

1 **Analysis of behavioral changes associated with deep non-perforating septic**
2 **pododermatitis of the bovine foot**

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ABSTRACT

The detection of lame cows is important to improve animal welfare. Automated methods for lameness detection have the potential to facilitate recognition and monitoring of lame cows in large dairy herd. The goals of this study were to evaluate the suitability of different physiological and behavioral variables for automated detection of lameness in dairy cows housed in a loose stall. Lame cows suffering from a deep, non perforating septic pododermatitis (DNPSP) of one claw of one and the same hind limb (n=32; group L) and 10 non lame cows (group C) were included in this study. Ethological variables by direct visual observation, locomotor activity by tridimensional accelerometers, weight distribution between hind limbs by the 4-scale weighing platform, feeding behavior by the nose band sensor and heart rate variability by the POLAR[®] device were assessed. The ethological scores, the lying time and all parameters of the weighing platform (mean limb difference [Δ weight], limb weight ratio [LWR] and standard deviation of the weight applied on each limb [SD]) revealed significant differences between groups L and C. Such difference was not evident for variables of heart rate variability and feeding behavior. The lameness score of cows in group L was positively correlated with the lying time ($r = 0.56$) and Δ weight ($r = 0.60$), whereas it was negatively correlated with LWR ($r = -0.67$) and SD ($r = -0.46$). The receiver operating characteristic analysis showed the highest values for the variable SD, with an area under the curve (AUC) of 0.84, a sensitivity of 0.97 and a specificity of 0.80. The logistic regression of the combination of SD and Δ weight was the best predictor of cows being lame with an AUC of 0.96 and accounting for 59.25% of the variation in the likelihood of a cow being lame ($R^2 = 0.59$). It is concluded that the combination of the variables SD and Δ weight – both derived from the weighing platform - represents a valuable dataset for automated identification of lame cows suffering from a DNPSP of one individual hind limb, when compared with non-lame cows.

INTRODUCTION

Orthopedic disorders causing lameness belong to the most common and economically most relevant production diseases of dairy cattle worldwide (Bennett et al. 1999). Prevalence of lameness of dairy cattle in European countries and the United States ranges between 30% and 48% (Amory et al. 2008; Dippel et al. 2009b; Dippel et al. 2009a; Barker et al. 2010; Bicalho et al. 2009; L.A. Espejo et al.). Economic losses are caused, among others, by reduced milk yield and fertility, increased risk of culling, treatment costs and additional expenditure for extra labor (Willshire and Bell 2009; Ettema and Østergaard 2006; Bruijnis et al. 2010; L.E. Green et al. 2002; Kossaibati and Esslemont 1997; L.D. Warnick et al. 2001; Amory et al. 2008, E.J. Garbarino et al. 2004, 2004, Ettema and Østergaard 2006)

The view of the farmer

(Blowey and Edmonson 2000; Leach et al. 2010) found that many farmers were not aware of the financial consequences caused by lame animals, and that they did not realize the implications of the lameness problem on productivity and profitability of their dairy enterprise. In an investigation of 222 English dairy farms, 90% of the farmers did not judge lameness a big issue, although the average prevalence of lameness was 36 % (Leach et al. 2010). However, farmer's interest in good claw health is a decisive factor for low within herd lameness prevalence (Becker et al. 2014b).

Studies of Russell et al. (1982) revealed that the lesions causing lameness were located in the area of the feet in 88.3% of the cases, 84% of the foot lesions occurred in the hind feet and 85% of these lesions affected the outer claw. Digital dermatitis, heel horn erosion, sole ulcers and white line disease were shown to be the predominant claw lesions of dairy cows (Manske et al. 2002; Barker et al. 2010; Becker et al. 2014b; Becker et al. 2014a). Among the

mentioned claw lesions, sole ulcers represent those that are economically the most important (Willshire and Bell 2009).

Risk factors

The principal reasons for horn defects were particularly environmental factors such as poor floor conditions, concrete, rough slippery floors, poor quality of laying surfaces, not livestock-adapted cubicles, poor surface hygiene, nutrition that does not fulfil the animal requirements, overstocking, stress of the animals, lacking patience of the stockperson handling the cows, as well as improper claw trimming (Cook and Nordlund 2009; Rouha-Müller et al. 2009; Kofler 2013)

Signs of pain

Cows are a prey species and rather stoical, they show seldom signs of pain until the stimulus is severe (Anil et al. 2005; Hudson et al. 2008; O'Callaghan 2002). However, it has been shown, that there are slight behavioral changes present in lame cows. Hudson et al. (2008) showed that cows suffering from pain associated with lameness changed their behavior to reduce discomfort. These behavioral changes encompass for example decreased movement/locomotion, decreased feed intake, reduced mental responsiveness, decreased interaction with other animals, tooth grinding, poor coat condition, and changes in posture and gait (Whay 2002).

Recognition and treatment of lame cows are often insufficient in practice. Whay et al. (2002) revealed that farmers only detected 25% of lame cows. The mean time from the onset of lameness to clinical recovery by the farm personnel was 27 days (Tranter and Morris 1991).

In general, veterinary treatments and management decisions are more effective, the earlier they are initiated relative to the initiation of the disease (González et al. 2008). (L.E. Green et

