Focal sampling of cow lying behaviour for automated welfare assessment

G. Mattachini[1], E. Riva[1], C. Bisaglia[2], J. C. A. M. Pompe[3], G. Provolo[1]
[1]*Department of Agricultural and Environmental Sciences, Università degli studi di Milano, Italy*
[2]*Consiglio per la ricerca e la sperimentazione in agricoltura, Laboratorio di Treviglio, 24047 Treviglio, Italy*
[3]*Farm Technology Group, Wageningen University, 6708 PB Wageningen, the Netherlands*
gabriele.mattachini@unimi.it

Abstract

The objective of the current study was to determine the number of focal animals required to represent the daily lying behaviour of a herd of lactating dairy cows. The study was carried out at 3 commercial dairy farms. The lying time (h/d) and number of lying bouts (n/d) of 15 ± 3 focal dairy cows, continuously recorded by electronic recording devices, were analysed using a resampling method for sample size determination. Estimates of lying behaviour based on five or six cows per focal group provided an accurate estimate of the overall means for each focal group monitored. An accurate estimate of the daily lying behaviour on commercial dairy farms might be generated using continuous recording of at least 40% of the cows in the herd. The sampling methods applied to the automated monitoring systems are time- and labour-saving tools that can be used to assess cow comfort and welfare in relation to lying behaviour.

Keywords: Dairy cow, Lying behaviour, Automated measurement, Focal animal, Sampling

Introduction

Behaviour is one of the most commonly used and sensitive indicators of animal welfare (Haley *et al.*, 2001). Measures of lying behaviour are important measures of cow comfort and welfare, providing valuable information on how cows interact with their environment (Tucker *et al.*, 2004; O'Driscoll *et al.*, 2008). The duration and frequency of lying behaviour (particularly the time spent lying down, the frequency of lying bouts, the duration of individual lying bouts and the laterality) have been identified as sensitive measures of stall comfort and can be used as indicators to assess the welfare of lactating dairy cows (Fregonesi and Leaver, 2001). On-farm monitoring of cow behaviour can be time consuming and labour intensive, particularly when the number of animals per pen is high. Large numbers of animals per pen (the usual experimental unit) make it difficult to sample the entire herd continuously. Focal sampling – which involves observation of one or more sample individuals for a specified period of time – requires less effort when studying groups of animals than continuous monitoring of the entire population (Martin and Bateson, 2007). Methods of assessing behavioural activity have changed in recent years in favour of automatic sampling techniques (Rushen *et al.*, 2012). Recent developments in sensor technology have created new opportunities for automatic monitoring and recording of animal behaviour. Electronic data loggers can be used to measure lying behaviour accurately, including the total time spent lying down, the number of lying bouts (Müller and Schrader, 2003; McGowan *et al.*, 2007), the duration of each lying bout for individual cows (O'Driscoll *et al.*, 2008) and the laterality of their lying behaviour (Ledgerwood *et al.*, 2010). These devices can be quite inexpensive, although this depends upon the particular device being used (size of memory storage and power options). However, since at least one device is needed per animal, the total cost can be high when there is a large number of animals, which will create pressure to reduce sample sizes during a welfare assessment (Rushen *et al.*, 2012). The accuracy of focal-group sampling depends on group size, cohesiveness, animal activity, and design and management factors, and potentially introduces biases into data collection. Therefore, focal animal sampling should be validated and selected according to the objectives of a specific study. The objective of the current study was to determine the number of focal animals required to represent the daily lying behaviour of the herd mean of lactating dairy cows, using a resampling-based procedure (jackknife).

