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Influence of sample design and measurement period on sample size required to measure lying time in dairy cows

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An important indicator of the well-being of dairy cows is their daily lying time, which is known to be affected by both intrinsic and extrinsic influences. To attain sufficient accuracy of lying time measurements, it is recommend to collect the daily lying time of 30 cows for three consecutive days. For on-farm research, however, the number of available cows or measurable days may be subject to restrictions that preclude such a study design. The objective of the present study is to investigate the influence of measurement period, sample size, and intrinsic factors on measurement accuracy. Using a survey of daily lying times on four practice farms with a total of 170 cows on three consecutive days, the magnitude of variances and the effect of intrinsic influencing factors were estimated. A goodness-of-fit calculation for mixed models was then used to estimate the required sample sizes for alternative survey scenarios. Results permit the conclusion that a longer measurement period allows reducing the sample size without loss of accuracy.

Keywords
Sample design, sample size, dairy cow lying times

Daily time spent lying down is an important indicator of dairy cow welfare, as it is associated with lameness (Chapinal et al. 2009, Ito et al. 2010, Proudfoot et al. 2010, Solano et al. 2016) and standing time (Bernardi et al. 2009, Dippel et al. 2012, Galindo et al. 2000, Proudfoot et al. 2010). Assessing welfare based on lying times requires a comprehensive knowledge of intrinsic and extrinsic influences (Tucker et al. 2021). Lying time is influenced by extrinsic environmental factors, such as housing and grazing systems (Falk et al. 2012, O'Driscoll et al. 2010, 2019, Sepúlveda-Varas et al. 2014), stocking density (Charlton et al. 2014, Ito et al. 2014), animal to feedlot ratio (Crossley et al. 2017, Schrader 2001), floor and cubicle design (Chen et al. 2017, Drissler et al. 2005, Fregonesi et al. 2007, Jones et al. 2017, Schütz et al., 2019, Tucker et al. 2004), feeding (DeVries and von Keyserlingk 2005, Huzzey et al. 2006), milking frequency or system (Charlton et al. 2014, Österman and Redbo 2001, Westin et al. 2016) and housing climate (Enders et al. 2006).

Lying time is further influenced by intrinsic factors, having been shown to vary between herds (Ito et al. 2009) and within a herd from animal to animal (Ito et al. 2009, Scheibe 1987, Vasseur et al. 2012). Lying time has been correlated with lactation day (Bewley et al. 2010, Maselyne et al. 2017, Vasseur et al. 2012), milk quantity (Bewley et al. 2010, Norring et al. 2012, Vasseur et al. 2012)and parity (number of pregnancies) (Løvendahl and Munksgaard 2016, Munksgaard et al. 2020, Singh et al. 1993, Vasseur et al. 2012).

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Sampling strategies

Experiments in which individual environmental factors are varied under ceteris paribus conditions and the resulting daily lying time is measured are essential in many dairy farm studies. For such experiments, ITO et al. (2009) recommend measuring lying time over three consecutive days on at least 30 cows per treatment, but they do not specify the sampling procedure in further detail. Vasseur et al. (2012) show that considering lactation stage and parity reduces sampling requirements. Indeed, for a number of reasons – from accommodating small farms (Vasseur et al. 2012) to demonstrating animal-friendly husbandry (Rushen et al. 2011) to allocating financial resources – it is of interest to reduce the number of cows sampled. That prompted this study into how sampling design affects the number of cows and measurement period needed for acceptably accurate lying time measurements.

Ito et al. (2009) and Vasseur et al. (2012) investigated the reduction of measurement period to one day. Both studies were based on measurement series in which the lying times of many animals were collected for periods of ten consecutive days, and the data sets were then randomly reduced. A reduction was considered acceptable if a high proportion of the variance in the mean daily lying time of cows from the full sample could be explained by that of the subsample.

