Behavioural changes in dairy cows with lameness in an automatic milking system

Giuliana G. Miguel-Pacheco[a*]; Jasmeet Kaler[a]; John Remnant[a]; Lydia Cheyne[a]; Caroline Abbott[a]; Andrew P French[b]; Tony P Pridmore[c]; Jonathan N. Huxley[a]

[a]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, LE12 5RD, United Kingdom

[b]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, LE12 5RD, United Kingdom

[c]School of Computer Science, University of Nottingham, Jubilee Campus, Nottingham, NG8 1BB, United Kingdom

[*]Corresponding author:

Giuliana Giannina Miguel-Pacheco

Tel (44) 1159 516753

Fax (44) 1159 516415

svxgm@nottingham.ac.uk

Abstract

There is a tendency worldwide for the automation of farms; this has included the introduction of automatic milking systems (AMS) in the dairy industry. Lameness in dairy cows is highly prevalent and painful. These impacts potentially affect not only animal welfare, but also farm economies. Three independent observational studies were carried out to assess the impact of lameness on the behaviour of zero grazed high yielding Holstein cows managed in an AMS. The aim of the first study was to examine the impact of lameness on rumination time, the second study investigated differences between lame and sound dairy cows in total eating time and the third study assessed the impact of lameness on milking behaviour (frequency and time of visits to the AMS). In the first study data from 150 cows were used to analyse rumination (collected using rumination collars) for the 48hr following locomotion scoring. A multilevel linear regression demonstrated that lameness had a small but significant negative association (coefficient: -7.88 (SE: 3.93)) with rumination. In the second study the behaviour of eleven matched lame and sound pairs of cows at the feed face was analysed for 24 hours after locomotion scoring. Each feeding behaviour variable (total duration time, frequency of feeding bouts and length of bouts) was analysed using individual single level regression models. There was a significant negative association between total feeding time and lameness (coefficient: -73.65 (SE: 25.47)) and the frequency of feeding bouts and lameness (-9.93 (2.49)). Finally, the third observational study used 38 matched pairs of lame and sound cows. Data on the number and timings of visits to the AMS were collected for 24 hours after each locomotion score and analysed using a binomial logistic regression model. There was a significant difference in AMS visits between groups; lame animals visiting the robot less frequently than sound cows (median difference 0.50 milking visits; T = 256.0; N = 25; p = 0.01) and lame cows were 0.33 times less likely to visit the AMS between 24:01 and 06:00. Results from these studies reveal that lameness in an AMS

affected feeding behaviour, rumination and AMS visits. All of these impacts are likely to have negative consequences for farm profitability, but also implications for the health and welfare of the animals.

Keywords: Automatic milking system; lameness; rumination; feeding; milking visits.

1. Introduction

Automatic milking systems (AMS) were introduced to the dairy industry approximately 20 years ago. The number of installations is increasing rapidly, currently there are approximately 8000 farms with AMS around the world (Jacobs and Siegford, 2012). The most attractive farm benefits for the use of AMS are the freedom they provide farmers compared to conventional parlours and the opportunity to increase milking frequency resulting in an increase in milk production (Uetake et al., 1997; Meskens et al., 2001). Of equal importance, the cows may benefit from the freedom to control their activity, with the possibility of longer periods of lying and reduced stress at the time of milking because they are not gathered and crowded as they are in conventional parlours. Additionally, more frequent milking reduces udder pressure whilst at the same time reducing stress on the udder ligaments (Meskens et al., 2001; Osterman and Redbo, 2001).

As the dairy industry has developed over the last 50 years, there has been an increase in the prevalence of lameness worldwide, for example in the UK the prevalence was 36.8% (Barker et al., 2010), 28.5% in Canada (Ito et al., 2010) and between 28-33% in Chile (Tadich et al., 2010). Lameness is a sign of pain and discomfort at the level of the leg but more commonly at the level of the claw (Archer et al., 2010a). Affected animals show behavioural signs of being in pain such as reduction in mobility and alterations in behaviour. Due to discomfort and changes in behaviour it is not surprising that lameness has been associated with a reduction in milk production (Green et al., 2002; Archer et al., 2010b) and in reproduction success (Huxley, 2013).

