

Diversity in the magnitude of hind limb unloading occurs with similar forms of lameness in dairy cows

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The objective of the study was to evaluate the relationship of veterinary clinical assessments of lameness to probability estimates of lameness predicted from vertical kinetic measures. We hypothesized that algorithm-derived probability estimates of lameness would accurately reflect vertical measures in lame limbs even though vertical changes may not inevitably occur in all lameness. Kinetic data were collected from sound ($n = 179$) and unilaterally lame ($n = 167$) dairy cattle with a 1-dimensional, parallel force plate system that registered vertical ground reaction force signatures of all four limbs as cows freely exited the milking parlour. Locomotion was scored for each hind limb using a 1–5 locomotion score system (1 = sound, 5 = severely lame). Pain response in the interdigital space was quantified with an algometer and pain response in the claw was quantified with a hoof tester fitted with a pressure gage. Lesions were assigned severity scores (1 = minimal pathology to 5 = severe pathology). Lameness diminished the magnitude of peak ground reaction forces, average ground reaction forces, Fourier transformed ground reaction forces, stance times and vertical impulses in the lame limbs of unilaterally lame cows. The only effect of lameness on the opposite sound limb was increased magnitude of stance times and vertical impulses in unilaterally lame cows. Symmetry measures of the peak ground reaction forces, average ground reaction forces, Fourier transformed ground reaction forces, stance times and vertical impulses between the left and right hind limbs were also affected in unilateral lameness. Paradoxically, limbs with clinically similar lesion and locomotion scores and pain responses were associated with a broad range of load-transfer off the limb. Substantial unloading and changes in the vertical limb variables occurred in some lameness while minimal unloading and changes in vertical limb variables occurred in other lameness. Corresponding probability estimates of lameness accurately reflected changes in the vertical parameters of limbs and generated low probability estimates of lameness when minimal unloading occurred. Failure to transfer load off limbs with pain reactions, locomotion abnormalities and lesions explained much of the limited sensitivity in lameness detection with vertical limb variables.

Keywords: Dairy cow, force plate, lameness, vertical kinetic variables.

Lameness has emerged as an important welfare and costly production problem in the dairy industry (Green et al. 2002; Booth et al. 2004; Bicalho et al. 2007a). Losses derive from diminished milk yields, loss of reproductive efficiency and increased involuntary culling (Green et al. 2002; Sogstad et al. 2006; Bicalho et al. 2008). Financial surveys report the average cost of lameness to be more than \$400

per incidence (Greenough et al. 1997) that has probably increased owing to the increased prevalence from 11% to 14% in 1996 and 2007, respectively (USDA, 2008).

Visual methods of diagnosis have served as the detection method of choice even though visual systems such as those designed by Sprecher et al. (1997) have been shown to be disadvantaged by subjectivity, labour intensiveness, limited accuracy (Wells et al. 1993; Whay et al. 2003) and low reproducibility between observers (Bicalho et al. 2007b; Channon et al. 2009). Sensitivity was problematic in that 54% and 76% of cows visually diagnosed as lame in the

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front and hind limbs respectively actually possessed painful lesions (Bicalho et al. 2007b). These are compelling arguments for the development of automated, objective techniques that include ground reaction force plate systems (Rajkondawar et al. 2002), camera-based imaging (Flower et al. 2005) or a 4-balance system of dynamic limb loading, step and kick behaviour (Pastell et al. 2006). False negatives, however, plagued the force plate system (Bicalho et al. 2007b; Liu et al. 2009) whereas false positives eroded accuracy of the 4-balance system (Pastell et al. 2006). Camera-based imaging offers considerable input on kinematic measures of motion but the approach does not generate measures of the ground reaction forces in any dimension.

In spite of these problems, these technologies generated novel insights into biomechanical events of normal and painful limbs. Lameness produced inequality in kinetic (Scott, 1988; Rajkondawar et al. 2006) or kinematic (Flower et al. 2005, 2006a) variables. Lameness unloaded limbs and decreased the magnitude of peak ground reaction force (PGRF), average ground reaction force (AGRF), stance time (STIME), vertical impulse (VIMPULSE) and the area under the Fourier transformed curve of a ground reaction force signature (GRF ω) (Scott, 1988; Rajkondawar et al. 2006). The Fourier transform of GRF characterizes the vibrations introduced to the floor by converting the curve of force *v.* time to a curve of force magnitude *v.* frequency. Load transfer produced asymmetric weight bearing (Pastell et al. 2006, 2010), decreased propulsive and braking forces on the lame limb (Scott, 1988), decreased height of the stride arc, triggered three-point support (Flower et al. 2005) and diminished (Rajkondawar et al. 2006) or increased stance times (Flower et al. 2005). There are conflicting reports of decreased (Flower et al. 2005) and no change in velocity (Flower et al. 2007; Chapinal et al. 2009) with lameness. Integration of this knowledge into objective systems should improve the sensitivity and specificity of detection technologies.

The objective of the study was to evaluate the relationship of veterinary clinical assessments of lameness to probability estimates of lameness predicted from vertical kinetic variables. We hypothesized that changes in vertical kinetic variables in unilaterally lame cows would be accurately reflected as changes in probability estimates of lameness. Moreover, we predicted that changes in vertical kinetic variables were not inevitably present across all lameness.

