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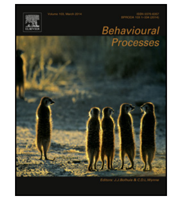

When continuous observations just won't do: Developing accurate and efficient sampling strategies for the laying hen

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ABSTRACT

Continuous observation is the most accurate way to determine animals' actual time budget and can provide a 'gold standard' representation of resource use, behavior frequency, and duration. Continuous observation is useful for capturing behaviors that are of short duration or occur infrequently. However, collecting continuous data is labor intensive and time consuming, making multiple individual or long-term data collection difficult. Six non-cage laying hens were video recorded for 15 h and behavioral data collected every 2 s were compared with data collected using scan sampling intervals of 5, 10, 15, 30, and 60 min and subsamples of 2 second observations performed for 10 min every 30 min, 15 min every 1 h, 30 min every 1.5 h, and 15 min every 2 h. Three statistical approaches were used to provide a comprehensive analysis to examine the quality of the data obtained via different sampling methods. General linear mixed models identified how the time budget from the sampling techniques differed from continuous observation. Correlation analysis identified how strongly results from the sampling techniques were associated with those from continuous observation. Regression analysis identified how well the results from the sampling techniques were associated with those from continuous observation, changes in magnitude, and whether a sampling technique had bias. Static behaviors were well represented with scan and time sampling techniques, while dynamic behaviors were best represented with time sampling techniques. Methods for identifying an appropriate sampling strategy based upon the type of behavior of interest are outlined and results for non-caged laying hens are presented.

Behavioral data collection is a type of assay. All assays require validation and refinement to meet specific objectives before results can be trusted as accurate; therefore, data collection protocols using behavioral observations should be subjected to similar rigorous scientific validation (Mitlohner et al., 2001). Determining the proper sampling interval (the length of time between consecutive observation sessions) and sampling duration (the length of time across which behavioral observations are recorded) for collecting behavioral data is highly dependent on the behavior of interest, and interval appropriateness is also dependent on the total duration of the observation period (Altmann, 1974). Different behaviors may not need to be collected at the same frequency since they may be performed for different durations of time. On one hand, if the sampling interval is shorter than the typical duration of the behavior, the same occurrence of a behavior may be recorded multiple times. Conversely, a sampling interval may be too large to capture the presence of the behavior, thus omitting it from the behavioral record.

Continuous observation (recording all events and states as they occur during a fixed period of time) is the most accurate way of determining animals' time budgets, and can provide a nearly perfect representation of resource use and behavior frequency and duration (Altmann, 1974). In particular, continuous observation can capture behaviors that are of very short duration, occur infrequently or are circadian, or are dynamic and difficult to identify from a still image (e.g., walking vs. standing). However, continuous observation is labor intensive and time consuming, making it difficult to collect data on multiple individuals or over long time spans. Further, statistically analyzing a continuous dataset can be challenging and mathematically difficult to support. Therefore, identifying behavioral sampling techniques for dynamic, static, or infrequent behaviors that will provide an accurate representation of data that would be collected by continuous observation is important for expedient and accurate behavioral data collection.

Data collection strategies can be influenced by factors including: individual circadian rhythms (e.g., dust bathing and egg laying), social interactions (e.g., aggression, copulation, grooming), and animal physiology (e.g., defecation, hunger, illness) (Abe et al., 1979; Vestergaard et al., 1997). Furthermore, sampling strategies may

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need to be assessed for species. Sampling strategies for behavioral data collection in domestic animals have been investigated for farmed foxes (Jauhiainen and Korhonen, 2005), young pigs (Arnold-Meeks and McGlone, 1986), feedlot cattle (Mitlohner et al., 2001), and female broiler chickens (Kristensen et al., 2007), yet no studies, to the authors' knowledge, have addressed sampling techniques for laying hens. However, identifying an appropriate sampling technique is not always a straightforward process.

D'Eath (2012a) highlights the complexity of identifying the impact of observer bias using multiple approaches to assess data from sow lameness scoring. Using sow lameness scoring, different statistical tests were used to identify how well two observers agreed in their score, whether observers were exhibiting a positive or negative bias with regard to the other observers, and whether observers were consistent in their scoring over time. More specifically, statistical investigations should identify how much different measures do differ (e.g., is there a bias? Is a behavior over or under-represented using this sampling technique), how strongly the two measures are associated (e.g., do the results from a continuous observation and a sampling technique increase or decrease at the same rate?), and how well they match or completely agree (D'Eath, 2012b). Even though this analysis was conducted for live sow lameness scoring, a similar approach can be applied to behavioral observations. These investigations can identify whether different sampling techniques are providing results that are similar to a continuous observation, whether the sampling techniques provide results that are biased, and whether the results from the sampling technique match the results of a continuous observation. Therefore, a comprehensive analysis should be used when identifying a sampling strategy to ensure that the strategy chosen is representative of the continuous sampling gold standard and the caveats of the selected strategy are understood.