An alternative approach is to determine the sample size required for an investigation. This requires both the variability of the characteristic under investigation and a precision requirement in the form of a desired standard error of a difference (Piepho et al. 2022). In the simple case of an experiment to investigate a normally distributed characteristic with two independent samples (one factor with two factor levels) with the same known variance, the sample size n per sample can be calculated from

$$n = 2\frac{\sigma^2}{\delta^2}\left(z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right)^2 \qquad \text{(Eq. 1)}$$

where σ^2 is the true variance of the characteristic of interest, δ^2 the mean difference of the characteristic of interest to be detected, and α and β are the errors of type 1 and type 2 (van Belle 2008, 2022). For many experiments in agricultural sciences, these simple case assumptions only apply to a limited extent because, for example, correlated errors or stratified samples are present in these experiments.

A standard approach to evaluating agricultural science experiments is to use mixed linear models (Piepho 2008). These allow modeling correlated observations using appropriate variance-covariance structures. As to the sample design for experiments with spatially correlated errors, Stroup (2002) presents an approach that estimates the necessary sample size for given design and variance components using a noncentral F-distribution. The approach is implemented using of a variety of different survey designs and subsequent dummy evaluation using a mixed linear model. Thus, only the data structure was created. Data were not simulated.

Recumbency measurements of a cow are more similar the smaller the temporal distance between the observations. This temporal dependence can also be accounted for in the evaluation model using a suitable variance-covariance structure. In the present work, the sample design approach developed for spatially correlated errors by Stroup (2002) will be used to estimate the required sample sizes for future experiments to influence the lying time of dairy cows. The required variance in daily lying time was estimated from data from four farms. Then, data sets with different number of measurement days and different number of cows were created. In addition, it was assumed that the treatments in the experiment were tested either on exactly the same cows or on different cows.

Animals, material and methods

When selecting the farms for study, care was taken to ensure the housing technologies used were as similar as possible (Table 1). All farms had high stalls with rubber mats. Three farms had slatted floors and the fourth had a combination of slatted and flat floors. Animal feeding space ratios ranged from 1.2 : 1 to 2.5 : 1, and animal lying space ratios ranged from 0.8 : 1 to 1.2 : 1. Herd sizes varied from 100 to 144 animals. The animals were milked twice daily.

Table 1: Husbandry characteristics of the study farms (A, B, C, D)

	Operation A	Operation B	Operation C	Operation D
Feeding aisle width in m	3.50	3.00–3.50	3.30	3.00
Gangway width in m	2.30	2.00–2.40	2.30	2.60
Lying surface length in m	1.85	1.70–1.75	1.65	1.75
Cubicle width in m	1.20	1.15	1.10	1.15
Neck rail design	rigid	flexible	rigid	rigid
Horizontal neck rail distance in m	2.10	1.95–2.15	2.00	1.95
Neck rail height in m	1.25	1.14–1.38	1.10	1.17
Head space wall in m	1.20	1.00	0.80	0.90
Head space opposite in m	0.70	0.70	0.80	0.70
Brisket board height in m	0.25	0.25	0.15	0.10
Gangway design	paved floor	slatted floor	slatted floor	slatted floor

Lactation time measurements were made between 5 March 2013 and 29 May 2013 on a total of 170 animals from the four farms in Schleswig-Holstein (Hein 2013). At least 30 cows per farm were randomly selected from the proportion of lactating cows and the lying time was recorded on three consecutive days. The animals were of the Holstein-Friesian breed. Annual herd yield was 8,238–10,676 kg milk cow^{-1} $year^{-1}$, inter-calving period 384–444 days, first calving age 25.6–28.4 months, live day yield 9.6–13.1 kg, and average useful life 3.9–4.8 years (Table 2).

Table 2: Farm-specific performance characteristics of the study farms (A, B, C, D)

	Operation A *n* = 37	Operation B *n* = 36	Operation C *n* = 34	Operation D *n* = 63
Annual milk yield in kg cow^{-1} $year^{-1}$	10,676	9,833	8,409	8,238
Intercalving period in days	421	444	384	400
First calving age in months	26.2	28.4	25.6	27.7
Daily milk yield in kg	13.1	11.0	9.6	10.7
Useful life in years	4.5	4.2	3.9	4.8
Mean total daily lying time in h [1)]	11.1	10.8	10.6	10.9

[1)] Without lame and rutting animals.