Materials and Methods

Cows and production units

Data were collected from cows located in two commercial dairy herds consisting of 550 and 1450 lactating Holstein dairy cows. The cows were housed in free stall barns with retractable curtains. In one facility, walkways between lying areas and along feed bunkers consisted of pre-cast slatted

concrete overlying a 2.5-m deep manure pit. Slats consisted of 5-cm slots separating 21-cm wide treads. Flooring between lying areas in the other facility was grooved cement with 1.2-cm deep \times 5-cm wide grooves spaced 10–12 cm apart and oriented diagonally to the direction of cow flow. Rubber mats covered the flooring in front of the feed bunkers. Lying areas in one herd consisted of rubber mats overlaid with wood shavings. Lying areas in the second herd consisted of sand bases overlaid with wood shavings. Cows in both herds were fed a total mixed ration three times a day formulated to meet lactation requirements of a 660-kg cow producing 38 kg of milk containing 3.5% fat daily (NRC, 2001). Diets consisted primarily of corn and alfalfa silage, grass hay, soybeans, cotton seed, ground, shelled corn, vitamins and minerals. Cows were milked 3 times a day in both herds and foot-trimmed two to three times a year by professional hoof trimmers. Routine trimming occurred at around 120–140 days in milk (DIM) and at the end of lactation. Lactating cows ($n = 15$ – 18 per week per farm) from each herd were randomly selected by a number generator without regard to locomotion status, production or parity. Data included single observations from 164 and 182 cows from each of the farms, respectively.

Data collection

Clinical evaluation of each cow. All locomotion scores, lesion diagnosis, lesion scores and pain responses were determined once a week by a single veterinarian for 15–18 cows. Note that all vertical variables were collected and the estimated probability of lameness determined from data collected during the 24 h immediately preceding the veterinary clinical examination.

Locomotion scores. Locomotion scores were established for each cow as previously described in detail by Rajkondawar et al. (2006) and modified from Sprecher et al. (1997) and Wells et al. (1993). Since the force plates simultaneously established data for all four limbs, locomotion examination by necessity consisted of an evaluation of each limb. Once a week, cows were observed at a stance and then while walking in a straight line. To facilitate identification of lame limbs, cows were circled to the right and left. All examinations were performed on the concrete alleyways (described in detail above) and coated with a thin layer of dry wood shavings. Locomotion observations were performed by observing the animal perpendicular, parallel, posterior and anterior to the line of travel. During locomotion evaluation, cows and limbs were observed for freedom of motion; left and right sided stride length; length of anterior and posterior swing phases; symmetry and arc of the foot flight; foot placement relative to body position and limb axis; foot rotation during weight bearing; symmetry of weight distribution at the walk and stance; and position of top line at a walk and stance. Note that in unilateral lameness, a score

of 1–5 assigned to the cow was synonymous with a limb score (Rajkondawar et al. 2006; Dyer et al. 2007).

Pain evaluation. Claw and interdigital integument pain was assessed as previously described in detail by Dyer et al. (2007). Pain reaction in the claw (P_c) was determined by compression using a hoof tester designed to transfer compression forces through a Dillon force gauge (Dillon model 'X' force gauge 250, Dillon Force Measurement Products and Systems, Fairmont MN, USA). Cows were initially adapted to the process of hoof compression by gentle application of pressure 4–5 times along an axis extending from the dorsal wall to the sole before pain determinations were performed along the axis extending between abaxial and axial walls. Increasing amounts of claw compression were applied to attain 711.68 N force (P_{max}) or until the cow no longer tolerated the compression (P_i) by showing a withdrawal response. This force generated a pressure of 459.74 N/cm² at the junction of the hoof with the arm of the hoof tester. Pressure attained at the onset of foot withdrawal was recorded only after animals reacted to 3 repeated compression tests along the same axis. Compression was always performed on the medial claw first followed by the lateral claw.

Pain reactions associated with lesions of the integument (P_{int}) was assessed using an algometer (44.48 N scale) with a blunt probe (Wagner Force Dial FDK 10, Wagner Instruments, Greenwich CT, USA) pressed against the integument. The probe was placed on the lesion surface or on the junction of the interdigital and plantar surface of the volar integument. Increasing amounts of force were applied to the integument or lesion to attain 44.48 N force or until the limb was withdrawn. The force of 44.48 N resulted in a pressure of 140.54 N/cm². Pain indices were calculated as P_i/P_{max} , where P_i was the pressure recorded upon limb withdrawal and P_{max} was 140.54 N/cm². Pressure attained at the onset of foot withdrawal was recorded only after animals reacted to 3 repeated pressure tests.

Lesion diagnosis and score. Claws and interdigital integument were cleaned, and examined by visual inspection and palpation. The digits and bulbs were separated for examination and the claws trimmed according to van Amstel et al. (2000). Lesion diagnosis and scores were established at the time of pain reaction responses and locomotion scoring by a single veterinarian following procedures described in detail by Rajkondawar et al. (2006). Mean maximal lesion score across a group of cows was calculated as the mean of the highest lesion severity score within a limb across all cows in a group. A veterinary classification of lame was declared for any locomotion score ≥ 3 .

