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# Evaluation of mechanical and thermal nociception as objective tools to measure painful and nonpainful lameness phases in multiparous sows

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## Abstract

The objective of this study was to quantify pain sensitivity differences using mechanical nociception threshold (MNT) and thermal nociception threshold (TNT) tests when sows were in painful and nonpainful transient lameness phases. A total of 24 mixed parity crossbred sows ( $220.15 \pm 21.23$  kg) were utilized for the MNT test, and a total of 12 sows ( $211.41 \pm 20.21$  kg) were utilized for the TNT test. On induction day (D0), all sows were anesthetized and injected with Amphotericin B (10mg/mL) in the distal interphalangeal joint space in both claws of one randomly selected hind limb to induce transient lameness. Three days were compared: (1) D-1 (sound phase, defined as 1 d before induction), (2) D+1 (most lame phase, defined as 1 d after induction), and (3) D+6 (resolution phase, defined as 6 d after induction). After completion of the first round, sows were given a 7-d rest period and then the procedures were repeated with lameness induced in the contralateral hind limb. During the MNT test, pressure was applied perpendicularly to 3 landmarks in a randomized sequence for each sow: 1) middle of cannon on the hind limb (cannon), 2) 1 cm above the coronary band on the medial hind claw (medial claw), and 3) 1 cm above the coronary band on the lateral hind claw (lateral claw). During the TNT test, a radiant heat stimulus was directed 1 cm above the coronary band. The data were analyzed using the MIXED procedure in SAS with sow as the experimental unit. Differences were analyzed between sound and lame limbs on each day. For the MNT test, pressure tolerated by the lame limb decreased for every landmark ( $P < 0.05$ ) when comparing D-1 and D+1. The sound limb tolerated more pressure on D+1 and D+6 than on baseline D-1 ( $P < 0.05$ ). Thermal stimulation tolerated by the sound limb did not change over the 3 d ( $P > 0.05$ ). However, the sows tolerated less heat stimulation on their lame limb on D+1 compared to D-1 levels ( $P < 0.05$ ). Both MNT and TNT tests indicated greater pain sensitivity thresholds when sows were acutely lame.

## Keywords

lameness, pain, sow, mechanical nociception, thermal nociception

## Disciplines

Agriculture | Animal Sciences | Large or Food Animal and Equine Medicine | Meat Science

## Comments

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*Validation of lameness nociception tools*

**Evaluation of mechanical and thermal nociception as objective tools to measure painful and non-painful lameness phases in multiparous sows**

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## ABSTRACT

The objective of this study was to quantify pain sensitivity differences using mechanical nociception threshold (MNT) and thermal (TNT) tests when sows were in painful and non-painful transient lameness phases. A total of 24 mixed parity crossbred sows ( $220.15 \pm 21.23$  kg) were utilized for the mechanical nociception threshold test and a total of 12 sows ( $211.41 \pm 20.21$  kg) were utilized for the thermal nociception threshold test. On induction day (D0), all sows were anesthetized and injected with Amphotericin B (10mg/mL) in the distal interphalangeal joint space in both claws of one randomly selected hind limb to induce transient lameness. Three days were compared (1) D-1 (Sound phase, defined as 1 d before induction), (2) D+1 (Most lame phase, defined as 1 d after induction) and (3) D+6 (Resolution phase, defined as 6 d after induction). After completion of the first round, sows were given a 7-d rest period and then the procedures were repeated with lameness induced in the contralateral hind limb. During the MNT test, pressure was applied perpendicularly to three landmarks in a randomized sequence for each sow: 1) middle of cannon on the hind limb (Cannon), 2) 1 cm above the coronary band on the medial hind claw (Medial claw), and 3) 1 cm above the coronary band on the lateral hind claw (Lateral claw). During the TNT test, a radiant heat stimulus was directed 1 cm above the coronary band. The data were analyzed using the MIXED procedure in SAS with sow as the experimental unit. Differences were analyzed between sound and lame limbs on each day. For the MNT test, pressure tolerated by the lame limb decreased for every landmark ( $P < 0.05$ ) when comparing D-1 and D+1. The sound limb tolerated more pressure on D+1 and D+6 than on baseline D-1 ( $P < 0.05$ ). Thermal stimulation tolerated by the sound limb did not change over the 3 days ( $P > 0.05$ ). However, the sows tolerated less heat stimulation on their lame limb on D+1

compared to D-1 levels ( $P < 0.05$ ). Both mechanical and thermal tests indicated greater pain sensitivity thresholds when sows were acutely lame.

**Keywords:** Lameness, pain, sow, mechanical nociception, thermal nociception

## INTRODUCTION

Sow lameness has been identified as an economical concern due to detrimental effects on reproductive performance, feed intake and overall longevity (Anil, et al., 2009; Fitzgerald et al., 2012), and a well-being challenge in regards to pain experienced by the sow and associations with increased piglet death due to crushing (Bonde et al., 2004). The United States Department of Agriculture (USDA; 2007) reported that lameness was the third most common reason producers cull gilts and sows from the breeding herd (15.2%), compared to age (36.6%) and reproductive failure (26.3%). It is unclear which types of lameness result in acute or chronic pain, but it is recognized that the duration and intensity of pain is an individual experience. Pain assessment is subjective in nature and pain recognition in animals is further complicated by the inability of animals to directly self-report their pain using a common language (Molony and Kent, 1997; Anil et al., 2002). For non-human animals previous work has used nociceptive threshold tests (Dyer et al., 2007; Tapper et al., 2013) to determine pain sensitivity caused by lameness. Tapper and colleagues (2013) used mechanical and thermal nociception threshold tests and found that the mechanical threshold test identified changes in pain sensitivity when applied to a transient lameness model in sows, but differences in responses to the thermal nociception test prior to lameness induction affected the utility of this tool. Therefore, the objectives of this study were to 1) evaluate a mechanical nociceptive threshold test as an objective pain assessment tool according to differences in response during sound and lame phases, and 2) evaluate a

thermal nociceptive threshold test as an objective pain assessment tool according to differences in response during sound and lame phases in multiparous sows.