In conventional parlours it has been observed that lame cows reduced their feeding time (Gonzalez et al., 2008; Gomez and Cook, 2010), increased their lying time (Ito et al., 2010) and modified their gait in order to access their needs (e.g. feed or social contact: Galindo and

Broom, 2002). In previous studies investigating the association between rumination and lameness, no definitive differences between lame and sound animals have been identified, possibly because rumination measurement was carried out using visual observations of behaviour over relatively short periods of time and / or across relatively small numbers of animals (Hassall et al., 1993; Singh et al., 1993; Almeida et al., 2008; Pavlenko et al., 2011). An AMS relies on the willingness of the cow to attend the robot by receiving a feed reward when milking (Prescott et al., 1998). Overall studies on lame cows in AMSs in other parts of the world have demonstrated that they visited the milking units less frequently compared to sound animals (Klaas et al., 2003; Bach et al., 2007; Borderas et al., 2008).

Technologies on AMS and other modern dairy farms are monitoring and recording increasing amounts of data on the behaviour of animals. These data have the potential to be used as early indicators for diseases such as lameness and allow us to better understand the secondary health and welfare consequences lameness may have on animals suffering from this painful condition. Three independent observational studies were carried out to assess the impact of lameness on the behaviour of zero grazed high yielding cows housed in an AMS. The aim of the first study was to examine the impact of lameness on rumination time, monitored continuously by rumination collars. The second study investigated differences between lame and sound dairy cows in total eating time over a 24 hour period. Finally, the third study assessed the impact of lameness on milking behaviour (frequency and time of visits to the AMS). In each study the null hypothesis stated that there was no difference in behaviour between lame and sound animals.

2. Materials and Methods

2.1. Animals and Housing

The studies were conducted on a 200 Holstein cows AMS unit, located in the midlands region of the UK, with an average milk yield per cow of approximately 11500 L per 305 days. All study protocols were reviewed and approved by the University of Nottingham's School of Veterinary Medicine and Science Ethical Review Committee before data collection began.

The unit consisted of four pens, each housing approximately 45 cows. Each pen consisted of three rows of free-stalls bedded with a thin layer of sawdust on a mattress base and one AMS (Lely Astronaut A3, Lely UK Ltd, St Neots, UK). Three of the four pens (Pen 2, 3 and 4) had 59 stalls and the remaining pen (Pen 1) contained 76 stalls. All walking and standing areas were covered with rubber matting (Kraiburg, Kitt LTD. UK); passageways were cleaned once per hour by automatic scrappers. Cows had free access to the AMS at any time; a maximum of 5 milking visits per cow per day was permitted. The maximum interval allowed between milking visits was set at 12 hours. Milking attendance was monitored twice a day (at approximately 07:00 and 15:00 h) and cows were selected if their visit frequency was inadequate (based on their days in milk, parity and yield). Selected individuals were identified and moved to the robot for milking.

Fresh feed was provided as a mixed ration once per day at approximately 08:30; ration was pushed up at 10:00, 12:00, 14:00, 17:00, 20:00 and 06:00. Feed was provided along one side of each pen (approximately 37m) and each pen contained two large water troughs. In addition, cows were provided with an individual concentrate ration (1.5kg/day) adjusted to the frequency of milking visits, in the AMS. If the cow produced more than 23L/day, an additional 0.16kg per each extra litre of milk was provided.

The farm had a lameness prevention and control plan in operation; all feet of all animals were trimmed every five months by a fully qualified foot trimmer. Additionally any animals that became lame were identified and treated as soon as possible by farm staff. Lactation cows walked through a foot bath containing 5% copper sulphate placed at the AMS exit for at least one day per week. Finally, the diet was fortified with 20mg of Biotin per cow per day to aid in the prevention of claw horn lesions.

