Environmental and cow-related factors affect cow locomotion and can cause misclassification in lameness detection systems.

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Changes in cow locomotion not related to lameness can cause false alerts in detection systems

Abstract

To tackle the high prevalence of lameness, techniques to monitor cow locomotion are being developed in order to detect changes in cows' locomotion due to lameness. Obviously, in such lameness detection systems, alerts should only respond to locomotion changes that are related to lameness. However, also other environmental or cow factors can contribute to locomotion changes not related to lameness and hence, might cause false alerts. In this study the effects of wet surfaces, dark environment, age, production level, lactation and gestation stage on cow locomotion were investigated. Data was collected at ILVO research farm (Melle, Belgium) during a 5 month period. The gait variables of thirty non-lame and healthy Holstein cows were automatically measured every day. In dark environments and on wet walking surfaces cows took shorter, more asymmetrical strides with less step overlap. In general, older cows had a more asymmetrical gait and they walked slower with more abduction. Lactation stage or gestation stage also showed significant association with asymmetrical and shorter gait and less step overlap probably due to the heavy calf in the uterus. Next, two lameness detection algorithms were developed to investigate the added value of environmental and cow data into detection models. One algorithm solely used locomotion variables and a second algorithm used the same locomotion variables and additional environmental and cow data. In the latter algorithm only age and lactation stage together with the locomotion variables were withheld during model building. When comparing the sensitivity for the detection of non-lame cows, sensitivity increased by 10% when the cow data was added in the algorithm (sensitivity was 70% and 80% for the first and second algorithm resp.). Hence, the number of false alerts of lame cows that were actually non-lame, decreased. This pilot study shows that using knowledge on influencing factors on cow locomotion will help in reducing the number of false alerts for lameness detection systems under development. However further research is necessary in order to better understand these and many other possible influencing factors (e.g. trimming, conformation) of non-lame and hence ‘normal’ locomotion in cows.

Keywords

Dairy cattle, lameness detection, locomotion, false alerts, gait variables

Implications

Lameness detection in dairy cattle is based on changes in locomotion variables of walking cows compared to the normal walk of this specific cow. However, this study shows that gait variables can also vary due to environmental (e.g. whether or not the walking surface is wet) or cow factors (e.g. gestation stage or growth of the calf). When implementing these influencing factors in the detection algorithms for lameness, the number of false alerts for lameness in non-lame cows decreased.

Introduction

During the last decades, the dairy industry has intensified in terms of keeping more cattle on fewer farms and more animals per caretaker. Consequently, the farmer’s time to monitor all individual cows drastically decreased. Sensors are being developed to support the farmers in their daily tasks, especially by monitoring the cows’ health so farmers can apply proper treatment or make thorough management decisions. As lameness is one of the most costly health problems in dairy cows also technology to detect lame cows is being investigated. Several sensor have been tested for their ability to register cow locomotion variables that are related to lameness; e.g. weight distribution (Pastell and Kujala, 2007), gait pattern (Maertens *et al.*, 2011) or posture pattern like arching of the back (Van Hertem *et al.*, 2014) (Van Nuffel *et al.*, 2015). Such lameness detection systems are based on the assumption that the lameness-relevant-variables change when a cow develops lameness e.g. shorter step length or more arching of the back. Next, these locomotion variables are combined in an algorithm that is used to alert the farmer if a cow shows a significant change in the variable and hence is becoming lame. However, not all changes in locomotion variables are related to lameness.

Indeed, several **environmental factors** such as flooring features are shown to alter locomotion characteristics. Phillips and Morris (2000) found that cows showed a different walking pattern on dry versus wetted concrete. The cows in their study reduced the arcs of the hind limbs on wet concrete suggesting the cows found a wet floor more slippery compared to dry concrete floor. The presence of slurry, particularly deep slurry, reduced the walking speed of cattle and altered their limb conformation during the support phase, giving them a different walking pattern from cows on dry or wetted concrete, probably to reduce the risk of slipping (Phillip and Morris, 2000). Cows studied by Telezhenko and Bergsten (2005) walked with longer strides and steps, but without speed difference on a continuous rubber floor compared to a slatted floor covered with rubber. Also, the acceleration of the legs was found to be lower on rubber flooring compared to concrete, indicating a smoother walking pattern (Chapinal *et al.*, 2011). The most impaired walking pattern was found on slippery concrete floor resulting in lower speed, shortened strides and a negative overlap. Similar results were also reported in the study by Rushen and De Passillé (2006), where a thin layer of slurry increased the slipping frequency and the number of strides, while it decreased the speed. These authors suggested that increasing the compressibility of the walk surfaces can improve cow locomotion independent of the roughness of the surface. For cows to walk normally, the optimal coefficient of friction has been reported to be between 0.4 N/N and 0.5 N/N (Phillips and Morris, 2001). Lower coefficients of friction cause cows to walk ‘stiffer and less confident’, i.e. quicker with shorter steps and less range of motion (van der Tol *et al.*, 2005). Higher coefficients of friction have been associated with longer swing phases combined with long strides to reduce friction (Phillips and Morris, 2001). Phillips *et al.* (2000) reported that the optimal light intensity for normal walking lies between 39 and 119 lux. The cows in their study were found to take shorter but quicker steps in a dark environment to increase their stability.

