

Behavioural evaluation of analgesic efficacy for pain mitigation in lame sows

MD Pairis-Garcia[‡], AK Johnson^{*†‡}, KJ Stalder[‡], CA Abell[‡], LA Karriker^{§¶}, JF Coetzee^{#¶}
and ST Millman[¶]

[†] 2356F Kildee Hall, Iowa State University, Ames, IA, USA

[‡] Department of Animal Science, Iowa State University, Ames, IA 50011, USA

[§] Swine Medicine Education Center, Iowa State University, Ames, IA 50011, USA

[#] Pharmacology Analytical Support Service, Iowa State University, Ames, IA 50011, USA

[¶] Veterinary Diagnostic and Production Animal, Iowa State University, Ames, IA 50011, USA

^{*} Department of Biomedical Science, Iowa State University, Ames, IA 50011, USA

* Contact for correspondence and requests for reprints: johnsona@iastate.edu

Abstract

Lameness in breeding swine has a large negative economic impact and is a welfare concern. Pain-related behaviour, such as postural changes, may be used to evaluate the presence and severity of pain in animals. The objective of this work was to determine the effects of flunixin meglumine (FM) and meloxicam (M) on postural changes in lame sows. Lameness was induced in 24 mature sows (*Sus scrofa*) using a chemical synovitis model. Three treatments were compared: FM (2.2 mg kg⁻¹; n = 24, intramuscular [IM]), M (1.0 mg kg⁻¹; n = 24, by mouth [PO]) and sterile saline (equivalent volume to FM; n = 24 [IM]), administered approximately 28 and 52 h after lameness induction. Behavioural data were collected in the home pen during 12-h periods and quantified using 15-min scan sampling on the day prior to (-24 h; Day -1) through +168 h post lameness induction. Frequency of behaviour was analysed by day using generalised linear mixed model methods. The frequency of standing postures significantly decreased and lying postures increased 24–72 h post lameness induction relative to baseline day. All postures returned to baseline frequencies by +168 h. Meloxicam-treated sows demonstrated lower frequencies of lying postures +48 and +72 h after lameness induction compared to saline-treated sows. Flunixin-treated sows did not differ in lying behaviours compared to saline-treated sows. No differences were noted in standing or sitting postures between treatments. The results of this study suggest that meloxicam mitigates pain sensitivity as demonstrated by higher frequency of standing and lower frequency of lying compared to saline-treated sows.

Keywords: animal welfare, behaviour, flunixin meglumine, lameness, meloxicam, swine

Introduction

Lameness is a major factor when culling females from the swine breeding herd (Engblom *et al* 2008; Anil *et al* 2009; Knauer *et al* 2012). Lameness was ranked as the third most common reason for culling sows, comprising 15% of cull sows marketed in the United States (Schenk *et al* 2010) with parity one to three sows (*Sus scrofa*) representing 10.5–14.9% of that population (Knauer *et al* 2012). Lameness prevalence in Finland, Denmark and England ranges from 8.8–16.9% (Bonde *et al* 2004; Heinonen *et al* 2006; Kilbride *et al* 2009). Lameness in breeding-aged swine has a large negative economic impact on livestock producers (Wells 1984) because it increases labour and veterinary costs (Pluym *et al* 2013) and shortens total sow productive lifetime (Stalder *et al* 2003). Lameness is recognised as a welfare concern because it is associated with the negative affective state of pain and has been identified as an animal-based measurement in The European Welfare Quality® (2011) and Pork Quality Assurance Plus® programmes (NPB 2013).

Lameness pain can be attributed to several aetiologies, including neurological deficits, hoof or limb lesions, mechanical-structural conformation, trauma, or metabolic and infectious disease (Wells 1984; Smith 1988; Main *et al* 2000). Dependent on the aetiology, pain associated with lameness can be severe, thus, appropriate pain management resulting from lameness is critical until a definitive diagnosis can be reached (Haley 2010). Changes to an animal's behavioural repertoire have been used to assess pain sensitivity in a variety of species including dairy cattle (*Bos taurus*) (O'Callaghan *et al* 2003; Ito *et al* 2010; Heinrich *et al* 2010; Blackie *et al* 2011; Alsaad *et al* 2012; Shearer *et al* 2013; Higginson *et al* in press), swine (Gregoire *et al* 2013), sheep (*Ovis aries*) (Stubsjøen *et al* 2009) and broiler chickens (*Gallus gallus*) (Weeks *et al* 2000).

