Title: Validating behavioral sampling techniques for lame sows administered flunixin meglumine and meloxicam

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Abstract: Lameness poses a welfare challenge for pigs as it is associated with pain. Monitoring changes in behavior is a useful tool for recognizing illnesses in animals, including lameness. Lame sows spend more time lying down compared to non-lame animals, but there is currently no practical way of recording these changes in behavior. The objectives of this study were to determine if scan sampling could detect behavioral changes in sows induced lame, and detect changes in behavior associated with pain mitigation in sows induced lame. Lameness was induced in 12 multiparous, crossbred sows using a chemical synovitis model. Standing, sitting and lying behaviors were evaluated using continuous sampling, as well as 5, 10 and 15 min scans ('scan sampling') for 12 h/d (0600-1800) over a 5-day period. Three pain control treatments

were evaluated: flunixin meglumine, meloxicam, and sterile saline administered approximately 28 and 52 h after lameness induction. No differences were found in lying and standing behavior in saline treated sows for all sampling methods ($P = 0.99$). Regardless of sampling method, standing time decreased in the days following lameness induction compared to the day before induction ($P < 0.01$). After treatment with flunixin meglumine, sows increased their standing behavior, but this change was only detected using the continuous sampling method ($P < 0.04$). All sampling methods were able to detect standing behavior changes among sows treated with meloxicam ($P < 0.01$). Results suggest that scan samples of 15 min or less are effective in detecting most behavioral deviations in sows treated with meloxicam or not treated at all.

Keywords: scan sampling, lameness, behavior, swine, nonsteroidal anti-inflammatory drug

Introduction

The welfare of farm animals is a key issue for producers, consumers, researchers and veterinarians. Definitions of animal welfare usually incorporate aspects of good health, absence of negative affective states, and living conditions that promote natural behaviors (Farm Animal Welfare Council, 1979; Fraser et al., 1997). To ensure good animal welfare is achieved on farms, assessments and auditing programs have been used as an objective means to evaluate the quality of life of farm animals. Measurements for assessment include animal-, resource-, and protocol-based measures (Webster, 2005; Blokhuis, 2008). Although the inclusion of all three parameters provides a better-rounded understanding of farm animal welfare, animal-based measures are considered the only direct indicator of an animal's welfare.

Animal-based measures often include an evaluation of painful states. Animal pain is defined as an aversive sensory experience that changes the animal's physiology and behavior

(Molony and Kent, 1997). Pain is a clinically important condition that adversely affects an animal's quality of life and, when left unmitigated, can result in distress and detrimental effects to the animal's physical health and overall welfare (Hellebrekers, 2000).

Lameness, defined as deviation in gait, may be a significant source of pain in sows, depending on the severity, chronicity and etiology of the lameness (Pairis-Garcia et al., 2015a). Pain associated with lameness is not only an animal welfare concern, but can also have severe economic impacts to the producer. Approximately 15% of the sow inventory is involuntary culled due to lameness (Schenk et al., 2010). Based on a total U.S. sow inventory at 5.98 million, approximately 897,000 sows are culled due to lameness resulting in ~\$43 million industry loss per year (\$87.14 per hundredweight).

Lameness in swine has been most commonly evaluated on-farm using a 5-point subjective scoring system (D'Eath, 2012), though some studies have suggested using a more succinct scoring system to decrease observer variation and increase consistency over time (D'Eath, 2012). Recognizing that scoring systems can be subjective in nature, developing and validating objective tools for lameness assessment are still required. A non-invasive tool to assess lameness includes visual observation of sow behavior. Sows spend nearly 80% of their time lying down (Elmore et al., 2010; Tuytens et al., 2008) and deviations in lying behavior may be a key indicator of discomfort or pain (Elmore et al., 2010). When lameness has been induced, sows spent less time standing and more time lying for up to 72 h after induction compared to non-lame sows (Pairis-Garcia et al., 2015a).

