Validation of Scan Sampling Techniques for Nursery Pig Feeder- and Nutritional Enrichment Use

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Summary and Implications

The objective of this study was to validate scan sample accuracy at various predetermined intervals relative to continuous observation of individual nursery pigs interacting with a feeder and nutritional enrichment. A total of 280 mixed-sex pigs, 19 to 24 days of age were randomly allocated to 28 pens. Pen was the experimental unit, and 7 pens were assigned to each of 4 treatments: (1) biscuit with maternal pheromone attractant (**MP**), (2) biscuit with sugary attractant (strawberry jam) (**JAM**), (3) biscuit with no attractant (positive control; **POS**), and (4) no biscuit (negative control; **NEG**). Each enrichment pen received 4 biscuits suspended from 2 ropes at the feeder for the first 7 days after weaning. The biscuit enrichments were added to the nursery pen at ~ the 15:00 hour on Day 0 and ~ the 08:30 hour on Days 1-6. Behavior observations were performed using continuous sampling of all nursery pigs in each pen during the first 30 minutes after biscuit placement on Days 0 to 6 (98 total hours, 3.5 hours/pen). The number of times each pig interacted with the feeder and biscuit was recorded as well as the duration of each interaction. These continuous observations were then used to create different data sets at varying sample times. The scan sample and continuous data were analyzed using the GLM procedure of SAS and behavioral frequency data were expressed as percentage of time (s) pigs were performing an identified behavior during the 30-minute observation periods. For enrichment use, there was no observed statistical difference between data collected using continuous observation, 10 second scans and 15 second scans when comparing continuous to 5 second scans, 10 second scans, 15 second scans and 30 second scans. For feeder use, there was no observed statistical difference between data collected using continuous observation and the 30 second scan sample when comparing continuous to 15 second scans, 30 second scans, 45 second scans and 60 second scans. In conclusion, 30 second scan sampling for feeder use and 10 second and 15 second scan sampling for enrichment use can be used, without being different to the continuous or "*gold standard*" methodology. These scan point validations are critical because they allow the observer to collect more total data in less time without giving up accuracy.

Introduction

Behavioral observations are a type of "assay" that is used to quantify animal biological responses. As with physiological measurements, behavioral observation methods should be selected based on the objectives of the study and validated. Behavior data includes *events*, which are relatively short in duration and are often counted as individual occurrences, and *states*, which are relatively long in duration. Furthermore, there are four types of sampling rules: *ad libitum*, focal animal, instantaneous scan and behavioral sampling. There are two recording rules, continuous and time sampling. Each sampling and recording rule have its advantages and challenges.

Continuous animal observation is considered the "*gold standard*", but typically results in a significant time commitment. Instead, if one can perform instantaneous scans of all animals of interest at validated pre-determined time points, and these data agree with the continuous methodology, then there is opportunity to collect accurate data faster. Therefore, the objective of this study was to validate scan sample accuracy at various predetermined intervals against continuous methodology for individual nursery pig interactions with a feeder and a biscuit enrichment.

Materials and Methods

All experimental procedures were approved by the Iowa State University Animal Care and Use Committee (IACUC#20-106).

Animals and housing

This experiment was conducted at the Iowa State University Swine Nutrition Farm (Ames, IA). A total of 280

mixed-sex pigs (Camborough 1050 X 337, PIC), 19 to 24 days of age (BW 5.8 ± 0.31 kg), were randomly allocated to 28 pens (10 pigs per pen). Each pen measured 2.4 m long x 1.2 m wide (~0.29 m2/pig), and pen floors were made of slatted PVC that opened to a manure pit below the pens. Each pen was equipped with a 4-hole, dry self-feeder measuring 0.61 m long and 2 nipple drinkers to provide pigs with ad libitum access to feed and water. Nursery room temperature was set at 30.5˚ C when the pigs arrived, and this was decreased on a schedule by approximately 0.25˚ C daily for 7 days post weaning. Pigs were individually identified using a livestock-safe marking stick to aid behavioral observations.