Behaviour commonly associated with lameness pain in swine include vocalisations, abnormal standing posture and/or gait, reluctance to move, decreased appetite and increased inactivity (Underwood 2002; Anil *et al* 2009).

Gregoire and colleagues (2013) found that severely lame sows spent less time standing and lay down earlier after feeding compared to moderately and non-lame sows. Validating postural changes in lame sows will be important for several on-farm reasons. First, these postures can be easily identified by the farmer and objectively assessed to detect changes in prevalence or severity of individual lameness. Secondly, as much of pork production is transitioning to group housing, understanding the behavioural time budgets and needs of lame sows will help improve facility design and management protocols to ensure all compromised animals have sufficient access to important resources such as food, water and a lying area.

Non-steroidal anti-inflammatory drugs (NSAIDs) are a common drug category used for pain management in animals and can be suitable for on-farm implementation as they are easy to administer, long-lasting and cost effective (Coetzee *et al* 2011). Flunixin meglumine and meloxicam are two common NSAIDs used in veterinary medicine. Previous work using broilers (Danbury *et al* 2000), dairy calves (Heinrich *et al* 2010; Schulz *et al* 2011; Pauly *et al* 2012; Coetzee *et al* 2014) and swine (Keita *et al* 2010; Hansson *et al* 2011; Reiner *et al* 2012; Kluviens-Poodt *et al* 2013) demonstrated NSAID efficacy for mitigating painful states on the basis of behaviour outcomes. Therefore, the objective of this study was to determine the effects of flunixin meglumine and meloxicam on mitigating lameness pain as measured by standing, lying and sitting in sows experiencing lameness associated with a chemical synovitis model.

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, Eighth 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. As lameness induction resulted in transient states of pain, the experiment was designed to allow each sow to serve as her own control (baseline measurements were defined as control data for each sow) thus reducing the total number of sows required while maintaining the number of experimental animals required to detect a statistical difference. Investigators established humane end-point criteria in which any sow that was unable to access water for 12 h, access food for 24 h or progressed to non-weight-bearing lameness for 48 h was removed from the study and humanely euthanised. No sows met these criteria during this study. All sows were acclimated to housing and handling for seven days prior to trial initiation.

Study animals and housing

Twenty-four multiparous (mean parity 6; range 2–9), non-pregnant, crossbred Newsham maternal cull sows were obtained from a commercial farm in Iowa (bodyweight 241.4 [\pm 15.5] kg). A power analysis was conducted to determine sample size per treatment for this study. Utilising

preliminary data and published peer-reviewed studies (Kotschwar *et al* 2009), we determined that to detect differences in behaviour at two standard deviations from the mean using α 0.01, a total of ten non-bred pigs were required thus resulting in a total of 30 sows (ten sows per treatment). However, data collected and analysed by Mohling and colleagues (2014a,b) demonstrated that differences between sound and lame states were detectable utilising 24 sows, thus we reduced the number of total sows for the project to 24.

All sows underwent a physical examination (evaluation of integument, cardiovascular and respiratory system) and a lameness evaluation prior to selection by a trained veterinarian with expertise in sow lameness. Lameness was evaluated prior to selection of sows from the farm using the following criteria: i) sow not moving freely using all four legs while walking; ii) weight shifting during walking or standing; or iii) non-weight bearing on any leg (Pairis-Garcia *et al* 2014a). Physical examination and lameness evaluation were also conducted between each round (total of three rounds per trial in which sows received one of three treatments per round) during the trial to confirm no observable residual lameness was present. Lameness was assessed by observing the sow walking on a non-slip mat measuring 4.3 m in length. Enrolled sows had to move freely using all four legs and demonstrate no weight-shifting or non-weight bearing prior to the start of the trial and between each round.

