The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK
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Validation of scan sampling techniques for behavioural observations of pastured lambs

AN Pullin[†], MD Pairis-Garcia*[†], MR Campler[†] and KL Proudfoot[‡]

[†] Department of Animal Sciences, The Ohio State University, 2029 Fyffe Road, Columbus, Ohio 43210, USA
[‡] Veterinary Preventive Medicine, The Ohio State University, 1920 Coffey Road, Columbus, Ohio 43210, USA
* Contact for correspondence and requests for reprints: pairis-garcia.1@osu.edu

Abstract

*The study of farm animal behaviour is a critical tool for assessing animal welfare. Collecting behavioural data with continuous sampling or short scan sampling intervals (eg every 60th second) is considered ideal as this provides the most complete and accurate dataset; however, these methods are also time and labour intensive. Longer sampling intervals provide an alternative in order to increase efficiency, but these require validation to ensure accurate estimation of the data. This study aims to validate scan sampling intervals for lambs (*Ovis aries*) housed on pasture. Grazing, lying, standing, drinking, locomotion, and mineral consumption were evaluated from six pens of crossbred lambs (six lambs per pen) for 15 h. Data from 1-min instantaneous scan sampling were compared with data from instantaneous scan sampling intervals of 5, 10, 15, and 20 min in two statistical tests: generalised linear mixed model and regression analysis. Using the mixed model, the percentage of time each behaviour was performed did not differ amongst sampling intervals for all behaviours except grazing, which was statistically different at 20-min intervals. Using regression analysis, lying and grazing estimations were accurate up to 20-min intervals, and standing was accurate at 10- and 20-min intervals only. Locomotion, mineral consumption, and drinking demonstrated poor associations for all tested intervals. The results from this study suggest that a 10-min instantaneous scan sampling interval will accurately estimate lying, grazing, and standing behaviour for lambs on pasture. This validation will assist with the efficiency of future data collection in lamb behaviour and welfare research.*

Keywords: *animal welfare, lamb, pasture, scan sampling, sheep, validation*

Introduction

The study of farm animal behaviour is considered an integral parameter for assessing animal welfare (Gonyou 1994). Understanding the behavioural needs and preferences of farm animal species provides the basis for scientists to investigate the impact of animal management strategies on behavioural deviations and overall welfare of that individual or group (Gonyou 1994). To date, the most common methods to evaluate behaviour in livestock species include continuous or scan sampling. Continuous sampling is considered the gold standard for collecting behavioural data as it provides the most complete and accurate dataset (Lehner 1992); however, this method is time and labour intensive, particularly in studies with large sample sizes and a high number of behaviours recorded, and may not be feasible due to technological or logistical limitations. To avoid this problem, researchers often rely on scan sampling methodology by recording behaviours at selected time-points within a sample period and estimating a proportion of time the animal spent performing a specific behaviour (Martin & Bateson 2007). In particular, short scan sampling intervals (eg every 60th second) are especially similar to continuous observation for certain behaviours (Mitlöhner *et al* 2001; Miller-Cushon & DeVries 2011). However, even a short scan interval can prove to be inefficient and identifying a longer, appropriate scan sample technique that provides accurate data in an efficient time-period could be subjective. Therefore, recent research has focused on validating scan sampling techniques among a variety of farm animal species, including laying hens (Daigle & Siegford 2014), broiler chickens (Kristensen *et al* 2007), dairy calves (Miller-Cushon & DeVries 2011), dairy cows (Endres *et al* 2005; Ledgerwood *et al* 2010; Kitts *et al* 2011), feedlot cattle (Mitlöhner *et al* 2001), and pigs (Arnold-Meeks & McGlone 1986; Whalin *et al* 2016). Despite these recent publications, the authors are unaware of research validating scan sampling techniques for lambs in a pasture setting.

In the United States, over 60% of sheep flocks are primarily managed on pasture (ie any fenced area specifically cultivated to raise forage or browse; USDA 2012). This trend is similar internationally with the majority of sheep raised in either pasture or rangeland systems (Nowak *et al* 2008). Therefore, most sheep behavioural research has been conducted on pasture systems, including work evaluating behavioural deviations due to influences of stocking density (Lin *et al* 2011), flock dynamics (Bojkovski *et al* 2014),

Table 1 Behavioural ethogram.