## MATERIALS AND METHODS

The project was approved by the Iowa State University Institutional Animal Care and Use Committee. The experiments were conducted over two trials; trial one occurred from July to August, 2011 and trial two from October to November, 2011. The investigators established humane endpoint criteria following lameness induction such that any sow that progressed to non-weight bearing lameness by 12 h and did not approach water by 12 h or feed by 48 h were removed from the study and humanely euthanized. One sow was removed in trial 2 during the second round prior to lameness induction because she was unable to stand for complete data collection of the nociception tests.

### *Animals and housing*

For the mechanical nociception tests a total of 24 ( $220.15 \pm 21.23$  kg) non-bred clinically normal, mixed-parity, crossbred sows were purchased from a producer in Iowa. To avoid confounding injury due to aggression, each sow was housed individually in concrete pens 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 (2.4 m length x 2 cm height x 1.4 m width) was provided for comfort. Pens were set up in two rows with a central aisle and allowed for nose to nose contact between sows.

Sows had *ad libitum* access to water via one nipple water drinker (Trojan Specialty Products Model 65, Dodge City, KS) that was positioned over a grate. Sows were hand-fed in their home

pens, receiving 2.3 kg of feed in the morning and 0.46 kg in the afternoon. On each data collection day, the morning ration was given in the modified gestation stall to facilitate standing behavior and any remaining ration was given in the home pen. Feed was composed of ground corn, soybeans, and nutrients formulated according to Swine NRC guidelines with no antimicrobials. A total of 6.8 ml (15 mg) of Matrix (Intervet/Schering-Plough, Millsboro, DE, USA) was added to the morning ration daily to prevent estrus cycle initiation. Facilities and sows were inspected by caretakers at 0730 and 1530 daily.

### *Induction of lameness*

Feed and water were withheld 18 h and 1 h respectively prior to anesthesia to reduce vomiting and aspiration risk. All sows were restrained in a standing position using a pig snare and then anesthetized using Xylazine (4.4 mg/kg; Anased<sup>®</sup>, Lloyd Laboratories, Shenandoah, IA, USA), Ketamine HCl (2.2 mg/kg; Ketaset<sup>®</sup>, Fort Dodge Animal Health), and Tiletamine HCl (4.4 mg/kg; Telazol<sup>®</sup>, Fort Dodge Animal Health) administered intramuscularly. Dosages were based on recommendations by Jean and Anderson (2012). Palpebral reflex were tested to confirm insensibility following anesthesia administration. After sows were rendered insensible, the claws on the assigned limb were washed with water to remove obvious fecal contamination, scrubbed for 3 min with iodine based surgical scrub (Operand<sup>®</sup>, Aplicare Inc., Branford, CT, USA) using 10 x 10 cm sterile gauze pads, and rinsed with 70% isopropyl alcohol until no evidence of the surgical scrub remained. After cleaning, 10 mg amphotericin B (X-gen Pharmaceuticals, Inc., Big Flats, NY, USA) were injected into the distal inter-phalangeal joint (intra-articular space) of both claws in the assigned limb (Karriker et al., 2013). Throughout anesthesia, respiratory rate (measured by number of chest elevations resulting from inspiratory effort over 15 s), and rectal temperature were monitored every 15 min until sows returned to a standing posture unaided.

### ***Experimental design***

Sows were acclimated to the facility, tools and handling for approximately 10 d prior to study commencement. All sows were included in the treatment and control data such that they were compared to themselves before and after induction. The experimental design was a 3(days) x 2(limb) factorial arrangement and the sow was the experimental unit. This experimental design provided robust control of intra- and inter-animal variations in behavioral responses and limited the number of animals required. Using a random number generator, sows were randomly allocated to one hind limb for first lameness induction. Three *days* were compared, ***D-1*** (Sound phase, defined as 1 d pre-induction), ***D+1*** (Most lame phase, defined as 1 d post-induction) and ***D+6*** (Resolution phase, defined as 6 d post-induction) and two hind *limbs*: left hind vs. right hind. The days of D-1, D+1 and D+6 were selected based on previous experience with the amphotericin B lameness induction model that had been validated by Karriker and colleagues (2013). ***Trial*** was defined as either trial 1 which included sows 1-12 or trial 2 which included sows 13-24. ***Round*** was defined as the first or second lameness induction within trial. After completion of the first round, sows were given a 7-d rest period and then the procedures were repeated with lameness induced in the opposite hind limb for the second round (Figure 1).

### ***Nociception tests***

Nociception tests were completed in a modified gestation stall (0.61 m x 2 m) located outside of the sows' home pens. During the nociceptive threshold tests, sows were fed their morning ration of feed. Remaining feed ration not consumed in the gestation stall was given in the home pen. Prior to thermal nociceptive testing, both hind limbs were cleaned with water and dried to remove fecal matter. Nociceptive threshold tests were administered on each of the three days on

both hind limbs. The same technician performed all nociception tests on all sows during all test days. The technician conducting data collection was blind to treatment allocation. In addition, the technician was blind to the numeric output values during the pain sensitivity test assessment, with the device positioned to keep output in view of a second technician that recorded these values. .

