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# Thermal and Mechanical Nociception Threshold Tests as Objective Tools to Measure Painful and Non-Painful Lameness Phases in Multiparous Sows

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### Summary and Implications

The objective of this study was to quantify differences in thermal and mechanical nociception thresholds when sows are in painful and non-painful lameness states. Twelve, clinically healthy, mixed-parity, crossbred sows were used for each of the nociceptive threshold tests. The sow was the experimental unit and a cross-over design with a 2 (left and right hind limb) x 3 (days: D-1, D+1 and D+6) factorial arrangement of treatments were compared. On induction day (D0), 10 mg of amphotericin B were injected in the distal interphalangeal joint space in both claws of one hind limb. All sows served as their own control and treatment. After completion of the first round, sows were given a 7-d rest period and then the round was repeated with the opposite hind limb induced. All data were statistically analyzed using the PROC MIXED procedure in SAS. A  $P$  value of  $\leq 0.05$  was considered to be significant. No differences were observed for sows that had lameness induced in the left- vs. right hind limb for either the thermal ( $P = 0.68$ ) or mechanical ( $P = 0.97$ ) threshold tests. There were also no differences between first and second rounds of induction for the thermal ( $P = 0.18$ ) or mechanical ( $P = 0.28$ ) threshold tests. For the thermal nociceptive tests, there was a significant difference for the induced limb when comparing D-1 and D+1 ( $P < .0001$ ), indicating the lame hind limb tolerated less thermal nociception when in a most lame phase. Similarly, mechanical pressure tolerated by the lame hind limb decreased for every landmark ( $P < 0.05$ ) when comparing D-1 and D+1. Both tools showed a decreased tolerance of mechanical and thermal stimulation when in a

most lame phase, indicating potential for limb lameness detection in sows.

### Introduction

There are currently no analgesic drugs specifically approved for pain relief in livestock by the U.S. Food and Drug Administration (FDA). The FDA Guidance Document 123 for the development of effectiveness data for non-steroidal anti-inflammatory drugs (NSAIDs) states that “*validated methods of pain assessment must be used in order for a drug to be indicated for pain relief in the target species.*” As a result, identification and validation of robust, repeatable pain measurements are essential for the development and approval of analgesic drug regimens for use in food animals. The thermal nociception threshold (TNT) and mechanical nociception threshold (MNT) tests measure the minimum thermal and mechanical stimulation, respectively, to elicit an avoidance response. Both of these tests may be an objective and practical tool that could be used to determine the severity of lameness in sows. Therefore, the objective of this study was to quantify differences in TNT and MNT in multiparous sows in painful and non-painful lameness states.

### Materials and Methods

**Animals and housing:** This project was approved by the Iowa State University IACUC. For the TNT test, twelve, apparently healthy, mixed-parity, crossbred sows ( $211.41 \pm 20.21$  kg) were purchased from a commercial producer in Iowa. For the MNT test, twelve, apparently healthy, mixed-parity, crossbred sows ( $228.89 \pm 19.17$  kg) were purchased from a commercial producer in Iowa. To avoid confounding injury due to aggression, each sow was housed individually. Each sow was housed in a concrete pen providing  $5.1 \text{ m}^2$  and a 0.6 m deep concrete ledge along the rear wall of the pen where sows were fed. A rubber mat was provided for sow comfort. All sows were fed twice daily to meet their dietary requirements. Sows had *ad libitum* access to water via one nipple drinker that was positioned over a grate. Pens were set up in two rows with a central aisle and allowed for nose to nose contact with cohorts. Lights were on a 12:12 light dark cycle with light hours between 0600 and 1800. Sows were acclimated for 10 days before any treatments were applied. The research for the TNT test was conducted October-November, 2011; research for the MNT test was conducted July-August, 2011.

**Experimental design and treatments:** The sow was the experimental unit. A cross over design with a 2 (hooves:

right hind and left hind) x 3 (days: D-1, D+1 and D+6) factorial arrangement of treatments were compared. Three days were compared: D-1 (sound phase; defined as 1 day pre-induction of lameness); D+1 (most lame phase; defined as 1 day post-induction of lameness) and D+6 (resolution phase; defined as 6 days after the induction of lameness). All sows served as their own control and treatment. After completion of the first round of induction, sows were given a 7-day rest period and then a second round was conducted with the opposite hind hoof induced.

**Induction of Lameness:** All sows were restrained in a standing position using a humane pig snare and then anesthetized using a combination of Xylazine (4.4 mg/kg), Ketamine HCl (2.2 mg/kg), and Tiletamine HCl and Zolazepam in combination (Telazol<sup>®</sup>; 4.4 mg/kg) administered IM. The assigned claws to be injected were washed with mild soap and water to remove obvious fecal contamination, scrubbed for 3 minutes with iodine based surgical scrub using 10 x 10 cm sterile gauze pad, and rinsed with 70 % isopropyl alcohol until no evidence of the surgical scrub remains. Ten mg of amphotericin B were injected in the distal interphalangeal joint space in both claws of one hind limb. All sows were monitored continuously until fully recovered.

**Thermal nociception threshold (TNT) test:** A constant radiant thermal stimulus was applied to the coronary band on both rear hooves on the three treatment days. To prevent tissue damage, a 20 second maximum duration was set, after which the stimulus automatically turned off. Thermal nociception was stopped when the limb was withdrawn or when 20 seconds was reached (Figure 1).