In addition, Van Nuffel (2014) showed that the majority of the variation in walking variables is attributed to differences between cows (> 97 %) compared to within cows (< 3 %) suggesting that **cow specific features** can influence gait. These results were also found in Telezhenko (2009). Locomotion score has been reported to increase with age (Ward, 1999; Manske *et al.*, 2002; Bicalho *et al.*, 2008). As the size of the udder increases with age – reasonably independently of milk production –, the more bulky udders of mature cows can force the hind legs to circumvent the udder, preventing free movement of the hind legs (Greenough *et al.*, 1981; Boelling and Pollot, 1998) limiting their strides and steps (Van Dorp *et al.*, 2004; Telezhenko and Bergsten, 2005). Several researchers reported a significantly change in abduction of the hind legs caused by the change in volume of the udder (Flower *et al.*, 2006; Chapinal *et al.*, 2009; O’Driscoll *et al.*, 2010; O’Driscoll *et al.*, 2011). Especially during early (O’Driscoll *et al.*, 2010) and peak lactation, disruption from normal locomotion is caused by swinging of the hind legs around the distended udder to reduce the possible discomfort associated with increased milk accumulation (Gleeson *et al.*, 2007). Blackie *et al.* (2011) could not find any difference in stride length (both in front and hind limbs) or tracking up between week 1, 6 and 12 of lactation. Flower *et al.* (2006) showed that cows walk with longer strides, higher stride heights, shorter stride duration and faster walks after milking compared to before milking. This might be either due to their high motivation to go back to the barn or pasture for feeding, or to the different weight carried between the hind legs because of less udder distension after milking. Subjective assessment of tracking up and reluctance to bear weight also improved after milking. In their study on the effect of once or twice-daily milking on udder firmness Tucker *et al.* (2004) could not find a decrease in stride length caused by discomfort for cows with different udder firmness while walking towards the milking parlour.

In addition, Chapinal *et al.* (2009) suggested that the state of late pregnancy also needs to be considered when studying the walking pattern of cows as the weight of the fetus might influence how cows distribute their weight between their legs and hence influences how cows walk. After calving, a decrease in gait asymmetry was found together with an increase in arching of the back. In the first week after calving, cows often appear to be walking stiffer which may be attributed to discomfort in the hindquarters following calving (Blackie *et al.*, 2011).

All above-mentioned influencing factors of age, lactation stage, milking or gestation stage seem to be closely associated with the changes in volume and firmness of the udders. The only study on the variation of udder volume during gestation and lactation was found in goats (Linzell, 1965). The rate of mammary growth was highest in late pregnancy, probably due to an increase in extracellular fluid in the udder at term (in average doubling in the last 6 weeks). Udder volumes decreased drastically after milking after which an increase reoccurs until the next milking.

Finally, the motivation to walk away from an aversive stimulus (impatient milker or dominant cow) or towards food may also influence cow walking pattern in terms of speed related variables (Herrmann, 1997). Carvalho *et al.* (2007) reported that trimmed cows had a more confident and stable walk. This was confirmed by Aoki *et al.* (2006) who quantitatively indicated that walking characteristics improved after trimming by increasing walking rate, step length and stepping rate. Limb angles also showed less 'on tiptoe' locomotion after trimming. Maertens *et al.* (2011) illustrated that the effect of 'routine' trimming on the gait of cows was similar to that of a lesion and the associated treatment. Such change in walking might indeed be caused by the increased sensitivity of the hoof by removing the excessive hoof horn (Dyer *et al.*, 2007) or might be caused by slow changes in walking due to the development of long toes prior to the hoof trimming, leading to recovery of the normal speed and symmetry in stance time and step length within a few days. However, in a study of Chapinal *et al.* (2010), cows exhibited either no change or deterioration in walking after trimming.

Also, other disease-related causes such as mastitis or abomasal displacement might influence cow locomotion due to a general feeling of sickness or painful body parts (e.g. painful udder for mastitis) (Milne *et al.*, 2003; Leslie and Pettersson-Wolfe, 2012; Fitzpatrick *et al.*, 2013). As such, monitoring changes in cow locomotion might be of added value for the detection systems for other disease besides lameness.

As literature shows that cow and/or environmental factors do change the locomotion in non-lame cows, such changes might result in false alerts to the farmer. The percentage of false alerts for non-lame cows in lameness detection systems under development range from less than 5 % to 24 % (based on the papers reviewed in Van Nuffel *et al.* 2015), which would result in 25 to 125 of non-lame cows that are falsely alerted as lame every day in a herd of 500 cows. Such high numbers of cows that are falsely alerted as lame are not feasible in practice and would drastically reduce the confidence of the farmer in any lameness detection system. Hence, possible approaches to reduce the number of false alerts should be investigated.

One approach to reduce the number of false alerts is to take non-disease related factors that can change the cow locomotion into account in a detection algorithm. Based on the review of Van Nuffel *et al.* (2015), the majority (79 %) of the studies on lameness detection systems do not include any environmental or cow factor into the algorithm. Only those studies that used a combination of available sensor data that was present on farm (e.g. milk yield and other milking data, feeding behavior data), include some form of cow-related factors into their detection algorithm. However, performance of the algorithms do not seem to be improved compared to algorithms using solely locomotion variables.

