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Measuring the efficacy of flunixin meglumine and meloxicam for lame sows using nociceptive threshold tests

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Abstract

Lameness in breeding swine can cause severe pain leading to on-farm welfare issues and significant economic impacts. Non-steroidal anti-inflammatory drugs including meloxicam and flunixin meglumine are commonly used in veterinary medicine for their analgesic and anti-inflammatory properties. Pressure algometry and thermal sensitivity tests are non-invasive methods to quantify pain sensitivity using nociceptive thresholds to provoke withdrawal responses on lame and sound legs. The objective of this work was to determine the effects of these drugs on nociceptive thresholds in sows induced lame using pressure algometry and thermal sensitivity tests. Lameness was induced in 24 mature, mixed-parity sows using a chemical synovitis model and three treatments were compared: meloxicam (1.0 mg kg^{-1} PO), flunixin meglumine (2.2 mg kg^{-1} IM) and sterile saline (IM). Pressure algometry was measured on sound and lame rear legs with three replicates at three landmarks. Thermal sensitivity tests were done on sound and lame rear legs with three replicates using a thermal stimulus at one landmark. From 37 to 72 h after lameness induction, meloxicam- and flunixin meglumine-treated sows tolerated higher pressure algometer nociceptive thresholds compared to saline-treated sows. Changes in thermal nociceptive thresholds were evident at the T_{max} time-points for meloxicam administration and 72 and 168 h post lameness induction for flunixin meglumine-treated sows. In conclusion, flunixin meglumine and meloxicam administration mitigated pain sensitivity in lame sows post lameness induction when pain sensitivity was evaluated with pressure algometry. These analgesic drugs may be a key tool to manage pain associated with lameness.

Keywords: *animal welfare, flunixin meglumine, lameness, meloxicam, nociceptive threshold, swine*

Introduction

Pain has been defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP 2004). Lameness associated with painful joint lesions has been identified as a welfare challenge for confined sows (Elmore *et al* 2010) with lameness ranked as the third most common reason for culling sows, comprising 15% of cull sows marketed in the United States (Schenk *et al* 2010). Culling sows prior to completion of the third parity has been identified as an economic loss as pig producers are neither able to pay off individual sow costs nor capitalise on the benefits of higher sow retention rates (Stalder *et al* 2000, 2003).

Diagnosis of pain associated with lameness is a difficult process due to unique individual experiences with pain (Gaynor & Muir 2009) and differences noted in pain tolerance and reaction between species, breeds, sex, age, pain duration and stimulus severity (Matthew 2000). Danish animal welfare scientists and veterinarians reported that fractures, osteochondrosis dissecans (OCD), and infectious arthritis were ranked highest for pain severity for lameness in swine (Jensen *et al* 2012).

Nociceptive threshold testing, such as pressure algometry and thermal sensitivity tests, can be used for clinical evaluation of painful conditions and analgesic efficacy. Nociception is the process by which the detection, transduction, and transmission of a noxious stimulus to higher centres of the central nervous system occurs (Livingston 2006). Mechanical and thermal nociceptive thresholds (MNT and TNT) can be defined as the amount of pressure or heat stimulation necessary to produce a behavioural response indicative of pain sensitivity (Haussler *et al* 2007). Mechanical and thermal nociceptive threshold tests have been used as objective pain assessment tools in a variety of livestock animals including broilers (Hothersall *et al* 2011), dairy cattle (Veissier *et al* 2000; Herskin *et al* 2003, 2009; Dyer *et al* 2007; Heinrich *et al* 2010; Fitzpatrick *et al* 2013;

Higginson-Cutler *et al* 2013), sheep (Nolan *et al* 1987; Ley *et al* 1989; Stubsjøen *et al* 2009) and swine (Jarvis *et al* 1997; Sandercock *et al* 2009; Di Giminiani *et al* 2012; Janczak *et al* 2012; Tapper *et al* 2013).

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most common categories of drugs used to manage animal pain based on their anti-inflammatory, antipyretic and analgesic properties (Gaynor & Muir 2009). Flunixin meglumine is a common NSAID used in veterinary medicine and is currently labelled for pyrexia control associated with swine respiratory disease at 2.2 mg kg^{-1} dose administered intramuscularly (Intervet Schering Plough 2013). Meloxicam is a member of the oxicam family and is labelled in swine for the treatment of non-infectious locomotor disorders and mastitis-metritis-agalactia syndrome in some European countries at 0.4 mg kg^{-1} dose administered intramuscularly (Friton *et al* 2003). Neither drug is specifically labelled for swine pain management in the United States; any potential application must be considered and guided by a veterinarian in the context of the Animal Medicinal Drug Use Clarification Act (AMDUCA). The objectives of this study were to determine the efficacy of meloxicam and flunixin meglumine for pain mitigation in lame sows using pressure algometer and thermal sensitivity nociceptive threshold tests.

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 the 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 thus reducing the total number of sows required whilst maintaining population sizes large enough to achieve statistical power. 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.

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 [± 15.5] kg). All sows underwent a physical examination and a lameness evaluation prior to selection by a trained veterinarian in charge with expertise in sow lameness. Lameness was evaluated 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. Sows selected for the project were categorised as non-lame. Physical examination and lameness evaluation were also conducted between each round during the trial to confirm no observable residual lameness was present.