al. 2002) revealed that the milk yield decreased already from 4 months before until 5 months after individual cows were diagnosed clinically lame. Difficulty in early detection (L.A. Espejo et al.) and the economic impact has led to increased interest in automated methods for lameness detection (Chapinal et al. 2010a). Therefore, various automated tools were developed and tested with the goal to improving the assessment and early detection of lameness in dairy farms. Using the 4-scale weighing platform, Rushen et al. (2007) and Pastell et al. (2010) revealed that lame cows reduced the weight-bearing of the affected limb. Furthermore, lame cows as compared to non-lame cows showed a higher asymmetry of weight within each pair of limbs and had a greater standard deviation of the weight applied on ~~each~~ the affected and the contralateral limb over time (Chapinal et al. 2010a). The latter variable proved to be the most accurate predictor of whether a cow was lame or not (Chapinal et al. 2010a). The use of tridimensional accelerometers revealed that grazing lame dairy cows as compared to non-lame cows spent more time lying and had fewer lying bouts per day (Sepúlveda-Varas et al. 2014). Generally, lying bouts of lame cows lasted longer than of non-lame cows (Chapinal et al. 2010a; Sepúlveda-Varas et al. 2014; Yunta et al. 2012). Acute locomotion disorders lead to a decrease in (i) feed intake, (ii) number of meals, (iii) visits to the feeders and (iv) a considerable decrease in eating time (González et al. 2008). By using the noseband pressure sensor technology, it is currently possible to detect, differentiate and record eating and rumination behaviour automatically (Zehner et al. 2012; Braun et al. 2014). The analysis of heart rate variability (HRV) represents another suitable automated method to assess stress and welfare status in farm animals (Borell et al. 2007). Heart rate variability reflects the balance between sympathetic and parasympathetic tone and delivers information on the stress response of the autonomic nervous system (Borell et al. 2007; Mohr et al. 2002). In general, sympathetic activity tends to increase heart rate (HR) and decrease HRV, whereas parasympathetic activity tends to decrease HR and increase HRV (Tarvainen et al. 2014).

Nordmann et al. (2011) showed that lower values in HRV and higher values in HR are associated with higher levels of stress in goats.

It was the primary aim of this study to evaluate the suitability of various automated methods (measures of weight distribution, locomotor activity, feeding behavior and heart activity) and ethological variables for the assessment of altered behavior in cows associated with lameness caused by “deep, non perforating, septic pododermatitis” (DNPSP) of one individual hind claw. A further aim was to identify the combination of automated tools that allow for the most accurate prediction of the correct group allocation (non-lame versus lame) of individual cows.

MATERIALS AND METHODS

Cows and housing

The study was carried out between April 2013 and March 2014 on a commercial dairy farm with around 900 lactating German Holstein cows, located close to Chemnitz, Germany. Cows were housed in a group of 30-40 moderately lame cows per pen in a free stall with concrete slatted floor and rubber floor cubicles. Pluriparous German Holstein dairy cows ($n = 44$; parity = 3.09 ± 1.22 [mean $\pm$ Standard deviation (STD)]); days in milk [DIM] = 104.95 ± 47.03 ; body weight [BW] = 625.63 ± 69.91 kg; daily milk yield at day 1 = 34.40 ± 7.18 kg) were included into the study. Cows were milked twice daily in a carousel milking parlor, at approximately 02:00 pm and 02:00 am and fed a TMR diet once daily that was formulated to meet the requirements for lactating dairy cows. Water was available ad libitum from self-filling troughs. The experimental protocol was approved by the Animal Care Committee of the University of Leipzig (Landesdirektion Sachsen, Referat 24 - Veterinärwesen und Lebensmittelüberwachung, Pharmazie, GMP Inspektorat, Anzeigennummer: A 30/12, Registriernummer: 24-9168.21/4/30).

Selection of cows

Once every 4 weeks, 4 cows (1 non lame cow [control = group C] and 3 lame cows [lame = group L]) were selected and moved to the lame cow pen. Criteria for inclusion in group C were that cows were in $\geq$ second lactation, clinically healthy and not lame. Criteria for inclusion in group L were that cows were clinically healthy except for the presence of lameness and a DNPSP (sole ulcer or white line ulcer) of one claw of one and the same hind limb. Criteria for immediate exclusion of cows during the selection process were (i) presence of signs of systemic disease or (ii) painful orthopedic lesions outside the claw lesion of interest, (iii) treatment with NSAID/SAID within 28 days prior to the experiment, (iv) withdrawal period after antimicrobial treatment not faded, (v) pregnancy > 7 months, or (vi) > 180 DIM at the time of selection. One cow of group L was excluded from the study, because it did not adequately familiarize with the experimental procedure, and one cow of group C was excluded at the end of data acquisition during data validation (viewing of blinded videos and photographs), because it was lame (score 4/13). In total, 42 cows (10 of group C and 32 of group L) were included in the final statistical analysis.

Experimental procedure

At day 1, cows were selected and moved to the lameness pen according to the result of a general clinical and a thorough orthopedic examination including gait scoring and visual inspection of the claws with the cows restrained in a trimming chute. At day 2, clinical and orthopedic examinations were repeated, the cows were equipped with the electronic health monitoring instruments which remained in place for continuous recording until the end of the study (day 6) and included (i) two tridirectional accelerometers (Rumi Watch[®], Itin@Hoch, Liestal, Switzerland) one each attached to the right and left metatarsal regions, (ii) one noseband pressure sensor (Rumi Watch[®], Itin@Hoch, Liestal, Switzerland) attached to the head of the cow, and (iii) a heart activity sensor (Polar Team 2 pro, © Polar Electro Oy, Finland) attached to the thorax with a belt. From days 3 to 6, cows were gait scored and

ethological parameters collected daily and restrained in the squeeze chute of the weighing platform for measuring weight distribution among limbs twice daily. At day 4, examination of all claws in the trimming chute was repeated and functional claw trimming was performed in all 3 healthy limbs of group L and in 2 front limbs and 1 hind limb (selected randomly) of cows of group C. All clinical examinations were performed by the study veterinarians, while functional claw trimming was performed by a professional claw trimmer.

Data collection

General clinical examination. At daily clinical examination, the following health parameters were recorded: Posture, general behavior, rectal body temperature, heart rate, respiratory rate, rumen fill, rumen motility, swing- and percussion auscultation, abdominal shape, appearance and amount of feces. At initial examination (day 2), the glutaraldehyde test for semiquantitative analysis of blood fibrinogen and antibody concentration was additionally performed (Doll et al., 1985).

Examination of the claws and gait score. All claws were examined in the trimming chute and the claw lesions photographed and classified by 2 of 3 previously trained study veterinarians (Müller, Nechanitzky, Reckardt) according to (Starke et al. 2007). Gait scoring was performed daily at a fixed time between 08:00 am and 10:00 am, while walking down two times a 15-m-long by 1.5 m wide concrete slatted passageway, so that the gait could be assessed from the side and from behind. To ensure that the cows walked in a consistent manner, a handler walked behind the cows encouraging them to walk when necessary. Two of the 3 previously mention study veterinarians immediately assigned a collective gait score to each cow for each day, using a numeric rating system with a range from 0 to 13 (Offinger et al. 2013) where 0 = none lame and 13 = severely lame. Each cow was additionally videotaped (Handycam Sony® HDR-XR 155) once from her right side and once from behind for final

scoring during data validation at the end of the study. Cows were familiarized with the procedure during days 2 to 4 and the arithmetic mean of data from days 5 and 6 of each cow were used for further analysis.