2.2. General Experimental Procedures

2.2.1. Locomotion score

For all the experiments, locomotion scoring was carried out following the UK industry standard four point system (DairyCo, 2009): Score 0 a cow with good mobility, score 1 with imperfect mobility, score 2 with impaired mobility with a limb that is immediately identifiable and score 3 with severely impaired mobility. Trained observers locomotion scored all the cows in each pen once every 7 (±1) days with the exception of experiment 2, when locomotion scoring was carried out every 5 (±2) days and only in pen 2. Experiment 1 was entirely observational, identification and treatment of lame cows continued according to standard farm management procedures throughout the study period. In experiments 2 and 3, lame cows were treated within 48 hours of identification.

2.2.2. Milk production and weight data

Days in milk, parity, daily milk production and daily body weight data were recorded and stored on the farm management system. At the end of the observational study, all data was collected using T4C software (Lely, Netherlands).

2.3. Specific Experimental Procedures

2.3.1. Experiment 1

This experiment was design as an observational longitudinal study to investigate the impact of lameness on rumination. Cows were observed for 9 weeks between October and

December 2011; each week they were assigned a locomotion score to identify them as lame (Score 2 or 3) or control (Score 0 or 1). Each cow was fitted with a rumination collar (Qwes-HR, Lely WestNV, The Netherlands) which registered and recorded total rumination time, chews per bolus and time between boluses (Schirmann et al., 2009). Rumination data was collected for the following 48hr, starting at 24:00 h on the day of the locomotion score.

2.3.2. Experiment 2

This study was designed as a prospective case-control study to investigate the impact of lameness on feeding behaviour. It was conducted between July and November 2010 on cows in pen 2.

2.3.2.1. Case and control selection

Case cows were considered eligible for inclusion if they were severely lame (Score 3) and had been calved for at least 20 days. They were included if a matching control animal (Score 0 or 1) could be identified in the population. Matching criteria for control animals are outlined in Table 1.

2.3.2.2. Behaviour recording

Case-control pairs were identified individually using a small piece of fluorescent fabric attached using adhesive (Kamar glue, Kamar Inc) over the left flank and the rump. Two ceiling mounted CCTV cameras with low light capability were used to record the entire feed face for 25 hours.

Videos were watched by a single trained observer using VLC Media Player (version 1.1, VideoLAN, Paris); the first 30 minutes of footage was discarded to allow animals to settle following handling for identification. A feeding behaviour bout started when cow placed her head into the feeding area and started to chew or nose the feed. Any other behaviour such as throwing or playing with the feed was not included.

The feeding behaviour (number and duration of bouts) of case-control pairs was logged over a continuous 24 hour period. Total feeding duration and frequency of feeding bouts in a 24h period were calculated per cow. The mean feeding bout duration in a 24h period was calculated by dividing the total feeding duration by the frequency of feeding bouts per cow.

2.3.3. Experiment 3

This observational study compared the milking visit frequency and time of the milking visits to the AMS between lame and non-lame cows. It was designed as a case-control study and conducted between October and November 2011.

2.3.3.1. Case-control selection

After each locomotion score, case-control pairs were selected using the matching criteria outlined in Table 1 and blocked by pen. Lame cows could only be included in the study once; cows classified as controls could be used more than once, if they met the matching criteria for more than one lame animal.

2.3.3.2. AMS visit data

Data for each case-control pair was downloaded for a 24 hour period beginning at 12:01. Data collected included number of milking visits in the last 24 hours, time of each visit, the number of refusals (the robot refused to milk the cow because the minimum milking interval of 4 hours had not been reached) and the number of failures (the robot failed to attached the teat cups to the cow).