To avoid confounding injury due to 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 × 1.4 × 1.2 m (length × width × height) and had a solid concrete floor with a rubber mat (2.4 × 1.4 × 0.02 m). Metal fences (1.2 × 0.76 m; height × width) were affixed to the end of each home pen. Each pen was provided with a form of environmental enrichment, including chains and/or plastic toys attached to the pen gates. Sows were provided *ad libitum* access to water via one nipple drinker and hand-fed 2.7 kg of a custom-mixed diet of 14.8% CP TMR composed of ground corn, soybeans, and nutrients formulated according to Swine NRC guidelines (2012) with no antimicrobials. FDA-approved Matrix® (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 the methods previously described (Karriker *et al* 2013) and a repeated measures design compared responses up to seven days following lameness induction. The following treatments were administered twice during each round, 24 h apart: meloxicam (1.0 mg kg^{-1} per os), flunixin meglumine (2.2 mg kg^{-1} intramuscular) and sterile saline (equivalent volume administered intramuscular). Two trials consisting of 12 sows per trial were conducted for a total of 24 sows and each trial consisted of three rounds of treatment, with a different treatment administered for each round. Sows were assigned to three blocks (four sows per block) by bodyweight and each block was randomly allocated to one of three treatments for round one. A ten-day wash-out period was provided between rounds to avoid previous treatment carry-over effects. In round one, sows were randomly assigned to one of three treatments and lameness induction was assigned to either the left or right rear leg so that leg assignment and treatment were balanced. 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 all sows received all three treatments. Prior to subsequent treatment round, pressure algometry and thermal sensitivity tests were conducted, sows were gait-scored and blood was collected to determine any lameness and residual drug carry-over.

Treatments

Three treatments were administered: i) meloxicam (1.0 mg kg^{-1} per os administered in 8 g cookie dough with additional sterile saline injected intramuscularly; n = 24); ii) flunixin meglumine (2.2 mg kg^{-1} administered intramuscularly with 8 g cookie dough; n = 24); or iii) sterile saline (administered intramuscularly at an equivalent volume to

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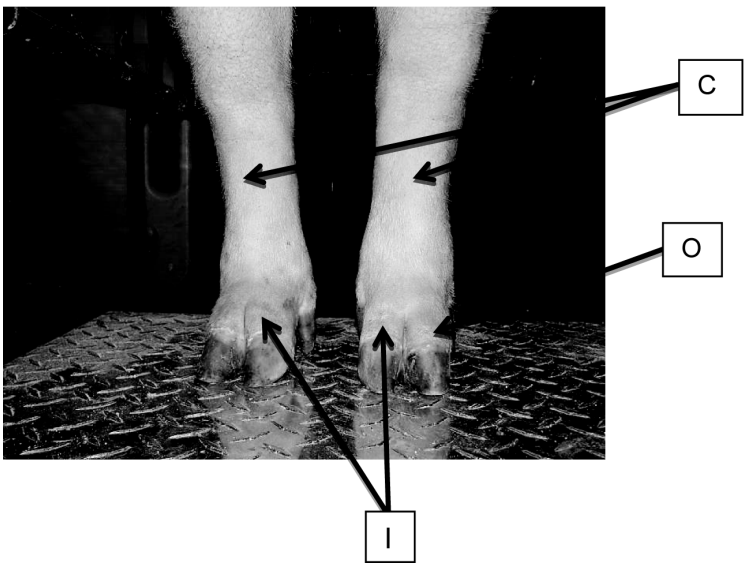
Figure 1

Pressure algometer landmark schematic. C = Middle of the cannon on the hind limb; O = 1 cm above the coronary band on the lateral hind claw; I = 1 cm above the coronary band on the medial hind claw.



flunixin meglumine with 8 g cookie dough; n = 24). Flunixin meglumine treatments were administered 27.5 and 51.5 h post induction and meloxicam 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. To control for observer bias, researchers were blinded to analgesic treatments, but could not be blinded to the trial day.

Pain sensitivity tests

Pain sensitivity tests were performed while sows were confined in a modified gestation stall (2.0 × 0.61 m; length × width) outside of the home pen, using methods previously described by Tapper and colleagues (2013). Sows were provided *ad libitum* access to feed by sprinkling feed into the stall feeder (≤ 1 kg feed per collection time) during testing to facilitate a relaxed standing posture. During acclimation, sows were trained to enter and stand in the testing stall where they received a portion of their standard feed to reinforce the behaviour. Acclimation was assessed by the sow's willingness to enter the stall feeder without human intervention, stand quietly and consume ration during data collection; at the end of acclimatisation all sows met this criteria. Both rear legs were rinsed with water and dried using paper towels to completely remove any dirt and dried faecal matter that might have been present. Scrubbing was not performed on the leg and if excessive dirt or faeces was present, the leg was soaked as to not cause a localised painful response. Pain sensitivity tests were performed at the same time of day to control for possible circadian behaviour and pain sensitivity patterns (Hastings 2010). The observer was blinded to the numeric output values during the pain sensitivity test assessment.