Ethological parameters assessed by direct observation. Starting at day 4, at least 2 hours after the last manipulation, each cow was observed for 30 min by an experienced observer who recorded the general behavior and the behavior involving the affected limb in group L or the behavior involving the limb that was excluded from claw trimming in group C, respectively. If the cow was lying at the intended beginning of the observation period, the cow was gently forced to get up, and the data collection was postponed for 5 minutes. The following parameters of the general behavior were registered: lying position; getting up behavior; dorsal line; trials to lay down; vigilance; comfort-behavior (grooming, cleaning of planum nasolabiale); vocalization (mooing, bruxism, groaning); increased respiratory rate; ear position. Variables were scored “positive”, if they occurred at least once during the observation period. To assess ethological data related to general behavior, a modified scoring system according to (List 2009) and (Feist 2004) was used, at which 0 reflected the physiological behavior and the scorings 1-3 were deviations from the physiological behavior (1 = slight; 3 = severe). To evaluate ethological data related to the affected limb, the strategy of behavior sampling with continuous recording was used (Martin und Bateson, P. P. G 2007). Every single movement, the cow made to reduce weight bearing of the affected limb was counted and added up to come up with a sum over the 30 minutes observation period. The arithmetic mean of data from days 5 and 6 of each cow was used for further analysis.

Locomotor activity. One tridimensional accelerometer (RumiWatch[®]; length 210 mm, width 55 mm, depth 29 mm, weight 130 g) each was attached with a strap to the metatarsus above the fetlock of both hind limbs. Acceleration was recorded at 10 Hz and raw data stored on the SD memory card with a capacity of 512 MB integrated in the data logger case. Cows were

familiarized with the loggers during days 2 and 3, and the arithmetic mean of one 12 hour period each of each variable and cow of days 4 and 5 was used for statistical analysis. The RumiWatch Converter[®] V0.7.2.17 (Just 2014) was used for calculation of the following variables of the locomotor activity from the raw data files (arithmetic mean of both data loggers): duration of lying and standing time and number of standing ups and lying downs.

Weight distribution between hind limbs. To measure weight distribution among limbs while the cow is standing, the method described by (Neveux et al. 2006) and (Chapinal et al. 2009a) was used. For this purpose, cows were restrained in a manual steel squeeze chute, mounted on a weighing platform (Itin und Hoch GmbH, Liestal, Switzerland). The platform contained 4 independent recording units (78 cm × 55 cm) with one hermetically sealed load cell (HBM, Hottinger Baldwin Messtechnik AG, Volketswil, Switzerland) each and covered with individual rubber mats (10 mm thickness). The registered weights were not affected by the position of the claws on the respective recording unit. Recording was manually started when the cow was in the right position, standing calm with every limb on the appropriate unit. If the measured total weight deviated for more than 5% from the originally measured total body weight of the cow, data collection was stopped automatically and was continued when the cow was in the right position, again, which was achieved by gentle manipulation of the cow. This way, data were excluded when a force closure was present. The total recording period per session lasted 5 minutes. Data were recorded at a sampling rate of 10 readings / second and recording unit. Cows had been familiarized with the weighing platform and the procedure by standing at least 3 times on the platform before the data collection started, and the arithmetic mean per cow was calculated from the second weighing of day 4, both weightings of day 5 and the weighing of day 6. Variables, calculated for the hind limbs and each 5 minute interval were mean weight applied on each limb, the standard deviation (SD) of the weight applied on each limb as a measure of weight shifting between hind limbs (Rushen et al. 2007),

the mean limb difference (Δ weight) calculated by subtracting the mean weight of the affected limb from the mean weight of the healthy limb, and the limb weight ratio (LWR) between the affected and the non-affected hind limb as an indicator of asymmetry in weight distribution within each pair of limbs (Pastell and Kujala 2007).

Feeding behavior. To continuously measure feeding behavior, the RumiWatch[®] nose band sensor was used, consisting of an oil-filled tube positioned over the back of the nose, a pressure sensor and a wireless transmitter which registered jaw movements with a frequency of 10 readings per second (Zehner et al. 2012). Data was exported to a personal computer, and the algorithm 0.9.6 of Rumi Watch[®] was used for calculation of the following feeding variables (Zehner et al. 2012): *ruminate time* (time, cows spent ruminating), *eat time* (time, cows spent eating), *ruminatechew* (number of rumination chews), *eatchew* (number of eating chews), *bolus* (number of rejected boli) and *chewsperbolus* (number of chews per bolus). Cows were familiarized with the nose band sensors on days 2 and 3, and the arithmetic mean of each variable collected during two 12 h periods of days 4 and 5 of each cow was used for statistical analysis.

Heart rate and Heart rate variability. To continuously measure heart activity, individual cows were fitted with the recording device POLAR[®] (Polar Team 2 pro, © Polar Electro Oy, Finland), consisting of two electrodes and one recorder/transmitter attached to a thorax belt. Data were collected at a sampling rate of 200Hz. The recorders/transmitters were replaced daily, because the battery capacity was sufficient for 36h only. The 24 h data of each cow was daily transferred via infrared transmission to a personal computer. Cows were familiarized with the thorax belts during days 3 and 4. Two 5 min periods per animal per day was analyzed, when the cow was lying for at least 5 minutes and when it was standing immediately after a lying period. For data analysis, Kubios[®] HRV software (Department of Applied Physics University of Kuopio, Finland) was used. To remove trend components,

data were detrended and artefact corrections were made following established procedures described by (Tarvainen, M.P., Niskanen, J.-P., 2012; Tarvainen et al. 2002). The following variables of heart activity were calculated: Mean heart rate (HR), beat-to-beat interval (RR), standard deviation of RR interval (SDRR), root mean square of successive RR differences (RMSSD), and the geometric means standards deviation 1 (SD 1) and 2 (SD 2). For calculation of SD 1 and SD2, the duration of each RR interval was plotted against the duration of the proceeding RR interval (Poincaré Plot). The software fitted an ellipse on the plot in order to parameterize the shape of the plot. The ellipse was according to the line-of-identity ($RR_j = RR_{j+1}$) at 45° to the X- axis. SD 1 can be considered to measure short term variability, mainly caused by parasympathetic activity, whereas SD 2 measures long term variability (Tarvainen, M.P., Niskanen, J.-P., 2012; Borell et al. 2007). The arithmetic mean of each variable per cow of days 5 and 6 was used for statistical analysis.