RumiWatch® pedometers were used to measure daily lying time (company ITIN + HOCH GmbH, Liestal, Switzerland). Before attaching the pedometers, the locomotion of the cows was checked to exclude lame cows from the study. Animals in heat were also excluded. Pedometers were attached on

the outside below the tarsal joint according to Ledgerwood et al. (2010). After an acclimation period of four days, measurements began.

Statistical approach

The aim of the evaluation was to determine the sample size necessary to detect a lying time difference between the treatments of 0.50 or 0.75 hours with a probability of 80 %. For this purpose, mixed models were first fitted to the real data. The variance components estimated from these were then used in simulations to determine the goodness of fit for varying data sets using the approach of Stroup (2002). The simulation approach is needed because formulas for determining power are not available for mixed models or for autocorrelated data (Stroup 2002, Piepho et al. 2022).

Estimation of variance components

Pedometer measurements of daily lying time from 170 cows across four herds were available. The cows could be divided into 12 subgroups by lactation stage (lactation day ≤ 100, 101–200, > 200) and parity (1, 2, 3, and >3). From these, four groups were formed by combining subgroups (Table 3). Four mixed models were fitted to the data and variance components estimated for herds and animal effects. The four models correspond to different approaches to sampling: Model 1 assumes a simple random sampling design. Models 2 to 4 used information on parity and lactation stage with different assumptions on variances and data type. E. g. Model 3 corresponds to a random sample in which parity and lactation stage are considered and the variances therefore correspond to variances observed within a simulated focus group (of one subgroup).

Table 3: Subdivision of cows into groups according to lactation stage and parity

Group 1	Group 2	Group 3	Group 4
Lactation stage ≤100	Lactation stage ≤100	Lactation stage >100	Lactation stage >100
Parity <3	Parity ≥3	Parity <3	Parity ≥3

In the first model (simple random sampling), the information on lactation stage and parity was ignored. The model can be described as follows:

$$y_{klm} = \mu + h_k + t_{kl} + e_{klm}, \quad \text{(Eq. 2)}$$

where y_{klm} is the daily lying time of the l-th cow from the k-th herd on the m-th day, μ is the intercept h_k is the random effect of the k-th herd, and t_{kl} is the random effect of the l-th animal. The term e_{klm} represents the normally distributed error of the observation y_{klm}.

It was assumed that consecutive measurements of a cow may be correlated. Therefore, models with and without first-order autocorrelation (AR(1); Gilmour et al. 1997) were tested. In Model 1, there are two terms that explain correlation of the data: The animal effect, which models a constant covariance between measurements of the same cow, and the autocorrelation, which models a decreasing covariance with time. Both effects can be modeled in a joint variance-covariance structure. The structure can be denoted as compound symmetry in the case without temporal covariance or as an AR(1) plus nugget structure in the case with temporal covariance i. e. with autocorrelation. The model without autocorrelation showed the smaller AIC (Wolfinger 1993) for Model 1. The reason for this is

that the estimation of an additional correlation parameter in the model with autocorrelation increases the AIC by 2. At the same time, the estimated autocorrelation was close to zero; therefore, the gain in likelihood was small. For that reason, the model without autocorrelation was used.

Model 1 was extended to include effects of the 12 subgroups and thus animal-specific information on parity and lactation stage. Both variables can influence daily lying time of animals (Vasseur et al. 2008). Hence Model 2, a random sample and mixed model, was developed; it can be described as follows::

$$y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + h_k + t_{kl} + e_{ijklm}, \quad \text{(Eq. 3)}$$

wobei y_{ijklm} is the daily lying time of the l-th cow from the k-th herd in der i-th parity and the j-th lactation period on the m-th measurement day and α_i, β_j and $(\alpha\beta)_{ij}$ are the fixed effects of the i-th parity, j-th lactation interval, and their interaction effects.