Environmental enrichment device

Six ingredients were used to make the biscuit: dried whey powder, corn starch, soybean oil, flour, sugar, and water. Ingredients were combined into a dough and stamped into 4 cm diameter cutouts with a 1 cm hole in the middle. Biscuits were baked at 190˚ C until golden brown. All biscuits were utilized in the nursery pens within 24 hours after baking. Two biscuits were threaded onto a 3 stranded, 0.5 cm diameter plain cotton rope, and the 2 biscuits were positioned on top of a 1 cm diameter flat washer (Figure 1). Each enrichment device was tied to the pen bars and secured in place with duct tape, so that the rope hung over the feeder and the biscuit hung at pig eye-level (Figure 2).

Figure 1. Enrichment device placement.

Figure 2. Nursery pig interacting with the nutritional enrichment and feeder.

Experimental design and treatment

The pen was the experimental unit. Pens were assigned to 1 of 4 treatments: (1) biscuit with maternal pheromone attractant (**MP**), (2) biscuit with sugary attractant (**JAM**), (3) biscuit with no attractant (positive control; **POS**), and (4) no biscuit (negative control; **NEG**). There were 7 pens allocated to each treatment. Each enrichment pen received 4 biscuits suspended from 2 ropes at the feeder for the first 7 days after weaning which is the "trial week". The biscuit enrichments were added to the nursery pen at ~ 15:00 hours on day 0 and ~ 8:30 hours on Days 1-6.

Behavioral data acquisition

Color video was continuously recorded in real time (30 fps) using cameras and a DVR Recorder. Cameras were mounted to the ceiling so that a single camera could capture images from 1 or 2 pens. The recording system was monitored daily to ensure proper video capture and view.

One observer (CS) was responsible for data collection. To control potential bias, the observer was masked to a variety of identifying factors on all video data, including treatment and pen number. Masking procedures involved assigning a random number to each 30-minute clip and presenting the video clips to the observer in a randomized sequence. One researcher (ES) with 2 years of behavioral research experience was responsible for observer training and served as the "*gold standard*".

Inter-observer reliability was calculated using an index of concordance, as a proportion of all agreements (A) and disagreements (D) in behavioral occurrences between observer and trainer, with the formula (A / [A+D]) * 100 ≥ 85%. Once the observer reached ≥ 85% reliability agreement with the trainer, data collection began. One randomly selected video clip was duplicated during data collection to confirm intra-observer reliability. The observer used a pre-determined ethogram (Table 1). Observations were performed using continuous sampling of all nursery pigs in the pen for the first 30 minutes after biscuit placement on Days 0 to 6 (98 total hours, 3.5 hours/pen).

Data calculations

All data (frequency and duration) of feeder and enrichment use were recorded in Google Sheets using continuous observations. The original continuous data was used to generate scan sampling data sets. For feeder use, 15-, 30-, 45-, and 60-second samples were created. For enrichment use, 5, 10, 15, and 20 second samples were created. These times were determined by watching a small portion of the video to gauge an estimate of frequency. This observation led to smaller sample times for the enrichment compared to feeder as the enrichment use was found to be shorter in duration.

The conversion of the continuous data to each scan sample was done in Google Sheets by generating the respective scan sample points based on the start time of the video clip. For example, 22:30:15 + 15 seconds = 22:30:30 = scan point 1 (Figure 3).

To generate the behavioral data set, scan sample points for feeder and enrichment use were created from the continuous data set. For example, does the generated time point 22:30:15 occur between the recorded start and end time of a behavior according to the reported continuous data?

Table 1. Ethogram for nursery pig feeder and enrichment use.