Behaviour	Description
Lying	At least 50% of the stomach or side in contact with ground; body not supported by all four legs
Grazing	Standing or walking with muzzle close to the grass (ie head is below shoulders)
Standing	Body supported by all four legs not in motion. Head is above shoulders
Locomotion	Body supported by four legs while in motion; excludes standing or walking while muzzle is in close contact with grass (ie grazing)
Mineral consumption	Standing with muzzle inside mineral feeder
Drinking	Standing with muzzle close to water (ie head is below shoulders)
Out of view	Lamb behaviour is unable to be identified because lamb is in blind spot of camera, blocked by another lamb or ewe, or head is not visible

restricted grazing time (Chen *et al* 2013), forage preferences (Villalba *et al* 2011), and mother-young bond (Dwyer & Lawrence 1999). These studies utilised scan sampling methodology to collect behavioural data for sheep on pasture (eg 1-min, 3-min, and 5-min intervals), but a validated technique for the chosen sampling interval was not referenced. As behaviour is a critical tool to evaluate animal welfare, refining behavioural recording methodology for lambs housed on a pasture system is critical for future sheep research. Therefore, the objective of this study was to validate the accuracy of four different instantaneous scan sampling intervals (ie 5-min, 10-min, 15-min, and 20-min intervals) when compared to a 1-min instantaneous scan sampling interval for lambs housed on pasture. In this case, a 1-min instantaneous scan sampling technique was chosen for comparison due to its demonstrated similarity to continuous sampling in ruminant species (Mitlöhner *et al* 2001; Miller-Cushon & DeVries 2011) and the inability to collect continuous data from the video recordings.

Materials and methods

The Ohio State University Institutional Animal Care and Use Committee approved the protocol for this study. The animals were cared for in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.

Study animals and housing

The study was conducted at the Ohio Agricultural Research and Development Center Sheep Unit in Wooster, Ohio, USA (40º 43'44.71 N, 81º 54'4.25'W) in July 2015. Animals were housed on established pasture dominant in fescue forage, which utilised rotational grazing with an average stocking density of 26,265 kg bodyweight per hectare.

Thirty-six 60-day old Hampshire × Dorset and Suffolk × Dorset crossbred twin lambs (*Ovis aries*) (17.7 [± 2.0] kg) were blocked by sex and bodyweight and randomly assigned to one of six pastures with six lambs per pasture. Animals had *ad libitum* access to water and minerals throughout the trial (VitaFerm Sheep Mineral, BioZyme® Inc, St Joseph, Missouri, USA).

Behavioural measurements

Behaviour was recorded with one of six colour wireless outdoor IP video cameras per pasture (Foscam, Model F19805P, Houston, Texas, USA) recording at 30 frames per second. Each camera was positioned centrally in front of the pasture at a height of 2.4 m from the ground. All video was captured digitally utilising portable laptops with external USB hard drives. Video output was viewed during recording with Foscam software (V4.1) to ensure picture clarity and camera positioning prior to the behavioural recording session. Video was recorded continuously for 15 h (0600–2100h) in five pastures and for 12 h (0600–1800h) in one pasture (n = 6 pastures; 87 total hours). Behavioural data (Table 1) were collected using a 1-min instantaneous scan sampling technique (ie every 60th second; Altmann 1974; Martin & Bateson 2007) for all animals in each pasture by one trained observer using Windows Media Player (Version 12, Microsoft, Redmond, Washington, USA). Due to video image quality, it was not possible to individually identify animals at all times for continuous focal animal sampling. As such, a 1-min instantaneous scan sampling technique was chosen as the standard for comparison, and previous validation literature has demonstrated that 1-min scan intervals are similar to continuous observation in ruminant animals (Mitlöhner *et al* 2001; Miller-Cushon & DeVries 2011).

Statistical analysis

The experimental unit in this study was the pasture (n = 6). Two statistical methods were used to assess the sampling intervals: generalised linear mixed model and regression analysis. All observations from the 1-min instantaneous scan sampling technique for each behaviour in each pasture were summed, then divided by the total possible observations (ie 15 h × 60 min h^{-1} × 6 lambs = 5,400 total observations) to create one percentage of time spent performing each behaviour per pasture. To calculate percentages for each sampling interval (5-min, 10-min, 15-min, 20-min), data were extracted from the 1-min dataset every fifth, 10th, 15th, and 20th min. Differences in the percentage of time each behaviour was performed between the five different instantaneous scan sampling techniques (1-min, 5-min, 10-min, 15-min, 20-min) were identified using a Generalised Linear Mixed Model (PROC GLIMMIX) in SAS version 9.4 (SAS Institute Inc, Cary, NC, USA). The model included sampling technique as a fixed effect and pen as a random effect. The means for each sampling technique were compared to the 1-min interval using contrast statements. A sampling interval was considered adequate if it was not statistically different from the 1-min interval.