2.4. Data Analysis

For all three experiments, downloaded data was managed in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA). Descriptive analysis and statistical analysis, where required, was carried out using Stata/SE 12.0 (Stata Corp 2011, USA). Multilevel and single level regression models were built using MLwiN version 2.25 (Centre for Multilevel, Modeling, University of Bristol). Level of significance was set as $P \leq 0.05$ for all the experiments. Results from multilevel models are presented as follows (Coefficient (SE)).

2.4.1. Experiment 1

The rumination data was not normally distributed and contained outliers. The Fourth Spread test (Devore, 2000) was used and extreme outliers were deleted. A multilevel linear regression model was built in order to study the association between rumination and lameness status. The model had the following form (Eq. 1):

$$y_{\mathrm{ijk}} = \beta_{0ijk} + \beta_1 x_{1\mathrm{ijk}} + \beta_2 x_{2\mathrm{ijk}} + \beta_3 x_{3\mathrm{ijk}} + \beta_4 x_{4\mathrm{ijk}} + \beta_5 x_{5\mathrm{ijk}} + e_{ijk} \text{ (Eq. 1)}$$

The outcome variable (y) was rumination that was averaged across the 2 days after the locomotion score in each observation week. The three levels of the model were AMS pen (k), cow (j) and observation week (i). β_0 was the intercept fixed at each level. β represents the regression coefficient and the predictor variables are represented by x. x_1 represents lameness status (0 = no lame, 1 = lame and 2 = lame and treated), x_2 stands for milk production (2 categories), x_3 days in milk (3 categories), x_4 for parity (4 categories), x_5 for weight (3 categories) and e stands for the random error. The model fit was checked by graphical analysis of normal distribution of residuals at level 2 (cow) and level 3 (observation week).

2.4.2. Experiment 2

Eleven case-control pairs were observed over seven separate recording periods. Data from one pair of cows was excluded; animals lost their markers and could not be identified on the recording. Therefore, data from ten pairs of cows were available for analysis.

Independent single level linear regression models were built for each feeding behaviour variable, controlling for parity. Model took the following form (Eq. 2):

$$y_{\mathrm{i}} = \beta_0 + \beta_1 x_{1\mathrm{i}} + \beta_2 x_{2\mathrm{i}} + \beta_1 x_{3\mathrm{i}} + \beta_1 x_{4\mathrm{i}} + \beta_1 x_{5\mathrm{i}} + \beta_1 x_{6\mathrm{i}} + e_i \text{ (Eq. 2)}$$

Where y was the outcome variable (total feeding duration, frequency of feeding bouts or mean of feeding bout), i the pair ID level, β_0 was the intercept fixed at level i. β was the regression coefficient and the predictor variables were represented by x. x_1 represents the case-control ID variable, $x_{(2\text{-}6)}$ stands for parity as categorical variable (5 categories) and e stands for the random error.

2.4.3. Experiment 3

The dataset of 38 pairs included AMS pen ID (1 to 4), cow ID, case (lame-1) or control (sound-0), locomotion score, parity, daily milk production (last 24 hours) and days in milk (DIM). Each visit to the AMS was allocated to one of four time periods (12:01 - 18:00; 18:01 - 24:00; 24:01 - 06:00 and 06:01 - 12:00). Parity, daily milk production and DIM were normally distributed; the Mean Paired test was used to compare data between groups. The total number of milking visits was not normally distributed and could not be successfully transformed; therefore Wilcoxon Signed-rank test was used to compare data between groups. Refusals data was analyzed using the Two Sample Proportion Test.

A multilevel binomial logistic regression model was carried out to compare the odds of the milking visits to the AMS at specific time periods between case and control groups. The model was set with 3 levels (AMS pen=k, cow ID=j and visit ID model=i) and the outcome was defined as whether cows visited the robot during a particular time period (visit Y/N). Visit ID for cases (1-4) and controls (5-8) were the explanatory variables were added as fixed effects. AMS pen (1-4) was also added as a fixed effect. The model was as follows (Eq. 3):

$$\text{Logit}(\pi_{ijk}) = \beta_0 x_{0ijk} + \beta_1 x_{1ijk} + \beta_2 x_{2ijk} + e_{ijk} \text{ (Eq.3)}$$

Where π was visit/no visit to the AMS, β_0 was the intercept fixed at each level, β represented the regression coefficient of each explanatory variables and the predictor variables were represented by x. x_1 represented robot (4 variables) and x_2 represented visit ID (8 variables). The random error is represented by e.