Pressure algometry

A hand-held pressure algometer (Wagner Force Ten™ FDX 50 Compact Digital Force Gage, Wagner Instruments, CT, USA) with a 1 cm^2 flat rubber tip was used to quantify MNTs in kilograms of force (Kgf) as calculated by the instrument. In an attempt to standardise the procedure and reduce variability associated with handler application of the device, the technicians trained in the application of the pressure algometer practised applying the force at a rate of approximately one Kgf s^{-1} on a static surface for 10-s periods during the seven-day acclimation period and immediately prior to data collection daily. Furthermore, the technician was blinded to the numeric output values during the pain sensitivity tests. An additional technician served as the recorder and was assigned to collect the output data. During data collection, pressure algometry was applied at the landmarks at approximately one Kgf s^{-1}. The maximum force applied was 10 Kgf, after which the recorder said 'Stop' and pressure was removed. Pressure was applied perpendicularly to three landmarks in a randomised sequence for each sow: i) middle of cannon on the rear leg (C); ii) 1 cm above the coronary band on the lateral rear claw (O); and iii) 1 cm above the coronary band on the medial rear claw (I; Figure 1). The outer and inner landmark represented where the drug was injected to induce lameness and were included as pain landmarks. The cannon landmark was included as a control landmark. The landmark sequences were repeated in triplicate on the right rear leg followed by the same sequence repeated in triplicate on the left rear leg. When a foot-lift response was observed, pressure was immediately removed, and the peak pressure representing the MNT recorded.

Table 1 Pressure algometer and thermal nociceptive threshold data sampling time-points.

Time (h)	Event/treatment application	Data sampling time-point
–24		Baseline
0	Lameness induction[†]	
24		Day 1 pre-treatment
27.5	Flunixin treatment[‡]	
28.5	Meloxicam treatment	Day 1 T_{max}[§] flunixin
30.5		Day 1 T_{max} meloxicam
36		Day 1 half-life[#]
48		Day 2 pre-treatment
51.5	Flunixin treatment	
52.5	Meloxicam treatment	Day 2 T_{max} flunixin
54.5		Day 2 T_{max} meloxicam
60		Day 2 half-life
72		Day 3
168		Recovery
312		Baseline for next round

[†] Lameness induced using a chemical synovitis model (Karriker *et al* 2013).

[‡] 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). Flunixin treatments administered 3.5 h after pre-treatment data collection. Meloxicam treatments administered 4.5 h after pre-treatment data collection. Saline treatments randomly administered either 3.5 or 4.5 h after morning data collection.

[§] T_{max} defined as the time in which drug reaches it maximum concentration. T_{max} for meloxicam-treated sows was 2 h after drug administration (unpublished data). T_{max} for flunixin-treated sows was 1 h after drug administration (Pairis-Garcia *et al* 2013). Sows treated with saline had data collected randomly at either 1 or 2 h after drug administration.

[#] Half-life defined as the time in which the drug reaches half of its maximum concentration. Half-life for all three treatments was 8 h after drug/saline administration (37 and 60 h post-induction for flunixin and meloxicam, respectively) (unpublished data; Pairis-Garcia *et al* 2013).

Thermal sensitivity

For consistent data collection across all treatments and trial days, the thermal sensitivity test immediately followed the pressure algometer test and measured the latency for a sow to withdraw her rear leg in response to precise, focused radiant heat stimulation. The analgesia meter (IITC Plantar Analgesia Meter, IITC Life Science Inc, Woodland Hills, CA, USA) was set at a constant 80% beam intensity; emitting 200°C. Prior research by the authors (Tapper *et al* 2013) determined that tissue damage did not occur when using a 20-s maximum duration. Thermal measurements were taken in triplicate 1 cm above the coronary band on the lateral side of the right rear leg, followed by the left rear leg. The latency for the sow to withdraw her leg in response to the stimulus was recorded.

Data time-point collection schedule is described in Table 1. Data for the pressure algometer and thermal sensitivity test were collected at the following time-points: –24 h (baseline), 24 h (Day 1 pre-treatment), 28.5 and 30.5 h (T_{max} for day 1), 36 h (Half-life for day 1), 48 h (Day 2 pre-treatment), 52.5 and 54.5 h (T_{max2} for day 2), 60 h (Half-life2 for day 2), 72 h (Day 3), 168 h (Recovery) and 312 h (Baseline for next round). The T_{max} is defined as the time in which the drug reaches its maximum concentration and half-life is defined as the amount of time it takes for the drug concentration to be reduced by one half. These values for flunixin meglumine were calculated in a previous pharmacokinetic experiment (Pairis-Garcia *et al* 2013). The T_{max} and half-life for meloxicam were calculated using data from a previous pharmacokinetic experiment conducted in our laboratory (unpublished data). The T_{max} for flunixin meglumine and meloxicam were 1 and 2 h after drug administration, respectively. As the T_{max} for meloxicam and flunixin meglumine were different, measurements collected at this time could not be directly compared. For both NSAIDs, half-life was 8 h after drug administration.

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 using Proc-Univariate. PROC MIXED procedures of SAS was used to analyse response differences between sound and lame legs (Response). The pressure algometer and thermal sensitivity test statistical models included the fixed effect of treatment, round, time-point, leg injected, treatment by time-point interaction, and treatment by time-point by leg injected interaction. Sow within group by trial interaction was included as a random effect and replicate within round by time-point by leg injected interaction was included as a repeated statement. An auto-regressive correlation was used for the repeated statement. A P-value of < 0.05 was considered to be significant for the MIXED analysis of variance and when separating means. Fixed effect least square means were separated using the PDIFF option in SAS and data were expressed as LS means (± SEM).

