–5 based on gait and posture abnormalities. (Sprecher et al., 1997). Greater than 72%, sensitivity and specificity were reported relative to VLS categories 1–4 on a five-point scale, with 100% specificity and 50% sensitivity for lameness scores categorized as five (Ghotoorlar et al., 2012). Results from scientific literature suggest that force plate measures are highly correlated with the gold standard VLS (Ghotoorlar et al., 2012). The ability to use a force plate system aids in eliminating staff training required to perform VLS system while maintaining comparable sensitivity and specificity.

Force plate validation for use in a research setting is crucial to create a platform to assess lameness in an unbiased, reproducible system. Amphotericin B is an anti-fungal pharmaceutical that produces transient arthritis and synovitis following an intra-articular injection (Kotschwar et al., 2009; Schulz et al., 2011; Coetzee et al., 2014). This model has been previously utilized in other species for analgesic evaluation and lameness detection modalities (Kotschwar et al., 2009; Schulz et al., 2011; Coetzee et al., 2014). The lameness reproducibility and consistency created when using amphotericin B make it an appropriate model system when validating the ability to accurately detect lameness using the force plate.

The objective of this study was to validate the embedded microcomputer-based force plate system to determine the minimum time required to detect individual limb induced synovitis in dairy cattle.

Material and methods

This project was approved by Iowa State University's Institutional Animal Care and Use Committee (IACUC 19-163). Cows were group housed in a free-stall barn meeting the requirements outlined in the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). All cows were fed a total mixed ration formulated to meet or exceed the National Research Council recommendations for lactating dairy cattle (NRC, 2001). All cows were provided ad libitum access to water. Milking occurred in a parlor at 0800 and 2000.

Study animals

Study animals were determined to be healthy, free from current and previous lameness (VLS = 0 on a zero to four-point scale), and not treated for lameness within 30 days prior to enrollment (Table 1). Three lactating Holstein cows were enrolled in this study, having 745, 780, and 773 kg individual BWs.

Lameness induction

In the sequential study design, three cows were first measured on the force plate for 15 min prior to lameness induction to evaluate baseline non-lame values for each individual limb, which were recorded as day 0. After the initial force plate evaluation that occurred to assess each animal prior to beginning, the study cows were visually examined and determined free from clinical signs of foot rot and digital dermatitis. To facilitate intra-articular injections, cows were restrained in a tilt chute and the hair around left lateral claw was clipped on the proximal surface of the coronary band. The surface was then aseptically prepared with alternating scrubs of chlorhexidine and 70% isopropyl alcohol. Lameness was induced by injecting 25 mg in a 3 mL volume of amphotericin B (X-Gen Pharmaceuticals Inc., Big Flats, NY) using a 21 $\times$ 1.5-inch needle into the lateral distal interphalangeal joint of the left rear (**LR**) leg (Schulz et al., 2011). Needle placement was confirmed using anatomical landmarks, joint fluid retraction, and injection ease. Twelve hours after induction, cows were walked onto the force plate to measure the weight each animal was willing to place on each limb. The right front (**RF**), left front (**LF**) and right rear (**RR**) feet were controls. Force plate measurements were repeated every 24 h for 7 days postamphotericin B injection.

Visual lameness score and visual analog score

The VLS was recorded at each time point using a modified approach to a previously described method (Sprecher et al., 1997) which assigned a 0–4 score at each time point for the seven-day time period (Table 1). To further characterize the degree of lameness, a visual analog score (**VAS**) was simultaneously used to analyze grade of lameness as previously described (Flower and Weary, 2006). Briefly, the VAS scoring system consisted of a 10 cm line drawn on paper ranging from 0 cm for no lameness to 10 cm for non-weight bearing lameness. To determine VLS and VAS, cows were assessed on a flat, concrete surface by allowing them to walk for 20 meters, then turned, and walked back to the initial starting point. The investigator who monitored cow movement was blinded to the experimental design.

Force plate

Design

The force plate design, embedded electronics, and software were adapted from Sun et al. (2011). Overall force plate dimen-

Table 1
Modified visual lameness scoring (VLS) system[1] used for evaluating induced lameness severity in lactating dairy cattle.