3. Results

3.1. Experiment 1

A total of 174 cows were observed during the study. Thirteen animals were excluded because they did not have at least 2 consecutive locomotion scores and a further 11 because they had either missing data or they suffered other disease conditions (e.g. mastitis) during the observation period. Therefore statistical analysis was performed on the remaining 150 cows with a total of 1057 locomotion scores.

The 150 animals (mean ±SD; parity = 2.5 ±1.5; DIM = 147.1 ±110.1; daily milk production = 38.11 ±9.6 L) had a mean body weight of 652.13 (±75.4) kg and a mean total rumination of 508.8 (±93.1) minutes in 24hrs. In total 110 cows were observed lame and 40 were never lame during the observation period. From these 110 cows, 40 cows were lame at least once, 42 were lame two or three times, 26 were lame between 5 to 8 times and 2 cows were identified as lame throughout the 9 weeks study period.

The results of the multilevel linear regression model are outlined in Table 2. Lameness had a small but significant ($P \leq 0.05$) negative association with rumination; rumination was reduced by 7.9 minutes per day, in the two days following a lame locomotion score. Parity and days in milk affected rumination; cows in third or higher parity ruminated more than primiparous cows ($P \leq 0.05$) and cows greater than 130 days in milk ruminated more than those less than 130 days in milk ($P \leq 0.05$). AMS pen did not have any significant effect on the model. There was random variability between cows (Coefficient: 5081.41 (SE: 619.45)) and between observation weeks (1997.87 (93.90)).

3.2. Experiment 2

As expected due to matching there were no significant difference in DIM, daily milk production and body condition scoring between lame and sound cows.

The results from the linear regression model confirmed a significant negative association between total feeding time and lameness (Coefficient: -73.65 (SE: 25.47)) and the frequency of feeding bouts and lameness (-9.93 (2.49)). Lame cows spent less time feeding (191.7 ±20.33 minutes / 24h) and had fewer feeding bouts (16.3 ±1.68 bouts/24h) than sound cows (Feeding time: 263.7 ±16.62 minutes / 24h; feeding bouts: 26.6 ±2.43 bouts/24h) (Figure 1). The mean duration of feeding bouts in a 24h period was 12.5 minutes (±1.4) for lame cows and 10.48 minutes (±0.91) for sound cows (Figure 1), the difference was not significant. For parity, the only significant positive association observed was between the mean length of a feeding bout and cows in 5^{th} (7.19 (2.64)) and 9^{th} (10.093 (2.64)) parity. Pair ID presented a significant ($P<0.01$) random variability for each of the three analysis (Total feeding time: 2929.85 (926.50); mean length of feeding bout: 5.69 (1.80) and frequency of feeding bouts 26.14 (8.27)).

3.3. Experiment 3

A total of 38 case-control pairs were enrolled in the observation period. Two cows were used twice as controls in the pair matching. As expected due to matching there were no significant difference in parity, DIM and daily milk production between lame and sound cows.

The total number of visits to the AMS for lame cows was 164 and for the control group was 140, from which refusals represented 25.6% of visits for the former and 22.9% for the latter (NS). In the 24 hour observation periods, 5 lame cows and 4 control cows overdue for milking were directed through the robot.

Lame cows visited a mean of 2.8 times per 24 hours (Range 1-4); control cows visited a mean of 3.2 times (Range 2-5). The difference was highly significant ($z = -2.706$, $p < 0.001$). Results of the logistic regression model demonstrated that after controlling for the effect of AMS pen, lame cows were significantly less likely to visit the AMS between 24:01 and 06:00 when compared to control animals (Table 3).