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Table 2 Least square means and standard errors for percentages of behaviour performed by six pastures of cross-bred lambs as measured by different sampling techniques (values are percentage of total duration for 15 h).

	Scan sampling method						
	1-min	**5-min**	**10-min**	**15-min**	**20-min**	**SEM**	***P*-value**
Behaviour							
Lying	35.3	35.4	35.6	35.9	35.6	0.3	$P > 0.05$
Grazing	39.3	38.9	38.1	39.0	37.0*	0.5	$P < 0.01$
Standing	6.3	6.3	6.7	6.4	6.7	0.4	$P > 0.05$
Locomotion	1.2	1.5	1.1	1.6	1.1	0.5	$P > 0.05$
Mineral	1.0	0.9	0.9	0.8	0.9	0.2	$P > 0.05$
Drinking	0.1	0.1	0.1	0.2	0.1	0.1	$P > 0.05$
Out of view	17.0	17.2	17.5	16.8	18.0	0.6	$P > 0.05$

* Indicates statistical difference ($P < 0.05$) between the results from the 1-min instantaneous scan sampling method and the tested interval. *P*-value represents overall *P*-value of the model.

A regression analysis was conducted to identify accuracy and bias of the behavioural values from each sampling technique (5-min, 10-min, 15-min, 20-min) when compared to the 1-min instantaneous scan sample data using linear regression (PROC REG) in SAS version 9.4 (SAS Institute, Cary, NC, USA). A sampling interval was considered to accurately estimate the behaviour if the following criteria were met: $R^2 \geq 0.9$, slope not statistically different from 1 ($P > 0.05$), and intercept not statistically different from 0 ($P > 0.05$; Ledgerwood *et al* 2010). The combination of these values reflects the strength of association (R^2), linear relationship (slope), and over- or under-estimation of the duration values of each behaviour (intercept; Ledgerwood *et al* 2010; Daigle & Siegford 2014).

Results

Differences in the mean percentage of time each behaviour was performed among the various sampling intervals is shown in Table 2. Values from the four intervals assessed did not differ ($P > 0.05$) from the values obtained from the 1-min sampling technique for all behaviours except grazing. The percentage of time estimated for grazing at the 20-min interval was statistically different from the 1-min interval ($P = 0.008$).

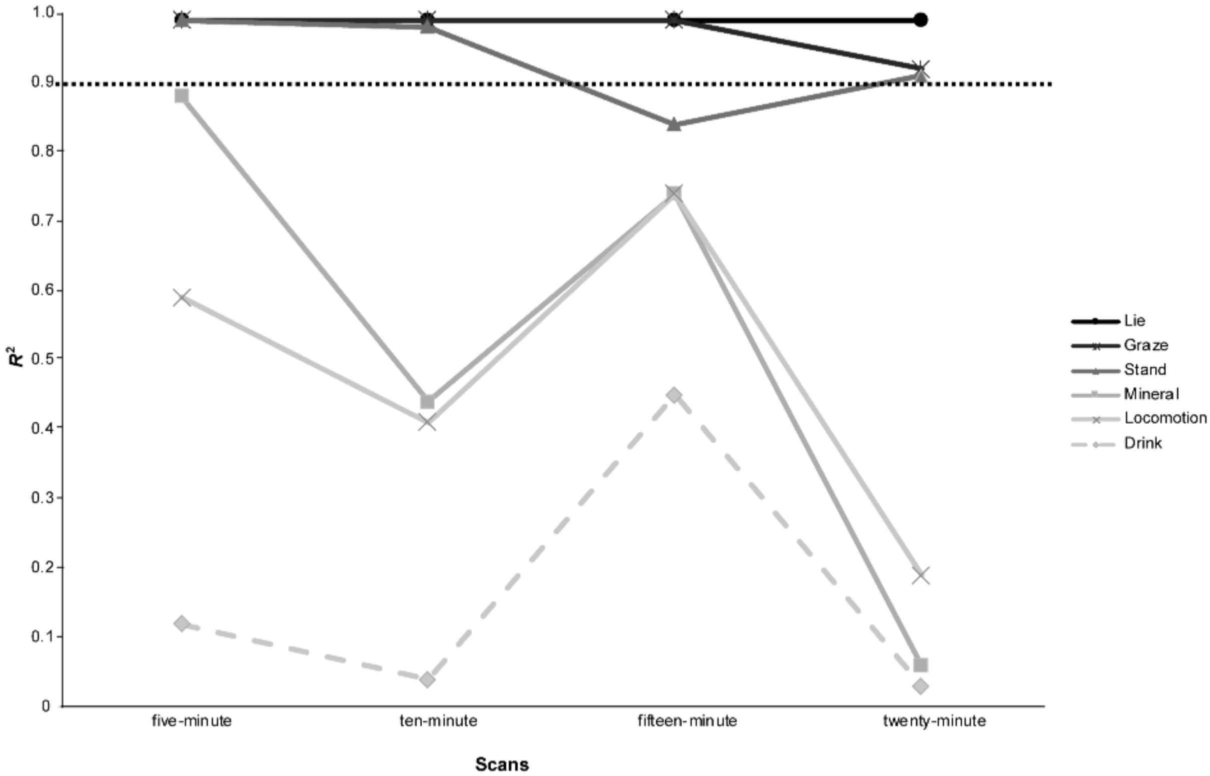
Using regression, the relationship between each sampling interval assessed compared to the 1-min sampling technique is illustrated in Figure 1 for all behaviours. Both lying and grazing behaviours met all three criteria (R^2, slope, and intercept) at 5, 10, 15, and 20-min instantaneous scan sampling intervals. Standing behaviour met only R^2 criteria at the 5-min interval (slope = 1.22; $P = 0.027$; intercept = –1.31; $P = 0.037$) but met all three criteria at 10 and 20-min intervals. Mineral consumption, locomotion, and drinking did not meet the R^2 criteria, but did meet slope and intercept criteria, for all sampling intervals tested in this study.