VLS	Clinical Signs
0	Normal stance and walking
1	Mild lameness; stands with flat back; walks abnormal
2	Moderate lameness; stands and walks with arched back
3	Lame; arched back standing and walking; favors 1+ limbs
4	Severely lame; arched back; non-weight bearing on 1 limb

[1] Adapted from Sprecher et al. (1997).

sions were 1.84 × 0.75 × 0.10 m (L × W × H), and 9.5 mm thick aluminum plating was used to construct the force plate (Sargent Metal Fabricating, Ames, IA). The top surface of each plate was coated with an epoxy overlay (AC3900-AR, Hog Slat, Newton Grove, NC) to provide footing that is similar to concrete flooring. Each quadrant had dimensions of 0.914 × 0.368 m (L × W). A single point load cell (500 kg; Model RL1250, Rice Lake Weighing Systems, Rice Lake, WI) was secured between the top and bottom force plate parts. Analog output from the load cell was 2.0 mV ± 0.02%, which was linearly proportional to the applied load. The load cell capacity was calculated to be 490.6 kg using the approach outlined by Sun et al. (2011) with an assumed 226 kg per limb live load (max 907 kg BW cow). Therefore, a 500 kg capacity load cell for each quadrant was selected for this force plate. The signal conditioning circuit and hardware were adapted from the previous design, in which, the amplified load cell signal was read into an embedded data acquisition and control board (USB-1408FS, Measurement Computing Corporation, Norton, MA). The software (Visual Basic v19, Microsoft Corp., Redmond, WA) was updated for compatibility with the current operating system.

Validation

Each force plate quadrant load cell and accompanying platform were verified using the following procedure. Seven 22.7 kg (50 lb) weights and two 11.3 kg (25 lb) weights were assembled and verified on a benchtop scale (Model R71MD35, Ohaus Corporation, Parsippany, NJ) to establish their "true" weight. One 50 lb weight with traceability was verified by the National Institute of Standards and Technology (NIST; US Department of Commerce, Gaithersburg, MD) for accuracy of the benchtop scale. Each quadrant was first independently calibrated by placing (1) 181.4 kg total weight at the center of each quadrant and (2) zeroed with no weights present via the software interface.

For each quadrant, an increasing-decreasing procedure was performed for validating purposes and to estimate hysteresis effects. Hysteresis is a Type A evaluation of measurement uncertainty. It is measured and defined as a maximum difference between the load cell's full range traverse as it upscales and downscales in readings (Heckert, et al., 2002).

$$S_{hysteresis} = Max|Y_{upscale} - Y_{downscale}|/\sqrt{3}$$

Each quadrant was separated into three sections for validation testing (interior, center, and exterior section after on-farm installation). A 22.7 kg weight was placed on one of the sections; the output weight was recorded for 30 seconds by the software, then this was repeated by incrementally adding 22.7 kg weights. Once the total static 181.4 kg weight load was achieved, the total weight was incrementally decreased by 22.7 kg and confirmed until no weight was on the quadrant. This procedure was repeated for each of the three sections within the quadrant.

Linear regression was performed for each quadrant section and the overall quadrant, using all three sections of data. Hysteresis was evaluated for each quadrant. The root mean square error for each quadrant and the sections within the quadrant were calculated (Table 2).

Animal validation

At each data collection time point, each force plate quadrant was independently calibrated by placing 181.4 kg in the center of the quadrant and with no weight applied via the software interface. The trial animals were then loaded on the force plate and weight distribution was recorded for each limb every second for 15 min each day for a seven-day period following lameness induction. Exclusion criteria included individual limb weight less than 45.5 kg or total weight less than 454.6 kg. These values would indicate that the foot was not appropriately positioned on the force plate.

Statistical analysis

For this pilot study, a sample size calculation was used to minimize the amount of induced arthritis-synovitis associated pain on the least number of animals, while still being able to determine a difference between limbs. A prior study using a similar force plate design using swine as the model system was used to conduct an A-priori power calculation to determine the population size (Sun et al., 2011). The force plate in this study was modeled after its design and therefore, the assumption was made that it would closely represent the effect seen in cattle. By using the dataset provided in the previous study, d = 3.13, α = 0.1 and power = 0.86 were used. Evaluation of covariance between animals and fixed effects of foot, time and foot × time were evaluated. Arithmetic mean, SD and range of the VLS and VAS across the seven-day time period were assessed and Tukey-Kramer HSD was used to compare means for individual animals to establish that locomotion deficits were induced.

Descriptive statistics were obtained using PROC MEANS (SAS v9.4, SAS Inst. Inc., Cary, NC, USA). These parameters included mean weight, SD of weight, skewness (asymmetry of weight distribution), 5th and 95th percentile weight per quadrant, range between 5th and 95th percentile for each cow, on each quadrant, per injected foot, per 24 h. The 5th percentile was selected as it is more robust than a smaller percentile for the minimum weight applied as values of 0 kg were recorded when shifting between limbs. Alternatively, the 95th percentile was chosen as the most appropriate measurement for maximum weight applied due to extreme values occurring when shifting within the animal holding chute. Alternatively, skewness is the measure of how greatly the probability distribution of a random variable deviates from the normal distribution. It is equivalent to three times the difference between the mean and median, divided by the SD.