Vertical limb movement variables. Kinetic evaluation was performed with two metal, parallel biomechanical force plates supported by four load cells/plate. Left and right force

Table 1. The limb movement variables (LMV) used in the study

LMV	Units	Description
PGRF	Nondimensional	Peak ground reaction force (GRF) normalized by the animal's dynamic weight of a tested limb
AGRF	Nondimensional	Average ground reaction force normalized by the animal's dynamic weight of a tested limb
STIME	S	Stance time is the time during which a limb is in contact with the floor
VIMPULSE	S	Impulse is the integral of the GRF normalized by the animal's dynamic weight with respect to time
GRF _ω	1/s	The area under the Fourier transformed curve of a GRF signature normalized by the animal's dynamic weight

plate dimension was 152 cm × 38 cm and the surfaces were covered with 5-mm thick rubber mats to avoid slipping. Load cells on each plate were calibrated with a known weight before use and thereafter twice a year. The system (Step Matrix, Bou-Matic, LLC, 1919 S. Stoughton Rd, Madison WI 53708, USA) was located in the return alley from the milking parlour to reduce any effect of mammary gland milk content on locomotion (Flower et al. 2006b). Cows walked freely across the plates. Limb movement variables (LMV) were determined from and considered valid when (1) only one cow occupied the plate, (2) time of passage across the plate was ≤ 6 s and (3) left and right limbs contacted only the left and right plates, respectively. Signatures of vertical ground reaction forces (GRF) by time for each limb were measured at a frequency of 200 Hz and stored in a data bank for download every 2 weeks. Signatures of GRF of hind and fore limbs were recorded as a function of time. GRF signatures were used to calculate the limb movement variables defined in Table 1. Each LMV was normalized by the dynamic weight of the cow.

Simultaneous, bilateral records of PGRF, AGRF, STIME, VIMPULSE, and GRF_ω of the hind limbs enabled calculation of pelvic limb symmetry measures for each LMV. Symmetry indices were calculated with reference to the affected side of the animal (Bockstahler et al. 2009):

$$SI = \frac{\chi^{lame} - \chi^{sound}}{\chi^{lame} + \chi^{sound}}$$

SI = symmetry index

χ^{lame} = LMV of lame limb

χ^{sound} = LMV of sound limb

In animals or groups with no lameness symmetry indices were calculated with reference arbitrarily set to the left side

of the animal as:

$$SI = \frac{\chi^{\text{left}} - \chi^{\text{right}}}{\chi^{\text{left}} + \chi^{\text{right}}}$$

SI = symmetry index

χ^{left} = LMV of left limb

χ^{right} = LMV of right limb

Accordingly symmetry indexes were calculated for PGRF (SPGRF), AGRF (SAGRF), STIME (STIME), VIMPULSE (SVIMPULSE), and GRF_{ω} ($SGRF_{\omega}$). Symmetry indices provided a measure of equality of the magnitude of an LMV in the lame limb compared with the magnitude of the same LMV in the opposite sound limb. Symmetry indices closer to 0 indicated equality of the particular LMV under question in the pelvic limbs. In all instances, increased lameness was expected to generate indices of greater negative value.

Distribution of vertical variables, symmetry indices, lesion score, pain reaction and locomotion score by true positive, true negative, false positive and false negative diagnostic outcomes for clinical and vertical variable assessments.

Diagnostic outcomes were established as true positive (TP) when clinical and predicted probability of lameness from kinetic variables declared lameness, true negative (TN) when clinical and force plate assessment declared absence of lameness, false positive (FP) when clinical assessment declared absence of lameness and the force plate assessment declared presence of lameness, and false negative (FN) when clinical assessment declared lameness and force plate assessment declared absence of lameness. Vertical variables, symmetry indices, lesion score, pain reaction and locomotion score of all cows were distributed according to the appropriate TP, FP, FN and FP group in the diagnostic approaches generated for each cow.

Statistical methods

The effect of lameness in one hind limb on the vertical limb movement variables in the lame as well as the opposing sound hind limb was evaluated using multiple analysis of variance (MANOVA) using SAS procedure GLM with MANOVA (Khatree & Naik, 1999; SAS Institute, 2004). For all unilaterally lame cows, LMV were grouped by locomotion score of the lame limb creating LMV data sets for the lame limbs (locomotion score = 1–5) and the corresponding LMV data sets for opposite sound limbs (locomotion score = 1). To assess the effect of increasing locomotion score on the magnitude of the vertical variables in the lame and opposite sound hind limb, the magnitude of the vertical variables of the lame and sound limbs were plotted as a function of increasing locomotion score. The effect of unilateral lame limb locomotion score on the symmetry variables, SPGRF, SAGRF, STIME, $SGRF_{\omega}$, or SVIMPULSE was analysed by MANOVA. Pair wise differences between LMV or symmetry indices were evaluated with Tukey's Pair wise Multiple Comparisons Test (Khatree & Naik, 1999).

A lameness prediction model (Rajkondawar et al. 2002) developed with logistic regression (Hosmer & Lemeshaw, 2000) predicted the probability of lameness as a function of vertical LMV. The model was:

$$P(\text{Lameness} = 1) = \frac{e^{\beta_0 + \sum \beta_i LMV_i}}{1 + e^{\beta_0 + \sum \beta_i LMV_i}}$$

The β coefficients are estimated by appropriate statistical methods and LMV_i is the i th LMV measurement (PGRF, AGRF, GRF_{ω} , STIME, and VIMPULSE defined in Table 1) and e^{β_0} was the x-axis intercept. For all unilaterally lame cows, LMV were grouped by lame limb locomotion score creating LMV data sets for lame limbs (locomotion score = 1–5) and the corresponding LMV data sets for sound limbs (locomotion score = 1) opposite the lame limb. The effect of unilateral locomotion score (locomotion score) on model-predicted probability of lameness was evaluated by MANOVA the lame limb and the opposite sound limb. Pair wise differences between LMV means were evaluated with Tukey's Pair wise Multiple Comparisons Test.