Results

Transient synovitis model

Prior to anaesthesia lameness induction and at the start of each subsequent round, all sows were clinically sound, defined as the ability to move freely using all four legs, showing no evidence of weight-shifting activities, non-weight-bearing, or reluctance to walk or stand on any leg. Peak lameness was observed on day one pre-treatment after lameness induction and all sows developed clinical signs of lameness including weight-shifting and reluctance to walk or stand on the injected leg. No sows became non-weight-bearing during the trial. No differences were observed between baseline day and baseline for next round for pressure algometry and thermal sensitivity test responses (Figure 2[a], 3[a]). Blood analysis (data not shown; Pairis-Garcia *et al* 2013) confirmed

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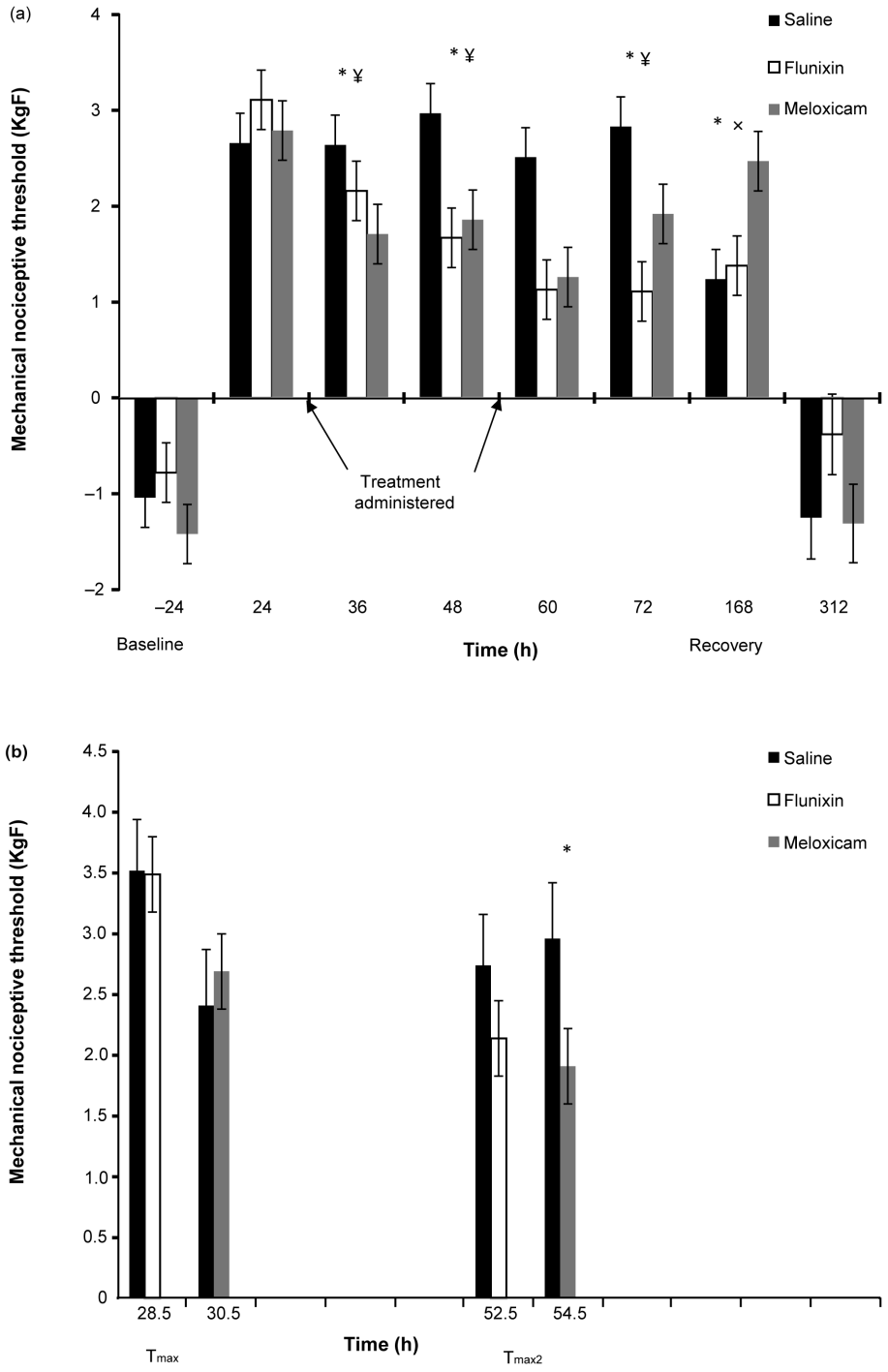
Figure 2