To further improve the performance of lameness detection systems by reducing the number of false alerts for non-lame cows, the following research questions were investigated: (1) Do environmental (wet walking surface, dark environment) and/or cow related factors (age, parity, production level, lactation and gestation stage) affect the locomotion of non-lame cows? And if so, (2) can the number of false alerts be decreased by including these influencing factors into the detection algorithms? The latter will be investigated by comparing the performance of two detection algorithms: one solely based on cow-locomotion data and a second algorithm, based on cow locomotion data combined with additional cow and environmental data.

Materials and methods

Experimental set-up

Data for this experiment was collected at ILVO research farm (Melle, Belgium) where the Holstein Friesian cows were housed in a deep litter barn with straw bedding and had access to pasture from approximately mid of April until the end of November. The cows were milked twice a day in a 2 x 3 auto-tandem milking parlour. The average milk yield was about 9000 litre per cow per year. In the retour alley after the milking parlour, the locomotion of individual cows was measured using the Gaitwise after milking (Maertens *et al.*, 2011). Gaitwise measures spatial (e.g. step length), temporal (e.g. stance time) and force related gait variables of claw-floor interactions of cows walking over the measurement zone. The ten gait variables measured by the Gaitwise system are summarised in Table 1 and were calculated as explained in Maertens *et al.* (2011).

Simultaneous to the Gaitwise measurements, video recordings were stored for locomotion scoring of the cows by a trained observer (K = 0.85). For locomotion scoring, a list of frequently used lameness attributes was used: non flexible joint movement, tender placement of the hoofs, arched back, low speed, irregular footfall in time or place, tracking up, abduction and head bobs. Finally, the locomotion was scored as ‘non-lame’ when the cow did not show any of these lameness attributes (locomotion score 1); ‘mildly lame’ if a lameness attribute was present (locomotion score 2) and as ‘severely lame’ if a single lameness attribute showed a clear impediment in locomotion or multiple lameness attributes were present (locomotion score 3) (Van Nuffel *et al.*, 2009). In order to have useful video footage, artificial lighting was present during the measurements. Locomotion scoring was performed on the videos acquired on Mondays and Thursdays.

All cows were motivated to return to the pasture or barn to find food. The cows were used to the Gaitwise system and were visually observed to walk over it in an undisturbed way. The cow and environmental factors considered in this study are summarized in Table 2. The factors ‘wet walking surface’ and ‘dark environment’ were recorded by the observer based on the video footage. All other factors were obtained from the farm records. Data were collected during the summer for a measuring period of 5 months.

Cows

Experiment 1

For the first experiment, only cows that did not show any signs of lameness during the 5 month measuring period were selected according to the following criteria: (1) not reported for mastitis, lameness or any other health problem during the measurement period by the animal care taker and farm records; (2) scored as ‘non-lame’ by the trained observer during the measurement period and (3) not trimmed during or 14 days before the measurement period. Based on these criteria, measurements of 30 cows were withheld for further analyses. To avoid possible confounding effects associated with morning versus evening milking routine, only morning measurements were considered. Selection according to this criteria resulted in a total of 951 measurements (from 30 cows, over a period of 5 months with an average of 2 measurements a week due to the frequency of locomotion scoring). Within this group, 12 cows were in their first parity, 6 were in their second parity and 12 were in the third or higher parity.

Experiment 2

To check the effect of cow and environmental factors on the performance of a lameness detection model, 100 cow measurements for every lameness status (n=300) were randomly selected during the 5 month measurement period. The lameness status of these 100 non-lame, 100 mildly lame and 100 severely lame cows were based on the videos that were scored by the trained observer.

Statistical analysis

Experiment 1

To determine the effect of environmental and cow data on the gait variables (dependent variables), linear mixed models were built with time as repeated variable within subject cow to correct for repeated measurements on each cow. The covariance between the repeated measurements was modelled using the Autoregressive structure (AR1). Rain, darkness, age, parity, production, days in milk (DIM) and gestation stage were added as independent variables (see Table 1). First, univariate associations were tested. Statistical significance in this step was assessed at $P < 0.25$. Next, Pearson correlations between all significant variables were calculated to discover multicollinearity. As expected, high correlations were found between age and parity ($R^2 = 0.83$), between DIM and gestation stage ($R^2 = 0.69$) and between milk production and DIM ($R^2 = 0.72$) and gestation stage ($R^2 = 0.65$), respectively. Age and DIM were withheld to be tested in further model building when combinations of these variables were significant in the univariate analyses. In the next step, multivariate models were fit for all dependent variables using a backwards stepwise regression. Statistical significance was assessed at $P < 0.05$. Least squares means were calculated to report mean values and standard deviations in tables. Finally, model fitting of the final models was done by visual inspection of the normal probability plots of the residuals. All analyses were performed in SAS 9.4 (SAS Institute Inc, NC, USA).