### ***Mechanical nociception threshold test***

A total of 24 sows were tested using the pressure algometer (MNT) was adapted from Tapper et al. (2013). A hand-held pressure algometer (Wagner Force Ten™ FDX 50 Compact Digital Force Gage, Wagner Instruments, CT, USA) with a 1 cm<sup>2</sup> flat rubber tip was used to quantify mechanical nociceptive threshold (MNT) in kilograms of force (kgf). Pressure was applied perpendicularly to three landmarks in a randomized sequence for each sow: 1) middle of cannon on the hind limb (Cannon), 2) 1 cm above the coronary band on the medial hind claw (Medial claw), and 3) 1 cm above the coronary band on the lateral hind claw (Lateral claw; Figure 2). 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. 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. When a limb withdrawal response was observed, pressure was immediately removed, and the peak pressure representing the MNT was recorded.

### ***Thermal nociception threshold test***

A total of 12 sows were tested using the thermal nociceptive threshold (TNT) test immediately followed the MNT test. The TNT measured the latency for a sow to withdraw her hind limb in response to precise, focused radiant heat stimulation. The TNT test procedures using the

analgesia meter were adapted from Tapper and colleagues. (2013). 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. Thermal measurements were taken in triplicate 1 cm above the coronary band on the lateral claw of the right hind limb, followed by the left hind limb (Figure 2). Once the machine was 7.62 cm from the landmark the thermal stimulus was activated. The latency for the sow to withdraw her limb in response to the stimulus was then recorded. To prevent tissue damage, a 20 second maximum duration was set, after which the analgesia meter automatically turned off.

### ***Statistical analysis***

Data collected was initially tested for normality using PROC Univariate in SAS 9.3 (SAS Inst. Inc., Cary, NC). Both thermal and mechanical nociception data were not normally distributed; therefore data were analyzed with PROC Glimmix using a gamma distribution. Results did not differ from the Mixed procedure and since the measures were continuous, both thermal and mechanical nociceptive data were fit to the Mixed model in SAS. A *P* value of < 0.05 was considered significant and PDIFF was used to determine differences between days, replicates and landmarks. Thermal nociceptive and mechanical nociceptive data were analyzed separately.

To assess differences between days over the landmarks on the lame and sound limbs, a model including the main effects of landmark (medial claw, lateral claw or cannon), replicate (first, second or third completion of landmark order), trial, landmark order, limb (defined as either the left or right hind limb) and the 3-way interaction of day\*limb treatment\*landmark (limb treatment defined as either lame or sound) was used. A separate code was used to analyze round,

trial and limb induced. This model included the main effects of replicate, landmark, round, trial, LMorder (order of anatomical landmark application), limb and the interactions of day\*limb treatment and day\*landmark. To assess differences between replicates a separate code was used, this model included the main effects of replicate, landmark, trial, LMorder, limb, and the interaction of replicate\*landmark and day\*limb treatment\*landmark. Sow within trial\*day, sow within trial\*round and landmark order within day were fitted as random effects for all 3 codes. A repeated measures statement of replicate within round\*day\*landmark\*limb treatment was also used for all 3 codes.

The thermal nociceptive model included the main effects of replicate, round, limb and the two-way interaction of day\*limb treatment to determine the differences between days for the lame and sound limb. Trial was not measured as the TNT test was only conducted during trial 2. A random statement of sow within day and sow within round was used. A repeated measures statement of replicate within round\*day\*limb treatment was used. Least Square Means provided estimates, standard error, and p values for variable interactions and effect comparisons. A *P* value of < 0.05 was considered significant and PDIFF was used to determine differences.

To determine differences between rounds of induction for 3 days over 3 landmarks for the lame hind limb, the interaction of round\*day\*limb treatment\*landmark was used.

## RESULTS

### *Nociception tests*

#### *Mechanical nociceptive threshold (MNT) test*

There were no differences observed when lameness was induced in the right- or left-hind limb ( $5.37 \pm 0.20$  kgf vs.  $5.15 \pm 0.21$  kgf;  $P = 0.34$ ) or between the first and second trial ( $5.04 \pm 0.23$  kgf vs.  $5.48 \pm 0.23$  kgf;  $P = 0.16$ ). Differences were observed between the first and second rounds within trial ( $5.52 \pm 0.2$  kgf vs.  $5.00 \pm 0.21$  kgf;  $P = 0.04$ ).

When comparing pressure over the 3 days, pressure tolerated by the lame limb decreased for all 3 landmarks ( $P < 0.05$ ) between D-1 and D+1. Pressure tolerated by the sound limb increased for all 3 landmarks between D-1 and D+1 ( $P < 0.05$ ). For the lame limb on D+6, more pressure was tolerated on D+6 compared to D+1, but was still different than D-1 (Table 1).

Since values had not returned to sound phase levels on D+6, the D-1 round 1 and D-1 round 2 were compared for the hind limbs. Hence, we compared the baseline (D-1) values for round 1 and round 2 by limb treatment to confirm that the sound limb remained sound and the lame limb became sound during the wash-out period. For the MNT test day\*limb treatment\*landmark was used to determine differences between the 2 days (round 1 D-1 and round 2 D-1) within landmarks for the lame and sound limbs. Similarly, limb treatment\*day was used for the TNT test. When comparing the lame limb, differences were observed at all 3 landmarks ( $P < 0.0012$ ; Figure 3). When comparing the sound limb, differences were not observed for the cannon and lateral claw ( $P > 0.08$ ), however the medial claw differed ( $P = 0.013$ ; Figure 4). Because of differences between rounds for the lame limb, further assessment was done within all landmarks, over 3 days comparing round 1 and round 2. Results revealed no significant differences (except for the cannon on D-1) between round 1 and round 2 over the 3 days ( $P > 0.05$ ; Table 2).

When comparing the 3 replications within the 3 landmarks, less pressure was tolerated on the first replication for the cannon and lateral claw compared to the second replication ( $P < 0.05$ ).

However, there was no difference in pressure tolerated by the sow for these 2 anatomical locations between the second and third replications ( $P > 0.05$ ; Figure 5).