The mean weight distribution ($\bar{X}$) as a proportion of each individual cow's total weight (Q_{Total}) was evaluated. Proportion comparisons occurred for each limb (Q_x).

$$\bar{X} = \frac{Q_x}{Q_{LF} + Q_{RF} + Q_{LR} + Q_{RR}} = \frac{Q_x}{Q_{Total}}$$

The output data were analyzed using a mixed model method (PROC MIXED, SAS v9.4, SAS Inst. Inc., Cary, NC, USA). Day relative to lameness induction, time period, foot, and injected foot were included as fixed effects and cow within replicate was included as a random effect in the model used to analyze these data. The mixed linear model utilized to evaluate these data is described as follows:

$$Y = Di + Tj + F + I + R(S) + e$$

$$Y = \mu + D_i + T_j + F_k + (TF)_{jk} + I_l + S_m + e_{ijklm(n)}$$

Table 2
Linear regression performed for each section of a quadrant (center, interior, exterior) and the overall quadrant of the embedded microcomputer-based force plate to determine RMSE using 22.7 kg weight recorded at 30 s increments to a final weight of 181.4 kg. The increasing-decreasing procedural estimation of hysteresis effects was performed prior to dairy cow lameness model validation.

Item	Quadrant			
	Left front	Right front	Left rear	Right rear
RMSE (kg)				
Center	0.87	1.18	0.88	1.02
Interior	3.17	0.59	1.91	1.05
Exterior	0.68	0.91	0.68	1.18
Overall	2.30	0.99	1.38	1.10
Hysteresis (kg)				
Overall	2.88	2.93	7.92	2.47
Hysteresis (%)				
Overall	0.58	0.59	1.58	0.49

where Y is the dependent variable (the percentage of the cow's weight applied on each foot recorded by the respective quadrant of the force plate), μ is the overall mean, D_i was the day relative to lameness induction, T_j was the time period, F_k was the foot of measurement, I_j was the foot that was injected with amphotericin B, S_m was the random cow replicate, $N(0, \sigma_m^2)$, and e_{ijklm} $N(0, \sigma^2)$, was the error term. The cumulative model includes time increments beginning at 0 s. Each time period was compared using mean, standard error, 5%, 95%, range, and skewness of the cow's weight distribution with Bonferroni adjustment at α = 0.05.

To confirm baseline soundness, least squares means ± standard error from day 0, 2 min cumulative force plate recordings from each $\bar{X}$ (normalized foot) were compared using Wilcoxon-Kruskal-Wallis Rank Sums chi-square approximation between cows and between feet to ensure equal weight distribution prior to study commencement and consistency between cows.

The differences in LSM for the rolling 60 s weights recorded for 2, 3, 4, 5, and 10 m were based on the PROC MIXED model (SAS v9.4, SAS Inst., Cary, NC). Least squares means for the effects in the model were compared using the option probability of differences.

Results

Force plate design

A computer aided design program (Inventor Professional, Autodesk Inc., San Rafael, CA USA) drawing is shown in Fig. 1. Each of the four quadrants of the embedded microcomputer-based force plate system were divided into three parts based on the quadrant's location from the center of the collection of quadrants when combined due to its length and offset location of the load cell. The exterior portion was furthest from center or outermost third of the plate (Fig. 1, C). The center portion was the center of the quadrant itself (Fig. 1, B) and the interior portion (Fig. 1, A) was the section that is placed next to the other force plate when placed together. The load cells were placed between the center and exterior portions of each plate to inhibit lifting that could result when a cow's foot placement is near the plate's edge.

Visual lameness scores and visual analog scores

The overall arithmetic mean, SD and range of the VLS and VAS across the seven-day period were assessed and Tukey-Kramer Honest Significant Difference was used to compare means for individual animals to establish that locomotion deficits were induced (Table 3). Plots of VLS and VAS for each individual animal across the course of the study are displayed to demonstrate the varying degree of lameness (VLS = 0–4) over time by amphotericin B due to its reversible induction of arthritis-synovitis when injected into the joint (Fig. 2).

Validation

For all three quadrant sections (interior, center, exterior), as well as, the overall quadrant calibration, the resulting R^2 value was greater than 0.99. The quadrant sections and the overall quadrant RMSE are listed in Table 2. For interior quadrant sections, a 136 kg maximum weight was used for calibration. A slight hysteresis was observed in each force plate quadrant (Table 2), which was caused by the interior quadrant section in each case.

Output statistics

Based on exclusion criteria of individual limb weight less than 45.5 kg or total weight less than 454.6 kg, the percentage of