Behaviors can be observed and scored using several sampling and recording methodologies. Sampling methods include continuous (recording the animal constantly over time), and scan sampling (recording behavior at specific intervals). Although the continuous

sampling method provides the most accurate behavioral repertoire of an individual, it is a labor intensive and time-consuming process. No research to our knowledge has determined if scan sampling methods can be used to detect changes in, behavior associated with lameness, nor if these methods are sensitive, enough to detect behavioral changes associated with pain mitigation. Therefore, the objectives of our study were to determine 1) the ability of three scan sampling methods to detect the same changes in standing, lying and sitting behaviors in sows induced lame compared to a continuous sampling method, and 2) evaluate the ability of three scan sampling methods to detect behavioral changes associated with pain mitigation in sows induced lame.

Materials and Methods

The protocol for this study was approved by the Iowa State University Animal Care and Use Committee. The animals were cared for in accordance with the United States Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, 8th edition. This work was performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) at Iowa State University College of Veterinary Medicine.

Animals and housing

Twelve multiparous (mean parity 6.0; range 2.0-9.0), non-pregnant, crossbred Newsham maternal cull sows were obtained from a commercial farm in Iowa (BW 241.4 ± 15.5 kg; mean $\pm$ SD). All sows underwent a physical examination (i.e., an evaluation of integument, cardiovascular, and respiratory systems) and a lameness evaluation prior to enrollment in the study by a trained veterinarian with expertise in sow lameness (for a detailed description of this scoring system see Pairis-Garcia et al., 2014; 2015a). Lameness was induced in each sow three times throughout the study; hence, the physical examination and walking lameness evaluation

where each sow walked at her own pace over a non-slip mat measuring 4.3 m in length were conducted between each lameness induction. No sow was induced if she demonstrated any observable lameness from previous inductions; therefore, all sows had no observable signs of lameness at the start of the induction. No sow on trial demonstrated any observable residual lameness at the time of induction; therefore, all sows were induced lame three times.

To avoid confounding lameness with injury resulting from aggression, each sow was housed in an individual pen; however, sows could see, smell, hear and have nose-to-nose contact with other sows. Each pen measured 3.7 m length x 1.4 m width x 1.2 m height and had a solid concrete floor with a rubber mat (2.4 m length x 0.02 m height x 1.4 m width). Metal fences (1.2 m height x 0.76 m width) were affixed to the end of each pen. Each pen was provided with environmental enrichment including chains and/or plastic toys attached to the pen gates.

Sows were provided *ad libitum* access to water via one nipple and hand-fed a custom mixed diet of 14.8% crude protein total mixed ration composed of ground corn, soybeans, and nutrients formulated according to Swine National Research Council guidelines (NRC, 2012) to meet or exceed non-gestating sow nutrient requirements. Matrix[®] (FDA approved; 0.22% Altrenogest; Intervet/Schering-Plough, Milsboro, Delaware, United States of America- Dose: 6.8 ml-15 mg) was added to one kg of feed daily to prevent estrus initiation.

Lameness induction

To induce sows, they were snared and then anesthetized intramuscularly using Xylazine (4.4 mg/kg) (Anased[®], Lloyd Laboratories, Shenandoah, Iowa, United States of America), Ketamine HCl (2.2 mg/kg) (Ketaset[®], Wyeth, Madison, New Jersey, United States of America), and Tiletamine HCl and Zolazepam HCl (4.4 mg/kg) used in combination (Telazol[®], Wyeth, Madison, New Jersey, United States of America). Once anesthetized, the sow's claw was cleaned

with mild soap and water, and then scrubbed for 5 min with iodine based surgical solution (Operand[®], Aplicare Inc., Branford, Connecticut, United States of America) using 4 x 4 sterile gauze pads. To rinse, 70% isopropyl alcohol was used on the foot until no surgical solution remained. Approximately 10 min after anesthetized, sows were positioned in lateral recumbency and injected with 15mg of amphotericin B (X-gen Pharmaceuticals, Inc., Big Flats, New York, United States of America) into the intraarticular space of the left or right rear medial distal interphalangeal joint (Karriker et al., 2013). During the first round, sows were randomly assigned to either the left or right rear leg. In the second round, the opposite rear leg was induced, and in the final round the original leg was re-induced (Pairis-Garcia et al., 2015a).