Statistical analysis

Statistical analysis was performed with NCSS statistic package (NCSS 9. NCSS, LLC. Kaysville, Utah, USA) using the arithmetic mean per cow of the 10 non-lame and 32 lame animals. Descriptive statistics showed that all variables were normally distributed. To elucidate differences of the control group versus the two types of claw horn defects (sole ulcer versus whit line ulcer) as well as the control group versus all cows with claw horn defects, ANOVA was carried out taking each numeric variable as the outcome and the classification lame/non-lame as the independent or grouping variable. Only the variables that showed differences in the ANOVA were then used as independent variables for further analysis, taking the classification lame/non-lame as the binary outcome. Significance level was set at $\alpha = 0.05$. In order to evaluate how good each independent variable or combinations of variables could be used for an automated detection of lame cows with a DNPS, we performed logistic regression models and a Receiver Operating Characteristic (ROC) analysis. ROC analysis

renders specificity, sensitivity and area under the curve (AUC) for each model (Pastell et al. 2010); the higher these values, the better the model. Combinations of variables which were highly or moderately correlated ($r > 0.2$) were avoided in the models, as by definition, model covariates need to be independent. Correlations were checked using Pearson correlation coefficients.

RESULTS

Cows. The lactation number of cows of group L (mean $\pm$ STD = 3.45 ± 1.20) was significantly ($P < 0.001$) higher as compared with group C (mean = 2; selection criteria). Cows of groups C and L, however, were not significantly different concerning the following variables: days in lactation, milk yield, glutaraldehyde test, body condition score, withers height and body weight. Data are given in table 1.

Gait Scores, Claw Lesions and Ethological Parameters. Cows of group L had a gait score of 5.17 ± 1.54 ranging from 2 to 9, while all cows of group C had a gait score of 0 (selection criteria; $P < 0.001$). None of the cows of group C showed any claw lesion. In group L, a sole ulcer was present in 15 cows, and a white-line ulcer in 17 cows. The general ethological score of group L (6.70 ± 2.83) was significantly higher ($P < 0.001$) as compared with group C (3.30 ± 1.75), and the ethological limb score of group L (62.75 ± 31.90) was also significantly higher ($P < 0.001$) as compared with group C (16.60 ± 15.71 ; table 1). The results of the ROC analysis and the goodness of fit of logistic regression models of the ethological scores are given in table 2.

Locomotor Activity. Lamé cows spent significantly more ($P = 0.049$) time lying (12.96 ± 2.63 h/day) and less time ($P = 0.049$) standing and walking (11.04 ± 2.63 h/day), respectively, than non-lamé cows (11.00 ± 2.79 h; 13.01 ± 2.79 h; table 1). The number of standing ups and lying downs was not significantly different between groups ($P = 0.342$; $P = 0.284$). The

results of the ROC analyses and the goodness of fit of logistic regression models of the significant variables standing time and lying time are given in table 2.

Weight Distribution. Three variables of weight distribution between hind limbs generated from the weighing platform revealed significant differences between groups (table 1). The SD ($P < 0.001$) and Δ weight ($P < 0.001$) were significantly higher in group L as compared with group C, while the LWR was significantly lower ($P < 0.001$) in group L as compared with group C. The ROC analyses revealed the highest sensitivity (0.97) for the variable SD with a specificity of 0.80 and an AUC of 0.84 at a cut-off of value 22.82 kg (table 2; figure 1).

Feeding Behavior and Heart Activity

There was a trend evident ($P = 0.068$) that cows of group L showing shorter lower feeding time than cows of group C. Neither the other variables of the feeding behavior nor of the heart activity revealed significant differences between the two study groups (data not shown).

Correlations between Variables

Correlations between variables that revealed to be significantly different between groups L and C are given in table 3. Pearson correlation coefficients exceeding 0.7 included: standing time versus lying time and LWR versus Δ weight. The correlations between SD and the other two variables collected from the weighing platform (LWR and Δ weight) were rather low ($r < 0.2$). In cows of group L, lying time ($r = 0.56$; figure 2a) and Δ weight ($r = 0.70$; figure 2b) were both positively correlated with the lameness score, while SD and LWR were both negatively ($r = -0.46$; figure 3a; $r = -0.67$ figure 3b) correlated with the lameness score of cows of group L.

Logistic Regression for Prediction of Lameness

The model considering the data of SD and Δ weight was the best predictor of cows being lame, accounting for 59.25 % of the variation in the likelihood of a cow being lame ($R^2 = 0.59$), with the greatest AUC (0.96) and a sensitivity of 0.94 and a specificity of 0.80 (table 2). The combination of the variables SD and lying time revealed the considerably lower values ($R^2 = 0.39$; AUC = 0.84).

DISCUSSION

The results of this study showed that cows with lameness caused by deep, non-perforating pododermatitis affecting one individual foot when compared with non-lame cows were automatically detected with high sensitivity (0.94) and specificity (0.80) by the use of the weighing platform, evaluating the variables SD and Δ weight. While the duration of lying time, as determined with three-directional accelerometers attached to the hind limbs, was significantly higher in cows of group L as compared to group C, feeding behavior and parameters of the HRV did not allow differentiating between lame and control cows.

The study was performed during a one year period. In order to eliminate unequally distributed effects of season (environmental temperature, humidity, light) and feeding on the two experimental groups, each time, 3 lame cows entered the study, these cows were accompanied by 1 control cow. Comparison of the groups L and C revealed no differences concerning the production data of the cows except for the lactation number. The latter was higher in cows of group L, as only pluriparous cows were allowed to enter the study, and only cows in 2nd lactation were selected for group C, but cows in 2nd and higher lactation were selected for group L. This was done, because parameters of metabolism of clinically healthy 2nd lactation cows were simultaneously collected and evaluated for a concurrent study.