4. Discussion

Lame dairy cows managed in an intensive AMS in the UK demonstrated a reduction in visits to the AMS, total rumination time, total feeding time and frequency of feeding bouts. Additionally, lame cows visited the milking unit less at night (24:01-06:01) compared to their sound herd mates. To the authors' knowledge this is the first study to investigate the association using rumination data collected continuously using collars over a prolonged period of time. Lame animals ruminated for significantly shorter period of time each day, compared to their sound herd mates, although the difference was small (~8 minutes / day). In agreement with previous studies, primiparous cows ruminated less than multiparous cows (Soriani et al., 2012). The effects of parity and days in milk were large compared to the impacts of lameness (Table 2).

The reason for the small but significant reduction in rumination time observed in lame animals was not identified in this study. In our study investigating the association between feeding behaviour and lameness on the same unit (experiment 2), lame animals ate for significantly shorter periods of time each day over significantly fewer meals. These findings are in agreement with previous studies conducted in other parts of the world in cows managed in a range of different systems (Bach et al., 2007; Gonzalez et al., 2008; Gomez and Cook, 2010). The observed reduction in total rumination time could be associated with a reduction in total dry matter intake (associated with the reduction in total feeding time) and therefore lower fibre content in the rumen. However a previous study has demonstrated that lame cows may compensate for the reduction in total feeding time by increasing their feed intake rate (Gonzalez et al., 2008). Alternatively the change in feeding behaviour observed in lame animals may adversely affect rumen function e.g. consuming the total daily dry matter intake over fewer meals, at an increased rate, may decrease rumination. Finally the discomfort / stress associated with lameness may directly affect rumen function via central depression of the centres controlling rumination, previous work has demonstrated that rumination is negatively associated with higher levels of cortisol (Bristow and Holmes, 2007; Almeida et al., 2008).

Lame cows visited the AMS less frequently than matched, sound animals; the reduction in visits was significant between midnight and 6am. These findings are in agreement with other authors (Klaas et al., 2003; Bach et al., 2007; Borderas et al., 2008). The pain and discomfort caused by lameness (Whay et al., 1997) may have reduced the cow's willingness to attend the AMS. In conventional parlours, lame cows are often the last to enter the milking unit (Hassall et al., 1993) and tend to walk more slowly (Chapinal et al., 2010). It can be postulated that the lame cows visited the AMS less because of the discomfort associated with standing and walking to the unit. If lame cows do not visit the AMS as frequently as their non-lame counterparts, particularly if they do it once a day, they are at increased risk of suffering discomfort from high fill, udder tension and intra-mammary infections (Gleeson et al., 2007).

The significant reduction in overnight visits to the AMS is less easy to explain. As herd and diurnal animals, cows tend to visit the AMS between 08:00 and 19:00 hours (Wagner-Storch and Palmer, 2003). The reduction in overnight visits may be associated with feeding behaviour. On this unit TMR was pushed up to the cows between 6am and 8pm. Pushing up is often associated with an increase in feeding activity i.e. it actively encourages animals to stand and visit the feed face (Personal Observation), and previous work has demonstrated that high yielding cows have higher motivations for feed than for being milked (Prescott et al., 1998). Once standing it seems plausible that animals are then more likely to visit the AMS. It is logical to assume that the increased pressure placed on the feet during rising and standing, is painful in lame animals. It seems possible that the absence of TMR being pushed up overnight decreases the likelihood that lame animals will be motivated to stand and visit the feed face and hence they are also less likely to visit the AMS.

Voluntary attendance to the milking unit is one of the principal benefits of AMS as it reduces the staff costs associated with conventional milking (Meskens et al., 2001). If daily voluntary visits to the AMS fall below an intervention threshold cows must be fetched and encouraged through the milking unit manually, increasing farm labour requirements. The process of fetching and tightly penning animals in a waiting area behind the robot can be a stressful process even on farms with a good stockmanship. Therefore, reduction in visits to the AMS may not only impact on profitability through losses in milk production and increased labour requirements but also be detrimental for cow welfare.