The fixed effects for lactation interval and parity define 12 means, for which the combination of lactation intercept and parity results in 12 subgroups. The term e_{ijklm} is the normally distributed error of the observation y_{ijklm} and all other terms are defined analogously to Model 1. For Model 2, a first-order autocorrelation was fitted for the error effects of one cow on consecutive days. Again, the model without autocorrelation showed better fit via smaller AIC. In Model 2, part of the variance of the animals from Model 1 is explained by the information on parity and intercept.

Model 2 was modified to fit heterogeneous group- or subgroup-specific error variances. A model with subgroup-specific means and subgroup-specific variances leads to very similar means and variance estimates compared to the separate evaluation of the data within the respective subgroups. The estimated values are not completely identical because when all subgroups are evaluated together, the variances for herd and for animals are estimated from all the data. In contrast, if data for a single subgroup are evaluated, the variances are estimated only from the data of that subgroup. It can be shown that model fitting with subgroup-specific means (12 means), group-specific variances (four variances, Table 3) and with an autocorrelation of measurements per cow resulted in the best fit for Model 3.

In Model 4, the factor lactation period was replaced by the metric variable "lactation day" and a second order polynomial function was fitted. A second-order polynomial was sufficient because for this model the lack-of-fit test for deviations from the polynomial was no longer significant. In Model 4, the factor β_j from Model 3 was replaced as follows:

$$\beta_j = \beta_1 x_{hijklm} + \beta_2 x^2_{hijklm}, \quad \text{(Eq. 4)}$$

where β_1 and β_2 are the parameters for the linear and quadratic terms of the regression when the lactation day is increased by one day and x_{hijklm} is the h-th lactation day of the l-th cow from the k-th herd in der i-th parity in the j-th lactation period on the m-th measurement day. The model can be described as follows:

$$y_{hijklm} = \mu + \alpha_i + \beta_1 x_{hijklm} + \beta_2 x^2_{hijklm} + \beta_{i1} x_{hijklm} + \beta_{i2} x^2_{hijklm} + h_k + t_{kl} + e_{hijklm} \quad \text{(Eq. 5)}$$

The four additional parameters in equation (4) result from two parameters for the main effect β_j and another two more for the interaction effects $(\alpha\beta)_{ij}$. For all four models, variance components were estimated for herd, animal, and error. Subgroup-specific means were fitted for Models 2, 3 and 4. For Models 3 and 4, a group-specific error variance (Table 3) was estimated.

Simulating data for varying research situations

The estimated variance components were used to determine the required sample size for future studies using the approach of Stroup (2002) where the structure of the data sets with different numbers of animals per treatment and measurement days per animal and treatment were simulated for two treatments and the power was calculated for these data sets using a non-central F-distribution for a given mean difference of 0.50 and 0.75 hours. Power is the probability of being able to detect a difference of interest in lying time between two treatments within an experiment. The smallest number of animals leading to a power > 0.8 was sought. It was assumed that the number of cows per treatment and the number of measurement days per cow were constant. One, three or ten measuring days were assumed in the data sets. Two scenarios were simulated: (i) a paired sample, where treatments were tested on the same cow in sequential order and (ii) an unpaired sample, where treatments were tested on different animals. In the paired sample, animals were simulated to be fitted with pedometers twice in a randomized temporal sequence and measured each time for the assumed measurement period. For the power calculation, only the structure of the data, the variance components and the difference to be proved are needed. Arbitrarily, therefore, the observations (and thus the mean of the observations) of the first treatment were set to zero, and the observations of the second treatment (as well as their mean) were increased by 0.50 and 0.75 hours, respectively. The simulation was performed in SAS.

Results

Estimation of variance components

Variance components were estimated for all four models (Table 4). The variance component of the herds was estimated as a very small value and bounded to zero by the REML method used in the software program. As a result, the variance parameter was dropped from all further calculations (e. g., AIC). The variance component of the error was similar for all four models. An autocorrelation was estimated only for Models 3 and 4.