Materials and methods

<u>Farms and animals</u>

This study was conducted at 3 commercial dairy farms between April 2010 and July 2011. Two dairy farms (A and B) were located in Friesland (Netherland) where animals were milked in an automatic milking system (AMS) and feeding was carried out by an automatic feeding system (AFS). In both farms, barns were E-W oriented and featured a loose-housing layout with a total of 141 and 129 cubicles with rubber mats covered with sawdust, and 61 and 85 feeding places, for Farm A and B respectively. The milking area, in both barns, consisted of two AMS units and a closed waiting area in front of the unit entrance. One-way gates provided selectively guided cow traffic. At the time of the study, barn A housed 107 lactating Holstein-Friesian cows (parity 2.4 ± 1.3, milk yield 33.0 ± 6.6 kg/d, days in milk 187 ± 99.7; mean ± SD) during the first monitored period (focal group 1) and 109 lactating Holstein-Friesian cows (parity 2.7 ± 1.5, milk yield 31.5 ± 10.5 kg/d, days in milk 138.3 ± 110.9; mean ± SD) in the second monitored period (focal group 2). Ninety-seven lactating Holstein dairy cows, 45 primiparous and 52 multiparous (parity 2.1 ± 1.4, milk yield 28.3 ± 11.2 kg/d, days in milk 188.4 ± 128.0; mean ± SD), subdivided into two homogeny groups (focal group 3 and 4) were used in the study at farm B. The third commercial dairy farm (C) was located at the Institute of Animal Sciences of the Volcani Center in Bet-Dagan (Israel). The cows were housed in a loose-covered pen, milked 3 times a day and fed twice daily. At the beginning of the data collection period, the barn housed a group of 92 lactating Israeli Holstein cows (focal group 5) with 215.4 ± 167.4 days in milk (mean ± SD).

<u>Behavioural recordings</u>

The lying behaviour of 73 focal dairy cows, randomly selected and subdivided into focal groups 1 to 5 (14.6 ± 3.2; mean ± SD, ranging from 11 to 19 cows; see Table 1), was continuously recorded for 3 to 16 days by electronic recording devices (Afimilk Pedometer Plus tag, Hobo Pendant G data logger, IceTag 2D). The data loggers, previously validated for recording standing and lying behaviour in dairy cows (McGowan *et al.*, 2007; Ito *et al.*, 2009; Higginson *et al.*, 2010) were programmed to record daily lying time (h/d) and number of lying bouts per day (n/d).

Table 1: Parity, milk production and days in milk of 5 focal groups monitored

Farm	Focal Group	Cow (n)	Day (n)	Parity mean ± SD	Milk Yield mean ± SD	DIM mean ± SD
A	1	12	3	2.7 ± ١,٤	33.6 ± ٥,٦	191 ± ١٠٧
A	2	11	16	3.0 ± 1.7	31.3 ± 11.5	213 ± 130
B	3	15	16	2.4 ± 1.1	29.6 ± 12.1	177 ± 121
B	4	16	16	2.4 ± 1.7	31.8 ± 10.8	200 ± 150
C	5	19	11	2.3 ± 1.5	/	218 ± 163

The HOBO Pendant G (Onset Computer Corporation, Pocasset, MA) is a waterproof 3-channel data logger. This data logger uses an internal 3-axis accelerometer with a range of ± 3 g. The data loggers were attached to the lateral side of the left or right hind leg of the cows using Vet-flex such that the x-axis was perpendicular to the ground and pointing towards the back of the cow (dorsal direction). The data loggers were programmed to record g-force at 1 min intervals following the procedure of Ito *et al.*, (2009). The g-force readings from the x-axis were used to evaluate lying and standing behaviour (Ledgerwood *et al.*, 2010).

The IceTag 2D (IceRobotics, Edinburgh, UK) is an electronic sensor device based on accelerometer technology that measures and determines the percentage of time the cows spent lying and standing for each recorded second (McGowan *et al.*, 2007). IceTag was attached to the lateral side of the left or right hind leg above the fetlock by means of a strap with a buckle. Lying behaviour, per-minute basis, was classified for each recording following the IceTag-recorded intensity thresholds for lying and standing (Trénel *et al.*, 2009). For both data loggers (Hobo Pendant G data logger and IceTag 2D) we followed the approach by Endres and Barberg (2007) and ignored lying bouts shorter than two minutes.