The lameness scoring system chosen in this study was previously described by (Offinger et al. 2013). It was preferred to more frequently used lameness scoring systems such as the one

described by (Sprecher et al. 1997) or the numerical rating system by (Flower and Weary 2006), because it allowed for a much more detailed differentiation among various degrees of lameness (scoring range of 1-13 versus 1-5). This was judged to be relevant, because the correlation between lameness score of lame cows and automated variables of lameness that were significantly different between the 2 groups was of major scientific interest.

Deep, non-perforating septic pododermatitis was chosen as the lameness causing foot pathology in this study, because it occurs in dairy cattle with a high incidence rate (Manske et al. 2002, Somers et al. 2003, 2003, Holzhauer et al. 2008; Becker et al. 2014a), frequently affecting only one hind foot, and often responsible for lameness (Zahid et al. 2014).

Interestingly, the behavior of cows with sole ulcers and white line disease was not different from each other, and, therefore, all lame cows of this study were combined in one single group (group L). It can be concluded that these 2 claw pathologies cause a similar degree of pain.

It was decided for this study to only evaluating variables of automated behavior description with the RumiWatch[®] system that had previously been scientifically validated. This explains, why only the accelerometer variables standing- and lying time and number of standing ups and lying downs were evaluated (Just 2014). The pedometer algorithm available at the time of data evaluation was neither validated for the number of steps nor designated to differentiate between limb movements when the cow was standing and such associated with walking.

Therefore, the variable “step”, as provided by the software of RumiWatch[®] was not used in this study. Two 12-hour instead of two 24-hour data intervals were used in this study, in order to avoid the evaluation of intervals during which the behavior of experimental cows was disturbed by external manipulations such as lameness scoring or forcing the cows to entering the weighing platform.

378 The ethological scoring systems proposed in this study allowed for good (focusing on general
379 behavior) to excellent (focusing on the affected limb) differentiation of cows between groups.
380 The design of the ethological scoring system, focusing on the affected limb, was very simple
381 and may theoretically well be applied and implemented by farmers. It represented merely the
382 sum over 30 minutes observation of every single movement, the cow made to reduce weight
383 bearing of the affected limb. In practice, this scoring system, however, does not seem to be
384 applicable, because data collection is very time consuming. Furthermore, the variables of the
385 weighing platform are focusing on similar behavioral changes, and evaluation is automated.
386 Alternatively, the development of video-analysis-software that automatically recognizes
387 alterations in the behavioral patterns of the limbs may be pushed forward in the future to
388 become a practical solution for lameness detection and animal monitoring.

389 The lying time of lame cows was found to be significantly longer, as compared with the
390 control cows. This was made-up by the duration the lying bouts, as the number of position
391 changes (standing ups and lying downs) was not significantly different between groups. This
392 finding is in agreement with previous studies showing similar results (Chapinal et al. 2009b;
393 Chapinal et al. 2010a; Sepúlveda-Varas et al. 2014; Janssen 2011) and in partial agreement
394 with Yunta et al. (2012) who found that lame cows had longer lying bouts than non-lame
395 cows, but total daily lying time was not affected by lameness. In contrast to (Chapinal et al.
396 2010b), the current study design allowed to detect a positive correlation between gait score on
397 the one hand and lying time on the other hand. In agreement with the recent literature, it may
398 be concluded that lying time and duration of lying bouts, as retrieved from the data of a single
399 pedometer per cow may be valuable additional co-variables for the automated detection of
400 lame cows. The potential of tridimensional accelerometers attached to the limbs of cows for
401 detection of lameness, however, may currently not at all be exhausted. Further development

seems warranted, as only validated variables of the RumiWatch[®] pedometers (lying versus standing behavior, but not the walking behavior) were evaluated in the current study.

The weighing platform revealed to be the most valuable individual tool for distinguishing lame from non-lame cows in the current study. This is in general agreement with previous findings described by (Chapinal et al. 2010a) and (Pastell et al. 2010). Nevertheless, sensitivity and specificity were higher in the current study as previously described. This may be explained by the fact that only cows with a very specific pathology restricted to one individual hind foot were included in group L and only completely non-lame cows in group C of the current study. In comparable studies, groups L and C were less narrowly defined (Pastell et al., 2010). Evaluation of the correlations between various variables showed unexpected and novel results. Firstly, the variable SD showed only minor correlation with both the variables Δ weight and LWR. Secondly, SD of lame cows was negatively correlated with the lameness score. The latter result may appear as a surprise. However, taking a closer look, it may well be explained: the variable SD is a measure of leg load variability or weight shifting (Rushen et al. 2007). If only one foot is affected, slight lameness may be accompanied by more intensive weight shifting as compared with severe lameness. Lesions causing severe lameness may be so painful that loading weight on this particular foot is avoided to a high degree, and weight bearing is constantly shifted to the contralateral healthy foot. (Pastell and Kujala 2007) already revealed that cows in a severe, painful stage of disease constantly lifted the affected limb to relieve pain. The latter is reflected by the positive correlation between the lameness score of lame cows and Δ weight as found in this study. Furthermore, (Pastell and Kujala 2007) showed that the lameness detection rate may be improved by combining the results of several measurement sessions of the weighing platform, taken from one and the same cow. In the current study it was taken advantage from this finding, and the results of 4 sessions performed in 12-hour-intervals were combined to come

up with 1 mean value for each variable. It remains unclear, whether a 4-scale weighing platform incorporated into a milking robot would yield similar results as compared to the stand-alone type used in this study. Behavior specifically associated with the milking procedure may potentially adversely affect the results.

(González et al. 2008) found a significant decrease in feed intake of cows with acute locomotion disorders, especially during the main feeding time. This finding is supported by a study by (Yunta et al. 2012) who found that lame cows stood up 13 min later and lay down 19 min earlier than non-lame cows relative to the time when the ration was delivered. In line with the findings of the above cited recent literature, we found a trend ($P = 0.068$) that cows of group L spent less time feeding as compared to group C.