Behavior	Description
Feeder use	The head is <u>above</u> the feed pan and <u>down</u> in the feeder. As soon as the pig shifts attention to something besides the feeder, the behavior ends. If the pig takes its head out of the feeder for less than 3 seconds, but still has its attention focused on the feeder, the behavior continues. If 3+ seconds pass with the head not in the feeder, the behavior ends.
Enrichment use	Enrichment use begins when the pig touches the enrichment device with their snout. As soon as the pig shifts attention to something besides the biscuit, the behavior ends. If the pig stops touching the rope for less than 3 seconds, but still has its attention focused on the biscuit, the behavior continues. If 3+ seconds pass without touching the biscuit, the behavior ends. If the pig shifts their focus off the biscuit, the behavior ends. If the pig starts paying attention to/touching the other hanging biscuit, the behavior ends and a new one begins.

A less than or equal to equation was generated in Google Sheets to convert the continuous data into each of the scan points. This equation allowed researchers to input their scan point and the equation automatically checked if that point was found between or equal to the start or end point of an observed behavior. If the scan point was between or equal to the start or end point the equation generated a 1 for yes and a 0 for no. For example, scan point 22:30:30, between the start time 22:29:55 and end time 22:31:01, generates 1 for yes (Figure 4).

Statistical Analysis

All analyses were performed using the GLM procedure of SAS (SAS Inst. Inc. Carry, NC) software for parametric data analysis. All behavioral frequency data were expressed as percentage of total seconds in the 30-minute observation period and were subjected to a square root arcsine transformation process to achieve a normalized distribution. Transformed data for validating the behavioral scan sampling period was analyzed as a completely randomized design to achieve least squares means. The total number of possible interactions when watched continuously were compared against the percentage of time pigs were performing the behavior in scan sample for 5, 10, 15, and 30 second intervals for enrichment use and 15, 30, 45, and 60 seconds for feeder use. A P-value of ≤ 0.05 was considered significantly different

Results and Discussion

For nutritional enrichment use, there was no observed difference between data collected using continuous observation (~11% of observed time) and data collected using 10 second instantaneous scans (~11% of observed time) and data collected using 15 second instantaneous scans (~7% of observed time). Even though there was no statistical difference between continuous and 15 second scan, 10 second scans were considered more accurate because the least square means were closer to that of continuous data. However, nutritional enrichment use was over-reported with 5 second scans and under-reported with 30 second scan relative to continuous data.

For feeder use, there was no observed difference between data collected using continuous observation and the 30 second scan sample. The 15 second interval was found to overrepresent the number of times animals were using the feeder and the 45 and 60 second intervals under-reported the amount of times pig were using the feeder (Table 2).

In conclusion, 30 second scan sampling for feeder use and 10 second scan sampling for enrichment use can be used without being different to the continuous or "*gold standard*" methodology. These scan point validations are critical because they allow the observer to collect more total data in less time without giving up accuracy. These scan sampling methods are set to be used in further studies using this sample population. Furthermore, the overall validation process can be used to determine accurate time points for studies using different animals.

Table 2. Least squares mean, standard errors and *P*-values for percentages of interaction for nursery pigs[1] housed in 28 pens from day 0 to day 6 at the ISU swine nutrition farm under varying scan sample times.

Behavior (%)			Scanning time, (sec)			
	Continuous	5	10	15	30	*P*-values
Enrichment use[2]	10.60 ± 1.44^a	22.48 ± 1.44^b	11.21 ± 1.44^a	7.46 ± 1.44^a	3.77 ± 1.44^c	<0.001
			Scanning time, (sec)			*P*-values
Behavior (%)	Continuous	15	30	45	60	
Feeder use[2]	6.20 ± 0.70^a	12.78 ± 0.70^b	6.35 ± 0.70^a	4.23 ± 0.70^c	3.32 ± 0.70^c	<0.0001

[1] The pigs used in this study were Camborough (1050) X 337, PIC, Hendersonville, TN ranging in age from 19-24 days of age

[2] Twenty-eight pens were used separated between 4 treatments with 7 pens per treatment: biscuit with maternal pheromone attractant, biscuit with sugary attractant, biscuit with no attractant, and no biscuit

[a, b, c] Different superscripts within rows are different ($P \leq 0.05$)

Figure 3. Google Sheet equation showing the addition of 30 seconds to determine the scan point.

Figure 4. Google Sheet showing the less than or equal to/greater than or equal to equation.