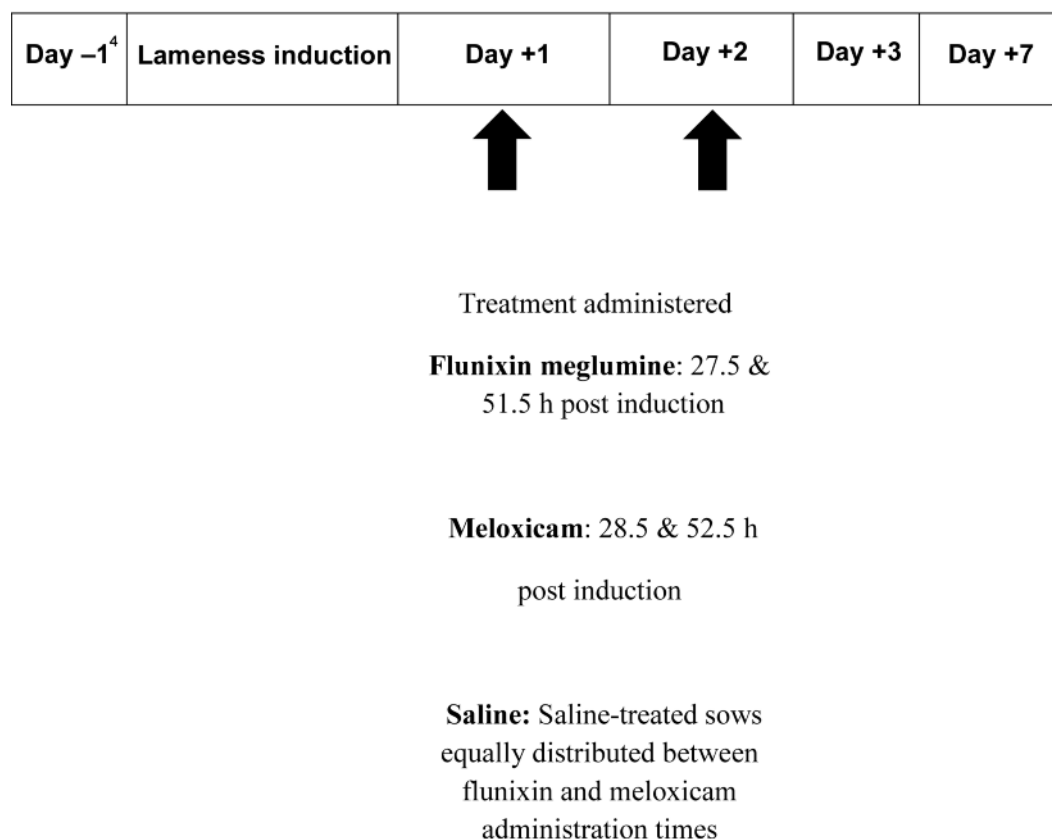
To avoid confounding injury resulting from aggression, each sow was housed in an individual pen; however, sows could see, smell, hear and have nose-to-nose contact with other sows. Each pen measured 3.7 \times 1.4 \times 1.2 m (length \times width \times height) and had a solid, concrete floor with a rubber mat (2.4 \times 1.4 \times 0.02 m; length \times width \times height). Metal fences (1.2 \times 0.76 m; height \times width) were affixed to the end of each home pen. Each pen was provided with environmental enrichment, including chains and/or plastic toys attached to the pen gates. During this trial, data were collected on sows which required them to be removed from the home pen for a 30-min period of time for a total number of eight data collection time-points.

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 (NRC) guidelines (2012) to meet or exceed non-gestating sow nutrient requirements. Matrix® (FDA approved; 0.22% Altrenogest, Intervet/Schering-Plough, Milsboro, USA, DE-Dose: 6.8 ml–15 mg) was added to 1 kg of feed daily to prevent oestrus initiation.