Data gained by means of the POLAR system proved not to be useful for the differentiation between lame and non-lame cows. Even though (Borell et al. 2007) and (Buck M. et al. 2013) showed that the analysis of parameters of the HRV are suitable for detecting acute stress in cattle, this method was not suitable to distinguish between non-lame and lame cows under the conditions of this experimental setting. It may be hypothesized that the basic stress level of the cows of this particular pen may have been generally elevated; the daily composition of this pen was highly variable, as every newly recovered cow was immediately replaced by a lame cow from another pen.

CONCLUSIONS

It is concluded from the results of this experimental field study that the combination of the variables SD and Δ weight – both derived from the weighing platform - represents a valuable dataset for identification of lame cows suffering from a DNPS of one individual hind limb, when compared with non-lame cows. Variables of feeding and of HRV are of minor value in this context. It has to be stressed, however, that the comparison of cows suffering from

DNPSP with cows showing lameness of other origin or involving both hind limbs was not done.

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REFERENCESLiteraturverzeichnis

Amory, J. R.; Barker, Z. E.; Wright, J. L.; Mason, S. A.; Blowey, R. W.; Green, L. E. (2008): Associations between sole ulcer, white line disease and digital dermatitis and the milk yield of 1824 dairy cows on 30 dairy cow farms in England and Wales from February 2003–November 2004. In: *Preventive Veterinary Medicine* 83 (3-4), S. 381–391. DOI: 10.1016/j.prevetmed.2007.09.007.

Anil, Leena; Anil, Sukumarannair S.; Deen, John (2005): Pain detection and amelioration in animals on the farm: issues and options. In: *J Appl Anim Welf Sci* 8 (4), S. 261–278. DOI: 10.1207/s15327604jaws0804_3.

Barker, Z. E.; Leach, K. A.; Whay, H. R.; Bell, N. J.; Main, D.C.J. (2010): Assessment of lameness prevalence and associated risk factors in dairy herds in England and Wales. In: *Journal of Dairy Science* 93 (3), S. 932–941. DOI: 10.3168/jds.2009-2309.

Becker, J.; Steiner, A.; Kohler, S.; Koller-Bähler, A.; Wüthrich, M.; Reist, M. (2014a): Lameness and foot lesions in Swiss dairy cows: I. Prevalence. In: *Schweizer Archiv für Tierheilkunde* 156 (2), S. 71–78. DOI: 10.1024/0036-7281/a000553.

Becker, Johanna; Reist, Martin; Steiner, Adrian (2014b): Factors influencing the attitudes of cattle veterinarians, farmers, and claw trimmers towards the pain associated with the treatment of sole ulcers and the sensitivity to pain of dairy cows. In: *The Veterinary Journal* 200 (1), S. 38–43. DOI: 10.1016/j.tvjl.2014.01.016.

Bennett et al. 1999: Estimating the costs associated with endemic diseases of dairy cattle, zuletzt geprüft am 14.10.2014.

480 Bicalho, R. C.; Machado, V. S.; Caixeta, L. S. (2009): Lameness in dairy cattle: A debilitating
481 disease or a disease of debilitated cattle? A cross-sectional study of lameness prevalence and
482 thickness of the digital cushion. In: *Journal of Dairy Science* 92 (7), S. 3175–3184. DOI:
483 10.3168/jds.2008-1827.

484 Blowey and Edmonson (2000): Blowey_the environment and mastitis_2000, zuletzt geprüft
485 am 09.07.2014.

486 Borell, Eberhard von; Langbein, Jan; Després, Gérard; Hansen, Sven; Leterrier, Christine;
487 Marchant-Forde, Jeremy et al. (2007): Heart rate variability as a measure of autonomic
488 regulation of cardiac activity for assessing stress and welfare in farm animals — A review. In:
489 *Physiology & Behavior* 92 (3), S. 293–316. DOI: 10.1016/j.physbeh.2007.01.007.

490 Braun, Ueli; Tschoner, Theresa; Hässig, Michael (2014): Evaluation of eating and rumination
491 behaviour using a noseband pressure sensor in cows during the peripartum period. In: *BMC*
492 *Vet Res* 10 (1), S. 195. DOI: 10.1186/s12917-014-0195-6.

493 Bruijnis, M.R.N.; Hogeveen, H.; Stassen, E. N. (2010): Assessing economic consequences of
494 foot disorders in dairy cattle using a dynamic stochastic simulation model. In: *Journal of*
495 *Dairy Science* 93 (6), S. 2419–2432. DOI: 10.3168/jds.2009-2721.

496 Buck M. et al. (2013): Influence of manure scrapers on dairy cows in cubicle housing
497 systems. In: *Livestock Science* 158 (1-3), S. 129–137. DOI: 10.1016/j.livsci.2013.10.011.

498 Chapinal, N.; Passillé, A. M. de; Rushen, J. (2009a): Weight distribution and gait in dairy
499 cattle are affected by milking and late pregnancy. In: *Journal of Dairy Science* 92 (2), S. 581–
500 588. DOI: 10.3168/jds.2008-1533.

501 Chapinal, N.; Passillé, A. M. de; Rushen, J.; Wagner, S. (2010a): Automated methods for
502 detecting lameness and measuring analgesia in dairy cattle. In: *Journal of Dairy Science* 93
503 (5), S. 2007–2013. DOI: 10.3168/jds.2009-2803.

504 Chapinal, N.; Passillé, A. M. de; Rushen, J.; Wagner, S. A. (2010b): Effect of analgesia
505 during hoof trimming on gait, weight distribution, and activity of dairy cattle. In: *Journal of*
506 *Dairy Science* 93 (7), S. 3039–3046. DOI: 10.3168/jds.2009-2987.

507 Chapinal, N.; Passillé, A. M. de; Weary, D. M.; Keyserlingk, M.A.G. von; Rushen, J.
508 (2009b): Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy
509 cows. In: *Journal of Dairy Science* 92 (9), S. 4365–4374. DOI: 10.3168/jds.2009-2115.

510 Cook and Nordlund (2009): The influence of the environment on dairy cow behavior, claw
511 health and herd lameness dynamics. In: *The Veterinary Journal* 179 (3), S. 360–369. DOI:
512 10.1016/j.tvjl.2007.09.016.