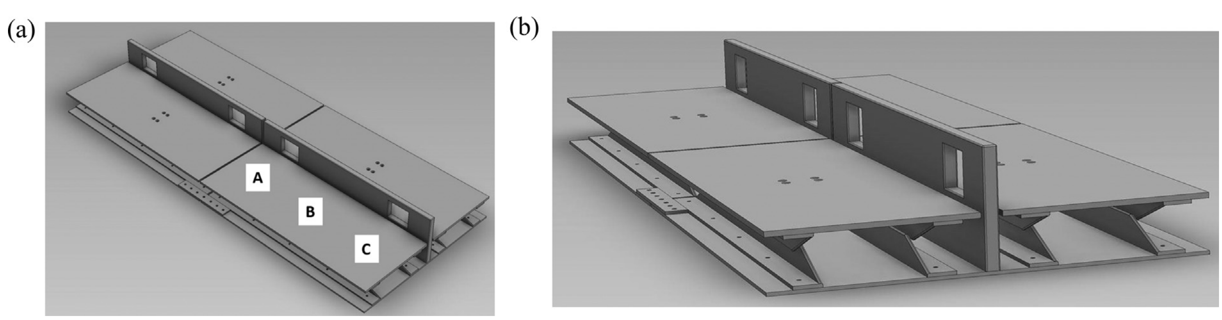

Fig. 1. Embedded microcomputer-based force plate system design drawn in computer aided design program (Inventor Professional, Autodesk Inc., San Rafael, CA, USA), aerial and isometric views, used to detect induced lameness in lactating dairy cattle. The letter (A) depicts the referenced interior portion, (B) the center portion, and (C) the exterior portion of the force plate when describing validation locations. This is repeated in each quadrant.

Table 3
Cow parameters of lameness degree indicated by locomotion scores displayed as arithmetic mean, SD, and range. Locomotion scores for lactating dairy cattle were evaluated across a seven-day time period utilizing visual lameness score (VLS) and visual analog score (VAS). Cow arithmetic mean comparison across time for all pairs using Tukey-Kramer Honest Significant Difference.

	Arithmetic Mean (SD), [range]		
Parameters	Cow 1	Cow 2	Cow 3
VLS	1.93 (1.33), [0, 4][a]	2.8 (0.94), [0, 4][a,b]	1.4 (1.06), [0, 4][b]
VAS	3.9 (3.15), [0, 8.2][a]	6.41 (2.30), [0.1, 9.5][b]	2.25 (2.21), [0, 7.5][b]

[a,b] Arithmetic means within a row with different superscript letters differ ($P \leq 0.05$).

excluded points are included as percentage of the total recordings for each individual animal (Table 4).

The initial soundness for each cow used in this study was evaluated by comparing 2 min cumulative LSM ± SE from each foot on day 0 (prior to lameness induction). The results for LF, RF, LR and RR were 0.26 ± 0.05, 0.25 ± 0.03, 0.24 ± 0.022, and 0.25 ± 0.011, respectively. Wilcoxon-Kruskal-Wallis Rank Sums chi-square approximation comparisons for these values displayed no difference when comparing means by cow ($P = 0.93$) and when comparing mean differences between feet within an individual cow ($P = 0.86$).

The LSM (±SE) when cows stood for a cumulative 2, 3, 4, 5, and 10 min on the force plate where the LR foot was injected to create lameness is shown in Table 5. There were no statistical weight differences in the LR limb values at 2, 3, 4, 5, and 10 min. Therefore, the minimum standing time required for valid measurements is no more than 2 min. The ability to detect differences between the lame and non-lame limbs at the aforementioned 2 min time course are presented in Table 6. Results demonstrate that significant differences ($P = <0.0001$) occur when comparing the force plate weights for each non-injected limb LSM (±SE) for each foot to the LR foot where artificial lameness was induced by injecting amphotericin B.

The evidence for the lameness induction concept and visual lameness detection is shown in Fig. 3. When evaluating the average BW proportion placed on each limb as a function of the cow's total BW, cows placed equal BW on all four limbs prior to lameness induction (day 0). Day 1 to day 7 is associated with the LR foot synovitis model effects on the left LR limb that was subjected to induced lameness relative to non-lame limbs (LF, RF, RR). Fluctuations across time were evident in all limbs, but significant changes were only observed when comparing the LR limb values to the other non-injected limbs.

The LSM comparisons between all four limbs are shown in Fig. 4. The parallel nature of the curve visually depicts that no significant LSM limb differences were observed between the 2-, 3-, 4-, 5-, and 10 min evaluation time points. This was confirmed by comparing foot by time LSM using Student's t-test (Table 5).

Discussion

Lameness creates both economic and welfare impacts on the dairy industry. The use of the embedded microcomputer-based force plate system could aid researchers in early lameness detection in dairy cattle by providing objective BW distribution assessments. This system has shown to be analogous to alternative lameness detection methodologies such as VLS, yet requires less time for lameness detection and eliminates training required for research staff (Ghotoorlar et al., 2012). Additionally, inter- and intra-observer variability has been reported to reduce VLS scoring reliability (Thomsen et al., 2008). Comparatively, the force plate allows for an objective quantitative weight distribution measurement from individual animals over time. The ability to detect subtle BW distribution changes will provide more accurate diagnosis and detection when mild lameness occurs. Detecting mild lameness cases is critical for successfully identifying the affected limb and applying earlier mitigation strategies to prevent mild lameness from evolving into severe lameness. Preventing lameness progression from mild to severe is often the difference between culling and maintaining a productive cow within the herd. Based on comparisons of cumulative detection minutes, detecting cow lameness requires a cow to spend a minimum of 2 min relative to longer time periods tested on the embedded microcomputer-based force plate system where standing BW measures are recorded for all four limbs in an amphotericin B induced synovitis model.