Discussion

The objective of this study was to determine the accuracy of four different scan sampling intervals (ie 5, 10, 15, and 20-min intervals) when compared to behavioural estimates collected using 1-min instantaneous scan sampling for lambs in a pasture setting. Two statistical methods were utilised to validate the sampling intervals: generalised linear mixed model and regression analysis.

Using a generalised linear mixed model, the comparison of mean percentages of behaviour yielded no differences between sampling techniques for all behaviours except grazing. The percentage of time that lambs spent for standing, lying, locomotion, mineral consumption, and drinking had similar estimates across all sampling intervals up to 20-min. The percentage of time that grazing behaviour was performed had similar estimates across sampling intervals up to 15-min. The results from this statistical test alone prompt a recommendation of 15-min instantaneous scan sampling to retrieve similar values compared to a 1-min sampling technique for all tested behaviours. These results are supported by previous methodologies utilising 15-min scan intervals in grazing cattle behaviour research (Senft *et al* 1985; Hart *et al* 1993), and they are similar to other publications that have utilised a generalised linear mixed model to validate sampling intervals for standing and feeding behaviours at 15-min scans for feedlot cattle (Mitlöhner *et al* 2001) and sows (Whalin *et al* 2016). However, these results should be viewed with caution, as the standard error in the model was high, particularly for behaviours that were performed at low frequency (locomotion, mineral feeding, drinking). Thus, the sample size used in this study (n = 6) may have been too small to detect real differences between the 1-min and other sampling techniques for these variables.

Figure 1



Strength of association (R^2) between the percentage of time each behaviour was performed using 1-min instantaneous scan sampling compared to 5, 10, 15 and 20-min scan intervals. A sampling interval is considered to have a strong association with the 1-min data if R^2 is $\geq$ 0.9, which is represented by the dotted line at 0.9 R^2.

Using a regression analysis, accuracy and bias could be validated regarding the percentage of time each behaviour was performed between different sampling techniques when compared to the 1-min scan sample. Both lying and grazing behaviours met all three criteria up to 20-min scans, whereas standing behaviour met all three criteria at 10 and 20-min scans only. Standing time (not grazing) had a high R^2 for 5-min, but the slope was not demonstrative of a linear relationship and the negative intercept value indicates that data were underestimated at this interval. It is unclear why standing time did not meet all three criteria for 5 and 15-min scans; a possible explanation is the presence of an outlier (lambs in one pasture had almost double the amount of standing time than all other pastures in the 15-min scans), however, there was no explanation for this outlier that warranted its removal. Nonetheless, the results from the regression analysis suggest that 20-min scan intervals would accurately estimate lying, grazing, and standing behaviours for lambs housed on pasture-based systems. This suggested interval is longer than findings from previous validation studies utilising only correlation coefficients, which recommend 10-min scan sampling intervals for feeding behaviour in lactating cows (Endres *et al* 2005) and dairy heifers (Kitts *et al* 2011), and utilising regression criteria to recommend 5-min scan sampling intervals for calf feeding behaviour (Miller-Cushon & DeVries 2011). The difference in findings between our study and others may be due to our animals being in an extensive environment as opposed to a stall, where sheep reportedly perform long durations of grazing and idling for the majority of the day (1.6 h grazing bouts and 28.8 min idling bouts for 61.2 and 23.1% of a 14-h day, respectively; Pokorná *et al* 2013). If a behavioural bout is long in duration, then longer scan sampling intervals are able to be utilised to report accurate data. Other differences that may arise between our studies and others is that our behavioural collection utilised the scan of a group as opposed to a focal animal. Consequently, individual animal differences do not contribute to variation in our data that may have resulted in a more conservative time interval.