513 Dippel, S.; Dolezal, M.; Brenninkmeyer, C.; Brinkmann, J.; March, S.; Knierim, U.;
514 Winckler, C. (2009a): Risk factors for lameness in freestall-housed dairy cows across two
515 breeds, farming systems, and countries. In: *Journal of Dairy Science* 92 (11), S. 5476–5486.
516 DOI: 10.3168/jds.2009-2288.

517 Dippel, Sabine; Dolezal, Marlies; Brenninkmeyer, Christine; Brinkmann, Jan; March,
518 Solveig; Knierim, Ute; Winckler, Christoph (2009b): Risk factors for lameness in cubicle
519 housed Austrian Simmental dairy cows. In: *Preventive Veterinary Medicine* 90 (1-2), S. 102–
520 112. DOI: 10.1016/j.prevetmed.2009.03.014.

521 Doll et al., 1985: Der Glutaraldehyd-Test beim Rind - seine Brauchbarkeit für Diagnose und
522 Prognose innerer Entzündungen., zuletzt geprüft am 11.01.2015.

523 E.J. Garbarino; J.A. Hernandez; J.K. Shearer; C.A. Risco; W.W. Thatcher (2004): Effect of
524 Lameness on Ovarian Activity in Postpartum Holstein Cows*, zuletzt geprüft am 17.04.2014.

525 Ettema and Østergaard (2006): Economic decision making on prevention and control of
526 clinical lameness in Danish dairy herds. In: *Livestock Science* 102 (1-2), S. 92–106. DOI:
527 10.1016/j.livprodsci.2005.11.021.

528 Feist, Melanie (2004): Untersuchungen zum Schmerzausdrucksverhalten bei Kühen nach
529 Klauenoperationen. (2004). (2004), zuletzt geprüft am 19.12.2014.

530 Flower and Weary (2006): Effect of Hoof Pathologies on Subjective Assessments of Dairy
531 Cow Gait, zuletzt geprüft am 27.06.2014.

532 González, L. A.; Tolkamp, B. J.; Coffey, M. P.; Ferret, A.; Kyriazakis, I. (2008): Changes in
533 Feeding Behavior as Possible Indicators for the Automatic Monitoring of Health Disorders in
534 Dairy Cows. In: *Journal of Dairy Science* 91 (3), S. 1017–1028. DOI: 10.3168/jds.2007-0530.

535 Holzhauer, M.; Hardenberg, C.; Bartels, C J M (2008): Herd and cow-level prevalence of sole
536 ulcers in The Netherlands and associated-risk factors. In: *Preventive Veterinary Medicine* 85
537 (1-2), S. 125–135. DOI: 10.1016/j.prevetmed.2008.01.004.

538 Hudson, C.; Whay, H.; Huxley, J. (2008): Recognition and management of pain in cattle. In:
539 *In Practice* 30 (3), S. 126–134. DOI: 10.1136/inpract.30.3.126.

540 Janssen (2011): Einfluss von Klauenlahmheiten bei Milchkühen auf Futteraufnahmeverhalten
 541 und Energiestoffwechsel sowie Auswirkungen der chirurgischen Behandlung und zusätzlicher
 542 Ketoprofenapplikation, zuletzt geprüft am 03.03.2015.

543 Just, Ladina (2014): Publikation, zuletzt geprüft am 12.01.2015.

544 Kofler (2013): KOFLER_Was kosten lahme Kühe_30-10-13, zuletzt geprüft am 07.07.2014.

545 Kossaibati and Esslemont (1997): The costs of production diseases in dairy herds in England.
 546 In: *The Veterinary Journal* 154 (1), S. 41–51. DOI: 10.1016/S1090-0233(05)80007-3.

547 L.A. Espejo; M.I. Endres; J.A. Salfer: Prevalence of Lameness in High-Producing Holstein
 548 Cows Housed in Freestall Barns in Minnesota, zuletzt geprüft am 17.04.2014.

549 L.D. Warnick; D. Janssen; C.L. Guard; Y.T. Gröhn (2001): The Effect of Lameness on Milk
 550 Production in Dairy Cows, zuletzt geprüft am 23.04.2014.

551 L.E. Green; V.J. Hedges; Y.H. Schukken; R.W. Blowey; A.J. Packington (2002): The Impact
 552 of Clinical Lameness on the Milk Yield of Dairy Cows, zuletzt geprüft am 17.04.2014.

553 Leach, K. A.; Whay, H. R.; Maggs, C. M.; Barker, Z. E.; Paul, E. S.; Bell, A. K.; Main, D.C.J.
 554 (2010): Working towards a reduction in cattle lameness: 1. Understanding barriers to
 555 lameness control on dairy farms. In: *Research in Veterinary Science* 89 (2), S. 311–317. DOI:
 556 10.1016/j.rvsc.2010.02.014.

557 List (2009): Einsatz von nichtsteroidalen Antiphlogistika im Rahmen des multimodalen
 558 Schmerzmanagements beim Rind, unter besonderer Berücksichtigung der Wirkung von
 559 Carprofen in Kombination mit einer Lokalanästhesie der Flanke. (2009), zuletzt geprüft am
 560 25.06.2014.

561 Manske, Thomas; Hultgren, Jan; Bergsten, Christer (2002): Prevalence and interrelationships
 562 of hoof lesions and lameness in Swedish dairy cows. In: *Preventive Veterinary Medicine* 54
 563 (3), S. 247–263. DOI: 10.1016/S0167-5877(02)00018-1.

564 Martin, Paul (2007): *Measuring behaviour*. 3. ed. Cambridge: Cambridge Univ. Press.

565 Martin, Paul R.; Bateson, P. P. G (2007): *Measuring behaviour. An introductory guide*. 3rd
 566 ed. Cambridge, New York: Cambridge University Press.

567 Mohr, Elmar; Langbein, Jan; Nürnberg, Gerd (2002): Heart rate variability. In: *Physiology &*
 568 *Behavior* 75 (1-2), S. 251–259. DOI: 10.1016/S0031-9384(01)00651-5.

569 Neveux et al. (2006): Hoof Discomfort Changes How Dairy Cattle Distribute Their Body
570 Weight, zuletzt geprüft am 17.11.2014.