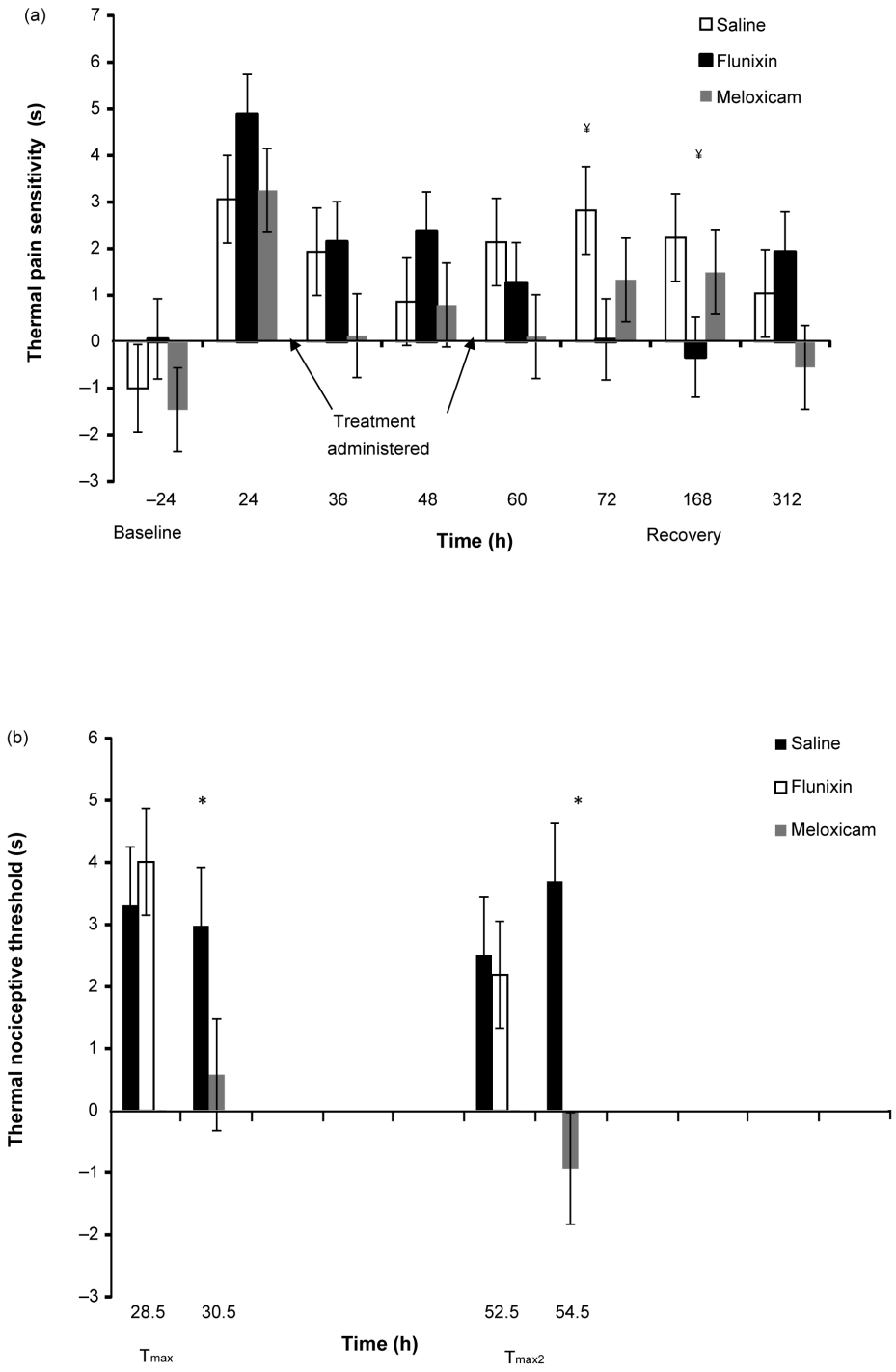


Showing (a) LS means (± SEM) of mechanical nociceptive thresholds (KgF) for difference in pressure[1] tolerated on the sound and lame leg of lame-treated sows. [1] A pressure algometer was used to determine mechanical nociceptive thresholds. The test was administered in triplicate for all landmarks and all time-points. [2] Treatments: 1) meloxicam (M; 1.0 mg kg^{-1} per os in cookie dough; n = 24); 2) flunixin meglumine (FM; 2.2 mg kg^{-1} intramuscular [IM]; n = 24); or 3) saline (S; equivalent volume to FM administered IM; n = 24). ¥ Denotes difference between saline- and flunixin meglumine-treated sows ($P < 0.05$); × Denotes difference between meloxicam- and flunixin meglumine-treated sows ($P < 0.05$); * Denotes difference between saline- and flunixin meglumine-treated sows ($P < 0.05$).

Showing (b) LS means (± SEM) of mechanical nociceptive thresholds (KgF) for difference in pressure[1] tolerated on the sound and lame leg of lame-treated[2] sows at T_{max}[3]. [1] A pressure algometer was used to determine mechanical nociceptive thresholds. The test was administered in triplicate for all landmarks and all time-points. [2] Treatments: 1) meloxicam (M; 1.0 mg kg^{-1} per os in cookie dough; n = 24); 2) flunixin meglumine (FM; 2.2 mg kg^{-1} intramuscular [IM]; n = 24); or 3) saline (S; equivalent volume to FM administered IM; n = 24).

[3] The T_{max} is defined as the time in which the drug reaches its maximum concentration after administration. The T_{max} and T_{max2} for flunixin meglumine was at 28.5 and 52.5 h after lameness induction. The T_{max} and T_{max2} for meloxicam was 30.5 and 54.5 h after lameness induction. * Denotes difference between saline- and meloxicam-treated sows ($P < 0.05$).