Table 4: Variance component estimates for total lying time in hours for the trait lying time using four different models

Cause of variance	Model			
	(1)	(2)	(3)	(4)
Herd	0	0	0	0
Animal	4.1732	3.1122	2.6722	2.6517
Autocorrelation	-	-	0.1758	0.1665
Error	2.1458	2.1466	2.1838	2.1569

The variance among animals is greatest for Model 1. For unpaired samples, therefore, the number of cows needed to be observed increases rapidly for Model 1, compared to other models. In the following, Model 1 is not considered further.

Estimated sample sizes, number of measurement days and power based on the simulations

Power increases as the minimum difference needed increases. For example, for 25 animals with paired sampling and a measurement period of three days, the power increases from 0.55 for 30 minutes to 0.88 for 45 minutes (Figure 1). If the number of animals is increased from 25 to 30, the power increases from 0.88 to 0.93 for the same measurement period (three days) and the same mean difference to be proven (45 minutes). For an extended measurement period of ten days, a power of 0.90 can be achieved with 18 and 8 animals in paired samples with a mean difference to be proven of 30 and 45 minutes, respectively.

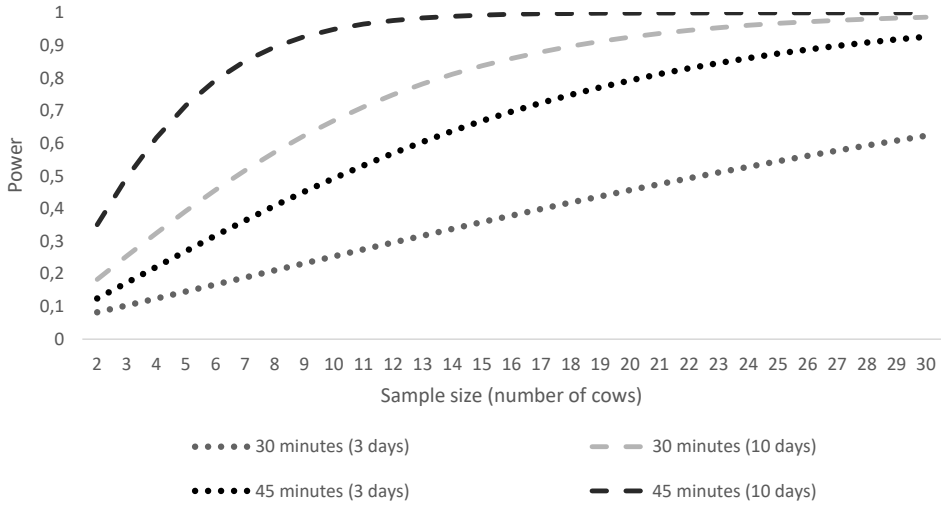

Figure 1: Power and sample size for studies on dairy cow lying times for treatment mean differences of 30 and 45 minutes using model 2 (random sample and mixed model) with paired samples

In general, the differences in sample sizes between Models 2, 3, and 4 were smaller than the differences due to the measurement period or the sample design (Figure 2). The relative differences between Model 2 and Models 3 and 4 result from the assumed autocorrelation of 0 and about 0.17, respectively, making the benefit of more measurement days greater for Model 2 than for Models 3 and 4. In agreement with Ito et al. (2009), a measurement period of three days resulted in 21–24 cows in paired samples for a difference to be proven of 45 minutes, while for a difference of 30 minutes, 46–52 cows per sample are needed. For a measurement period of 10 days, 14–17 or 8–9 animals are needed, depending on the model and accuracy. Under the same condition but using unpaired testing, the range of sample sizes is estimated at 41–210 and 19–93 cows, respectively. Increasing the difference to be proven from 30 to 45 minutes resulted in roughly halving the required sample size.