The Pedometer Plus tag (afimilk, Kibbutz Afikim, Israel) provides information relating to lying time and lying bouts by means of a posture sensor including an omni-directional tilt switch to sense a tilt in orientation above an operating angle. From this information, the device calculates a number of behavioural parameters, including the rest time (the time that the cow is lying down) and the rest bout (the number of lying bouts). The Pedometer Plus tag was attached with a strap to the lateral side of the leg above the fetlock, between the knee and the hoof. The recorded data were analysed by the Afifarm software which calculates daily lying time (h/d) and number of lying bouts per day (n/d) for each cow monitored.

Data analyses: sample size determination using the visual jackknife

To determine how the sample size in focal sampling affects the estimates for lying behaviour of cows in each of the five focal groups, the lying time and number of lying bouts were analysed using a sample size determination method, derived from a resampling-based procedure (namely, jackknife). This approach is based on intensive use of the sample data by systematically taking sub-samples of the original data set, and calculating mean and standard deviation for each of subsamples. The software (SISSI – Shortcut In Sample Size Identification, ver. 1.01; Confalonieri *et al.*, 2007) was used to generate virtual samples and matrices of the means and standard deviations for the lying time and number of lying bouts for each of 5 focal groups. When analysing the trends in the means for increasing values of *N-k* (*N* is the total number of observations; *k* is the number of observations not used by the jackknife), the optimum sample size is considered to be the *N-k* value for which the variability between the means does not significantly decrease with increasing sample size (Confalonieri *et al.*, 2007). Specifically, four weighted linear regressions are performed for the generated means (the first uses the highest values of the $N\text{-}k \leq (N\text{-}k)'$; the second uses the lowest values; the third uses the highest values of $N\text{-}k \geq (N\text{-}k)'$; and the fourth uses the lowest values). A global index (SR^2) is calculated by summing the coefficients of determination of the four regressions. By repeating the steps for all the possible *(N-k)'* it is possible to identify the optimum sample size, i.e. *(N-k)'* where SR^2 is the highest. The process stops when the next sample size does not produce an SR^2 that is larger than 5% of the previous value.

Results

Means and standard deviations were obtained for all the generated virtual sub-samples for each of five focal groups and were plotted on two charts, with the values of (N-k) on the X-axis and the means and standard deviations on the Y-axis. This allows a visual representation of how the means and standard deviations for the samples generated vary with increasing sample size of focal group (Figures 1 and 2, for lying time and number of lying bouts, respectively).