### ***Thermal nociceptive threshold (TNT) test***

There were no differences observed when lameness was induced in the right- or left-hind limb ( $9.48 \pm 0.83$  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 days, thermal stimulation tolerated by the sound limb did not change ( $P > 0.05$ ). The sows tolerated less heat stimulation on their lame limb on D+1 compared to D-1 ( $P < 0.05$ ; Table 1). For the lame limb on D+6, more thermal stimulation was tolerated on D+6 compared to D+1, but was still different than D-1 (Table 1).

Since values had not returned to sound phase levels on D+6 (D-1 during round 1), data collected on D-1 (round 1) and D-1 (round 2) were compared. The lame limb was observed to resolve lameness prior to the second round (Figure 5). The sound limb increased thermal tolerance entering round 2 compared to the beginning of round 1 (Figure 5).

When comparing the 3 replications, the sows tolerated more thermal stimuli on the first replication compared to subsequent replications ( $P < 0.04$ ). There was no observed difference in tolerance of the thermal stimuli between the second and third replication (Figure 6;  $P = 0.89$ ).

## **DISCUSSION**

### ***Mechanisms of nociception***

Nociception may be defined as the “physiologic component of pain processing involving the transduction, transmission and modulation of signals generated by stimulation of peripheral

nociceptors” (Lamont, 2008). Primary sensory neurons located in outlying tissues relay mechanical, chemical and thermal input from these sites to the dorsal horn of the spinal cord. Type A $\beta$ -fiber myelinated sensory neurons are low-threshold mechanoreceptors involved in tactile perception while unmyelinated C-fibers and thinly myelinated A $\delta$ -fibers transduce nociceptive and thermal stimuli (Costigan and Woolf, 2000). A $\delta$ -fibers can further be subdivided into 2 classes, type I and type II nociceptors. Type I nociceptors respond to mechanical and chemical stimuli and have relatively high heat thresholds, however these nociceptors will sensitize to heat stimuli when the tissue is injured. Type II A $\delta$  nociceptors have a lower thermal threshold but a high mechanical threshold (Basbaum et al., 2009). Type C fibers are reactive to heating and cooling, low threshold mechanical stimulation, as well as to some algogenic substances (Almeida et al., 2004). However, this experiment was not designed to directly measure which receptors were activated.

### ***Mechanical nociceptive threshold (MNT) test***

In our study, sows had a decreased MNT over all 3 landmarks on the lame limb on D+1 compared to D-1, indicating that the tool differentiated between sow responses during sound and lame states. The mechanical nociceptive threshold (MNT) is the minimum pressure that produces a response, and a lower threshold would correspond to increased pain sensitivity experienced by the sow being lame. Pressure algometry has been used in human studies to distinguish between painful and non-painful states (Tunks et al., 1995; Giesbrecht and Battie, 2005). Use of the pressure algometer to measure MNTs in healthy patients found that pain sensitivity measurements were reliable between consecutive testing days (Nussbaum and Downes, 1998) and had good inter-rater and test-retest reliability (Tunks et al., 1995). There have been several studies using mechanical nociception tests to quantify painful and non-painful states using

lameness as a model in sheep (Ley et al., 1989), dairy cattle (Dyer et al., 2007) and most recently in lame sows (Nalon et al., 2013; Tapper et al., 2013). These studies concluded that the mechanical nociceptive tests were able to quantify decreased mechanical thresholds when animals were in painful lameness states.

Interestingly in this study, the cannon location was considered the control anatomical site and the expectation was that no pressure changes would have been recorded due to pain sensitivity.

Anatomical investigation by Karriker and colleagues (2013) reported that an injection of meat marking dye remained localized in the distal interphalangeal joint space, though future studies to determine possible circulatory levels of amphotericin B in the animal should be conducted.

Findings from our study are consistent with Tapper and others (2013) who observed a decrease in pressure tolerated on the cannon landmark following lameness induction. Similarly, nociception thresholds decreased in association with corneal scarification and ulceration in calves, but differences were observed at the calf level post-treatment and not between particular landmarks (Dewell et al., 2014). The authors recommend using the sows' sound day data as the control given the centralized pain sensitivity responses that were observed resulting in day versus landmark effects.

In our experimental design we utilized 3 days, D-1 (sound phase), D+1 (most lame phase) and D+6 (resolution phase). These time points were modeled after Karriker and colleagues (2013) who tested sows in sound and lame states using an embedded force plate and GAITFour pressure mat to validate an amphotericin B induced transient lameness model. These researchers concluded that lameness had not resolved by D+5, but resolved by D+7 relative to induction. Results of the current study, using pressure algometer, indicated that lameness was resolving, but had not returned to D-1 levels for both sound and lame limbs by D+6. Furthermore, when sows

began round 2 (D-1) they tolerated less pressure on the same lame limb suggesting resolution may not fully occur until after day 13 (D-1 of round 2). Tapper and colleagues (2013) found no differences between baseline values when comparing multiple trials of the MNT test.

To assess differences between rounds, we compared landmarks over all 3 days between lame limbs for round 1 and round 2. This analysis evaluated the limb entering induction for each round (left hind in round 1 compared to right hind in round 2 or right hind in round 1 compared to left hind in round 2) to assess differences between lame limbs pre-induction. Although not significant, many of the MNTs were lower during round 2 than in round 1. One possible explanation for this is that the sows viewed the application of the pressure algometer as an aversive experience and acquired learned avoidance response as the study continued, therefore decreasing MNTs in round 2. However, if sows found the procedure to be aversive, a linear relationship including significant decreases in all landmarks between rounds would be expected. Therefore, aversion to the mechanical nociception procedure could be possible, but it is not likely the cause of the decreased threshold during round 2.