Experiment 2

In the second experiment, two lameness detection algorithms were developed. Mildly lame and severely lame cows were grouped together in a lame-cow group. The first algorithms was solely based on gait variables as measured by the Gaitwise, the second algorithm was based in the gait variables combined with cow and environmental variables that had a significant effect on cow locomotion in the first experiment. The gait variables for the first model were selected using a general linear model. Significant variables were then used to construct a linear discriminant analysis (LDA) model to predict the lameness status. Similarly, the second LDA model was constructed by adding extra environmental and cow data to the selected significant gait variables using a general linear model. All statistical analyses were performed using SAS (version 9.4, SAS Institute Inc., NC, USA).

Results

Experiment 1

On average 31.7 ± 8.6 measurements were successfully collected per cow during the measurement period.

The factors associated with the specific gait variables included in the final model are summarised in Tables 3, 4 and 5. Darkness significantly increased the asymmetry in step length and step overlap in the final model. In rainy weather, cows took shorter and more asymmetrical steps. In general, older cows had a more asymmetrical gait and they walked slower, with more abduction. Parity showed a significant increase in asymmetry in step length. Lactation stage, calculated as days in milk, showed significant association with more asymmetric step lengths and shorter steps, and less step overlap. As illustrated in Figure 1, step overlap decreased with increasing number of days in milk and became negative towards the end of the lactation. Cows with a negative step overlap place their hind limbs after the imprint of the fore limb that has just been lifted, hence, there is no step overlap. As every cow has her own way of walking, there is a large variation in the regression lines for the step overlap as a function of DIM for the different cows, as can be seen in Figure 2.

Experiment 2

From the randomly selected cows, lactation stage data of 4 cows was missing (1 mildly lame cow and 3 severely lame cows), hence these cows were omitted from this experiment resulting in 296 measurements. The variables that were selected during the modelling procedure for the first model using solely the gait variables were asymmetry in stride length, asymmetry in stance time and asymmetry in stride time together with stride length, stance time, step overlap and abduction. In the second model all cow and environmental factors were added to the dataset, but only lactation stage (DIM) and age were withheld by the algorithm during model building. The results of both the algorithm using solely gait variables and the results of the algorithm that combines gait variables with cow-factors are summarised in Tabel 6 and 7 respectively.

Discussion

In the present study, the effect of environmental and cow related factors on locomotion variables acquired for non-lame, healthy cows was investigated. A dark environment was found to have a significant influence in terms of more asymmetry in step length and less step overlap compared to locomotion during natural day light. Although the light intensity of the artificial light that was present during dark periods was not measured, these results suggest that more artificial light might be needed for the cows to show similar walking behaviour as performed during natural daylight. In rainy weather and hence on wet walking surface, cows did take shorter and more asymmetrical steps compared to non-rainy weather. Based on the video footage, cows were noticed to walk slowly with their head down in the rainy and windy weather. Based on these observations, the shorter and more asymmetrical strides can explain their adaptations to a slower and more cautious locomotion to reduce the risk of slipping or the lower motivation to walk through rainy and windy weather. The present results are in line with those of the studies of Phillips and Morris (2000) and van Der Tol *et al.* (2005), in which wet or slippery surfaces were significantly associated with reduced speed assuming that the rainy weather condition in this study, did change the slipperiness of the rubber flooring of the measurement zone.

Older cows walked slower, with more abduction and in general more asymmetry compared to the younger and hence more agile cows. Although udder size was not measured in this study, the reduction in speed and step overlap and the increase in abduction with increasing age can – besides the decrease in agility of older cows – most likely be attributed to an increase in udder size with age, as reported by other researchers (Boelling and Pollot, 1998; Telezhenko and Bergsten, 2005). Larger udders might force cows to swing their hind legs around the udder resulting in more abduction and larger udders might also hinder the hind legs in the forward movement resulting in shorter steps with the hind legs and hence, less step overlap.

Due to the high correlation of production level with DIM and gestation stage, production was not withheld in the final model. During univariable testing however, both stride length and step overlap were significantly decreased with increased production. Under the assumption that the size of the udders is positively correlated with the production level, the shorter strides and less step overlap could be assigned to the larger udders. In literature, however, no clear evidence can be found between production level and size of the udder. Also, measurements were specifically performed after milking to prevent possible impacts of the filling of the udder on the locomotion of the cows.