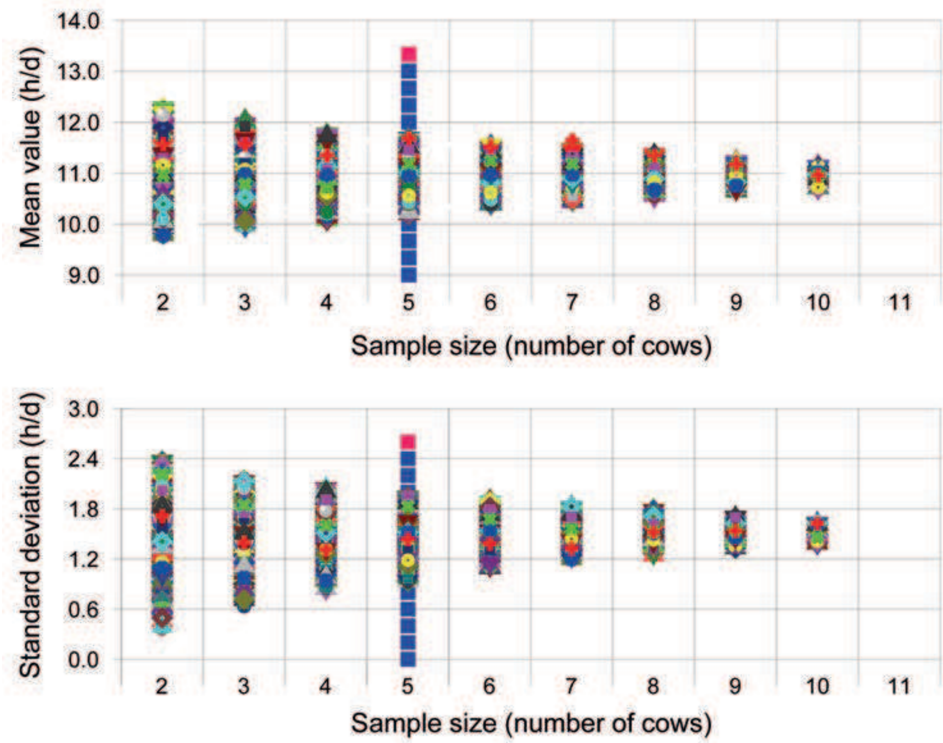

Figure 1: Daily lying time (focal group 1) – Means (h/d) and standard deviations (h/d) of the generated populations of subsamples (various symbols and colours) when the jackknife is applied for different k values. The sample size (*N-k*) values are shown on the X-axis, with *k* from (*N-2*) to 1. *k* is the number of observations not used by the jackknife; *N* is the total number of observations (12 cows). The automatically computed sample size is indicated by the vertical series of blue dots

Figure 1 shows that the differences between the means and standard deviations for daily lying time in the populations of subsamples generated for focal group 1 decrease for a sample size of up to five cows. These differences, obtained with this resampling method, continue to slightly decrease with larger sample sizes (more than five cows). For this focal group, five cows can be considered the optimum sample size, obtained by an automatic sample size determination procedure, based on the variability between the means.

The same considerations apply to the number of lying bouts in focal group 4 (Figure 2). The mean values chart shows that the range of the plot is similar for sample sizes larger than six cows, while differences in standard deviation are greater in samples smaller than six cows. The accuracy of estimates of daily lying time (Figure 1) and lying bouts (Figure 2) decreased when estimates were based on fewer cows per focal group.

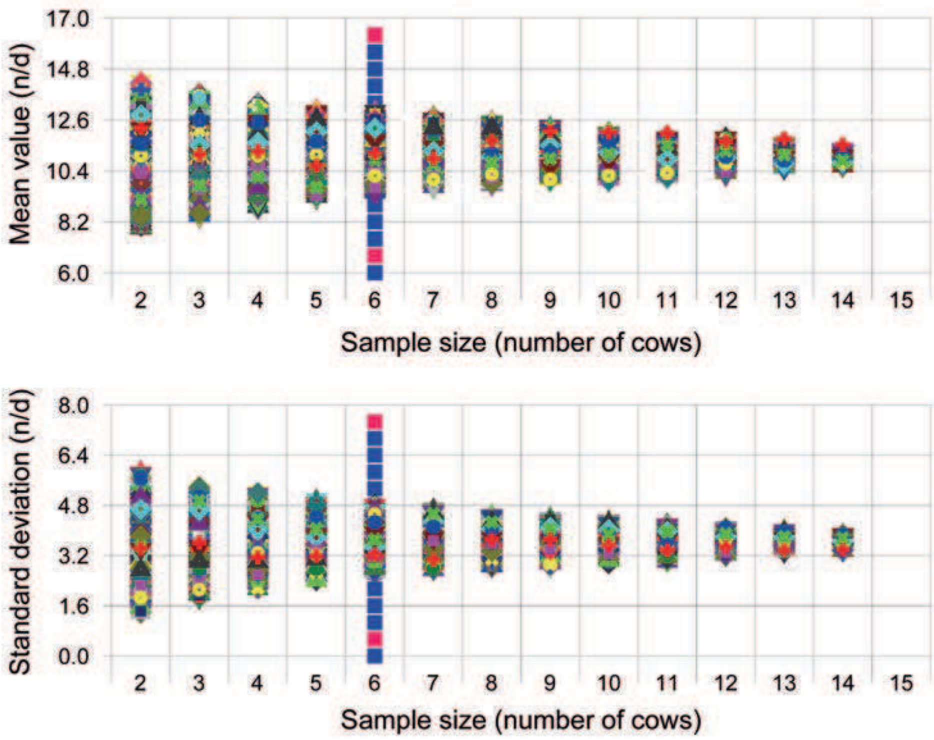

Figure 2: Number of lying bouts per day (focal group 4) – Means (n/d) and standard deviations (n/d) of the generated populations of subsamples (various symbols and colours) when the jackknife is applied for different k values. The sample size (*N*-*k*) values are shown on the X-axis, with *k* from (*N*-*2*) to 1. *k* is the number of observations not used by the jackknife; *N* is the total number of observations (16 cows). The automatically computed sample size is indicated by the vertical series of blue dots