Experimental design

Lameness was induced by injecting amphotericin B into the distal interphalangeal joint according to methods previously described by Karriker and colleagues (2013) and responses up to seven days following lameness induction were compared. Each round lasted for 312 h. During a round a sow received a different treatment. In round one, sows were randomly assigned to one of the three treatments (flunixin

Figure 1



Data collection schematic to determine drug¹ efficacy for pain mitigation in lame² sows³ using three postures (standing, sitting and lying postures) for a 12-h period⁵.

¹ Treatments: i) meloxicam (M; 1.0 mg kg⁻¹ per os in cookie dough; n = 24); ii) flunixin meglumine (FM; 2.2 mg kg⁻¹ intramuscular [IM]; n = 24); or iii) saline (S; equivalent volume to FM administered [IM]; n = 24). ² Lameness induced by injecting amphotericin B into the distal interphalangeal joint of the sow (Karriker *et al* 2013). ³ Twenty-four multiparous (mean parity 6; range 2–9), non-pregnant, cross-bred Newsham maternal cull sow. ⁴ Day before lameness induction (Day -1), 24 h after lameness induction prior to treatment (Day +1), 48 h after lameness induction prior to treatment (Day +2), 72 h after lameness induction (Day +3) and 168 h after lameness induction (Day +7). ⁵ Postures evaluated each day between 0600–1800h.

meglumine, meloxicam and saline) and lameness induction was assigned to either the left or right rear leg. In round two, sows were randomly assigned to one of the remaining two treatments and lameness was induced in the rear leg that was sound in the previous round. By the last round, sows received the treatment they had not been administered in round one or two and the leg induced lame on the first round was induced again. The NSAID treatments were administered twice, during each round, 24 h apart (Figure 1).

An *in vivo* measurement to assess lameness pain in swine is difficult to perform using computer-based modeling due to inter-animal variation with drug metabolism and response. Sows were the target species for this study and have different metabolic capabilities, physiologic coping capabilities, and different mechanisms of drug metabolism than younger swine previously evaluated for meloxicam drug efficacy for lameness (Friton *et al* 2003). Lameness represents 15% of sows currently culled on-farm, thus repre-

senting approximately 87,255 sows of the entire US inventory of breeding stock at 5,817,000 in 2012 (NHF 2013). Utilising 24 sows for this study represents an extremely small percentage (0.004%) of the breeding stock in the US but results from this study can be applied, in turn, to a much larger population nationally and globally. This inducible lameness model facilitates the collection of data on the same non-bred pig when both sound and lame, thus increasing study power by reducing inter-individual variation. The choice to induce lameness three times in one sow was based on the importance of objectively comparing and evaluating differences between meloxicam and flunixin meglumine as well as differences between treatment and no treatment. In addition, sows only went through one round of lameness induction in which they were not provided with an analgesic and no sows met the criteria for humane endpoints. A ten-day wash-out period was provided between rounds to avoid previous treatment carry-over effects. This

Table 1 Postural ethogram when determining drug¹ efficacy for pain mitigation in lame² sows³ over a 12-h period⁴.

Postures	Description
Standing	Assuming or maintaining an upright position on extended legs. Includes all actions where all four feet are in contact with the ground
Sitting	Posterior portion of the sow's body is in contact with the pen or the ground of the pen. Anterior portion of the body is supported by the front two legs in extension
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
Unknown	Any time in which the sow and her behaviour cannot be clearly defined, including any events in which the camera or camera screen has malfunctioned, the sow is outside of her pen or when the sow's head is not visible. Blue screen or camera not functioning

¹ Treatments: i) meloxicam (M; 1.0 mg kg⁻¹ per os in cookie dough; n = 24); ii) flunixin meglumine (FM; 2.2 mg kg⁻¹ intramuscular [IM]; n = 24); or iii) saline (S; equivalent volume to FM administered [IM]; n = 24).

² Lameness induced by injecting amphotericin B into the distal interphalangeal joint of the sow (Karriker *et al* 2013).