**Figure 1. Thermal Nociception Threshold (TNT) test.**



**Mechanical nociception threshold (MNT) test:** A hand-held pressure algometer with a 1 cm<sup>2</sup> flat rubber tip was used to quantify mechanical nociceptive thresholds (MNTs) in kilograms of force (kgf). The application rate for all sows on all landmarks was approximately 1 kgf/second. The maximum force applied was 10 kgf for a 10 second period to prevent tissue damage. Pressure was applied perpendicularly to 3 landmarks in a randomized sequence for each sow 1) Cannon: middle of cannon on the hind limb,

2) Medial claw: 1 cm above the coronary band on the medial hind claw and 3) Lateral claw: 1 cm above the coronary band on the lateral hind claw. The randomized landmark sequence was repeated in triplicate on the right hind limb followed by the same sequence repeated in triplicate on the left hind limb. When a limb-lift response was observed or if 10 kgf over 10 second was reached pressure was immediately removed, and the peak pressure representing the MNT was recorded (Figure 2).

**Figure 1. Mechanical Nociception Threshold (MNT) test on lateral claw landmark.**



### Statistical Analysis

Data were analyzed using SAS for parametric data. PROC UNIVARIATE was applied to all of the tools to test for normalcy. Both the TNT and MNT tests were not normal. However, results did not differ from the MIXED procedure and since the variables are continuous, both data sets were fit to the MIXED model. The TNT test model included replicate (defined as the first, second or third application of the tool), round, leg (defined as the limb induced lame for that round, either left or right hind) and the interaction of day\*limb status (defined as either the lame or sound hind limb). Sow within day and sow within round were fitted as random effects with replicate within round\*day\*limb status as a repeated effect. The MNT test model included replicate (first, second or third completion of landmark order), landmark order (order of landmarks within a replicate), leg (defined as the limb induced lame for that round, either left or right hind), and the interaction of day\*limb status\*landmark (landmark defined as cannon bone, medial claw, or lateral claw). Sow within day, sow within round and landmark order within day were fitted as random effects. Replicate within round\*day\*landmark\*limb status was fit as a repeated effect. A separate model including replicate, round, landmark order, leg, and the interactions of day by limb status and day by landmark was used to assess differences between rounds of induction for MNT. A  $P$  value of  $\leq 0.05$  was considered significant and PDIFF was used to determine differences for both the MNT and TNT tests.

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### Results and Discussion

**TNT test:** There were no differences observed when the right- or left-hind limb was induced lame ( $9.48 \pm 0.83$  sec vs.  $9.95 \pm 0.83$  sec;  $P = 0.68$ ) or between the first and second rounds of induction ( $8.90 \pm 0.82$  sec vs.  $10.53 \pm 0.83$  sec;  $P = 0.18$ ). When comparing thermal sensitivity over the 3 treatment days, thermal stimulation tolerated by the sound limb did not change ( $P > 0.05$ ). However, the sows tolerated less heat stimulation on the lame limb on D+1 compared to D-1 ( $P < 0.05$ ; Table 1).

**MNT test:** There were no differences observed when the right- or left-hind limb was induced lame ( $5.02 \pm 0.28$  and  $5.03 \pm 0.28$ ;  $P = 0.97$ ) or between first and second rounds of induction ( $5.24 \pm 0.28$  and  $4.82 \pm 0.28$ ;  $P = 0.28$ ). No differences were seen for landmark order ( $P = 0.20$ ). Pressure tolerated by the lame limb decreased for every landmark ( $P < 0.05$ ) on D+1 compared to D-1. The sound limb did not vary in tolerance threshold between D-1 and D+1 ( $P > 0.05$ ; Table 1). Both tools showed a decreased

tolerance of mechanical and thermal stimulation when in a most lame phase, indicating potential for limb lameness detection in sows.

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**Table 1. Comparison of treatment days (D-1, D+1 and D+6) for the sound and lame hind hooves.**

Limb status	Landmark	Day		
		D-1	D+1	D+6
<b>Thermal nociception threshold (TNT) test (sec)</b>				
Sound	Lateral claw	$10.25 \pm 0.86^a$	$9.87 \pm 0.89^a$	$11.60 \pm 0.87^a$
Lame	Lateral claw	$11.99 \pm 0.86^a$	$5.02 \pm 0.89^b$	$9.55 \pm 0.87^c$
<b>Mechanical nociception threshold test (kgf)</b>				
Sound	Cannon bone	$6.51 \pm 0.37^a$	$6.05 \pm 0.38^a$	$6.89 \pm 0.38^a$
	Medial claw	$6.26 \pm 0.37^a$	$6.60 \pm 0.38^{ab}$	$7.36 \pm 0.38^b$
	Lateral claw	$5.30 \pm 0.37^a$	$6.16 \pm 0.38^{ab}$	$6.98 \pm 0.38^b$
Lame	Cannon bone	$6.51 \pm 0.37^a$	$3.82 \pm 0.38^b$	$3.61 \pm 0.38^b$
	Medial claw	$6.94 \pm 0.37^a$	$0.94 \pm 0.38^b$	$1.86 \pm 0.38^b$
	Lateral claw	$6.41 \pm 0.37^a$	$0.93 \pm 0.38^b$	$1.39 \pm 0.38^b$

<sup>ab</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).