The sample size in this study was based on a-priori power calculation to minimize the number of animals that might experience pain or stress as a result of induced arthritis-synovitis. This is a limitation to the pilot study, but determination of the minimum time required for standing to detect differences in weight distribution aids in decreased stress to animals enrolled in future studies where the force plate is implemented. To date, the 2 min time point has proven useful in mitigating the impact of exclusion data due to an animal adjusting to the confinements of the force plate. Additionally, implementation of the force plate in a field setting, like a milking parlor, would not impose any restrictions due to an average standing time of 5 min for the milking procedure. Further work could be completed to determine if the minimum time a cow needs to stand on the force plate can be reduced further and still accurately determine lameness. For this study, cow lameness severity was monitored across a seven-day time period to evaluate the BW proportion that a cow was willing to place on each limb and measured on each force plate quadrant. The ability to

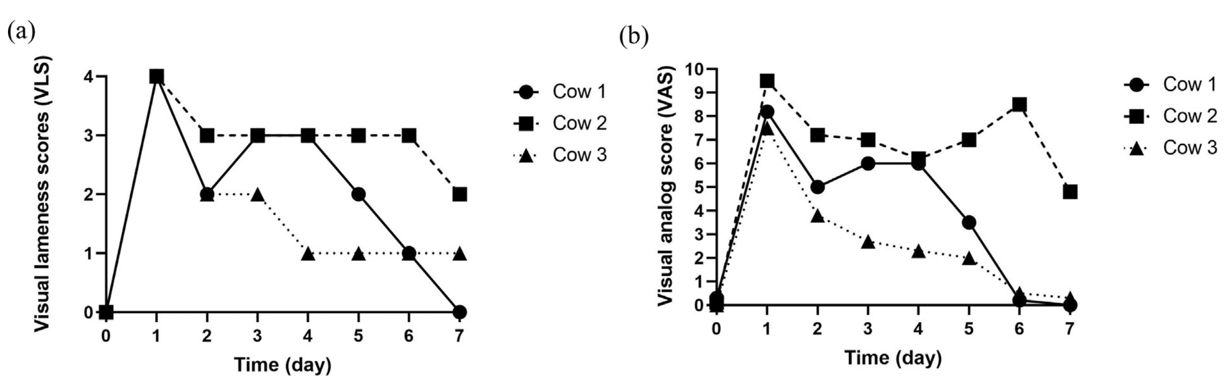

Fig. 2. (A) Visual lameness scores (VLS) and (B) visual analog score (VAS) across time for three lactating dairy cattle for seven days. Lameness scoring began prior to induction ($T = 0$) with amphotericin B in the left hind distal interphalangeal joint.

Table 4
Individual cow data eliminated based on total weight less than 454.6 kg (A) or weight of an individual limb of less than 45.5 kg while standing on the microcomputer-based force plate system (B) across the seven-day period.

Item	Eliminated data			Available data		
	n	%		*n*	%	Total
(A) Total weight less than 454.6 kg						
Cow						
1	553	11.51		4 253	88.49	4 806
2	1 227	19.71		4 997	80.29	6 224
3	1 357	19.53		5 590	80.47	6 947
Total	3 137	17.45		14 840	82.55	17 977
Item	Q1	Q2	Q3	Q4	Total weight	Total
(B) Individual limb weight less than 45.5 kg						
Cow						
1	4 751 (98.86%)	4 796 (99.79%)	4 767 (89.49%)	4 767 (99.19%)	4 639 (96.53%)	4 806 (100%)
2	6 209 (99.76%)	5 973 (95.97%)	6 068 (84.83%)	6 068 (97.49%)	5 586 (89.75%)	6 224 (100%)
3	6 932 (99.78%)	6 913 (99.51%)	6 806 (81.73%)	6 806 (97.97%)	6 815 (98.10%)	6 947 (100%)
Total	17 892 (99.53%)	17 682 (98.36%)	17 641 (84.88%)	17 641 (98.13%)	17 040 (94.79%)	17 977 (100%)

Q1 = Left front, Q2 = Right front, Q3 = Left rear, Q4 = Right rear.

Table 5
Average (least squares means) estimate ± SE and associated *P* values of the weight (kg) applied by the left rear (LR) foot across time (2-, 3-, 4-, 5-, and 10 min intervals) after inducing synovitis of the distal interphalangeal joint with amphotericin B in lactating dairy cattle (*n* = 3).