A clear picture of the behavior of any one individual animal living either alone or within a group can be difficult to obtain. However, examining the response of individual animals is important to assessing their welfare, or quality of life, which must be done at an individual level. For example, egg-laying hens (*Gallus gallus domesticus*) are increasingly housed in larger groups in complex environments. Even though substantial previous research has been performed investigating the behavior and resource use of non-cage laying hens, the methodological approaches have varied widely (Table 1). Future behavioral data collection on these and other animals would benefit from a systematic investigation into the impact of sampling technique on the quality of the data obtained through different sampling techniques. Therefore, there is a need to address how sampling techniques are determined, what factors impact their ability to be more or less accurate and examine the impact of different sampling techniques on the representation of continuous observation.

By identifying the strengths and weaknesses of different sub-sampling techniques for different types of behaviors, researchers may be able to expedite data collection, understand what effects their sampling technique may have on interpretation of results, collect data that can be statistically analyzed, estimate tradeoffs between efficiency and accuracy, and have information that provides a good representation of continuous observation. The results of this study provide a framework for how to approach these unique problems using specific behavioral data from laying hens. Therefore, our objectives were to compare and validate instantaneous scan sampling and time sampling methods with continuous observation of behavior, using non-cage laying hens as a model, to identify efficient and accurate sampling techniques for static, dynamic, and infrequent behaviors.

1. Materials and methods

1.1. Animals and housing

Data were collected from laying hens housed in an experimental non-cage system at the Michigan State University Poultry Center. Prior to the start of the study, all protocols were submitted to and approved by the Michigan State University Institutional Animal Care and Use Committee. Three identical rooms (6 m × 4.5 m) at Michigan State University Poultry Teaching and Research Center were used. Each room was furnished in the same configuration with nest boxes, perches, tube feeders, and a water line with nipples. Sixteen nest boxes (each 0.4 m long × 0.3 m wide × 0.3 m high) in an 8 × 2 configuration were mounted 0.3 m above the ground on one wall. Perches consisted of a three-level wooden rail structure (with each rail 6 m long and ~5 cm in diameter with a flat top and rounded sides and bottom) and mounted over a 1 m × 6 m slatted area at a height of 0.53, 0.76, and 0.99 m from the ground. The perches were mounted to the wall at a slope of 45° with a 40 cm distance between each wooden rail. Room floors were covered with ~8 cm of wood

Table 1
Overview of different types of instantaneous scans (IS) and time sampling (TS) techniques utilized in previous research to assess behavior (B), location (L), aggression (A), and resource use (R) of commercially housed chickens.

Author	Sampling technique	Species	Parameters assessed
Estevez et al. (2002)	15 min TS	Laying hens	A
O'Connor et al. (2011)	15 min TS	Laying hens	A
Oden et al. (2000)	18, 20 min TS/day	Laying hens	A
Cordiner and Savory (2001)	30 min TS twice daily	Laying hens	A
Hughes et al. (1997)	60 min IS and 30 min TS	Laying hens	A and L
Webster (2000)	8 min IS	Laying hens	B
Webster and Hurnik (1994)	8 min IS	Laying hens	B
van Liere et al. (1990)	15 min IS	Laying hens	B
Shimmura et al. (2008)	10 min IS across 2 h 3×/day	Laying hens	B and R
Shimmura et al. (2007)	10 min scan for 4 h twice daily	Laying hens	B and R
Olsson and Keeling (2000)	2 min, 4 min, and 15 min IS	Laying hens	B and R
Tanaka and Hurnik (1992)	5 min IS	Laying hens	B and R
Albentosa and Cooper (2005)	60 min IS	Laying hens	Cage height preference
Keeling and Duncan (1991)	15 min IS	Laying hens	Flock activity
Widowski et al. (1992)	5 min IS	Broilers	L
Mallapur et al. (2009)	5 min TS for 1 h	Broilers	L
Leone and Estévez (2008)	1 min IS and 15 min TS	Broilers	L and A
Kristensen et al. (2007)	15 min IS	Broilers	L and B
Channing et al. (2001)	2 ISs/day	Laying hens	L and B
Donaldson and O'Connell (2012)	120 min IS	Laying hens	L and R
Cordiner and Savory (2001)	15 min and 30 s IS	Laying hens	R