Every 15 min while anesthetized the heart rate, respiratory rate, and rectal temperature were monitored until sows returned to a sternal position unaided (Karriker et al., 2013).

Treatments

Twelve sows were randomly assigned to three blocks (4 sows per block), and within each block sows were randomly allocated to one of three treatments for round one: 1) Flunixin meglumine (FM; 2.2 mg/kg administered intramuscularly (IM) n=12), 2) Meloxicam (M; 1.0mg/kg by mouth (PO) administered in 8 g cookie dough; n=12) or, 3) Saline (S; administered IM at an equivalent volume to flunixin meglumine; n=12). In round two, sows were randomly assigned to one of the remaining two treatments. By the third round, sows received the treatment they had not been administered in round one or two (Pairis-Garcia et al., 2015a). The trial included three rounds, each lasting 312 h.

Flunixin meglumine treatments were administered 27.5 and 51.5 h post induction and meloxicam was administered 28.5 and 52.5 h post lameness induction. Drugs were administered twice during each round to ensure that the drug effect lasted for a sufficient enough time to allow

for a full day's observation (based on previous data assessing drug concentration and elimination rates; Pairis-Garcia et al., 2013; 2015b). Half of the saline-treated sows had the saline administered at 27.5 and 51.5 h post lameness induction to match sows receiving flunixin meglumine; the remaining half of the saline-treated sows received saline at 28.5 and 52.5 h after lameness induction to match sows receiving meloxicam.

Behavioral measurements

Sows were filmed in their pens continually over a 12 h period (0600-1800) on the day before lameness induction (day -1), as well as day +1, +2 (first pain mitigation treatment), +3 (second pain mitigation treatment) and +7 after lameness induction. Sows were not evaluated on day 0, as sows were anesthetized that day. Video was recorded using one 12 V color Close Circuit Television (CCTV) camera (Model WV-CP484, Matsushita Co. Ltd.) positioned centrally in front of the pen (2.9 m from pen front) using an elbow bracket at a height of 2.8 m from the floor. Video was captured digitally utilizing a Noldus portable laptop (Noldus Information Technology, Wageningen, The Netherlands). Twelve color Panasonic cameras (WV-CP484, Kadoma, Japan) were fed into a multiplexer, which allowed the image to be recorded using a PC with HandiAvi (v4.3, Anderson's AZcendant Software, Tempe, Arizona, United States of America) at 30 frames/s. A computer screen was used to view the DVR output to ensure picture clarity and camera positioning prior to each behavioral recording session. Behaviors evaluated are described in Table 1.

All behavioral data was collected using a continuous sampling method by one trained observer using the Observer software (The Observer, Ver. 5.0.25, Noldus Information Technology, Wageningen, The Netherlands). This data set was used to determine sow behavior

at 5, 10 and 15 min scans. To control for observer bias, the observer scoring the video was blind to analgesic treatments.

Sampling Methods

The video data were divided into 1 min intervals. In each minute, the proportion of time the sow was standing, lying and sitting was recorded with the sum of the 4 proportions (sitting, standing, lying, and other) totaling 100%. The behaviors were summarized using 4 sampling methods: continuous, 5-, 10- and 15- min scans. For the continuous sampling method, the data were first collected by watching the sows continuously, then, the average proportion of time spent performing each behavior was calculated. The 5 min scan used every fifth 1 min interval to calculate the average proportion of time the behavior was performed. The 10 min scan used every tenth 1 min interval, and the 15 min scan used every fifteenth 1 min interval. Therefore, if 60 min were recorded, the continuous sampling method would use 60, 60 sec, observations to calculate the average proportion, the 5 min scan would use 12, 60 sec, observations, the 10 min scan would use 6, 60 sec, observations, and the 15 min scan would use 4, sec, observations. The average proportion calculated using each method was the dependent variable used to examine sow behaviors during different lameness states.