Another explanation for the threshold decreases between rounds was due to tissue injury caused by the induction model. Tissue injuries can result in greater sensitivity to painful stimuli (hyperalgesia) and stimuli that are usually non-noxious may evoke pain (allodynia; Andrew and Greenspan, 1999). Whay and colleagues (1998) assessed the nociceptive thresholds of 42 sound- and 53 dairy cows displaying hind-claw lameness. Cows found to display unilateral hind-claw lameness (n=42) were re-evaluated at 28 days after lameness treatment. The lame cows had lower nociceptive threshold compared to the sound cows on day 1 and also at retesting on day 28. The authors concluded that lame cows were in a hyperalgesia state. Similarly, Nalon and colleagues (2013) assessed mechanical nociception threshold on the metatarsi and metacarpi of

all sow limbs to determine if hyperalgesia occurred with naturally occurring lameness. Two tools were used, one being a hand-held probe and the other being a limb-mounted wireless actuator. Twenty-eight pregnant sows were investigated, of which 14 were moderately lame and 14 were not lame. Results showed that mechanical thresholds were lower in limbs affected by lameness compared to normal limbs. The authors concluded that lame sows had lower mechanical thresholds in the lame limbs, revealing local sensitization to noxious stimuli (hyperalgesia). Therefore, for future studies, the authors of the present study recommend adding additional data collection points between D+6 and the beginning of the second round of induction to provide a better understanding of the lameness resolution phase and possible hyperalgesia.

In this study, 3 replications for the 3 landmarks were performed. Interestingly, the first replication for the cannon and lateral claw tolerated less pressure than subsequent replications. A possible explanation for less pressure tolerated on the first replication could be that the sow was startled from the initial contact on these 2 landmarks, however, if a true startle response had occurred one would expect differences in all 3 landmarks. To limit a possible startle response, future studies using the pressure algometer should consider desensitizing the sow to this touch and pressure by spending time palpating the area of application. Similar to the research done by Nalon and colleagues (2013), use of a wireless tester could clarify if a startle response is due to the human interaction or the application of the algometer. Results of this research showed the hand-held probe yielded lower MNTs than the wireless actuator for lame limbs in sows. The authors noted that using the probe possibly results in a higher predictability of the tool application due to the sows' ability to see the operator approaching the limb and react faster. Furthermore, refinement on the number of replications needed per landmark to be tested should be considered.

### ***Thermal nociceptive threshold (TNT) test***

In the current study, sows did not show differences over the 3 days when the limb was sound, however they tolerated less thermal stimulation on D+1 compared to D-1. The thermal nociceptive threshold (TNT) test measures latency for a withdrawal response to precise, focused radiant heat. Tapper and colleagues (2013) evaluated pain sensitivity in sows using the TNT test when in sound and lame states when provided analgesic drugs. The authors reported that the TNT test detected differences between all treatment groups on the sound phase treatment day and was therefore not an effective test with the methodology used in that study. The authors noted that residual water from cleaning each leg may have altered the conduction during the testing which therefore might explain possible differences between their research and the current study, in which residual water was gently blotted with paper towels.

In the current study, the lame limb was beginning to show resolution by D+6. Further data analysis in this study showed that when sows began the second round they tolerated more thermal stimulation on the sound limb but there was no difference for the lame limb, indicating that lameness had resolved prior to the second round induction. Similarly to the mechanical nociception threshold test, it is recommended to add additional data collection points between D+6 and the beginning of the second round to provide a more accurate view of lameness resolution when using this lameness model.

In this study sows tolerated more thermal heat on the first- compared to the second- and third replication. It could be speculated that sows are experiencing hyperalgesia similar to what was discussed for the pressure algometer. Hyperalgesia can be measured by comparing the difference in latencies to thermal nociception between a control and injured condition (Galbraith et al.,

1993). Previous research using rats and mice have quantified that thermal sensitivity can distinguish pain sensitivity and quantify hyperalgesia (Hargreaves et al., 1988; Chen et al., 1999). A secondary consideration is that the heat damaged the tissue, therefore decreasing the tolerance of heat after the first replication. To assess heat damage, terminal studies could be completed to evaluate cell damage at the testing site. Other methods include testing other heat sensitivity tools. Thermal sensitivity using a laser technique has been developed by Herskin and colleagues (2009) who validated a laser-based method to measure thermal nociception in group-housed pigs. Two experiments observed behavioral responses toward cutaneous nociceptive stimulation from a computer-controlled CO<sub>2</sub>-laser beam applied to the caudal part of the metatarsus on the hind limbs or the shoulder region of gilts. Increasing the power output led to decreasing latency to respond (moving, lifting or kicking limb;  $P < 0.001$ ) when testing the hind limbs. Increasing the power output led to gradually decreasing latency to respond (moving shoulder, moving body, muscle twitching, rubbing shoulder;  $P < 0.0001$ ) when testing the shoulder. The authors concluded that behavioral responses to nociceptive cutaneous laser stimulation are a valid non-invasive measure of nociception in group housed gilts. Use of the laser-based method has also been validated for use in dairy cattle (Veissier et al., 2000; Herskin et al., 2003). Herskin and colleagues (2003) evaluated a laser-based method to measure thermal nociception in dairy cows. Increasing the power output decreased latencies to respond ( $P < 0.01$ ); therefore, behavioral responses to a laser stimulus seem to be a valid measure of nociception in dairy cows.

In the current study, the thermal test utilized only one landmark (lateral claw) because of application restrictions when sows were in the gestation stall. The authors suggest refinement on the number of landmarks and the number of replications per landmark to be tested. Finally, in the present study, thermal testing always took place after mechanical testing. Future studies should

assess both nociceptive tools separately to diminish possible residual effects of the mechanical test on the thermal test.