Model accuracy was assessed as model sensitivity and specificity defined as $\frac{TP}{TP+FN}$ and $\frac{TN}{FP+TN}$ respectively for which FP = false positive, FN = false negative, TP = true positive and TN = true negative. The effects of unilateral lameness on claw pain, interdigital integument pain, locomotion score, mean maximum lesion score, PGRF, AGRF, STIME, GRF_{ω} , VIMPULSE, and the respective symmetry indices were determined across TP, FP, TN, and FN outcomes. For purposes of analysis, left limbs of the TN and FP non-painful, sound animals were arbitrarily assigned to the data set containing the painful limbs of unilaterally lame (TP and FN) animals. The right limbs of the TN and FP non-painful, sound animals were arbitrarily assigned to the data set containing the non-painful limbs from unilaterally lame (TP and FN) animals. The clinical findings, the LMV and the respective symmetry indices were grouped by TP, FP, TN and FN outcomes and evaluated by MANOVA for outcome. Pair wise differences between LMV, symmetry, claw pain, interdigital integument pain, locomotion score and mean maximum lesion score means were evaluated with Tukey's Pair wise Multiple Comparisons Test.

The research protocol was approved by the Animal Care and Use Committees for the University of Maryland, Baltimore County and the University of Delaware.

Results

The population of cattle ($n=346$ cows) consisted of 179 bilaterally sound cows (locomotion score = 1 in LH and RH) and 167 unilaterally lame cows (locomotion score >1 in LH or RH). Across the entire population, locomotion score inversely affected the magnitude of PGRF (Fig. 1), AGRF (Fig. 2), GRF_{ω} (Fig. 3), STIME (Fig. 4) and VIMPULSE (Fig. 5) of the lame limb in unilaterally lame cattle (Figs 1–5) ($P \leq 0.001$). PGRF, AGRF and GRF_{ω} of mildly (score 3),

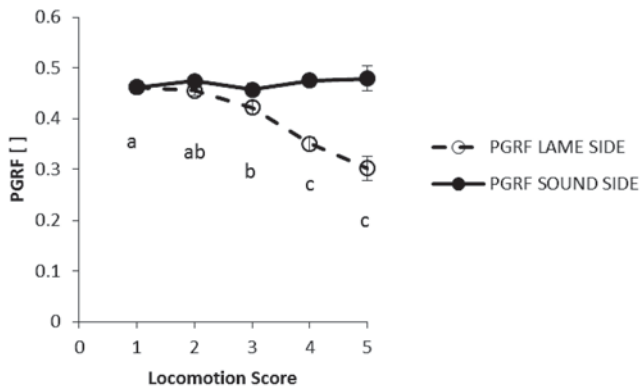


Fig. 1. Effect of limb locomotion score (note that limb locomotion score is cow locomotion score in unilaterally lame cows) on peak ground reaction force (PGRF) in lame (open circles, locomotion score 1–5) and sound (closed circles, locomotion score=1) limbs of unilaterally lame cows. Data are depicted as mean ± SEM (*n* = 346). Within lines, means without a common subscript letter differ significantly (*P* ≤ 0.05).

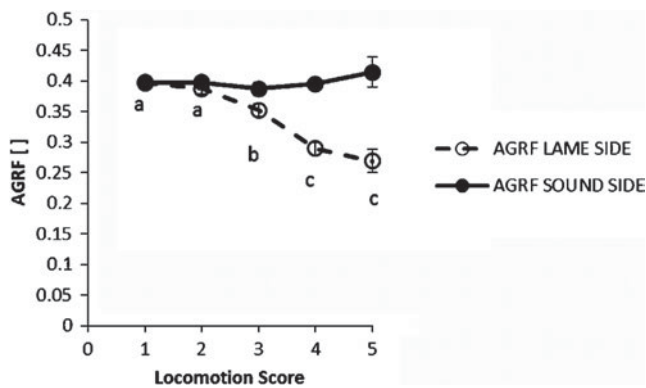


Fig. 2. Effect of limb locomotion score (note that limb locomotion score is cow locomotion score in unilaterally lame cows) on average ground reaction force (AGRF) in lame (open circles, locomotion score 1–5) and sound (closed circles, locomotion score=1) limbs of unilaterally lame cows. Data are depicted as mean ± SEM (*n* = 346). Within lines, means with different subscripts differ significantly (*P* ≤ 0.05).

moderately (score 4) and severely (score 5) lame cows was smaller than AGRF, PGRF and GRF_ω of locomotion score 1 sound cows (*P* ≤ 0.05). PGRF, AGRF, GRF_ω, STIME and VIMPULSE of moderately (score 4) and severely (score 5) lame cows was smaller than those recorded in cows lacking visible lameness (score 1 and 2) or cows with mild lameness (score 3) (*P* ≤ 0.05). AGRF and GRF_ω of mildly lame cows (locomotion score 3) was smaller than AGRF and GRF_ω of locomotion score 1 and 2 cows (*P* ≤ 0.05). No differences existed between PGRF, AGRF, STIME, VIMPULSE and GRF_ω for cows lacking visible locomotion abnormalities (locomotion score 1 and 2) (*P* ≥ 0.05).

Across the sample population the magnitude of PGRF, AGRF and GRF_ω of sound limbs in populations of unilaterally

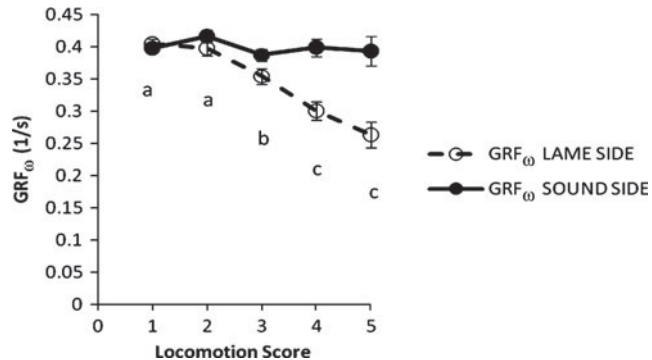


Fig. 3. Effect of limb locomotion score (note that limb locomotion score is cow locomotion score in unilaterally lame cows) on peak ground reaction force (PGRF) changes in lame (open circles, locomotion score 1–5) and sound (closed circles, locomotion score=1) limbs of unilaterally lame cows. Data are depicted as mean ± SEM (*n* = 346). Within lines, means with different subscripts differ significantly (*P* ≤ 0.05).