Increasing lactation stage, calculated as days in milk, showed significant associations with more asymmetrical and shorter strides with less step overlap compared to earlier in lactation. Again, these finding could be allocated to the udder size although no measurements were performed with full udders or during peak lactation (as most cows are used for feeding experiments during the first months of lactations and hence are housed in a barn where no Gaitwise measurements could be performed). However, due to the high correlation between lactation stage and gestation stage ($R^2 = 0.69$), the stage of gestation might be a better explanation for the more asymmetrical, shorter – and hence slower – locomotion with less step overlap. The presence of a growing – and hence heavier – foetus in the uterus of the cows can hinder the gait of the cows, mainly the step overlap. Step overlap even tended to be negative during the last months of the gestation, meaning that the imprints of the hind legs did not reach the imprints of the front legs (Figure 1). Even though – except for one cow – step overlap in every cow declined towards the end of the gestation period, a large variation between cows was noticed (Figure 2). Only 3 % of the variation in step overlap could be explained by DIM, so other influencing factor – besides those included in this study – should be accounted for. One possible explanation might be that cows tend to walk more carefully towards the end of the gestation. Carrying weight has been linked to slower and shorter strides in equine and human gait (Martin and Nelson, 1986; Pascoe *et al.*, 1997; Wincler *et al.*, 2001). Hence, the presence of heavier calves by the end of the gestation period might be the explanation for the slower and more asymmetrical locomotion towards the end of the lactation (cfr. gestation), as suggested by Chapinal *et al.* (2009). In the majority of pregnancies in dairy cattle, the foetus is carried in the right uterine horn. Also, more calves in these right-sided pregnancies are male and thus often heavier than those in the left horn (Foote *et al.*, 1959; Morrow *et al.*, 1968; Giraldo *et al.*, 2010; Gharagoslou *et al.*, 2013). Unequal distribution of extra weight during pregnancies might indeed induce a more asymmetric gait.

Cows in early lactation enrolled in this study did show less asymmetry in their stride lengths compared to further in lactation, which might be similar to the decrease in asymmetry and arching of the back after calving that was found in the study of Chapinal *et al.* (2009). However, no analysis on the difference in asymmetric gait variables before versus after calving of individual cows was done in this study due to the lack of gait data before calving (dry cows are housed in different barn) and data after calving as most of the cows where enrolled in feeding experiments after calving and could therefore not measured by the Gaitwise. As the Gaitwise system is based on measurements of claw-floor variables, no automated measurements of arched back were performed.

As several cow and environmental factors that were selected into the final model influence the locomotion of non-lame cows, a pilot study was performed to investigate the effect of adding these influencing factors to a lameness detection system on the number of false alerts. This added value was investigated by comparing the performance of two lameness detection models: One using solely the measured gait variables and a second one using the measured gait variables combined with the influencing factors of experiment 1. During the Linear Discriminant analysis using solely the cow gait variables, three variables of asymmetry (in stride length, stride time and stance time) and stride length, step overlap and abduction were withheld for model building. Out if the 100 non-lame cows based on the reference scoring of the trained observer, 29 cows were misclassified as lame by the model (Table 6).

When also the cow and environmental data were added to the dataset for model building, 10 non-lame cows that were falsely alerted as lame by the first model, were now correctly scored as non-lame resulting in an increase in sensitivity for the classification of non-lame cows from 71% to 81% (Table 6 and Table 7). In the second model, only lactation stage (DIM) and age were withheld as additional variables to the measured gait variables. Similar to the approach used in the first experiment, both gestation stage and parity were not used in this model due to the high correlation with lactation stage and age resp. Due to the lack of any relation between production and cow-gait variables, also production was omitted from the dataset. None of the environmental variables (wet surface or dark environment) seemed to be of added value based on this pilot study. Hence, in this pilot study, adding information on the lactation stage and the age of the cows decreases the number of false alerts. However, other approaches to combine environmental and cow factors into a model with lameness-related-variables might be more suited as the correlation between these variables within cows should be taken into account during model building.

Conclusion

The effect of cow and environment factors on the gait variables of non-lame, healthy cows has been investigated using measurements of 30 cows during 5 months. Cows tend to walk with smaller strides, less step overlap and more asymmetry in stride length towards the end of the lactation or the end of the gestation and when they get older. In dark environments and on wet walking surfaces cows took shorter, more asymmetrical strides with less step overlap. During model building, age and lactation stage were withheld into the model based on cow locomotion variables and the sensitivity for detecting non-lame cow increased from 71% to 81%. Hence, the number of false alerts of lame cows that were actually non-lame, decreased.

The tested factors of this pilot study were limited and a follow up study with more factors and more cows, during all days of lactation and gestation could provide more information about the influence of these factors on cow locomotion.

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References

Aoki Y, Kamo M, Kawamoto H, Zhang JG and Yamada A 2006. Changes in walking parameters of milking cows after hoof trimming. Animal Science Journal 77, 103-109.

Blackie N, Bleach E, Amory J and Scaife J 2011. Impact of lameness on gait characteristics and lying behaviour of zero grazed dairy cattle in early lactation. Applied Animal Behaviour Science 129, 67-73.

Boelling D and Pollot GE 1998. Locomotion, lameness, hoof and leg traits in cattle: I. Phenotypic influences and relationships. Livestock Production Science 54, 193-203.

Carvalho V, Bucklin R, Shearer J, Naas I, Neto M, Souza S and Massafera V 2007. Dairy Cattle Linear and Angular Kinematics during the Stance Phase. Agricultural Engineering International: the CIGR Ejournal. Vol. IX, 1-10.

Chapinal N, de Passille AM, Pastell M, Hanninen L, Munksgaard L and Rushen J 2011. Measurement of acceleration while walking as an automated method for gait assessment in dairy cattle. Journal of Dairy Science 94, 2895-2901.

Chapinal N, de Passille AM and Rushen J 2009. Weight distribution and gait in dairy cattle are affected by milking and late pregnancy. Journal of Dairy Science 92, 581-588.