Statistical analysis

Behavioral data were analyzed using SAS software version 9.3 (SAS Institute Inc. 2011). Data were screened for normality by plotting a predicted residual plot and a quantile-quantile plot. Data were normally distributed. To determine differences in sampling methods for each treatment (saline, flunixin meglumine, meloxicam) a generalized linear mixed model method (PROC MIXED) was used. The model included sampling method (continuous, 5 min

scan, 10 min scan, and 15 min scan), day relative to lameness induction (d -1, +1, +2, +3 and +7) and drug as a fixed effect. Sow within day was included as a random effect. A P -value of < 0.05 was considered significant when evaluating MIXED model effects. When a fixed effect was a significant source of variation, different levels within the fixed effect were separated using the PDIFF option in SAS.

Results

Effect of sampling method when given saline

There was no effect of sampling method (continuous, 5-, 10- and 15-min scans) on standing, lying or sitting postures for sows administered saline (Table 2; $P = 0.99$). Standing and lying behaviors differed between days for sows given saline (Table 2; $P < 0.01$). Compared to the baseline (d -1) and d +7 sows increased their time spent lying for the first 3 d after induced lame. Time spent sitting was not different between days.

Effect of sampling method when given pain mitigation by day

There was no effect of sampling method (continuous, 5-, 10- and 15-min scans) on standing, lying or sitting postures for sows administered meloxicam (Table 3; $P > 0.05$). Regardless of sampling method, standing and lying behaviors were different between days for meloxicam treated sows (Tables 3; $P < 0.01$). Sows receiving the meloxicam treatments spent more time lying during the first 3 d after lameness induction compared to baseline (d -1) and d +7. Sows receiving flunixin meglumine spent less time standing on d -1 compared to all other days. However, this difference was only noted when using the continuous sampling method (Table 4; $P < 0.04$). There were no differences in sitting behaviors between days among sows receiving any of the treatments.

Discussion

The first objective of this study was to determine if scan sampling could detect the same changes in behavior as the continuous sampling method for sows induced lame. No differences were noted in standing, lying or sitting behaviors using 5-, 10- or 15-minute scans compared to continuous observation for sows administered saline. These results agree with previous work demonstrating that 15-min scan samples provided good estimates of lying and standing behavior in sows (Pairis-Garcia et al., 2015a), dairy cattle (Mattachini et al., 2013), and beef cattle (Mitlöhner et al., 2001). In the cattle studies, the 15-min scan sampling technique captured statistically similar behavioral frequencies as the continuous sampling method (Mattachini et al., 2013; Mitlöhner et al., 2001). Similar to our study, both of the scan sampling studies involving cattle first recorded behavioral data continuously before comparing the data to various scans (e.g. 5-, 10-, or 15-min) (Mattachini et al., 2013; Mitlöhner et al., 2001). Fifteen-min scan samples have also been utilized successfully in the pig literature as Pairis-Garcia et al. (2015a) used 15-min scan samples to record the sitting, standing, and lying of sows induced lame. .

The results from our study provide researchers with a more realistic option to observe behavioral changes associated with lameness; scan samples offer more flexibility to the researcher and the time to implement additional components or variables to the data set that can further enhance the understanding of lameness and pain. For example, the researcher could consider adding behaviors or examining more long-term effects (increased time spent watching videos) with the increase in time saved.

The second objective of this study was to determine the ability of the three scan sampling methods to detect changes in postural behaviors of sows given pain mitigation after lameness