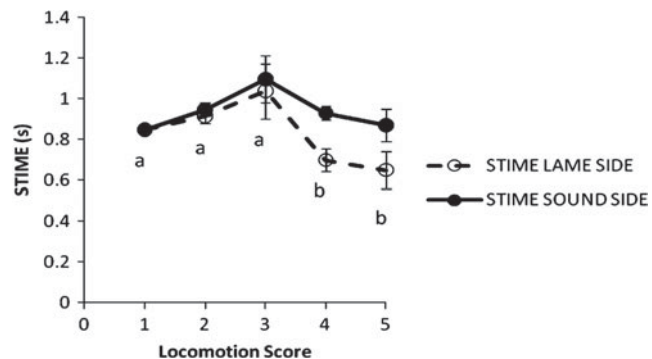


Fig. 4. Effect of limb locomotion score (note that limb locomotion score is cow locomotion score in unilaterally lame cows) on stance time (STIME) of limbs lame (open circles, locomotion score 1–5) and sound (closed circles, locomotion score=1) limbs of unilaterally lame cows. Data are depicted as mean ± SEM (*n* = 346). Within lines, means with different subscripts differ significantly (*P* ≤ 0.05).

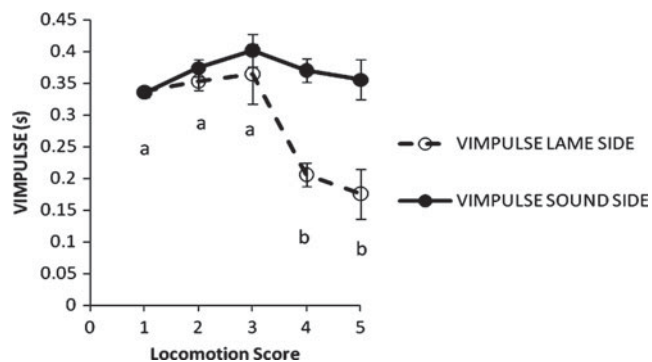


Fig. 5. Effect of limb locomotion score (note that limb locomotion score is cow locomotion score in unilaterally lame cows) on vertical impulse (VIMPULSE) of lame (open circles, locomotion score 1–5) and sound (closed circles, locomotion score=1) limbs of unilaterally lame cows. Data are depicted as mean ± SEM (*n* = 346). Within lines, means with different subscripts differ significantly (*P* ≤ 0.05).

Table 2. Peak ground reaction force (PGRF), average ground reaction force (AGRF), Fourier transformed ground reaction forces (GRF_{ω}), stance time (STIME), vertical impulse (VIMPULSE), claw pain reaction (P_c), interdigital integument pain reaction (P_{int}), limb locomotion score (note that limb locomotion score is cow locomotion score in unilaterally lame cows) and maximum lesion severity score for lame hind limbs of lame animals (TP and FN) and the left hind limb in sound animals (TN and FP) within TP, TN, FP, FN groups. Data are presented as mean \pm SEM

Variable	Group†			
	TP (n=54)	TN (n=215)	FP (n=27)	FN (n=50)
PGRF	0.37 ^a \pm 0.020	0.47 ^b \pm 0.004	0.38 ^a \pm 0.022	0.48 ^b \pm 0.021
AGRF	0.30 ^a \pm 0.015	0.40 ^b \pm 0.004	0.31 ^a \pm 0.015	0.41 ^b \pm 0.017
STIME	0.83 \pm 0.089	0.93 \pm 0.041	0.86 \pm 0.056	0.93 \pm 0.064
GRF_{ω}	0.31 ^a \pm 0.016	0.42 ^b \pm 0.005	0.29 ^a \pm 0.019	0.40 ^b \pm 0.018
VIMPULSE	0.25 ^a \pm 0.033	0.37 ^b \pm 0.012	0.27 ^{ab} \pm 0.024	0.35 ^b \pm 0.025
P_c	0.671 ^a \pm 0.035	0.978 ^b \pm 0.004	0.978 ^b \pm 0.009	0.677 ^a \pm 0.04
P_{int}	0.847 ^a \pm 0.046	0.975 ^b \pm 0.007	0.989 ^b \pm 0.01	0.850 ^a \pm 0.048
Locomotion score	3.7 ^b \pm 0.1	1.3 ^a \pm 0.0	1.3 ^a \pm 0.1	3.0 ^c \pm 0.1
Lesion score	4.1 ^a \pm 0.2	1.1 ^b \pm 0.1	1.4 ^b \pm 0.3	4.1 ^a \pm 0.2

† TP=true positive, TN=true negative, FP=false positive, FN=false negative

‡ Means within rows without a common superscript letter differ significantly ($P \leq 0.05$)