571 Nordmann, Eva; Keil, Nina Maria; Schmied-Wagner, Claudia; Graml, Christine; Langbein,
572 Jan; Aschwanden, Janine et al. (2011): Feed barrier design affects behaviour and physiology
573 in goats. In: *Applied Animal Behaviour Science* 133 (1-2), S. 40–53. DOI:
574 10.1016/j.applanim.2011.04.016.

575 O'Callaghan, K. (2002): Lameness and associated pain in cattle - challenging traditional
576 perceptions. In: *In Practice* 24 (4), S. 212–219. DOI: 10.1136/inpract.24.4.212.

577 Offinger, J.; Herdtweck, S.; Rizk, A.; Starke, A.; Heppelmann, M.; Meyer, H. et al. (2013):
578 Postoperative analgesic efficacy of meloxicam in lame dairy cows undergoing resection of the
579 distal interphalangeal joint. In: *Journal of Dairy Science* 96 (2), S. 866–876. DOI:
580 10.3168/jds.2011-4930.

581 Pastell, M.; Hänninen, L.; Passillé, A. M. de; Rushen, J. (2010): Measures of weight
582 distribution of dairy cows to detect lameness and the presence of hoof lesions. In: *Journal of*
583 *Dairy Science* 93 (3), S. 954–960. DOI: 10.3168/jds.2009-2385.

584 Pastell and Kujala (2007): A Probabilistic Neural Network Model for Lameness Detection. In:
585 *Journal of Dairy Science* 90 (5), S. 2283–2292. DOI: 10.3168/jds.2006-267.

586 Rouha-Mülleder, Cornelia; Iben, Christine; Wagner, Elisabeth; Laaha, Gregor; Troxler, Josef;
587 Waiblinger, Susanne (2009): Relative importance of factors influencing the prevalence of
588 lameness in Austrian cubicle loose-housed dairy cows. In: *Preventive Veterinary Medicine* 92
589 (1-2), S. 123–133. DOI: 10.1016/j.prevetmed.2009.07.008.

590 Rushen, Jeffrey; Pombourcq, Emilie; Passillé, Anne Marie de (2007): Validation of two
591 measures of lameness in dairy cows. In: *Applied Animal Behaviour Science* 106 (1-3), S. 173–
592 177. DOI: 10.1016/j.applanim.2006.07.001.

593 Russell, A.; Rowlands, G.; Shaw, S.; Weaver, A. (1982): Survey of lameness in British dairy
594 cattle. In: *Veterinary Record* 111 (8), S. 155–160. DOI: 10.1136/vr.111.8.155.

595 Sepúlveda-Varas, P.; Weary, D. M.; von Keyserlingk, M A G (2014): Lying behavior and
596 postpartum health status in grazing dairy cows. In: *J. Dairy Sci.* 97 (10), S. 6334–6343. DOI:
597 10.3168/jds.2014-8357.

598 Somers et al. (2003): Prevalence of Claw Disorders in Dutch Dairy Cows Exposed to Several
599 Floor Systems, zuletzt geprüft am 03.01.2015.

600 Sprecher et al., 1997: PII: S0093-691X(97)00098-8, zuletzt geprüft am 11.01.2015.

601 Starke, A.; Heppelmann, M.; Beyerbach, M.; Rehage, J. (2007): Septic arthritis of the distal
602 interphalangeal joint in cattle: comparison of digital amputation and joint resection by solar
603 approach. In: *Veterinary surgery* : VS 36 (4), S. 350–359. DOI: 10.1111/j.1532-
604 950X.2007.00257.x.

605 Tarvainen, Mika P.; Niskanen, Juha-Pekka; Lipponen, Jukka A.; Ranta-aho, Perttu O.;
606 Karjalainen, Pasi A. (2014): Kubios HRV – Heart rate variability analysis software. In:
607 *Computer Methods and Programs in Biomedicine* 113 (1), S. 210–220. DOI:
608 10.1016/j.cmpb.2013.07.024.

609 Tarvainen et al. (2002): An advanced detrending method with application to hrv analysis -
610 Biomedical Engineering, IEEE Transactions on. Tarvainen, M.P., Ranta-aho, P.O.,
611 Karjalainen, P.A, zuletzt geprüft am 11.01.2015.

612 Tarvainen, M.P., Niskanen, J.-P., 2012: Kubios HRV Version 2.1 Users Guide. Online
613 verfügbar unter http://kubios.uef.fi/media/Kubios_HRV_2.1_Users_Guide.pdf, zuletzt geprüft
614 am 19.12.2014.

615 Tranter and Morris (1991): A case study of lameness in three dairy herds. In: *New Zealand*
616 *Veterinary Journal* 39 (3), S. 88–96. Online verfügbar unter
617 <http://www.ingentaconnect.com/content/tandf/nzvj/1991/00000039/00000003/art00002>.

618 Whay, H. (2002): Locomotion scoring and lameness detection in dairy cattle. In: *In Practice*
619 24 (8), S. 444–449. DOI: 10.1136/inpract.24.8.444.

620 Whay et al. (Hg.) (2002): Farmer perception of lameness prevalence. Proceedings of the 12th
621 International Symposium on Lameness in Ruminants.

622 Willshire and Bell 2009: Willshire and Bell 2009, zuletzt geprüft am 09.07.2014.

623 Yunta, C.; Guasch, I.; Bach, A. (2012): Short communication: lying behavior of lactating
624 dairy cows is influenced by lameness especially around feeding time. In: *J. Dairy Sci.* 95 (11),
625 S. 6546–6549. DOI: 10.3168/jds.2012-5670.

626 Zahid, Umar Nazir; Randhawa, Swaran Singh; Hussain, Syed Ashaq; Randhawa, Sarnarinder
627 Singh; Mahajan, Vishal; Dua, Kirti (2014): Claw Lesions Causing Clinical Lameness in
628 Lactating Holstein Frisian Crossbred Cows. In: *Veterinary Medicine International* 2014 (4),
629 S. 1–8, zuletzt geprüft am 03.03.2015.

630 Zehner et al. (Hg.) (2012): Validation of a new health monitoring system (RumiWatch) for
631 combined automatic measurement of rumination, feed intake, water intake and locomotion in
632 dairy cows. Proceedings of international conference of agricultural engineering CIGR-Ageng.
633 Online verfügbar unter http://wcigr.ageng2012.org/images/fotosg/tabla_137_C0438.pdf.