5. Conclusion

The observational studies described here demonstrate that lameness in high yielding cows managed in an AMS affects feeding behaviour, rumination and visits to the AMS. All of these impacts are likely to have negative consequences for farm profitability, but also implications for the health and welfare of the cows. Further studies are required in order to maximise the use and benefits of the technologies available in AMSs as a tool to measure and monitor the health status of cows.

Acknowledgements

Giuliana Miguel-Pacheco is funded by a Scholarship awarded by the University of Nottingham International Office; the authors gratefully acknowledge their support. The authors wish to thank Arjen van der Kamp (Research & Field Experience Farm Management Support) and Moyna Bierma (T4C Support & Development) from Lely International (Maassluis, Netherlands) for their technical support.

References

Almeida, P.E., Weber, P.S.D., Burton, J.L., Zanella, A.J., 2008. Depressed DHEA and increased sickness response behaviors in lame dairy cows with inflammatory foot lesions. Domestic Animal Endocrinology 34, 89-99.

Archer, S., Bell, N., Huxley, J., 2010a. Lameness in UK dairy cows: a review of the current status. In Practice 32, 492-504.

Archer, S.C., Green, M.J., Huxley, J.N., 2010b. Association between milk yield and serial locomotion score assessments in UK dairy cows. Journal of Diary Science 93, 4045-4053.

Bach, A., Dinares, M., Devant, M., Carre, X., 2007. Associations between lameness and production, feeding and milking attendance of Holstein cows milked with an automatic milking system. Journal of Dairy Research 74, 40-46.

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. Journal of Diary Science 93, 932-941.

Borderas, T.F., Fournier, A., Rushen, J., De Passille, A.M.B., 2008. Effect of lameness on dairy cows' visits to automatic milking systems. Canadian Journal of Animal Science 88, 1-8.

Bristow, D.J., Holmes, D.S., 2007. Cortisol levels and anxiety-related behaviors in cattle. Physiology & Behavior 90, 626-628.

Chapinal, N., De Passillé, A.M., Rushen, J., Wagner, S., 2010. Automated methods for detecting lameness and measuring analgesia in dairy cattle. Journal of Diary Science 93, 2007-2013.

DairyCo, 2009. Mobility Score-Instructions.

Devore, J.L., 2000. Overview and Descriptive Statistics, Probability and statistics for engineering and the sciences, Duxbury, Pacific Grove, CA.

Galindo, F., Broom, D.M., 2002. The effects of lameness on social and individual behavior of dairy cows. Journal of Applied Animal Welfare Science 5, 193-201.

Gleeson, D.E., O'Brien, B., Boyle, L., Earley, B., 2007. Effect of milking frequency and nutritional level on aspects of the health and welfare of dairy cows. Animal 1, 125-132.

Gomez, A., Cook, N.B., 2010. Time budgets of lactating dairy cattle in commercial freestall herds. Journal of Dairy Science 93, 5772-5781.

Gonzalez, L.A., Tolkamp, B.J., Coffey, M.P., Ferret, A., Kyriazakis, I., 2008. Changes in feeding behavior as possible indicators for the automatic monitoring of health disorders in dairy cows. Journal of Dairy Science 91, 1017-1028.

Green, L.E., Hedges, V.J., Schukken, Y.H., Blowey, R.W., Packington, A.J., 2002. The impact of clinical lameness on the milk yield of dairy cows. Journal of Dairy Science 85, 2250-2256.

Hassall, S.A., Ward, W.R., Murray, R.D., 1993. Effects of lameness on the behaviour of cows during the summer. Veterinary Record 132, 578-580.

Huxley, J.N., 2013. Impact of lameness and claw lesions in cows on health and production. Livest Sci 156, 64-70.