induction. The choice to provide flunixin meglumine and meloxicam was based on pain management literature demonstrating that both flunixin meglumine and meloxicam result in pain mitigation associated with lameness (Friton et al, 2003; Schulz et al, 2011; Welsh and Nolan, 1995). In addition, for swine in the United States, both flunixin meglumine and meloxicam are drugs, which can be prescribed by a veterinarian and utilized and stored on-farm. Neither drug requires a Drug Enforcement Agency license and is safe for employees to administer on-farm. No differences between sampling methods were detected for standing, lying and sitting behaviors for sows treated with meloxicam after lameness induction. However, sows treated with flunixin meglumine decreased their standing time in the first day after lameness induction, but this finding was only detectable using the continuous sampling method. Differences in results between flunixin meglumine and meloxicam may be due to differences in drug efficacy as meloxicam is identified to be a more specific cyclo-oxygenase (COX) inhibitor as compared to flunixin meglumine (Beretta et al., 2005). Sows given flunixin meglumine had a small change in behavior after lameness induction, which may have not been sufficient change to be detected by the scan sampling methods. In contrast, sows provided meloxicam had a large change in behavior after lameness induction that may have been more easily detected by the scan sampling technique. Flunixin meglumine acts by decreasing prostaglandin synthesis by non-selectively inhibiting the enzyme COX (Houdeshell and Hennessey, 1977) while meloxicam (a member of the oxicam class) demonstrates effective anti-inflammatory, analgesic and antipyretic via selective COX 2 inhibition and good systemic absorption properties (Busch et al., 1998).

This result suggests that flunixin meglumine and meloxicam were efficacious at reducing pain-related behaviors on the second and third day after lameness induction, but not the first day. These results are most likely the result of the anti-inflammatory and analgesic properties of

flunixin meglumine and meloxicam (Mathews, 2002). Although the scan samples were not able to detect the reduction in standing time on the day after lameness induction, standing behavior on the remaining days (+2, +3, and +7), as well as lying and sitting time on all days were not different between sampling methods. Therefore, in conclusion, scan sampling methods can be utilized to detect changes in behavioral response administered a drug depending on the drug being evaluated.

These results may allow future researchers interested in lameness and lameness pain relief in sows to save research time, for they validate the use of a 15-min scan as an accurate portrayal of sow behavior. Additionally, these findings may be insightful animal-based measures for pig welfare audits. Future studies should determine the number of hours of observation time needed to accurately capture the sow's behavior with 15-min scans.

Conclusion

Lameness is a major welfare and economical concern for the U.S. swine industry. This study determined that practical scan sampling (5, 10 and 15 min) methods were generally able to detect changes in standing and lying behavior of lame sows just as well as the more labor-intensive continuous sampling method. Scan sampling may be a valuable tool for determining behavioral deviations due to lameness and can be applied to research as it is accurate and decreases the work for the investigator compared to the continuous sampling method.

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Table 1. Posture definitions (adapted from Pairis-Garcia et al., 2015a).

Measure	Definition
Standing	Assuming or maintaining an upright position on extended legs. Includes all actions where all four feet are in contact with ground.
Sitting	Posterior portion of the sow's body is in contact with or ground of the pen. The front two legs in extension support anterior portion of the body.
Lying	Lying with the majority (> 50%) of the sternum contacting the ground or lying with either the left or right side of body in contact with the ground.
Other	Any time the sow's behavior or posture cannot be clearly defined; sow no longer visible to the camera (e.g., sow was taken out of the pen, loss of camera footage, too dark to see the sow).

Table 2. Standing, lying, and sitting behavior LS means ($\pm$ SEM) for sows treated with saline using four sampling methods.