Chapinal N, de Passille AM and Rushen J 2010. Correlated changes in behavioral indicators of lameness in dairy cows following hoof trimming. Journal of Dairy Science 93, 5758-5763.

de Mol RM, Andre G, Bleume, EJB, van der Werf JTN, de Haas Y and van Reenen CG 2013. Applicability of day-to-day variation in behavior for the automated detection of lameness in dairy cows. Journal of Dairy Science 96, 3703-3712.

Dyer RM, Neerchal NK, Tasch U, Wu Y, Dyer P and Rajkondawar PG 2007. Objective determination of claw pain and its relationship to limb locomotion score in dairy cattle. Journal of Dairy Science 90, 4592-4602.

Fitzpatrick CE, Chapinal N, Petersson-Wolfe CS, DeVries TJ, Kelton DF, Duffield TF and Leslie KE 2013. The effect of meloxicam on pain sensitivity, rumination time, and clinical signs in dairy cows with endotoxin-induced clinical mastitis. Journal of Dairy Science 96: 2847–2856.

Flower FC, Sanderson DJ and Weary DM 2006. Effects of milking on dairy cow gait. Journal of Dairy Science 89, 2084-2089.

Foote WD, Tyler WJ and Casida LE 1959. Effect of some genetic and maternal environmental variations on birth weight and gestation length in Holstein cattle. Journal of Dairy Science 42, 305-311.

Gharagozlou F, Vojgani M, Akbarinejad V, Niasari-Naslaji A, Hemmati M and Youssefi R 2013. Parallel distribution of sexes within left and right uterine horns in Holstein dairy cows: Evidence that the effect of side of pregnancy on sex ratio could be breed-specific in cattle. Animal Reproduction Science 142, 101– 105.

Giraldo AM, Hylan D, Bondioli KR and Godke RA 2010. Distribution of sexes within the left and right uterine horns of cattle. Theriogenology 73, 496-500.

Gleeson DE, OBrien B, Boyle L and Earley B, 2007. Effect of milking frequency and nutritional level on aspects of the health and welfare of dairy cows. Animal 1, 125-132.

Hogeveen H, Kamphuis C, Steeneveld W and Mollenhorst H 2010. Review: Sensors and clinical mastitis – the quest for the perfect alert. Sensors 10, 7991-8009.

Kramer E, Cavero D, Stamer E and Krieter J 2009. Mastitis and lameness detection in dairy cows by application of fuzzy logic. Livestock Science 125, 92-96.

Leslie KE and Petersson-Wolfe CS 2012. Assessment and Management of Pain in Dairy Cows with Clinical Mastitis. Veterinary Clinics of North America: Food Animal Practice 28, 289-305.

Linzell JL 1965. Measurements of udder volume in live goats as an index of mammary growth and function. Journal of Dairy Science 49, 307-311.

Maertens W, Vangeyte J, Baert J, Jantuan A, Mertens KC, De Campeneere S, Pluk A, Opsomer G, Van Weyenberg S and Van Nuffel A 2011. Development of a real time cow gait tracking and analysing tool to assess lameness using a pressure sensitive walkway: The GAITWISE system. Biosystems Engineering 110, 29-39.

Martin P and Nelson RC 1986. The effect of carried loads on the walking patterns of men and women. Ergonomics 29, 1191–1202.

Miekley B, Traulsen I and Krieter J 2012. Detection of mastitis and lameness in dairy cows using wavelet analysis. Livestock Science 148, 227-236.

Miekley B, Traulsen I and Krieter J 2013. Mastitis detection in dairy cows: the application of support vector machines. Journal of Agricultural Science 151, 889-897.

Milne MH, Nolan AM, Cripps PJ and Fitzpatrick JL 2003. Assessment and alleviation of pain in dairy cattle with clinical mastitis. Cattle Practice 11, 289-293.

Mollenhorst H, Rijkaart LJ and Hogeveen H 2012. Mastitis alert preferences of farmers milking with automatic milking systems. Journal of Dairy Science 95, 2523-2530.

Morrow DA, Roberts SJ and McEntee K 1968. Latent effects of pregnancy on postpartum ovarian activity in dairy cattle. Journal of Animal Science 27, 1408-1411.

O'Driscoll K, Gleeson D, O'Brien B and Boyle L 2010. Effect of milking frequency and nutritional level on hoof health, locomotion score and lying behavior of dairy cows. Livestock Science 127, 248-256.

O'Driscoll K, Gleeson D, O'Brien B and Boyle L 2011. Does omission of a regular milking event affect cow comfort? Livestock Science 138, 132-143.

Pascoe DD, Pascoe DE, Wang YT, Shim DM and Kim CK 1997. Influence of carrying book bags on gait cycle and posture of youths. Ergonomics 40, 631-641.

Pastell ME and Kujala M 2007. A probabilistic neural network model for lameness detection. Journal of Dairy Science 90, 2283-2292.

Phillips CJC and Morris ID 2000. The locomotion of Dairy Cows on concrete floors that are dry, wet, or covered with slurry of excreta. Journal of Dairy Science 83, 1767-1772.