³ Twenty-four multiparous (mean parity 6; range 2–9), non-pregnant, crossbred Newsham maternal cull sows.

⁴ Postures evaluated each day between 0600–1800h.

wash-out period was determined using previous studies conducted in our laboratory assessing the pharmacokinetics of both meloxicam and flunixin meglumine (Pairis-Garcia *et al* 2013, 2014b). Prior to subsequent treatment round, pressure algometry, thermal sensitivity tests were conducted. Sows were gait scored to evaluate any residual lameness carry-over. A blood sample was collected from each sow prior to initiation of the following round to determine residual drug carry-over.

Treatments

Twelve sows were assigned to three blocks (four sows per block) and within each block treatment was randomly allocated to one of three treatments for round one: i) flunixin meglumine (FM; 2.2 mg kg⁻¹ administered intramuscularly; n = 24); ii) meloxicam (M; 1.0 mg kg⁻¹ PO administered in 8 g cookie dough; n = 24); or iii) saline (S; administered IM at an equivalent volume to flunixin meglumine PO; n = 24). To control for confounding effects of treatment administration route, sows treated with flunixin meglumine or saline were also given 8 g of cookie dough, while sows treated with meloxicam received an IM sterile saline injection. This ensured that all sows were handled in the same way, regardless of the treatment being administered. Data were collected at the T_{max} for both drugs. The T_{max} is defined as the time in which the drug reaches its maximum concentration and was chosen as a sample point based on the goal to collect data in a window of time in which the drug may be most effective

(Maddison *et al* 2008). Based on T_{max} values previously calculated by our laboratory (Pairis-Garcia *et al* 2013, 2014b), flunixin meglumine treatments were administered 27.5 and 51.5 h post induction and meloxicam was administered 28.5 and 52.5 h after lameness induction. Half of the saline-treated sows had treatment 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 their treatments at 28.5 and 52.5 h after lameness induction to match sows receiving meloxicam. Treatments 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. This choice was based on previous data collected by our laboratory assessing drug concentration and elimination rates (Pairis-Garcia *et al* 2013, 2014b). To control for observer bias, observers scoring the video were blind to analgesic treatments and day.

Behaviour measures

All sows were filmed in their home pens continually over a 12-h period (0600–1800h) for seven days. Video was recorded using one 12 V colour Close Circuit Television (CCTV) camera (Model WV-CP484, Matsushita Co Ltd, Japan) positioned centrally (2.9 m from pen front) using an elbow bracket at a height of 2.8 m from the floor. Video was captured digitally utilising a Noldus portable lab (Noldus Information Technology, Wageningen, The Netherlands). Twelve colour 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, AZ, USA) at 30 frames per second. A computer screen was used to view the DVR output to ensure picture clarity and camera positioning prior to each behavioural recording session. Three postures were collected using a 15-min instantaneous scan sample (Martin & Bateson 2007; Table 1). Fifteen-minute scan samples to detect differences in standing, lying and sitting postures during lame and non-lame states when lameness was induced using a chemical synovitis model in sows was validated previously in our laboratory (unpublished data). Any time the sow's behaviour could not be clearly defined the behaviour was marked as unknown. Behaviour observations were collected on Day -1 (day before lameness induction or baseline, 24 h after lameness induction prior to treatment (Day +1), 48 h after lameness induction (Day +2), 72 h after lameness induction (Day +3) and 168 h after lameness induction (D +7; Figure 1). All data were collected by two trained observers using the Observer software (The Observer, Version 5.0.25, Noldus Information Technology, Wageningen, The Netherlands). Inter-reliability training on a 2-h sample video was conducted to ensure a 95% agreement between observers. For the remaining data, each observer was assigned twelve sows to score for the study duration.