	Day relative to lameness induction				
	-1	+1	+2	+3	+7
Standing behavior, %					
Continuous	40.3 $\pm$ 4.5 ^a	21.1 $\pm$ 4.6 ^b	21.8 $\pm$ 4.5 ^b	19.3 $\pm$ 4.5 ^b	43.5 $\pm$ 4.6 ^a
5 min scan	44.4 $\pm$ 4.5 ^a	28.8 $\pm$ 4.6 ^b	22.2 $\pm$ 4.5 ^b	21.9 $\pm$ 4.5 ^b	44.9 $\pm$ 4.6 ^a
10 min scan	44.1 $\pm$ 4.6 ^a	24.4 $\pm$ 4.5 ^b	21.9 $\pm$ 4.5 ^b	21.5 $\pm$ 4.6 ^b	44.8 $\pm$ 4.6 ^a
15 min scan	43.9 $\pm$ 4.5 ^a	22.2 $\pm$ 4.6 ^b	22.5 $\pm$ 4.6 ^b	20.8 $\pm$ 4.6 ^b	44.9 $\pm$ 4.5 ^a
Lying behavior, %					
Continuous	46.9 $\pm$ 7.2 ^a	68.4 $\pm$ 7.2 ^b	71.4 $\pm$ 7.2 ^b	76.9 $\pm$ 7.2 ^b	50.7 $\pm$ 7.2 ^a
5 min scan	44.0 $\pm$ 7.2 ^a	63.3 $\pm$ 7.2 ^b	71.5 $\pm$ 7.2 ^b	74.8 $\pm$ 7.2 ^b	45.3 $\pm$ 7.2 ^a
10 min scan	44.3 $\pm$ 7.2 ^a	72.5 $\pm$ 7.2 ^b	71.6 $\pm$ 7.2 ^b	74.4 $\pm$ 7.2 ^b	45.2 $\pm$ 7.2 ^a
15 min scan	44.1 $\pm$ 7.2 ^a	66.3 $\pm$ 7.2 ^b	71.2 $\pm$ 7.2 ^b	76.2 $\pm$ 7.2 ^b	44.8 $\pm$ 7.0 ^a
Sitting behavior, %					
Continuous	4.7 $\pm$ 2.2	4.1 $\pm$ 2.2	1.8 $\pm$ 2.2	1.1 $\pm$ 2.1	3.4 $\pm$ 2.1
5 min scan	4.7 $\pm$ 2.2	4.6 $\pm$ 2.2	1.7 $\pm$ 2.2	0.9 $\pm$ 2.2	3.2 $\pm$ 2.2
10 min scan	4.6 $\pm$ 2.1	3.9 $\pm$ 2.2	1.6 $\pm$ 2.1	1.6 $\pm$ 2.1	3.4 $\pm$ 2.1
15 min scan	5.1 $\pm$ 2.2	4.2 $\pm$ 2.1	1.6 $\pm$ 2.2	1.1 $\pm$ 2.1	3.9 $\pm$ 2.1

^{a-b} Indicates differences between days within a row with a *P*-value <0.05.

Table 3. Standing, lying, and sitting behavior LS means ($\pm$ SEM) for sows treated with meloxicam using four different sampling methods.

	Day relative to lameness induction				
	-1	+1	+2	+3	+7
Standing behavior, %					
Continuous	42.2 $\pm$ 4.3 ^a	16.0 $\pm$ 4.3 ^b	27.5 $\pm$ 4.3 ^b	24.7 $\pm$ 4.3 ^b	42.3 $\pm$ 4.3 ^a
5 min scan	42.9 $\pm$ 4.3 ^a	17.3 $\pm$ 4.3 ^b	27.2 $\pm$ 4.3 ^b	24.6 $\pm$ 4.3 ^b	44.9 $\pm$ 4.3 ^a
10 min scan	41.7 $\pm$ 4.3 ^a	17.4 $\pm$ 4.3 ^b	28.4 $\pm$ 4.3 ^b	24.9 $\pm$ 4.3 ^b	44.3 $\pm$ 4.3 ^a
15 min scan	44.8 $\pm$ 4.3 ^a	18.9 $\pm$ 4.3 ^b	29.3 $\pm$ 4.3 ^b	25.4 $\pm$ 4.3 ^b	45.9 $\pm$ 4.2 ^a
Lying behavior, %					
Continuous	46.4 $\pm$ 5.7 ^a	71.1 $\pm$ 5.7 ^b	60.9 $\pm$ 5.7 ^b	67.7 $\pm$ 5.7 ^b	49.5 $\pm$ 5.7 ^a
5 min scan	46.6 $\pm$ 5.7 ^a	69.1 $\pm$ 5.7 ^b	55.5 $\pm$ 5.7 ^a	67.4 $\pm$ 5.7 ^b	46.4 $\pm$ 5.7 ^a
10 min scan	46.3 $\pm$ 5.7 ^a	69.5 $\pm$ 5.7 ^b	60.8 $\pm$ 5.7 ^b	66.8 $\pm$ 5.7 ^b	47.1 $\pm$ 5.7 ^a
15 min scan	45.1 $\pm$ 5.7 ^a	68.2 $\pm$ 5.7 ^b	60.2 $\pm$ 5.7 ^b	72.1 $\pm$ 5.7 ^b	44.6 $\pm$ 5.7 ^a
Sitting behavior, %					
Continuous	2.9 $\pm$ 3.3	7.5 $\pm$ 3.3	4.7 $\pm$ 3.3	3.4 $\pm$ 3.3	4.3 $\pm$ 3.3
5 min scan	2.9 $\pm$ 3.3	7.6 $\pm$ 3.3	4.5 $\pm$ 3.3	3.8 $\pm$ 3.3	4.5 $\pm$ 3.3
10 min scan	3.1 $\pm$ 3.3	7.7 $\pm$ 3.3	4.3 $\pm$ 3.3	3.6 $\pm$ 3.3	4.7 $\pm$ 3.3
15 min scan	2.5 $\pm$ 3.3	7.3 $\pm$ 3.3	4.2 $\pm$ 3.3	7.1 $\pm$ 3.3	5.9 $\pm$ 3.3