The mean values, standard deviations, range of variability (CV) and sample size for lying time and number of lying bouts for each focal group are reported in Table 2. Estimates of lying time (h/d) and number of lying bouts (n/d) based on five or six cows per focal group ($N - k$ value for which the variability between the means and standard deviations does not significantly decrease with increasing sample size) provided an estimate of the overall means.

Table 2: Means, standard deviations, range of variability (CV) and sample size[1] for daily lying time and number of lying bouts per day for each focal group (five) monitored

Focal Group	Lying time (h/d) mean ± SD	CV range < CV (%) <		Sample Size[1] $N-k$ value
1	10.94 ± 1.55	9.63	17.35	5
2	11.89 ± 1.83	10.78	18.36	5
3	11.32 ± 1.92	11.75	20.89	6
4	12.14 ± 1.86	10.5	19.67	5
5	9.07 ± 1.94	12.25	28.65	6
	Lying bout (n/d) mean ± SD	**CV range < CV (%) <**		**Sample Size $N-k$ value**
1	9.8 ± 3.7	28.64	41.88	7
2	9.4 ± 2.5	21.11	30.9	5
3	9.5 ± 2.1	14.46	27.56	5
4	11.1 ± 3.7	24.61	41.99	6
5	11.8 ± 2.5	14.43	25.8	5

[1]Automatically computed sample sizes ($N-k$ value) were obtained using a resampling-based procedure (jackknife)

Discussion and conclusions

Mitlöhner *et al.* (2001) showed that estimates of the percentage of time spent lying based on one to nine animals out of a group of ten were all similar, indicating that one focal animal for every ten was sufficient to estimate the group mean. However, Cook *et al.* (2005) sampled ten focal cows from a pen containing approximately 85 cows and found some differences between behavioural indices calculated from only the focal cows and the same indices based on all cows. Furthermore, most of the studies that have utilized automated devices to measure behaviour have sampled focal animals. Ito *et al.* (2009) established that 30 cows per farm provided a reasonable sample to detect variations in lying behaviour with approximately 90% accuracy, but that accuracy dropped to less than 60% when the sample size decreased to ten cows.

Endres and Barberg (2007) placed activity monitors on the legs of at least 15% of the cows in each herd to represent the entire group. Considering all possible combinations of cows for each group monitored, we found that a sample of at least six cows (40%) is necessary to provide an accurate estimate of lying behaviour for a group with an average size of 15 cows. In the present study, cows differed greatly in terms of lying time and number of lying bouts both between focal groups and among cows within each focal group, and it is necessary to sample and select cows to obtain a representative measure of the herd. The accuracy of focal-group sampling depends upon group size, cohesiveness, animal activity and management operations, thus potentially introducing biases into data collection. Several farm conditions contribute to and affect the social behaviour of a dairy herd, including the type of housing, the number of cows and the space allowance per cow. These factors largely explain the differences in automatically computed sample size (*N–k* value) obtained for the five focal groups monitored, for lying time and number of lying bouts. The number of cows in the group, the variability of lying behaviour among the cows and the different environmental and farm conditions may play a part in determining the sample size for focal sampling. Furthermore, the different aspects of lying behaviour (lying time and number of lying bouts) can also affect the sample size for focal sampling. Group definition and the method of selecting the animals in the "focal group" are crucial for valid behavioural sampling. Some authors have limited their selection to only high-yielding cows or systematically selected the cows based on the order they entered the milking parlour; other authors have selected the cows by limited random selection or on the basis of parity, stage of lactation, locomotion score, and/or health problems.