Time (min)	Time (min)				
	2	3	4	5	10
2		−2.62E−3 ± 0.013 (*P* = 0.8360)	−3.97E−3 ± 0.013 (*P* = 0.7539)	−5.05E−3 ± 0.013 (*P* = 0.6901)	7.25E−3 ± 0.013 (*P* = 0.5668)
3			−1.35E−3 ± 0.013 (*P* = 0.9152)	−2.43E−3 ± 0.013 (*P* = 0.8479)	4.63E−3 ± 0.013 (*P* = 0.7145)
4				−1.08E−3 ± 0.013 (*P* = 0.9320)	3.28E−3 ± 0.013 (*P* = 0.7953)
5					3.28E−3 ± 0.013 (*P* = 0.8618)
10					

characterize BW distribution fluctuation patterns over time makes it possible to differentiate specific lameness patterns recorded over time using the force plate. Further research is necessary to determine pattern differentiation and distribution when cows experience natural lameness events like digital dermatitis, sole ulcerations, and infectious pododermatitis. Due to the complexity of lameness, this was beyond the scope of this study.

The force plate could be modified in some ways to improve the ease of use in a research setting. Due to the large dimensions for each force plate quadrant and the slightly off-center load cell positioning, when weight beyond 136 kg was added at the edge furthest from the load cell, the force plate began to lift off the load cell, causing load cell readings to decrease. To account for this effect when validating using live animals, that section served as the interior to reduce the likelihood that a cow's limb would be placed in this location for a relatively long time while standing on the force plate. In future force plate designs, identifying a method to center the load cell beneath the plate would likely mitigate this issue.

Though the force plate is limited to vertical motion detection, changes in locomotion evaluation may not be necessary because the force plate evaluates weight distribution that is associated with subsequent gait changes in quadrupeds like dairy cattle. We feel that though kinematic data may be useful in some scenarios, it is not needed due to the accuracy of force plate measurements. Alternatively, in research for use in pain detection, a multimodal approach may be necessary due to the complexity of the pain model in cattle. In scenarios like this, the force plate system may benefit from the combination with cow locomotion evaluation using a pressure mat analysis (Pairis-Garcia et al., 2014; Kleinhenz et al., 2018; Kleinhenz et al., 2019), kinematics (Conte et al., 2014) and accelerometers (Higginson Cutler, 2012; Kokin et al., 2014). Further analysis is needed to determine the most effective combination for lameness-associated pain modalities to

Table 6
Average (least squares means) estimate ± SE of weight applied (kg) across a 2 min period and associated *P* value comparing amphotericin B induced synovitis of the left rear foot relative to non-lame feet (right front, left front, right rear) of lactating dairy cattle (*n* = 3).

Lameness induced foot	Non-lame feet		
	Right front	Left front	Right rear
Left rear	−0.1129 ± 0.013 (*P* < 0.0001)	0.1150 ± 0.013 (*P* < 0.0001)	−0.01058 ± 0.013 (*P* < 0.0001)

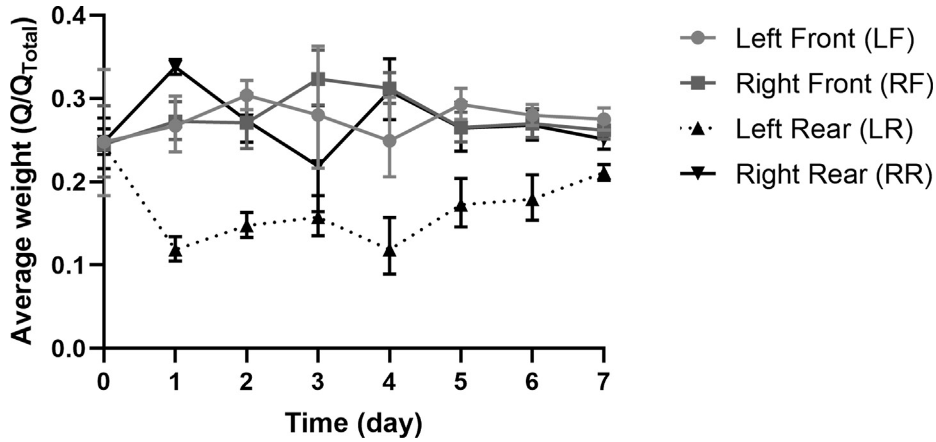

Fig. 3. Geometric mean with SD proportion of weight for three cows across a seven-day time period. Each weight (Q_x) was normalized to the total weight (Q_{Total}). Day 0 indicates baseline values for cows prior to amphotericin B left rear (LR) distal interphalangeal joint injection to induce lameness synovitis. Day 1 indicates 12 h postinduction.