^{a-b} Indicates differences between days within a row with a *P*-value <0.05.

Table 4. Standing, lying, and sitting behavior LS means ($\pm$ SEM) for sows treated with flunixin meglumine using four different sampling methods.

	Day relative to lameness induction				
	-1	+1	+2	+3	+7
Standing behavior, %					
Continuous	33.9 $\pm$ 4.3 ^a	21.1 $\pm$ 4.3 ^b	28.1 $\pm$ 4.3 ^a	23.7 $\pm$ 4.3 ^a	38.3 $\pm$ 4.3 ^a
5 min scan	34.3 $\pm$ 4.3	23.9 $\pm$ 4.3	30.7 $\pm$ 4.3	25.6 $\pm$ 4.3	40.1 $\pm$ 4.3
10 min scan	34.5 $\pm$ 4.3	24.6 $\pm$ 4.3	30.4 $\pm$ 4.3	25.1 $\pm$ 4.3	39.9 $\pm$ 4.3
15 min scan	34.4 $\pm$ 4.3	23.7 $\pm$ 4.3	29.6 $\pm$ 4.3	25.4 $\pm$ 4.3	38.1 $\pm$ 4.5
Lying behavior, %					
Continuous	57.2 $\pm$ 5.0	70.5 $\pm$ 5.0	64.4 $\pm$ 5.1	71.6 $\pm$ 5.0	51.3 $\pm$ 5.1
5 min scan	53.6 $\pm$ 5.0	67.5 $\pm$ 5.1	61.9 $\pm$ 5.1	69.1 $\pm$ 5.0	45.1 $\pm$ 5.1
10 min scan	54.9 $\pm$ 5.0	66.9 $\pm$ 5.1	61.9 $\pm$ 5.0	69.2 $\pm$ 5.0	45.2 $\pm$ 7.2
15 min scan	44.1 $\pm$ 7.2	66.3 $\pm$ 7.2	71.2 $\pm$ 7.2	76.2 $\pm$ 7.2	44.8 $\pm$ 7.0
Sitting behavior, %					
Continuous	4.6 $\pm$ 1.8	3.4 $\pm$ 1.8	1.8 $\pm$ 1.8	2.2 $\pm$ 1.8	4.6 $\pm$ 1.8
5 min scan	4.6 $\pm$ 1.8	3.8 $\pm$ 1.8	2.2 $\pm$ 1.8	2.7 $\pm$ 1.8	4.3 $\pm$ 1.8
10 min scan	4.4 $\pm$ 1.8	4.0 $\pm$ 1.8	2.0 $\pm$ 1.8	2.9 $\pm$ 1.8	4.4 $\pm$ 1.8
15 min scan	5.0 $\pm$ 1.8	4.0 $\pm$ 1.8	1.6 $\pm$ 1.8	2.1 $\pm$ 1.8	4.6 $\pm$ 1.8

^{a-b} Indicates differences between days within a row with a *P*-value <0.05.