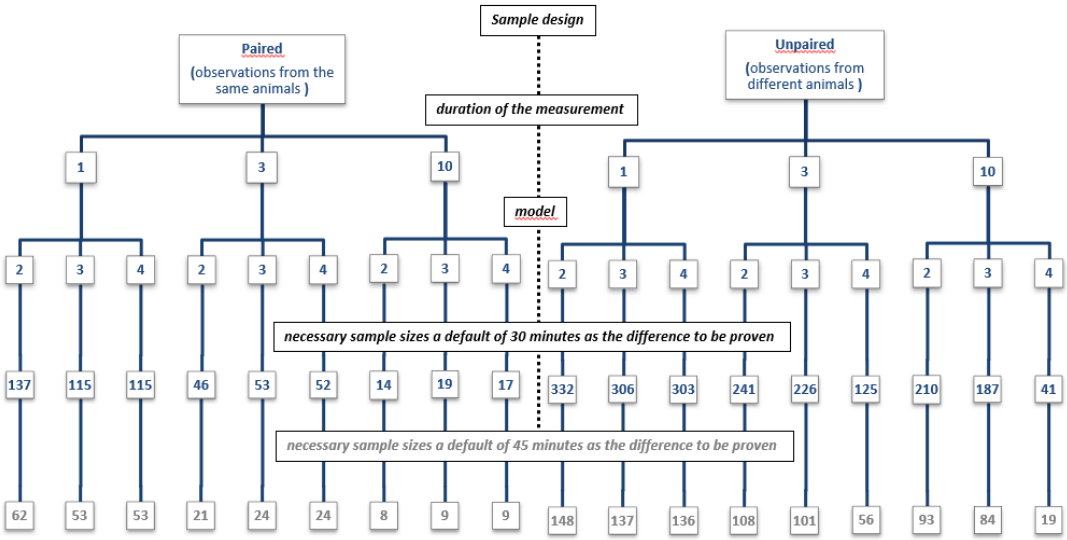

Figure 2: Sample sizes needed for studies on dairy cow lying behavior with a power of 80% and 30 or 45 minutes as the time difference to be proven for Models 2, 3, and 4

Influence of the measurement period on the sample size

Figure 3 shows that increasing the number of measurement days per cow reduces the sample size required to reach the same power, and further that additional measuring days have decreasing marginal benefits. Beyond a measurement period of about 15 days, hardly any gain is seen.

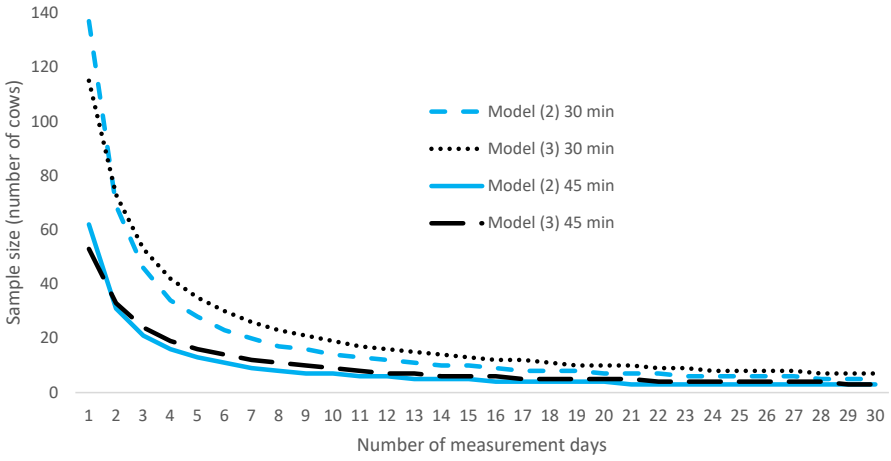

Figure 3: Relationship between increasing number of measurement days and sample size for treatment mean differences of 30 and 45 minutes with power > 0.8

Discussion

Results from this study agree with VASSEUR's (2012) finding that accounting for lactation stage and parity in experiments on the daily lying time of dairy cows allows the required number of cows in the sample to be reduced. Our results (Model 1) indicate that not accounting for these cow-specific factors necessitates much larger sample sizes. They also showed that even with a simple random sample, lactation stage and parity can be taken into account by a model.