Regardless of the cause, individual lying behaviours of cows housed together can be highly variable. In small and non-homogeneous groups, the behaviour of each individual cow has a greater effect on the total behaviour of the group (in terms of weighting), whereas in large groups or synchronized subgroups each individual cow has a smaller effect on the total behaviour of the group. This relationship was confirmed in the results of this study, where the number of cows required to estimate the behaviour of a focal group consisting on average of 15 cows was 40% (six cows), whereas Ito *et al.* (2009) found that for a group of 44 cows, the number of focal animals required to obtain a reasonable estimate of lying behaviour was approximately 25% of the herd (ten cows).

In conclusion, estimates of the daily lying behaviour of the herd mean can be generated using continuous recording of at least six focal cows out of 15. The sampling methods applied to the automated monitoring systems are time- and labour-saving tools that can be used to assess cow comfort as exemplified by lying behaviour.

Acknowledgements

Special thanks go to the farms for their significant contributions of collaborative effort, time and patience to this study. Thanks are also due to Wageningen University, Farm Technology Group and Institute of Agricultural Engineering, A.R.O., Volcani Center for supporting this research, and Wageningen UR Livestock Research for providing the animal activity monitoring sensors.

References

Confalonieri, R., Acutis, M., Bellocchi, G., and Genovese, G. 2007. Resampling-based software for estimating optimal sample size. *Environmental Modelling & Software,* 22, 1796-1800.

Cook, N.B., Bennett, T.B., and Nordlund, K.V. 2005. Monitoring indices of cow comfort in free-stall-housed dairy herds. *Journal of Dairy Science,* 88: 3876-3885.

Endres, M.I., and Barberg, A.E. 2007. Behavior of dairy cows in an alternative bedded-pack housing system. *Journal of Dairy Science,* 90: 4192-4200.

Fregonesi, J. A., and Leaver, J. D. 2001. Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. *Livestock Production Science,* 68: 205-216.

Haley, D. B., de Passillé, A.M., and Rushen, J. 2001. Assessing cow comfort: effects of two floor types and two tie stall designs on the behaviour of lactating dairy cows. *Applied Animal Behaviour Science,* 71: 105-117.

Higginson, J.H., Millman, S.T., Leslie, K.E., and Kelton, D.F. 2010. Validation of a new pedometry system for use in behavioural research and lameness detection in dairy cattle. In: *Proceedings of the First North American Conference on Precision Dairy Management,* pp 132–133, Toronto, Canada.

Ledgerwood, D.N., Winckler, C., and Tucker, C.B. 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. *Journal of Dairy Science,* 93: 5129-5139.

Martin, P., and Bateson., P. 2007. Measuring behaviour: An Introductory Guide. *Cambridge University Press,* Cambridge, U.K.

McGowan, J.E., Burke, C.R., and Jago, J.G. 2007. Validation of a technology for objectively measuring behaviour in dairy cows and its application for oestrous detection. In: *Proceedings of the New Zealand Society of Animal Production,* 67: 136-142.

Mitlöhner, F.M., Morrow-Tesch, J.L., Wilson, S.C., Dailey, J.W., and McGlone, J.J. 2001. Behavioral sampling techniques for feedlot cattle. *Journal of Animal Science,* 79(5) 1189-1193.

Müller, R., and Schrader, L. 2003. A new method to measure behavioural activity levels in dairy cows. *Applied Animal Behaviour Science,* 83: 247-258.

O'DriScoll, K., Boyle, L., and Hanlon, A. 2008. A brief note on the validation of a system for recording lying behaviour in dairy cows. *Applied Animal Behaviour Science,* 111: 195-200.

Rushen, J., Chapinal, N., and de Passillé, A.M. 2012. Automated monitoring of behavioural-based animal welfare indicators. *Animal Welfare*, 21: 339-350.

Trénel, P., Jensen, M.B., Decker, E.L., and Skjoth, F. 2009. Quantifying and characterizing behavior in dairy calves using the IceTag automatic recording device. *Journal of Dairy Science,* 92: 3397-3401.

Tucker, C.B., Weary, D.M., Fraser, D., 2004. Free-stall dimensions: effects on preference and stall usage. *Journal of Dairy Science,* 87, 1208-1216.