Figure 3



Showing (a) LS means (± SEM) of thermal nociceptive thresholds (S) for difference in heat stimulation[1] tolerated on the sound and lame leg of lame-treated[2] sows. [1] A thermal sensitivity test was used to determine thermal nociceptive thresholds. The test was administered in triplicate for all time-points. [2] Treatments: 1) meloxicam (M; 1.0 mg kg^{-1} per os in cookie dough; n = 24); 2) flunixin meglumine (FM: 2.2 mg kg^{-1} intramuscular [IM]; n = 24); or 3) saline (S; equivalent volume to FM administered IM; n = 24). ¥ Denotes difference between saline- and flunixin meglumine-treated sows ($P < 0.05$).

Showing (b) LS means (± SEM) of thermal nociceptive thresholds (S) for difference in heat stimulation[1] tolerated on the sound and lame leg of lame-treated[2] sows at T_{max}[3]. [1] A thermal sensitivity test was used to determine thermal nociceptive thresholds. The test was administered in triplicate for all time-points. [2] Treatments: 1) meloxicam (M; 1.0 mg kg^{-1} per os in cookie dough; n = 24); 2) flunixin meglumine (FM; 2.2 mg kg^{-1} intramuscular [IM]; n = 24); or 3) saline (S; equivalent volume to FM administered IM; n = 24). [3] The T_{max} is defined as the time in which the drug reaches its maximum concentration after administration. The T_{max} and T_{max2} for flunixin meglumine was at 28.5 and 52.5 h after lameness induction. The T_{max} and T_{max2} for meloxicam was 30.5 and 54.5 h after lameness induction. * Denotes difference between saline and meloxicam treated sows ($P < 0.05$).

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Table 2 Mean (± SEM) descriptive nociceptive threshold for a foot-lift response to a pressure algometry test† applied to saline-treated sows using a lameness induction model.

		Trial time-point‡				
Landmark§	**Leg#**	**Baseline**	**Day 1 Pre-treatment**	**Day 2 Pre-treatment**	**Day 3**	**Recovery**
Cannon	Lame	5.93 (± 0.34)	2.93 (± 0.34)	2.97 (± 0.34)	3.66 (± 0.34)	5.22 (± 0.34)
	Sound	5.42 (± 0.43)	4.35 (± 0.43)	5.26 (± 0.43)	5.82 (± 0.43)	5.46 (± 0.43)
Outer	Lame	5.70 (± 0.34)	1.12 (± 0.34)	0.94 (± 0.34)	1.07 (± 0.34)	3.01 (± 0.34)
	Sound	4.30 (± 0.43)	4.43 (± 0.43)	4.04 (± 0.43)	4.29 (± 0.43)	4.90 (± 0.43)
Inner	Lame	5.62 (± 0.34)	1.03 (± 0.34)	1.04 (± 0.34)	0.93 (± 0.34)	2.97 (± 0.34)
	Sound	4.47 (± 0.43)	4.35 (± 0.43)	4.61 (± 0.43)	4.11 (± 0.43)	4.64 (± 0.43)

† A foot-lift response was used to determine the mechanical nociceptive thresholds (MNTs in kg of force, Kgf) in response to a pressure algometry test.
‡ This test was administered in triplicate to 24 sows on baseline (–24 h prior to lameness induction), Day 1 (24 h post lameness induction; pre-treatment), Day 2 (48 h post lameness induction; pre-treatment), Recovery (72 h post lameness induction) and Recovery (168 h post lameness induction).
§ Pressure was applied perpendicularly to three landmarks in a randomised sequence for each sow: i) middle of cannon on the rear leg (Cannon); ii) 1 cm above the coronary band on the lateral rear claw (Outer); and iii) 1 cm above the coronary band on the medial rear claw (Inner).
# Lameness was induced using a chemical synovitis model (Karriker *et al* 2013) and lameness was induced on either the left or right rear leg.

Table 3 Mean (± SEM) descriptive nociceptive threshold for a foot-lift response to a thermal sensitivity test† applied to saline-treated sows using a lameness induction model.

	Trial time-point‡				
Leg§	**Baseline**	**Day 1 Pre-treatment**	**Day 2 Pre-treatment**	**Day 3**	**Recovery**
Lame	10.5 (± 0.70)	5.18 (± 0.70)	5.09 (± 0.70)	4.88 (± 0.70)	8.49 (± 0.70)
Sound	9.43 (± 0.93)	8.18 (± 0.93)	5.90 (± 0.93)	7.63 (± 0.93)	10.7 (± 0.93)

† A foot-lift response was used to determine the thermal nociceptive thresholds (TNTs [s]) in response to a thermal sensitivity test. Thermal measurements were taken in triplicate 1 cm above the coronary band on the lateral side of the right rear leg, followed by the left rear leg using a laser set at 80% intensity, emitting 200°C.
‡ This test was administered in triplicate to 24 sows on baseline (–24 h prior to lameness induction), Day 1 (24 h post lameness induction; pre-treatment), Day 2 (48 h post lameness induction; pre-treatment), Recovery (72 h post lameness induction) and Recovery (168 h post lameness induction).
§ Lameness was induced using a chemical synovitis model (Karriker *et al* 2013) and lameness was induced on either the left or right rear leg.

systemic drug levels were below the limit of detection in between rounds suggesting that ten days was a sufficient wash-out period for systemic drug clearance.