Both Vasseur (2012) and Ito et al. (2009) investigated reducing the measurement period to one day. Their studies focus on the correlation of the estimated lying time per cow at full and at assumed reduced measurement period. In contrast, the present study estimates the minimum sample sizes needed for a significance test to detect a given mean treatment difference to be proven with given variances and power. Results are in agreement with Ito et al. (2009) and showed that with measurements on the same 30 cows per treatment over three measurement days (hence paired samples), a difference considered relevant of 30 and 45 minutes can be proven to be significant. Similar accuracy can be achieved with fewer than 30 animals if the measurement period is extended.

Note that the estimated variance-covariance structure used herein is only an estimate of the true variance-covariance structure, so it is subject to error. This leads to overall smaller or larger sample sizes when the variance components are in truth smaller or larger. Moreover, the structure is based on three consecutive single-day measurements. From a technical point of view, it can be assumed that lie times of adjacent days could be both positively and negatively correlated. Therefore, no information about the correlation of measurements more than three days apart was available in the data, meaning it was extrapolated. In the current study, Models 3 and 4 assumed a positive correlation between consecutive measuring days. Therefore, a decreasing correlation with increasing time difference was assumed as this led to a better model fit. Additionally, this decrease follows a functional relationship (here a first order autoregressive function).

There are many other variance functions that can model a decrease in correlation with time. To keep the number of models described manageable, only two commonly used models (Smith et al. 2001) were used in this study, the compound symmetry and the first order autoregressive variance covariance structure. Autocorrelation parameter estimates were found to be close to zero, so that measurements a few days apart were nearly uncorrelated. However, if the autocorrelation is in fact larger, the results from this study underestimate the necessary sample size.

In paired samples, observations from the same animals are used to calculate the mean differences. Therefore, the animal effects cancel out in the difference calculation. The omission of the animal variance in the variance of a difference results in smaller required sample sizes. For unpaired samples, the variance of the animals results in larger sample sizes compared to paired samples. In the current simulation, the variance component estimates used for the variance of animals could include variability due to lactation stage, parity, or other intrinsic factors that were not explained by the subgroup means. In that case, only a part of the animal variance would be omitted in a paired sample.

The benefit of additional consecutive measurement days is reduced by a positive autocorrelation, as in this case consecutive days contain similar information. Autocorrelation allows predicting some part of the variance seen in lying times on a given day by those of previous days. Furthermore, the sample sizes estimated herein apply to paired samples when using a randomized experimental design, such as a cross-over design. In these designs, lying time is measured over successive periods and treatment means are adjusted by adjusting for period effects. Lying time variability resulting from changes in extrinsic factors between periods may reduce the accuracy of pairwise comparisons. In such cases, the required sample size would be larger than determined in this study.

In the case of research carried out as before-and-after studies, for example in the context of on-farm research, it must be realized that no true replicates exist. The reason for before-and-after comparisons is that it is rarely possible to work with control groups and randomized treatments on working dairy farms. However, the applicability of such studies is limited.

The use of pedometer-based measurement technology to determine lying times for individual animals is associated with costs and effort; the purchase of the pedometers (per pedometer approx. € 500) involves the highest one-off cost and the travel cost to the farms the greatest expenditure of time. For on-farm research, pedometer availability and travel time can be limiting. In that case, an efficient design is one that uses as few pedometers with as few trips to the farm as possible. For example, 14 cows over a measurement period of ten days can achieve accuracy comparable to the 30 cows over three measurement days recommended by Ito et al. (2009). While 140 instead of 90 measurements are performed, the investment in measurement equipment and the effort for the one-time attachment and removal of the pedometers are cut by more than half, while the travel time is unchanged. The results of the present study thus enable a more efficient implementation of on-farm research with pedometer-based measurement technology on lying behavior in dairy cows.

Conclusions

If the intrinsic influencing factors of lactation period and parity are taken into account in studies on the lying period of dairy cows using pedometer-based measurement technology, the study effort can be reduced while maintaining the same quality. Furthermore, a reduction in the sample size can be compensated for by an increase in the period of the measurement, which makes it possible to carry out on-farm research more efficiently.

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