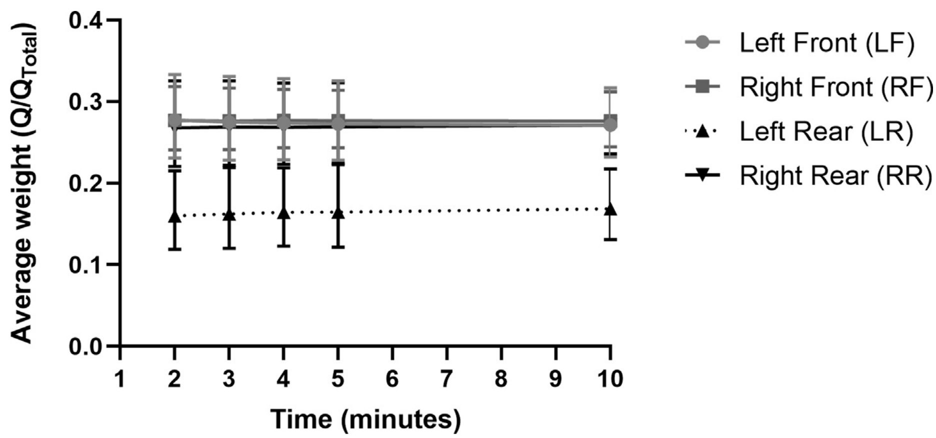

Fig. 4. Geometric mean with SD proportion of weight for three cows across standing on force plate for a 10 min period. Mean comparisons were determined at 2, 3, 4, and 10 min. Each weight measurement (Q_x) was normalized to the total weight (Q_{Total}). Cows received an injection of amphotericin B in the left rear (LR) distal interphalangeal joint to induce lameness via development of synovitis.

create a validated model for detecting lameness in the bovine species.

In conclusion, the embedded microcomputer-based force plate system to evaluate dairy cattle lameness was validated using an induced lameness model where amphotericin B was injected into the hind limb. It was determined that 2 min is the minimum time required when compared to longer testing intervals to differentiate significant differences between individual limb lameness induced synovitis and non-lame limbs when evaluated using the embedded force plate system. The embedded microcomputer-based plate system shows promise in improving detection of lameness severity in research modalities.

Ethics approval

The animal study was reviewed and approved by Iowa State University's Institutional Animal Use and Care Committee prior to the commencement of trial procedures (protocol number IACUC 19-163).

Data and model availability statement

The datasets generated for this study are available to reviewers or upon request.

Author ORCIDs

Rochelle Warner: https://orcid.org/0000-0002-9527-2644
Benjamin C. Smith: https://orcid.org/0000-0003-3299-3628
Kenneth J. Stalder: https://orcid.org/0000-0001-9540-681X
Locke A. Karriker: https://orcid.org/0000-0001-6003-1130
Brett C. Ramirez: https://orcid.org/0000-0002-3205-9723
Suppasit Plaengkaeo: https://orcid.org/0000-0001-9566-7331
Patrick J. Gorden: https://orcid.org/0000-0002-6096-0965

Author contributions

The study was designed by: **PJG, RW, LAK, KJS,** and **BCR**
Animal work was carried out by: **RW**
Data analysis was carried out by: **RW, KJS, BCS,** and **SP**
Manuscript was written by: **RW, PJG, BCR, BCS,** and **KJS**
Manuscript was reviewed, read and approved by: All authors.

Declaration of interest

The authors do not have any conflicts of interest to declare.

Acknowledgements

We would like to thank the staff from the Iowa State University Dairy Farm.

Financial support statement

This research was funded via a grant from the Iowa Livestock Health Advisory Council. This work is a product of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. IOW0400 (BCR and KJS) is sponsored by the Hatch Act and State of Iowa funds. The content of this article is however solely the responsibility of the authors and does not represent the official views of the USDA.

References

Brand, A., Noordhuizen, J.P.T.M., Schukken, Y.H., 1996. Herd health and production management in dairy practice. Wageningen Academic Publishers, Wageningen, Netherlands.

Coetzee, J.F., Mosher, R.A., Anderson, D.E., Robert, B., Kohake, L.E., Gehring, R., White, B.J., Kukanich, B., Wang, C., 2014. Impact of oral meloxicam administered alone or in combination with gabapentin on experimentally induced lameness in beef calves. Journal of Animal Science 92, 816. https://doi.org/10.2527/jas.2013-6999.

Conte, S., Bergeron, R., Gonyou, H., Brown, J., Rioja-Lang, F.C., Connor, L., Devillers, N., 2014. Measure and characterization of lameness in gestating sows using force plate, kinematic, and accelerometer methods. Journal of Animal Science 92, 5693–5703.

Cook, N.B., 2003. Prevalence of lameness among dairy cattle in Wisconsin as a function of housing type and stall surface. Journal of the American Veterinary Medical Association 223, 1324. https://doi.org/10.2460/javma.2003.223.1324.

Federation of Animal Science Societies, 2010. Guide for the care and use of agricultural animals in research and teaching. ADSA/ASAS/PSA, Champaign, IL, USA.

Flower, F.C., Weary, D.M., 2006. Effect of hoof pathologies on subjective assessments of dairy cow gait. Journal of Dairy Science 89, 139–146. https://doi.org/10.3168/jds.S0022-0302(06)72077-X.