Pressure algometry

Throughout the study, 4.6% of the response values were above the maximum pressure applied to 10 Kgf. Pressure tolerated on the sound and lame leg for saline-treated sows across all landmarks over the round can be found descriptively in Table 2. No differences were observed between sound and lame leg responses on baseline days between treatments ($P > 0.05$; Figure 2[a]). When comparing the pressure tolerated when sows were most lame (day 1 pre-treatment) to baseline, sound and lame leg responses differed at all landmarks ($P < 0.0001$). However, there were no treatment differences between sound and lame leg responses on day 1 pre-treatment ($P > 0.05$; Figure 2[a]). Thirty-seven hours after lameness induction (Half-life) and up to Day 3 after lameness induction, both flunixin meglumine and meloxicam sows tolerated greater pressure compared to saline sows ($P < 0.01$). When comparing flunixin meglumine- to saline-treated sows at T_{max} and T_{max2}, no differences were observed (Figure 2[b]). However, when comparing meloxicam- to saline-treated sows, differences were observed at T_{max2} (Figure 2[b]). Leg injected (Left leg: 2.12 [± 0.17]; Right leg: 1.60 [± 0.17] KgF) and round (Round 1: 2.36 [± 0.18]; Round 2: 1.99 [± 0.17]; Round 3: 1.79 [± 0.17] KgF) had an effect on MNT ($P < 0.001$).

Thermal sensitivity

Throughout the study, 9.5% of the response values were above the maximum 20 s the thermal test was applied. Time that thermal heat was tolerated on the sound and lame leg for saline-treated sows can be found descriptively in Table 3. There was no difference between sound and lame leg latency responses on baseline days between treatments ($P > 0.05$; Figure 3[a]). When comparing time in which heat stimulation was tolerated when sows were most lame (day 1 pre-treatment) to baseline, sound and lame leg latency responses differed ($P < 0.001$). However, there were no treatement differences between sound and lame leg latency responses on day 1 pre-treatment ($P > 0.05$; Figure 3[a]).

Sows administered flunixin meglumine tolerated heat stimulation longer compared to saline sows at 72 and 168 h after treatment administration ($P < 0.01$; Figure 3[a]), however this did not differ from meloxicam-treated sows. When comparing flunixin meglumine- to saline-treated sows at T_{max} and T_{max2}, no differences were observed (Figure 3[b]). However, when comparing meloxicam- to saline-treated sows, differences were observed at T_{max} and T_{max2} time-points (Figure 3[b]; $P < 0.001$). Leg injected (Left leg: 2.22 [± 0.50]; Right leg: 0.77 [± 0.48] s) and round (Round one: 2.88 [± 0.5]; Round two: 1.20 [± 0.49]; Round three: 2.66 [± 0.49] s) had an effect on TNT ($P < 0.001$).

Discussion

Transient synovitis model

This amphotericin B-induced lameness model produced a transient and reproducible synovitis of the distal interphalangeal joint for all sows. All sows were clinically sound prior to lameness induction for each round, showing no evidence of weight-shifting activities, non-weight bearing, or reluctance to walk or stand on any leg. Peak lameness was observed 24 h after induction with all sows demonstrating clinical lameness including weight-shifting and reluctance to walk. This coincides with results from previously published work assessing validity of amphotericin B-induced lameness model in swine (Karriker *et al* 2013; Tapper *et al* 2013) and cattle (Kotschwar *et al* 2009). Responses to pressure algometry and thermal sensitivity tests did not differ between baseline days at the start of each round confirming no lameness carry-over from previous rounds. In addition, blood collection tests on baseline days confirmed systemic drug levels were below the limit of detection.

Pain sensitivity tests

Both the MNT and TNT were easily applied and successfully demonstrated differences in pain sensitivity between baseline and all time-points up to recovery (168 h) regardless of treatment. This suggests both the pressure algometer and thermal sensitivity test are objective tools to assess pain sensitivity in this lameness induction model. The pressure algometry and thermal sensitivity results coincide with results by Tapper and colleagues (2013) demonstrating similar although slightly lower thresholds for both the sound and the lame leg. For the pressure algometer, 4.6% of all data for the pressure algometer reached the maximum pressure of 10 Kgf, as compared to Tapper and colleagues with 13.5%. However, 9.5% of all thermal sensitivity data resulted in the maximum 20 s duration as compared to 4.6% in Tapper and colleagues' work (2013). One possible explanation may be that Tapper and colleagues (2013) used a different veterinarian to perform the lameness induction protocol and different induction technique may have played a role. In our study, the pressure algometer was able to detect changes in pain sensitivity using a lameness induction model. In addition, this tool was also able to detect differences between treated and non-treated animals, confirming the sensitivity of pressure algometry as a tool to evaluate analgesic efficacy in laboratory settings. Our study contributes to the growing body of knowledge across livestock species supporting the use of pressure algometry to quantify pain sensitivity (Dyer *et al* 2007; Sandercock *et al* 2009; Stubsjøen *et al* 2009; Heinrich *et al* 2010; Hothersall *et al* 2011; Nalon *et al* 2013; Tapper *et al* 2013).

Unlike Tapper and colleagues (2013), our data demonstrate that the thermal sensitivity test is also an objective tool to assess pain sensitivity. No differences were noted between treatments at baseline. However, differences were observed from baseline through to recovery, suggesting that the thermal sensitivity test detected pain sensitivity associated with lameness. Improvement in methodologies, such as completely drying both legs prior to thermal heat application, may have been a reason why differences were seen between days in our study. Although this test detected differences from baseline through recovery, it was not sensitive enough to detect drug effects. Differences were only noted between saline- and meloxicam-treated sows when measurements were taken at the time meloxicam likely attained maximum drug concentration.