In conclusion, these results support the use of both TNT and MNT tests as assessment tools for detecting changes in pain sensitivity of sows experiencing transient lameness. Both tools detected a decrease in mechanical and thermal thresholds between sound and most lame phases indicating their potential use in the laboratory as well as for diagnosing lameness pain sensitivity and treatment interventions on farm. However, further research should be conducted with these tools to assess the resolution of lameness when using this lameness model.

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**Table 1** Comparison of days (D-1, D+1 and D+6) for the sound and lame hind limb using the mechanical nociception threshold (MNT) (kgf)<sup>1</sup> and thermal nociception threshold (TNT) tests (sec)

**<sup>1</sup>Mechanical Nociception Threshold test (kgf)**

<b>Limb status<sup>2</sup></b>	<b>Landmark<sup>3</sup></b>	<b>Day<sup>4</sup></b>		
		<b>D-1</b>	<b>D+1</b>	<b>D+6</b>
<b>Sound</b>	Cannon	6.58 ± 0.30 <sup>a</sup>	6.91 ± 0.30 <sup>ab</sup>	7.57 ± 0.30 <sup>b</sup>
	Medial claw	6.10 ± 0.30 <sup>a</sup>	6.96 ± 0.30 <sup>b</sup>	7.47 ± 0.30 <sup>b</sup>
	Lateral claw	5.51 ± 0.30 <sup>a</sup>	6.35 ± 0.30 <sup>b</sup>	6.68 ± 0.30 <sup>b</sup>
<b>Lame</b>	Cannon	7.03 ± 0.30 <sup>a</sup>	3.77 ± 0.31 <sup>b</sup>	4.33 ± 0.30 <sup>b</sup>
	Medial claw	7.34 ± 0.30 <sup>a</sup>	0.95 ± 0.31 <sup>b</sup>	2.08 ± 0.30 <sup>c</sup>
	Lateral claw	6.60 ± 0.30 <sup>a</sup>	0.92 ± 0.31 <sup>b</sup>	1.66 ± 0.30 <sup>b</sup>

**<sup>2</sup>Thermal Nociception Threshold test (sec)**

<b>Sound</b>	Lateral claw	10.25 ± 0.86 <sup>a</sup>	9.87 ± 0.89 <sup>a</sup>	11.60 ± 0.87 <sup>a</sup>
<b>Lame</b>	Lateral claw	11.99 ± 0.86 <sup>a</sup>	5.02 ± 0.89 <sup>b</sup>	9.55 ± 0.87 <sup>c</sup>

<sup>1</sup>Mechanical nociception threshold (MNT) test used to quantify mechanical nociceptive thresholds (MNTs) in kilograms of force tolerated by the sow on the lame and sound limbs (kgf).

<sup>2</sup>Thermal nociception threshold (TNT) test used to quantify the latency in seconds for a sow to withdraw her hind limb in response to radiant heat stimulation (sec).

<sup>2</sup>Treatment assigned to limb being measured; either sound or lame.

<sup>3</sup>Three landmarks used for MNT test: Cannon landmark defined as middle of cannon on the hind limb, Medial claw defined as 1 cm above the coronary band on the medial hind claw, Lateral

claw defined as 1 cm above the coronary band on the lateral hind claw. TNT test measurement on lateral claw of the hind limb 1 cm above the coronary band.

<sup>4</sup> D-1 (Sound phase, 1 d pre-induction), D+1 (Most lame phase, 1 d post-induction), and D+6 (Resolution phase, 6 d post-induction) days.

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

**Table 2** Comparison of round 1 and round 2 for the lame hind limb on days D-1, D+1 and D+6 using the mechanical nociception threshold (MNT<sup>1</sup>) (kgf) test to assess differences in pressure tolerance over the 3 landmarks.

Day <sup>2</sup>	Landmark <sup>3</sup>	Round of induction <sup>4</sup>		524
		1	2	525
D-1	Cannon	7.61 ± 0.37 <sup>a</sup>	6.44 ± 0.37 <sup>a</sup>	526
	Medial claw	7.36 ± 0.37 <sup>a</sup>	7.31 ± 0.37 <sup>a</sup>	527
	Lateral claw	6.92 ± 0.37 <sup>a</sup>	6.26 ± 0.37 <sup>a</sup>	528
D+1	Cannon	4.02 ± 0.37 <sup>a</sup>	3.52 ± 0.37 <sup>a</sup>	529
	Medial claw	1.04 ± 0.37 <sup>a</sup>	0.86 ± 0.37 <sup>a</sup>	530
	Lateral claw	1.11 ± 0.37 <sup>a</sup>	0.73 ± 0.37 <sup>a</sup>	531
D+6	Cannon	4.50 ± 0.36 <sup>a</sup>	4.17 ± 0.36 <sup>a</sup>	532
	Medial claw	2.06 ± 0.36 <sup>a</sup>	2.14 ± 0.36 <sup>a</sup>	533
	Lateral claw	1.52 ± 0.36 <sup>a</sup>	1.85 ± 0.36 <sup>a</sup>	534

535

<sup>1</sup>Mechanical nociception threshold (MNT) test used to quantify mechanical nociceptive thresholds (MNTs) in kilograms of force tolerated by the sow on the lame and sound limbs (kgf).

<sup>2</sup>D-1 (Sound phase, 1 d pre-induction), D+1 (Most lame phase, 1 d post-induction), and D+6 (Resolution phase, 6 d post-induction) days.

<sup>3</sup>Three landmarks used for MNT test: Cannon landmark defined as middle of cannon on the hind limb, Medial claw defined as 1 cm above the coronary band on the medial hind claw, Lateral claw defined as 1 cm above the coronary band on the lateral hind claw. TNT test measurement on lateral claw of the hind limb 1 cm above the coronary band.