Phillips CJC and Morris ID 2001. The locomotion of dairy cows on floor surfaces with different frictional properties. Journal of Dairy Science 84, 623-628.

Phillips CJC and Morris ID 2002. The ability of cattle to distinguish between, and their preference for, floors with different levels of friction, and their avoidance of floors contaminated with excreta. Animal Welfare 11, 21-29.

Phillips CJC, Morris ID, Lomas CA and Lockwood SJ 2000. The locomotion of dairy cows in passegeways with different light intensities. Animal Welfare 9, 421-431.

Rushen J and de Passille AM 2006. Effects of roughness and compressibility of flooring on cow locomotion. Journal of Dairy Science 89, 2965-2972.

Telezhenko E 2009. Measurement of spatial gait parameters from footprints of dairy cows. Animal 3, 1746-1753.

Telezhenko E and Bergsten C 2005. Influence of floor type on the locomotion of dairy cows. Applied Animal Behaviour Science 93, 183-197.

Tucker CB, Dalley DE, Burke J-LK and Clark DA 2004. Milking cows once daily influences behavior and udder firmness at peak and mid lactation. Journal of Dairy Science 90, 1692-1703.

van Der Tol PPJ, Metz JHM, Noordhuizen-Stassen EN, Back W, Braam CR and Weijs WA 2005. Frictional forces required for unrestrained locomotion in dairy cattle. Journal of Dairy Science 88, 615-624.

Van Dorp TE, Boettcher P and Schaeffer LR 2004. Genetics of locomotion. Livestock Production Science 90, 247-253.

Van Hertem T, Viazzi S, Steensels M, Maltz E, Antler A, Alchanatis V, Schlageter-Tello A, Lokhorst C, Romanini CEB, Bahr C, Berckmans D and Halachmi I 2014. Automatic lameness detection based on consecutive 3D-video recordings. Biosystems Engineering 119, 108-116.

Van Nuffel A 2014. Development of an automated detection system for lameness in cattle: The Gaitwise system. PhD for the requirement for the degree of doctor in Bioscience Engineering, Katholic University Leuven; Leuven (BE).

Van Nuffel A, Sprenger M, Tuyttens FAM and Maertens W 2009. Cow gait scores and kinematic gait data: can people see gait irregularities? Animal Welfare 18, 433-439.

Van Nuffel A, Vangeyte J, Mertens KC, Pluym L, De Campeneere S, Saeys W, Opsomer G and Van Weyenberg S 2013. Exploration of measurement variation of gait variables for early lameness detection in cattle using the GAITWISE. Livestock Science 156, 88-95.

Van Nuffel A, Zwertvaegher I, Van Weyenberg S, Pastell M, Thorup VM, Bahr C, Sonck B, Saeys W 2015. Review on Lameness detection in Dairy Cows: part 2. Use of Sensors to Automatically Register Changes in Locomotion or Behavior. Animals 5, 861-885.

Wickler SJ, Hoyt DF, Cogger EA and Hall KM 2001. Effect of load on preferred speed and cost of transport. Journal of Applied Physiology 90, 1548–1551.

Figure captions

Figure 1 Scatter plot and regression line for Step Overlap (m) against DIM (Days In Milk) (not corrected for clustering).

Figure 2 Regression lines for every individual cow based on Step Overlap (m) against DIM (Days In Milk).

Tables

Table 1. Definitions of the gait variables as measured by the Gaitwise system (Maertens et al., 2011)

Name (unit)	General definition*
Stride length (m)	Distance between two consecutive imprints of the same hoof
Stride time (s)	Time between two consecutive imprints of the same hoof
Stance time (s)	Time that the hoof is on the floor during one complete stride
Step overlap (m)	The lengthwise distance between the front hoof imprint and a subsequent imprint of the hind hoof on the same side
Abduction (m)	The sideways distance between the front hoof imprint and a subsequent imprint of the hind hoof on the same side
Asymmetry in step width (m)	Mean difference in step width between left and right hoof imprints
Asymmetry in step length (m)	Mean difference in step length between left and right hoof imprints
Asymmetry in step time (s)	Mean difference in step time between left and right hoof imprints
Asymmetry in stance time (s)	Mean difference in time that a hoof is on the ground between left and right hoof imprints
Asymmetry in relative pressure (/)	Mean difference in relative maximum force exerted by the legs between left and right hoof imprints

* Some definitions are based on spatial gait parameters from Telezhenko (2009)

Tabel 2. Summary of the environmental and cow factors considered in this study and their definitions and classification

Factor	Description / Definition	Classification / Unit
Wet walking surface	Cows walked over measurement zone during heavy rain and wind or during dry weather. Based on the observations, the measurement zone was visually wet during rain and cows lowered their head against the rain and wind.	0 = No rain 1 = Rain
Dark environment	Based on the observation, cows walked over the measurement zone during natural lighting (daylight) or during with artificial light in dark environments (from dusk till down)	0 = measurements performed during natural daylight; 1 = measurements performed with artificial light in a dark environment
Age	Age of the cows in years	Number of years
Parity	Number of calvings	1 (first parity), 2 (second parity), 2+ (third or more parity)
Production	Milk production during morning milking	kg milk
Days In Milk (DIM)	Number of days after calving (cfr. stage of lactation)	Number of days
Gestation stage	Number of days after successful artificial insemination	Number of days