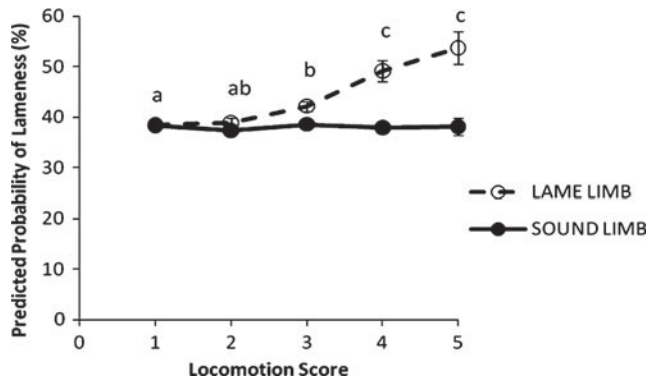


Fig. 6. Effect of limb locomotion score (note that limb locomotion score is cow locomotion score in unilaterally lame cows) on the probability of lameness. Model prediction of lameness in lame (open circles, locomotion score 1–5) and sound (closed circles, locomotion score=1) limbs in unilaterally lame cows. Data are depicted as mean \pm SEM ($n=346$). Within lines, means without a common subscript letter differ significantly ($P \leq 0.05$).

lame animals remained unchanged with increasing locomotion score (Figs 1–5, $P \leq 0.05$). The magnitude of STIME and VIMPULSE in the sound limb increased as the locomotion score of the lame limb approached 3 ($P \leq 0.05$) but returned to sound levels in locomotion scores ≥ 4 ($P \geq 0.05$).

Across the sample population, predicted probability of lameness increased with increasing limb lameness score (Fig. 6, $P \leq 0.001$). Predicted lameness probabilities for visibly lame cows (locomotion score=3, 4, and 5) were greater than sound cows (locomotion score=1) ($P \leq 0.05$). Increasing locomotion score in the lame limb had no effect on predicted probability of lameness in the opposite sound limb of unilaterally lame cows ($P > 0.05$).

Comparing model predictions with clinical predictions of lameness for each animal, however, revealed a small

sensitivity (51.92%) and a larger (88.84%) specificity of the predictive lameness model (Table 2). We explored the small sensitivity by distributing P_c , P_i , locomotion score, mean maximum lesion score, PGRF, AGRF, STIME, GRF_{ω} , VIMPULSE, and the respective symmetry indices across TP, FP, TN, and FN outcomes. P_c , P_i lesion severity and locomotion scores of the lame TP and FN groups were greater than those of the sound TN and FP groups (Table 2, $P \leq 0.05$). No differences occurred between P_c , P_i , maximal lesion severity scores and locomotion scores across the non-painful TN and FP groups or the painful TP and FN groups, respectively (Table 2, $P > 0.05$). Unexpectedly PGRF, AGRF, GRF_{ω} , and VIMPULSE of the lame, clinically painful TP group were all smaller than those of the other lame, clinically painful, FN group ($P \leq 0.05$). More surprisingly, AGRF, STIME, GRF_{ω} and VIMPULSE of the painful, clinically lame FN group were as great and similar to the pain-free, sound TN group ($P \geq 0.05$). AGRF, STIME, GRF_{ω} and VIMPULSE of the painful, TP group were as small and no different from the pain-free, FP group ($P \geq 0.05$). PGRF, AGRF and GRF_{ω} of the TN group were greater than the FP group ($P \leq 0.05$).

To further explore these results, we distributed the symmetry of the vertical variables across the clinically lame TP and FN and clinically sound TN and FP animals (Table 3). Symmetry indices of the lame TP and FN animals were smaller than the sound TN and TP animals ($P \leq 0.05$). In accord with the vertical variables, the lame animals that seemed clinically uniform were segregated into two (TP and FN) groups by the smaller symmetry indices (greater asymmetry) in the TP compared with the FN animals ($P \leq 0.05$). No differences occurred in the symmetry indices of the clinically sound TN and FP groups ($P \geq 0.05$).

We further explored the apparent lack of lateral load transfer from the lame to sound limbs (Fig. 1–6) by distributing sound limb P_c , P_i , locomotion score, mean maximum lesion score, PGRF, AGRF, STIME, GRF_{ω} and VIMPULSE across TP, FP, TN and FN outcomes (Table 4).

Table 3. Symmetry indices for peak ground reaction force (SI_PGRF), average ground reaction force (SI_AGRF), transformed ground reaction forces (SI_GRF ω), stance time (SI_STIME), vertical impulse (SI_VIMPULSE) vertical limb variables within TP, TN, FP, FN groups. Data are presented as mean \pm SEM

Variable	Group			
	TP (n = 54)	TN (n = 215)	FP (n = 27)	FN (n = 50)
SI_PGRF	-0.16 ^a \pm 0.020	0.00 ^b \pm 0.005	0.01 ^{bc} \pm 0.046	-0.05 ^c \pm 0.012
SI_AGRF	-0.16 ^a \pm 0.018	-0.01 ^b \pm 0.004	0.02 ^b \pm 0.040	-0.05 ^c \pm 0.012
SI_STIME	-0.17 ^a \pm 0.025	-0.01 ^{bc} \pm 0.007	0.03 ^b \pm 0.030	-0.05 ^c \pm 0.021
SI_GRF ω	-0.16 ^a \pm 0.022	-0.01 ^b \pm 0.006	0.02 ^b \pm 0.051	-0.06 ^c \pm 0.013
SI_VIMPULSE	-0.32 ^a \pm 0.035	-0.02 ^b \pm 0.008	0.05 ^b \pm 0.059	-0.10 ^c \pm 0.026

† TP = true positive, TN = true negative, FP = false positive, FN = false negative

‡ Within columns, means without a common superscript letter differ ($P \leq 0.05$); within rows, means without a common superscript letter differ significantly ($P \leq 0.05$)