Statistical analysis

Data were analysed using SAS software version 9.3 (SAS Institute Inc 2011). Data were analysed for normality by plotting a predicted residual plot and a quantile-quantile plot. Postures were summed by day and analysed as frequency per

count data. Data were analysed using generalised linear mixed model methods (PROC GLIMMIX) to compare postural frequency differences between treated sows (saline vs flunixin vs meloxicam) by trial day (Day -1 to Day +7). The statistical model included leg, day by treatment interaction, treatment round and day as fixed effects. Sow was included as a random effect. A *P*-value of < 0.05 was considered significant when evaluating GLIMMIX model effects. When the fixed effect was a significant source of variation, different levels within the fixed effect were separated using the PDIF option in SAS. The I-Link option was used to transform the LS mean and standard error values back to the original measurement units.

Results

When assessing saline-treated sows only, standing postures decreased and lying postures increased on Days +1 to 3 when compared to Day -1 baseline (*P* < 0.001; data not shown) with both lying and standing postural frequency returning to baseline day levels by Day +7 (*P* > 0.05). Sitting postures were not different for saline-treated sows when comparing the non-lame day (Day -1) to the most lame day (Day +2). When comparing differences in postural frequencies between treated sows (saline vs flunixin vs meloxicam) meloxicam-treated sows demonstrated decreases in lying postures on Day +2 and +3 compared to saline-treated sows (*P* < 0.04; Table 2). No differences were noted between flunixin meglumine- and saline-treated sows, however a trend toward more standing and less lying postures by flunixin-treated sows compared to saline-treated sows was found on Day +2 and +3 (Table 2).

Discussion

The objective of this study was to determine the effects of flunixin meglumine and meloxicam on mitigating lameness pain as measured by standing, lying and sitting in sows experiencing lameness associated with a chemical synovitis model. Postural changes have been used as a tool to study environmental and physiological challenges to the sow (Ringgenberg *et al* 2010).

Utilising 15-min scan samples successfully detected postural differences for both lame and non-lame sows and coincides with previous work which validated the use of 15-min scan samples in our laboratory. Sows that were not provided analgesia but did have induced lameness (saline-treated sows) exhibited reduced standing posture frequency and an increased lying posture frequency from Day +1 to Day +3. Sows treated with flunixin meglumine and meloxicam also demonstrated reduced standing posture frequency and increased lying posture frequency for these days, but deviations from baseline data were smaller compared to saline-treated sows. The results from our study are similar to previous reports from Gregoire and colleagues (2013) that noted lameness severity in sows decreased total standing time when evaluating standing over a 24-h period (non-lame: 14.5%; mildly lame: 13.7%, severely lame: 6.3%). The increased lying frequency observed among lame sows in this study may have resulted from their inability to change positions. Bonde and colleagues (2004) reported that lame sows had a greater uncontrolled lying down posture incidence (ie

Table 2 Difference in postural frequencies (\pm SEM) between treatments¹ from 24 lame² sows³ using 15-min scan samples over a 12-h period⁴.

Day ⁵	Posture	Flunixin meglumine	Meloxicam	Saline
Day -1	Standing	11.9 (\pm 1.5)	12.9 (\pm 1.5)	13.8 (\pm 1.6)
	Sitting	3.4 (\pm 0.7)	3.9 (\pm 0.8)	3.1 (\pm 0.7)
	Lying	27.8 (\pm 1.8)	24.2 (\pm 1.6)	26.2 (\pm 1.7)
Day +1	Standing	6.6 (\pm 1.1)	6.0 (\pm 1.0)	6.0 (\pm 1.0)
	Sitting	2.5 (\pm 0.6)	1.5 (\pm 0.5)	2.5 (\pm 1.0)
	Lying	35.3 (\pm 2.0)	32.9 (\pm 1.9)	35.4 (\pm 2.0)
Day +2	Standing	6.9 (\pm 1.1)	5.7 (\pm 1.0)	5.2 (\pm 1.0)
	Sitting	2.7 (\pm 0.6)	2.1 (\pm 0.6)	2.1 (\pm 0.6)
	Lying	33.2 (\pm 1.9) ^{ab}	31.5 (\pm 1.9) ^a	37.3 (\pm 2.1) ^b
Day +3	Standing	7.5 (\pm 1.1)	6.7 (\pm 1.1)	6.4 (\pm 1.1)
	Sitting	1.9 (\pm 0.5)	1.6 (\pm 0.5)	1.8 (\pm 0.5)
	Lying	34.9 (\pm 1.9) ^{ab}	31.4 (\pm 1.9) ^a	36.9 (\pm 2.1) ^b
Day +7	Standing	11.8 (\pm 1.4)	12.1 (\pm 1.5)	14.8 (\pm 1.7)
	Sitting	3.5 (\pm 0.7)	4.5 (\pm 0.9)	3.7 (\pm 0.8)
	Lying	23.7 (\pm 1.6)	22.1 (\pm 1.6)	25.6 (\pm 1.8)