<sup>4</sup>Round was defined as the first or second induction of lameness within trial

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

**Figure 1** Schematic depiction of a trial.

**Figure 2** Mechanical nociception threshold<sup>1</sup> landmark schematic. 1= Middle of the cannon on the hind limb; 2= 1 cm above the coronary band on the lateral hind claw; 3 = 1 cm above the coronary band on the medial hind claw. Thermal nociception threshold<sup>2</sup> test utilized the lateral hind claw only.

**Figure 3** For the lame limb, mechanical nociception threshold (MNT<sup>1</sup>) test comparison of round 1 (R1) and round 2 (R2) on D-1 for each of the landmarks (cannon, medial claw and lateral claw<sup>2</sup>) to determine sows resolved lameness prior to second round of induction.

**Figure 4** For the sound limb, mechanical nociception threshold (MNT<sup>1</sup>) test comparison of round 1 (R1) and round 2 (R2) on D-1 for each of the landmarks (cannon, medial claw and lateral claw<sup>2</sup>) to determine if sows remained sound on their sound limb prior to round 2 lameness induction<sup>3</sup>.

**Figure 5** Mechanical nociception threshold (MNT<sup>1</sup>) test comparing differences between the first (1), second (2) and third (3) replicate for each of the MNT landmarks (cannon, medial claw and lateral claw<sup>2</sup>) over all days.

**Figure 6** Thermal nociception threshold (TNT<sup>1</sup>) test comparison of round 1 (R1) and round 2 (R2) D-1 days for the lame and sound limbs to determine if sows resolved lameness on their lame limb and remained sound on their sound limb prior to the second round induction<sup>2</sup>.

**Figure 7** Thermal nociception threshold (TNT<sup>1</sup>) test comparing differences between the first (1), second (2) and third (3) replicate using the thermal nociceptive threshold test (seconds stimulus tolerated) over all days.

12 Sows enrolled  
Handling/Tools/Facility acclimation (~10 d)

Sows randomly assigned to left- or right-hind limb for Round 1 induction

***D-1 (Sound phase)***

Collected data

D0 (Induction of lameness)

No data collection

***D+1 (Most lame phase)***

Collected data

***D+6 (Resolution phase)***

Collected data

Sows repeat cycle after 7-d wash-out period  
(Round 2 induction on the opposite hind limb)

Completion of Round 2  
Sows were removed from the study

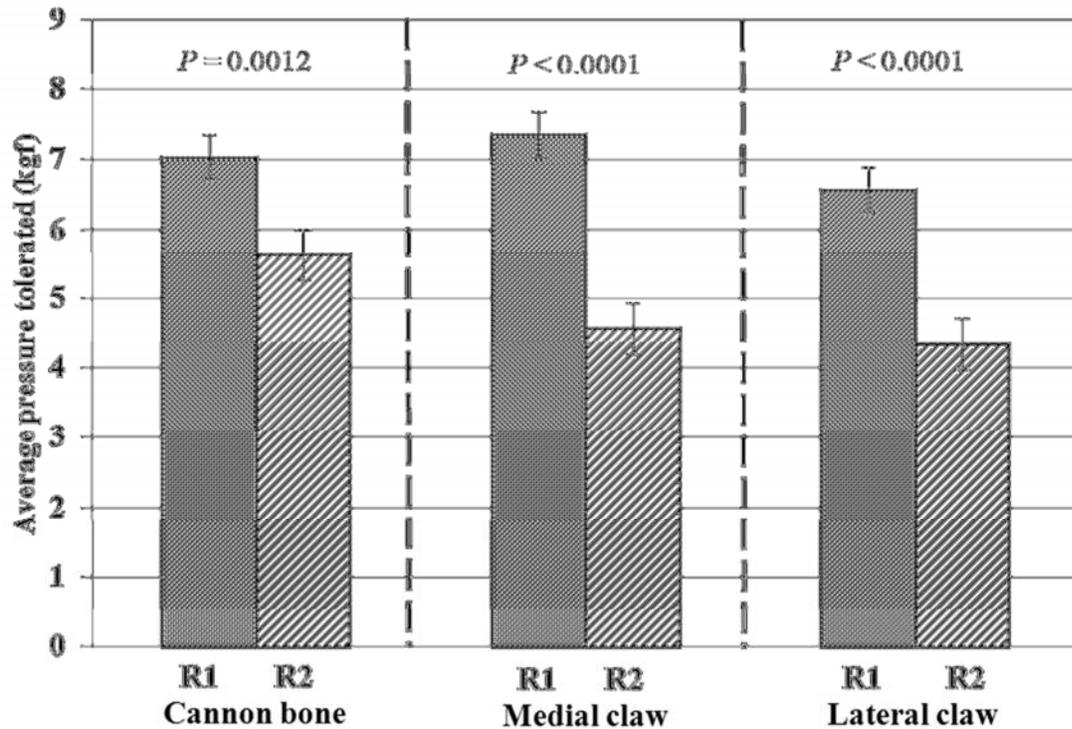
**1**

**2**

**3**

<sup>1</sup>Mechanical nociception threshold (MNT) test used to quantify mechanical nociceptive thresholds (MNTs) in kilograms of force tolerated by the sow on the lame and sound limb (kgf).