Table 4. Peak ground reaction force (PGRF), average ground reaction force (AGRF), Fourier transformed ground reaction forces (GRF ω), stance time (STIME), vertical impulse (VIMPULSE), claw pain reaction (P_c), interdigital integument pain reaction (P_{int}), limb locomotion score (note that limb locomotion score is cow locomotion score in unilaterally lame cows) and maximum lesion severity score for sound hind limbs of lame (TP and FN) animals and the right hind limb of sound animals (TN and FP) within TP, TN, FP, FN groups of unilaterally lame cows. Data are presented as mean \pm SEM

Variable	Group			
	TP (n = 54)	TN (n = 215)	FP (n = 27)	FN (n = 50)
PGRF	0.44 ^b \pm 0.012	0.47 ^a \pm 0.004	0.39 ^c \pm 0.018	0.49 ^a \pm 0.012
AGRF	0.37 ^a \pm 0.010	0.40 ^b \pm 0.003	0.34 ^a \pm 0.013	0.42 ^b \pm 0.010
STIME	0.95 \pm 0.043	0.90 \pm 0.025	0.80 \pm 0.038	0.93 \pm 0.031
GRF ω	0.37 ^b \pm 0.012	0.41 ^a \pm 0.004	0.321 ^c \pm 0.018	0.42 ^a \pm 0.011
VIMPULSE	0.35 ^a \pm 0.016	0.36 ^a \pm 0.008	0.27 ^b \pm 0.019	0.39 ^a \pm 0.016
P_c	0.942 ^a \pm 0.018	0.976 ^a \pm 0.005	0.975 ^a \pm 0.013	0.865 ^b \pm 0.031
P_i	0.945 \pm 0.027	0.977 \pm 0.007	1.000 \pm 0.000	0.977 \pm 0.012
Locomotion score	1.000 \pm 0.000	1.000 \pm 0.001	1.000 \pm 0.002	1.000 \pm 0.003
Lesion score	1.481 ^{ab} \pm 0.024	1.037 ^a \pm 0.010	1.111 ^{ab} \pm 0.025	2.04 ^b \pm 0.027

† TP = true positive, TN = true negative, FP = false positive, FN = false negative

‡ Means within rows without a common superscript letter differ significantly ($P \leq 0.05$)

Note the sound limbs across all groups of animals were indistinguishable by locomotion and P_c and P_i with the one exception of minimally elevated P_c and P_i in the sound limbs of the FN animals (Table 4, $P \leq 0.05$). Distribution of the different LMV across the TP, FP, TN and FN outcomes showed that the vertical variables of sound limbs in unilaterally lame TP and FN groups were all equal (Table 4, $P \geq 0.05$) or smaller ($P \leq 0.05$) in magnitude compared with the corresponding variable in the bilaterally sound TN group.

The distribution of lesion types in lame limbs was similar across TP, TN, FP and FN groups (data not shown). Note that the lateral but not the medial claws of the TP and FN animals exhibited pain responses (Table 5, $P \leq 0.05$).

Discussion

These results supported our hypothesis and extended results of earlier reports (Scott, 1989, Rajkondawar et al. 2006,

Weishaupt et al. 2006). PGRF, AGRF, STIME, GRF ω and VIMPULSE decreased with increased locomotion score in the pelvic limbs. In general, lame limb loading diminished with increasing lameness concordant with the increased frequency and magnitude of P_c and P_i with lameness (Dyer et al. 2007). Differences in limb loading between the lame and contralateral sound limbs also resulted in greater asymmetry across many hind limb kinetic variables (Weishaupt et al. 2006).

Stance duration in locomotion score 1 cows was greater than that reported by Flower et al. (2005) and changed erratically with increasing lameness. Cows with nonexistent (locomotion 2) and mild, visible locomotion changes (locomotion 3) increased stance times similarly to those reported for lame cows by Flower et al. (2005). Moderate to severe hind limb lameness shortened the duration of stance time less than that recorded for sound cows in this study and lame cows reported by Flower et al. (2005). It has been proposed that extended times of vertical force application accomplished a reduced rate of limb loading and lowered

Table 5. Lateral and medial pain reaction in claws (P_c) of lame hind limbs (TP and FN) and sound hind limbs (TN and FP). Data are presented as mean \pm SEM

Variable	Group†			
	TP ($n=54$)	TN ($n=215$)	FP ($n=27$)	FN ($n=50$)
Lateral P_c	0.672 ^{a‡} \pm 0.036	0.976 ^b \pm 0.005	0.968 ^b \pm 0.015	0.671 ^a \pm 0.039
Medial P_c	0.948 ^a \pm 0.01	0.998 ^a \pm 0.001	0.999 ^a \pm 0.001	0.975 ^a \pm 0.02

†TP=true positive, TN=true negative, FP=false positive, FN=false negative

‡Means within rows without a common superscript letter differ significantly ($P \leq 0.05$)

peak forces on painful limbs (Clayton et al. 2000, Flower et al. 2005, Weishaupt et al. 2006). At some point, however, this compensatory response evidently no longer lowered discomfort and the cattle simply decreased the duration of limb loading. These data are concordant with earlier observations in cattle (Rajkondawar et al. 2006) and horses (Clayton et al. 2000; Weishaupt et al. 2006). Other compensatory mechanisms may include limb abduction/adduction (O'Callaghan et al. 2003, Chapinal et al. 2009) and anterior load shifts (Scott, 1989, Flower et al. 2005).

Although the predictive probability of lameness increased with worsening locomotion score across the sample population of cattle, accuracy of the predicted probability of lameness in each cow was eroded by small sensitivity. We approached the problem assuming quantifiable P_c and P_i was a key determinative of lameness (Dyer et al. 2007). The assumption was supported by the finding that lesion P_c and P_i rather than lesion presence or distribution was associated with lameness in the clinically lame cows.