Galindo, F., Broom, D.M., 2000. The relationships between social behaviour of dairy cows and the occurrence of lameness in three herds. Research in Veterinary Science 69, 75–79. https://doi.org/10.1053/rvsc.2000.0391.

Ghotoorlar, S.M., Ghamsari, S.M., Nowrouzian, I., Ghidary, S.S., 2012. Lameness scoring system for dairy cows using force plates and artificial intelligence. Veterinary Record 170, 126. https://doi.org/10.1136/vr.100429.

Greenough, P.R., Weaver, A.D., 1997. Lameness in cattle. WB Saunders, Philadelphia, PA, USA.

Heckert, N.A., Filliben, J.J., Croarkin, C.M., Hembree, B., Guthrie, W.F., Tobias, P., Prinz, J., 2002. Handbook 151: NIST/SEMATECH e-Handbook of Statistical Methods. National Institute of Standards and Technology, Gaithersburg, MD, USA.

Higginson Cutler, J., 2012. Welfare in dairy cattle: Epidemiologic approaches for detection and treatment of lameness PhD thesis. University of Guelph, Ontario, Canada.

Kleinhenz, M.D., Gorden, P.J., Smith, J.S., Schleining, J.A., Kleinhenz, K.E., Rea, D., Coetzee, J.F., 2018. Evaluation of Transdermal Flunixin Meglumine on Experimentally Induced Lameness in Adult Dairy Cattle. Journal of Animal Science 96 (suppl. 2), 11. https://doi.org/10.1093/jas/sky073.019.

Kleinhenz, M.D., Gorden, P.J., Smith, J.S., Schleining, J.A., Kleinhenz, K.E., Juarez, J.R., Rea, D., Coetzee, J.F., 2019. Effects of transdermal flunixin meglumine on experimentally induced lameness in adult dairy cattle. Journal of Dairy Science 102, 6418–6430. https://doi.org/10.3168/jds.2018-15091.

Kokin, E., Praks, J., Veermäe, I., Poikalainen, V., Vallas, M., 2014. IceTag3D™ accelerometric device in cattle lameness detection. Agronomy Research 12, 223–230.

Kotschwar, J., Coetzee, J., Anderson, D., Gehring, R., Kukanich, B., Apley, M.D., 2009. Analgesic efficacy of sodium salicylate in an amphotericin B-induced bovine synovitis-arthritis model. Journal of Dairy Science 92, 3731–3743. https://doi.org/10.3168/jds.2009-2058.

McNeil, B., Díaz, J.C., Bruns, C., Stock, J., Millman, S., Johnson, A., Karriker, L.A., Stalder, K.J., 2018. Determining the time required to detect induced sow lameness using an embedded microcomputer-based force plate system. American Journal of Animal and Veterinary Sciences 13, 59–65. https://doi.org/10.3844/ajavsp.2018.59.65.

National Research Council, 2001. Nutrient requirements of dairy cattle. National Academy of Sciences, Washington, DC, USA.

O'Callaghan, K., 2002. Lameness and associated pain in cattle - challenging traditional perceptions. In Practice 24, 212. https://doi.org/10.1136/inpract.24.4.212.

Pairis-Garcia, M.D., Johnson, A.K., Stalder, K.J., Karriker, L.A., Coetzee, J.F., Millman, S.T., 2014. Measuring the efficacy of flunixin meglumine and meloxicam for lame sows using nociceptive threshold tests. Animal Welfare 23, 219–229. https://doi.org/10.7120/09627286.23.2.219.

Schulz, K.L., Anderson, D.E., Coetzee, J.F., White, B.J., Miesner, M.D., 2011. Effect of flunixin meglumine on the amelioration of lameness in dairy steers with amphotericin B-induced transient synovitis-arthritis. American Journal of Veterinary Research 72, 1431. https://doi.org/10.2460/ajvr.72.11.1431.

Sprecher, D.J., Hostetler, D.E., Kaneene, J.B., 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 47, 1179–1187. https://doi.org/10.1016/S0093-691X(97)00098-8.

Sun, G., Fitzgerald, R.F., Stalder, K.J., Karriker, L.A., Johnson, A.K., Hoff, S.J., 2011. Development of an embedded microcomputer-based force plate system for measuring sow weight distribution and detection of lameness. Applied Engineering in Agriculture 27, 475–482. https://doi.org/10.13031/2013.37063.

Thomsen, P.T., Munksgaard, L., Tøgersen, F.A., 2008. Evaluation of a lameness scoring system for dairy cows. Journal of Dairy Science 91, 119–126. https://doi.org/10.3168/jds.2007-0496.

Vermunt, J.J., 2005. The multifactorial nature of cattle lameness: A few more pieces of the jigsaw. The Veterinary Journal 169, 317–318. https://doi.org/10.1016/j.tvjl.2004.05.005.