Ito, K., Von Keyserlingk, M.A.G., LeBlanc, S.J., Weary, D.M., 2010. Lying behavior as an indicator of lameness in dairy cows. Journal of Diary Science 93, 3553-3560.

Jacobs, J.A., Siegford, J.M., 2012. Invited review: The impact of automatic milking systems on dairy cow management, behavior, health, and welfare. Journal of Dairy Science 95, 2227-2247.

Klaas, I.C., Rousing, T., Fossing, C., Hindhede, J., Sorensen, J.T., 2003. Is lameness a welfare problem in dairy farms with automatic milking systems? Animal Welfare 12, 599-603.

Meskens, L., Vandermersch, M., Mathijs, E., 2001. Literature review on the determinants and implications of technology adoption. Deliverable no. 1 of EU project 'Implications of the introduction of automatic milking on dairy farms', work package 1, Socio-economic aspects of automatic milking.

Osterman, S., Redbo, I., 2001. Effects of milking frequency on lying down and getting up behaviour in dairy cows. Applied Animal Behaviour Science 70, 167-176.

Pavlenko, A., Bergsten, C., Ekesbo, I., Kaart, T., Aland, A., Lidfors, L., 2011. Influence of digital dermatitis and sole ulcer on dairy cow behaviour and milk production. Animal 5, 1259-1269.

Prescott, N.B., Mottram, T.T., Webster, A.J.F., 1998. Relative motivations of dairy cows to be milked or fed in a Y-maze and an automatic milking system. Applied Animal Behaviour Science 57, 23-33.

Schirmann, K., von Keyserlingk, M.A.G., Weary, D.M., Veira, D.M., Heuwieser, W., 2009. Technical note: Validation of a system for monitoring rumination in dairy cows. Journal of Dairy Science 92, 6052-6055.

Singh, S.S., Ward, W.R., Lautenbach, K., Murray, R.D., 1993. Behaviour of lame and normal dairy-cows in cubicles and in a straw yard. Veterinary Record 133, 204-208.

Soriani, N., Trevisi, E., Calamari, L., 2012. Relationships between rumination time, metabolic conditions, and health status in dairy cows during the transition period. J. Anim. Sci. 90, 4544-4554.

Tadich, N., Flor, E., Green, L., 2010. Associations between hoof lesions and locomotion score in 1098 unsound dairy cows. Veterinary Journal 184, 60-65.

Uetake, K., Hurnik, J.F., Johnson, L., 1997. Behavioral pattern of dairy cows milked in a two-stall automatic milking system with a holding area. J. Anim. Sci. 75, 954-958.

Wagner-Storch, A.M., Palmer, R.W., 2003. Feeding behavior, milking behavior, and milk yields of cows milked in a parlor versus an automatic milking system. Journal of Dairy Science 86, 1494-1502.

Whay, H.R., Waterman, A.E., Webster, A.J.F., 1997. Associations between locomotion, claw lesions and nociceptive threshold in dairy heifers during the peri-partum period. Veterinary Journal 154, 155-161.

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt, H.F., Lesch, T.N., 1982. A dairy-cow body condition scoring system and its relationship to selected production characteristics. Journal of Dairy Science 65, 495-501.

Table 1. Matching criteria used for selection of case and control animal in experiment 2 and experiment 3.

Matching Criteria	Experiment 2		Experiment 3	
	Case	**Control**	**Case**	**Control**
Locomotion Score	3	0 or 1	2 or 3	0 or 1
Parity	-	-	1 2 >2	1 2 >2
Days in Milk (DIM)	20 – 180 days > 180 days	± 20 days ± 50 days	<19d 20 – 180 days > 180 days	<19d ± 20 days ± 50 days
Daily milk yield (Litres)	Any	+/- 5 litres	Any	+/- 5 litres
Body Condition Score*	Any	+/- 0.5	-	-

*BCS: one to five visual scale with inclusion of half points, assigned according to standard methodologies (Wildman et al., 1982).