We compared data across the TP and FN groups because both the TP and FN animals showed equal pain reaction responses across claw and interdigital locations. Surprisingly, only half the cows deemed clinically lame by pain reaction responses, lesion severity and locomotion score reduced vertical forces in the lame limb. Small vertical forces in this group (TP) rendered a large predicted probability of lameness consistent with the clinical diagnosis of lame (TP group). The remaining cows deemed clinically lame by pain reaction responses, lesion severity and locomotion score possessed vertical forces equal in magnitude to those of sound TN animals. These vertical forces rendered a small predicted probability of lameness inconsistent with the clinical diagnosis of lame (FN group). Clearly, clinical lameness produced two distinctly different sets of vertical variables because the magnitude of painful limb unloading varied substantially. In a sense the normal vertical forces of the lame limbs in the FN group 'blinded' the force plate system to the same lesions, pathology, P_c and P_i and locomotion scores associated with small vertical forces and large probability of lameness in the TP animals. These remarkable findings supported other (Scott, 1989) preliminary findings that changes in vertical variables were not an inevitable consequence of lameness in cattle. These differences probably explain the large coefficients of variability in vertical kinetic variables at greater locomotion scores (Rajkondawar et al. 2006).

To examine these phenomena further, we exploited within-cow comparisons of left and right limb loading manifested as vertical symmetry (Pastell et al. 2006, Weishaupt et al. 2006). Data across the population as well as the TP, FN, TN and FP groups indicated sound limbs could serve as internal loading controls in the vertical dimension because lateral load transfer off lame limbs did not impact loading in the vertical dimension of the opposite sound limbs. Symmetrical load bearing across hind limbs lacking visible lameness (locomotion score 1 and 2 and the TN cows) generated symmetry indices of zero. Shifts in weight bearing with visible lameness (locomotion score ≥ 3) led to symmetry indices of greater negative magnitude for PGRF, AGRF, GRF_{ov} and VIMPULSE concordant with lameness effects in equines and bovines (Clayton et al. 2000; Pastell et al. 2006; Weishaupt et al. 2006). Most notably, vertical symmetry divided the clinically homogenous population of lame cattle (TP and FN) into groups with large (TP) and small (FN) asymmetry. These data support the hypothesis that lame limbs were unloaded in the vertical dimension by clinically painful cows but the magnitude of vertical unloading was variable. Some lameness provoked vertical load transfer sufficient in magnitude to alter both absolute and relative measures of weight bearing. Other lamenesses provoked marginal amounts of vertical load transfer only detectable by relative measures of weight bearing across limbs of the same cow.

Explanations for these results remain speculative. Trivial explanatory variables such as changes in speed across force plates (Khumsap et al. 2001) were ruled out because time of passage through the system remained constant across locomotion scores (data not shown). It seemed unlikely that the algorithm computing the predicted probability of lameness was faulty because the small predicted probability of lameness in the FN group was exactly the computation expected from vertical variables recorded in these painful, visibly lame animals. An interesting and quite plausible explanation could be the abduction of painful limbs (O'Callaghan et al. 2003; Chapinal et al. 2009) by the FN animals. Abduction would transfer loading from painful lateral claws (van der Tol et al. 2003) to non-painful medial claws yet continue to sustain normal to near normal vertical limb loading. The finding that virtually all the P_c emanated from lateral rather than medial claws would enable lateral claw load transfer to the medial claw. One-dimensional vertical force plate systems would not record this form of

load redistribution and could be expected to generate small probability estimates of lameness in the face of altered locomotion, elevated P_c and P_i and severe lesion pathology. It may not be coincidental the 48.07% of painful, lame animals classified as sound by vertical variable measurements matched the 45% and 55% sensitivity and specificity of limb abduction as a diagnostic sign of sole ulceration (Chapinal et al. 2009). Lastly, even the lame TP and FN groups showed greater P_c and P_i than the sound TN and FP groups; the absence of P_c and P_i differences between the TP and FN animals eliminated pain as an explanatory variable for differences in limb unloading between these two groups.

Although the FP lowered specificity, the impact on accuracy was small compared with the FN group. Paradoxically the absence of pain, small mean maximal lesion score, sound locomotion score and similar lesion distributions in the non-painful, clinically sound FP and TN groups generated vertical forces and symmetry indices in the FP group that were smaller than the TN group, and identical to those of the painful, clinically lame TP group. We speculate that these animals randomly misstepped and generated vertical variables of aberrantly small magnitude.

In conclusion, the system and associated algorithm predicted lameness probabilities accurately from vertical variable inputs generated by the plates. It was also clear that lamenesses with similar lesion distribution, lesion severity, locomotion score and pain reaction produced more than one effect on vertical kinetic limb variables. In some cases large vertical loads were transferred off the limb whereas other times there was minimal vertical unloading of the limb. Together these observations conclusively established that automated methods of lameness detection integrated vertical variables into accurate and useful diagnostic outputs even though changes in vertical variables did not inevitably occur across all lameness. The results raise many important questions for future investigation. We proposed, but have not determined that some cows compensate for lameness through medial shifts in claw loading sufficient to alleviate lateral P_c while sustaining normal limb loading through the medial claw of otherwise painful limbs. Cows could also periodically change compensatory load-shifting to produce intermittent changes in the vertical variables of limbs. Alternatively, different types of compensatory load shifts may change transverse, propulsive and braking dimensions without effect on the vertical dimension. One or more of these compensatory mechanisms could hide considerable amounts of potentially costly, treatable lameness from detection systems restricted to the vertical dimension.

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