Differences between the left and right rear legs were observed for both thermal and pressure algometry; the right leg consistently tolerated less pressure or thermal stimulation compared to the left leg. These differences may be due to the order in which the measurements were applied with the right leg always being measured first. Anticipatory or pain-related fear behaviour has been acknowledged in human medicine as a cause for changes in pain sensitivity, often resulting in pain enhancement (Crombez *et al* 1999a,b). It is likely that the decrease in nociceptive thresholds on the right leg is due to the sow being startled by initial manipulation of the leg and anticipating or fearing pain onset. Nalon and colleagues (2013) found that when comparing nociceptive thresholds using a hand-held probe compared to a limb-mounted actuator, MNT were significantly lower with the hand-held probe. This may be due to the sow's reaction towards the operator approaching the limb, anticipating that stimulus and therefore reacting faster. It is possible that by the time that data were collected on the right leg, the sow may have become desensitised to this manipulation and presence of the observer resulting in higher nociceptive thresholds for the left leg. Further research is needed to determine if there is a true leg sensitivity difference or if this is a methodology artefact.

Round had an effect on the thermal sensitivity and pressure algometry tests. The TNT decreased during round two while the MNT decreased with each subsequent round. Janczak and colleagues (2012) evaluated the stability and repeatability of measuring MNT using a hand-held algometer in piglets not in pain. The authors of this study found several factors influencing MNT including habituation time, pig weight, testing week, days within test week and replication within the week. This study concluded that repeated measures can be used to evaluate changes in pain threshold in pigs; however habituation for at least several days is required to gain higher correlations among MNT responses (Janczak *et al* 2012). The sows used in this present study were acclimated to both tests for seven days prior to round one, but were not handled

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during the wash-out period prior to rounds 2 and 3. This may explain changes in response between rounds. There were no differences found between rounds when comparing baseline day, indicating that the difference in habituation does not explain the difference in response over rounds to these tests. It is possible that sows became sensitised to the lameness induction over rounds which resulted in increased responses during lameness. Further studies are needed to evaluate if there are changes to sow sensitivity and responses to lameness induction or amphotericin B.

Analgesic efficacy

Meloxicam and flunixin meglumine mitigated pain sensitivity between 36 and 72 h after lameness induction compared to saline-treated sows when using the pressure algometer test. Previous studies have also demonstrated flunixin meglumine and meloxicam efficacy for acute and chronic pain mitigation in cattle (Currah *et al* 2009; Heinrich *et al* 2010; Schulz *et al* 2011; Fitzpatrick *et al* 2013 Huber *et al* 2013), sheep (Welsh & Nolan 1995) and swine (Friton *et al* 2003; Reiner *et al* 2012; Kluviers-Poodt *et al* 2013). Our results agree with Schulz and colleagues (2011) who found flunixin meglumine to be efficacious in providing analgesia for steers induced lame using an amphotericin B model. However, our results differ from previously published data evaluating flunixin meglumine using the same transient lameness model in sows (Tapper *et al* 2013). Unlike Tapper and colleagues (2013), our experiment evaluated several additional time-points to assess pain sensitivity. The additional time-points and deliberate choice to collect data during the drug's T_{max} and half-life was based on the goal to collect data in a window of time in which the drug may be most effective. However, no studies, to date, have determined at what time either drug is maximally effective in sows. It is unknown if these additional time-points resulted in our ability to detect differences in pain sensitivity because: i) during these time-points the drug reached its maximum analgesic efficacy; or ii) additional time-points and increased enrolled sow numbers contributed to greater statistical power associated with a larger data set.

The analgesic effects of flunixin meglumine and meloxicam did not differ in the period between 36 and 72 h post lameness induction, although treatments could not be compared at T_{max} as T_{max} was different between meloxicam and flunixin meglumine. Meloxicam administered orally has several advantages over flunixin meglumine for use in the field including: i) cost (oral meloxicam administration costs approximately US$0.004 per kg bodyweight to administer at 1.0 mg kg^{-1}); ii) reduced stress (oral meloxicam does not require physical restraint for administration); iii) decreased macroscopic lesions of the muscle and fibrous tissue at drug injection sites (Magyan & Glavits 2007); and iv) decreased public health risk associated with accidental needle breakage into muscle (Chase *et al* 2008). As neither drug is specifically labelled for pain management for swine in the United States, administration of either product would be considered extra-label drug use (ELDU). This practice, which is regulated under the Animal Medicinal Drug Use Clarification Act (AMDUCA), requires that drugs be administered under the supervision or by a veterinarian with an established veterinary-client-patient relationship (Coetzee 2011).

Animal welfare implications and conclusion

Meloxicam and flunixin meglumine were effective in modifying pain sensitivity in lame sows evaluated using pressure algometry. Thermal sensitivity tests were also applied during this time but were only sensitive enough to detect changes in pain sensitivity immediately after drug administration. Our research suggests that meloxicam and flunixin meglumine are effective pharmaceutical interventions for pain mitigation associated with a chemically induced synovitis model. Further research evaluating the efficacy and optimising the dose regimen of these drugs in chronic or naturally occurring lameness on-farm should be investigated.

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