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

¹ Treatments: i) meloxicam (M; 1.0 mg kg⁻¹ per os in cookie dough; n = 24); ii) flunixin meglumine (FM; 2.2 mg kg⁻¹ intramuscular [IM]; n = 24); or iii) saline (S; equivalent volume to FM administered [IM]; n = 24). ² Lameness induced by injecting amphotericin B into the distal interphalangeal joint of the sow (Karriker *et al* 2013).

³ Twenty-four multiparous (mean parity 6; range 2–9), non-pregnant, crossbred Newsham maternal cull sow.

⁴ Postures evaluated on each day between 0600–1800h.

⁵ Day before lameness induction (Day -1), 24 h after lameness induction prior to treatment (Day +1), 48 h after lameness induction prior to treatment (Day +2), 72 h after lameness induction (Day +3) and 168 h after lameness induction (Day +7).

dropping herself on the floor) and it may be possible that sows will choose to remain lying for longer time-periods to avoid potentially painful lying down events. Alternatively, sows may be experiencing increased pain sensitivity when standing resulting in longer lying periods. Further behavioural evaluation analysing these transition behaviours and postures in addition to lying, bout duration and frequency are required to determine if this is the case.

Pharmacological tools for pain management on-farm include drugs such as meloxicam and flunixin meglumine. Based on lying frequency alone, data from the present study suggest that meloxicam-mitigated pain sensitivity 48 and 72 h after administration resulting in postural frequencies more similar to sows in their non-lame states when compared to the postural frequencies exhibited from saline-treated sows 48 to 72 h post treatment. In addition, although no differences were noted between flunixin- and saline-treated sows, sows treated with flunixin meglumine

exhibited reduced lying behaviour 48 to 72 h after lameness induction compared to saline-treated sows. These data are consistent with previously published results (Pairis-Garcia et al 2014a) that utilised the same sows but treatment effectiveness was evaluated using nociceptive threshold tests. That study utilised pressure algometry and thermal sensitivity tests, when both flunixin meglumine and meloxicam were used to mitigate pain. In addition, the data collected in this study also coincides with published findings that have reported successful drug efficacy evaluation when attempting to mitigate pain by using behavioural changes to assess pain relief in cattle (Heinrich et al 2010; Pauly et al 2012; Coetzee et al 2014). The present findings suggest that these behavioural postures show promise as a welfare assessment tool that can be implemented at the farm level that will benefit the swine industry because it is a quick, effective and economically feasible method to evaluate lameness and assess drug therapy for pain mitigation.

Conclusion

Utilising 15-min scan samples among sows where lameness was induced using a chemical synovitis model demonstrated differences in standing and lying frequencies when lame and non-lame. Lame sows demonstrated decreased standing frequency and increased lying frequency on +48 and +72 h (Day +2 and +3) after lameness induction regardless of treatment. However, sows administered meloxicam exhibited decreased lying frequency +48 and +72 h after lameness induction when compared to saline-treated (control) sows. The present findings suggest that meloxicam administration successfully mitigated pain sensitivity 48–72 h after lameness induction when pain mitigation evaluation was based on postural frequencies. No differences were noted in standing or sitting postures among sows from the different treatments suggesting changes in inactive postures, such as lying, may be a better indicator to determine drug efficacy intended to improve sow comfort and welfare.

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