Table 3: Final multilevel linear model describing the factors influencing specific gait variables with β = linear regression coefficient; SE = standard error; significant P-values are shown for the multilevel model, (SU = variables were significant ($P < 0.05$) at the univariable level but not at the multivariable level)

Factor		Asymmetry in step width				Asymmetry in step length			Asymmetry in step time			Asymmetty in stance time		
		β	SE	P-value		β	SE	P-value	β	SE	P-value	β	SE	P-value
Intercept		0.1623	0.0051			0.4246	0.0049		0.3637	0.0057		0.0049	0.0051	
Darkness[b]								0.028			SU			
	0				0	ref[a]								
	1				1	0.0056	0.0019							
Wet surface[c]				0.004				0.0043						
	0	ref[a]			0	ref[a]								
	1	0.0069	0.0022		1	0.0066	0.0021							
Age		0.0092	0.0010	<0.0001					0.0058	0.0011	<0.0001	0.0062	0.0010	<0.0001
Parity[d]				SU				<0.0001			SU			SU
	1				1	ref[a]								
	2				2	0.0258	0.0047							
	3				3	0.0086	0.0037							
Production														
DIM[e]				SU		0.0007	$2*10^{-5}$	0.0010			SU			SU
Gest. stage[f]				SU				SU			SU			

[a] ref = reference; [b] Dark environment → 0 or 1 = absence or presence of the influencing factor 'dark environment'; [c] Wet Surface → 0 or 1 = absence or presence of the influencing factor 'Wet walking surface'; [d] Parity → 1, 2 or 3 = first, second or third + parity; [e] DIM = Days in Milk; [f] Gest. Stage = gestation stage
beta= linear regression coefficient en SE= standard error

Table 4: Final multilevel linear model describing the factors influencing specific gait variables with β = linear regression coefficient; SE = standard error; significant P-values are shown for the multilevel model, (SU = variables were significant ($P < 0.05$) at the univariable level but not at the multivariable level)

Factor	Asymmetry in force			Stride length			Stride Time		
	β	SE	P-value	β	SE	P-value	β	SE	P-value
Intercept	10.07	0.7991		1.6621	0.0153		1.3746	0.02448	
Darkness[b]			SU						
Wet surface[c]			SU			0.0009			
0				ref[a]					
1				-0.02839	0.0076				
Age	0.9764	0.1611	<0.0001			SU	0.0281	0.0049	<0.0001
Parity[d]			SU			SU			SU
Production						SU			
DIM[e]			SU	-0.0003	$7*10^{-5}$	<0.0001			
Gest. stage[f]						SU	0.0005	0.0001	<0.0001

[a] ref = reference; [b] Dark environment → 0 or 1 = absence or presence of the influencing factor 'dark environment'; [c] Wet Surface → 0 or 1 = absence or presence of the influencing factor 'Wet walking surface'; [d] Parity → 1, 2 or 3 = first, second or third + parity; [e] DIM = Days in Milk; [f] Gest. Stage = gestation stage

beta= linear regression coefficient en SE= standard error

Table 5: Final multilevel linear model describing the factors influencing specific gait variables with β = linear regression coefficient; SE = standard error; significant P-values are shown for the multilevel model, (SU = variables were significant ($P < 0.05$) at the univariable level but not at the multivariable level)

Factor	Stance Time			Step Overlap			Abduction		
	β	SE	P-value	β	SE	P-value	β	SE	P-value
Intercept	0.9182	0.0152		0.0365	0.0083		0.0087	0.0025	
Darkness[b]						0.044			
0				Ref[a]					
1				-0.0074	0.0035				
Wet surface[c]						SU			
Age	0.0203	0.0031	<0.0001			SU	0.0049	0.0005	<0.0001
Parity[d]			SU			SU			SU
Production						SU			
DIM[e]			SU	-0.0001	$4*10^{-5}$	0.002			SU
Gest. stage[f]			SU			SU			

[a] ref = reference; [b] Dark environment → 0 or 1 = absence or presence of the influencing factor ‘dark environment’; [c] Wet Surface → 0 or 1 = absence or presence of the influencing factor ‘Wet walking surface’; [d] Parity → 1, 2 or 3 = first, second or third + parity; [e] DIM = Days in Milk; [f] Gest. Stage = gestation stage
beta= linear regression coefficient en SE= standard error

Table 6: Summary of classifications of non-lame and lame cows using solely cow-gait variables obtained from the Gaitwise system based on the training dataset

Actual Group	**Predicted group membership**		**Sensitivity**
	Non-lame	Lame	
Non-lame	71	29	***71 %***
Lame	45	151	***77 %***
Specificity	***77 %***	***71 %***	

Table 7: Summary of classifications of non-lame and lame cows using a combination of cow-gait variables obtained from the Gaitwise system and cow and environmental factors

Actual Group	**Predicted group membership**		**Sensitivity**
	Non-lame	Lame	
Non-lame	81	19	***81 %***
Lame	45	151	***77 %***
Specificity	***77 %***	***81 %***	