Of the six behaviours tested in this study, lying, grazing, and standing were the only ones that met all three criteria in the regression analysis for at least one of the intervals tested. This is likely due to these three behaviours collectively representing 80% of the daily time budget and thus being conspicuous events, which is a characteristic recommended by Martin and Bateson (2007) for instantaneous sampling. On the other hand, behaviours, such as drinking, mineral consumption and locomotion, collectively made up 2% of total time budget for pastured lambs. These behaviours had the largest standard errors relative to the estimated value in the generalised linear mixed model. In addition, none of these three behaviours met the R^2 criteria for the regression analysis, indicating all tested intervals had a poor association when compared with the 1-min standard. Martin and

Bateson (2007) state that instantaneous sampling is not appropriate for behaviours that are short in duration or are rare behaviour patterns, which may be characteristic of drinking, mineral consumption, and locomotion.

Sheep provided *ad libitum* water spent 0.2% of a 24-h day drinking (Al-Ramamneh *et al* 2012), which is similar to our results of 0.1%. Additionally, free-choice mineral intake in grazing ruminants is highly variable amongst individuals in terms of intake and number of visits, and 40% of visits reportedly occur in the evening after 2000h (Tait & Fisher 1996). In this case, our behavioural observations did not account for individual variation and occurred between 0600–2100h for five pens and 0600 and 1800h for one pen, which may not have been optimal for collecting mineral consumption behavioural data. Lastly, locomotion that does not include grazing behaviour in pastured sheep can vary from 1.8 to 2.8% of a 16-h day (Lin *et al* 2011), which is similar to our results of 1.2% within a 15-h day. The overall performance of these behaviours in the time budget indicates infrequent and rare behavioural patterns, which may be inappropriate for the instantaneous scan sampling intervals tested in this study. The association (R^2) of these behaviours drops at 10-min intervals, rises at 15-min intervals, and then drops again at 20-min intervals (Figure 1). An explanation for this is that it is likely a statistical anomaly given the overall disassociation with the 1-min data at all tested intervals. The results for drinking, mineral consumption, and locomotion should be interpreted with caution as there were outliers and, due to the small sample size, this caused inconsistencies in the data. Additionally, they were less normally distributed when the instantaneous scan sampling interval became longer. If an instantaneous scan sampling interval is longer in duration than the bouts that a behaviour is performed, then the accuracy of estimating that behaviour within the time budget will be poor (Miller-Cushon & DeVries 2011). Therefore, a shorter scan interval (eg 1-min) or continuous observation would be recommended to provide greater accuracy in estimating infrequent behaviours, such as drinking, mineral consumption, and locomotion.

When considering both statistical tests, the generalised linear mixed model recommends a 15-min scan interval for all behaviours and a regression analysis recommends a 20-min scan interval for three behaviours (eg lying, grazing, and standing). Combining the statistical tests provides the most robust analysis of intervals for the validation. Since standing behaviour is not accurate for a 15-min interval in the regression analysis, we ultimately conclude that a 10-min scan interval is supported by both tests to accurately estimate lying, grazing, and standing behaviours of lambs kept on pasture. Based on these results, evaluating lamb behaviour with an emphasis on grazing and activity patterns can be accomplished utilising 10-min scans as opposed to the more conservative scanning methodologies conducted in previous sheep research (3-min scan: Lin *et al* 2011; Chen *et al* 2013; 5-min scan: Key & MacIver 1980; Bojkovski *et al* 2014).

Animal welfare implications

The present study validates a 10-min scan sampling interval that can be used as a tool for collecting behavioural data for lambs in welfare-related research, particularly for the most common type of environment that lambs are housed on. This validated interval is higher than more conservative scan sampling intervals utilised in recent sheep research, which will improve the efficiency and accuracy of collecting behavioural data to contribute to future sheep welfare literature.

Conclusion

Ultimately the scan sampling methodology is determined by the researcher based on the behaviours of interest, logistics, and statistical approach to validating the sampling technique. The results from this study suggest that 10-min scan intervals can accurately estimate lying, grazing, and standing behaviours of lambs on pasture.

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