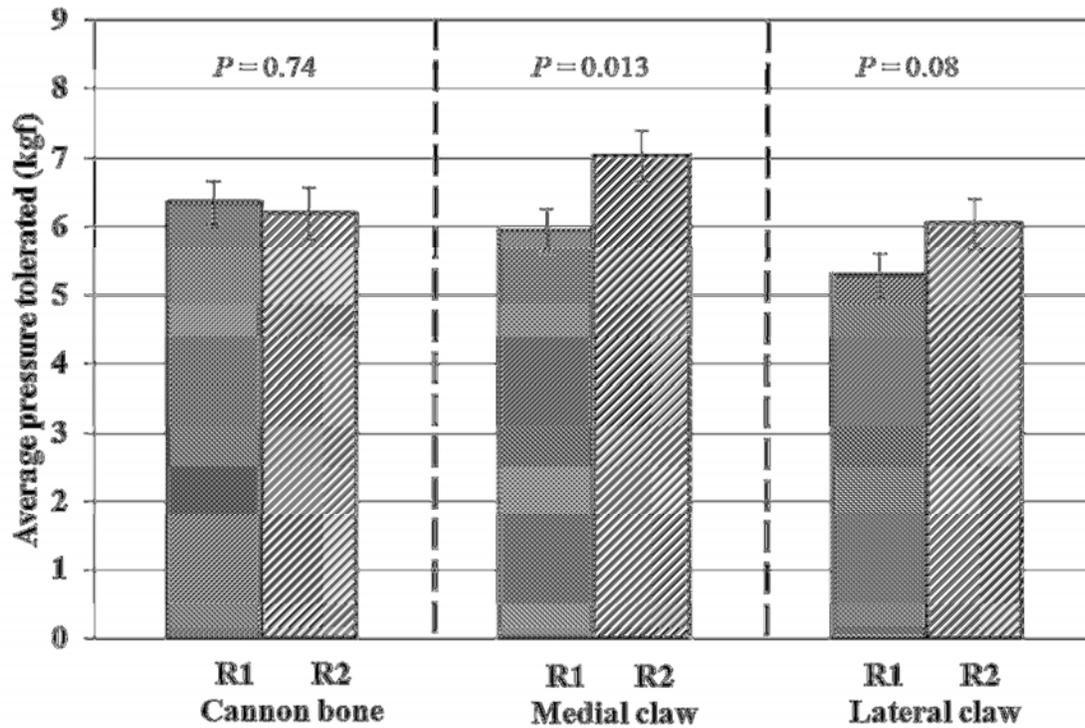
<sup>2</sup>Thermal nociception threshold (TNT) test used to quantify the latency in seconds for a sow to withdraw her hind limb in response to radiant heat stimulation (sec). TNT test measurement on lateral claw of the hind limb 1 cm above the coronary band.



<sup>1</sup>Mechanical nociception threshold (MNT) test used to quantify mechanical nociceptive thresholds (MNTs) in kilograms of force tolerated by the sow on the lame and sound limb (kgf).

<sup>2</sup>Three landmarks used for MNT test: Cannon landmark defined as middle of cannon on the hind limb, Medial claw defined as 1 cm above the coronary band on the medial hind claw, Lateral claw defined as 1 cm above the coronary band on the lateral hind claw.

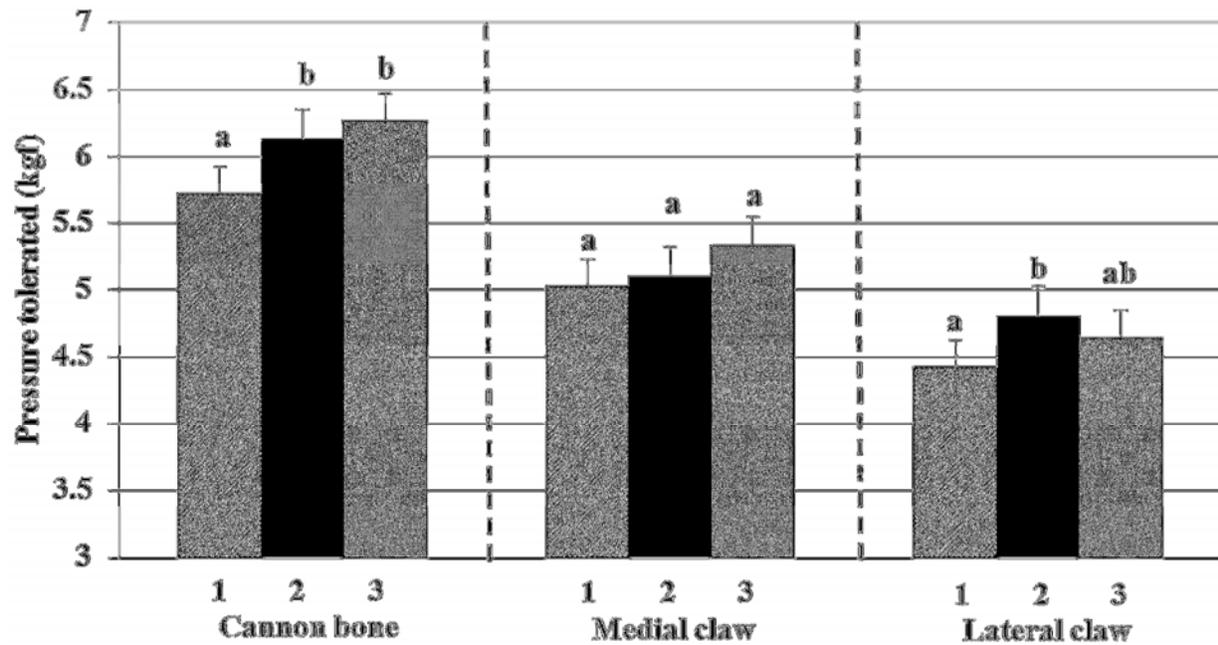
<sup>3</sup>P-values ( $P < 0.05$ ) represent differences between rounds (R1 and R2) within a landmark.



<sup>1</sup>Mechanical nociception threshold (MNT) test used to quantify mechanical nociceptive thresholds (MNTs) in kilograms of force tolerated by the sow on the lame and sound limb (kgf).

<sup>2</sup>Three landmarks used for MNT test: Cannon landmark defined as middle of cannon on the hind limb, Medial claw defined as 1 cm above the coronary band on the medial hind claw, Lateral claw defined as 1 cm above the coronary band on the lateral hind claw.

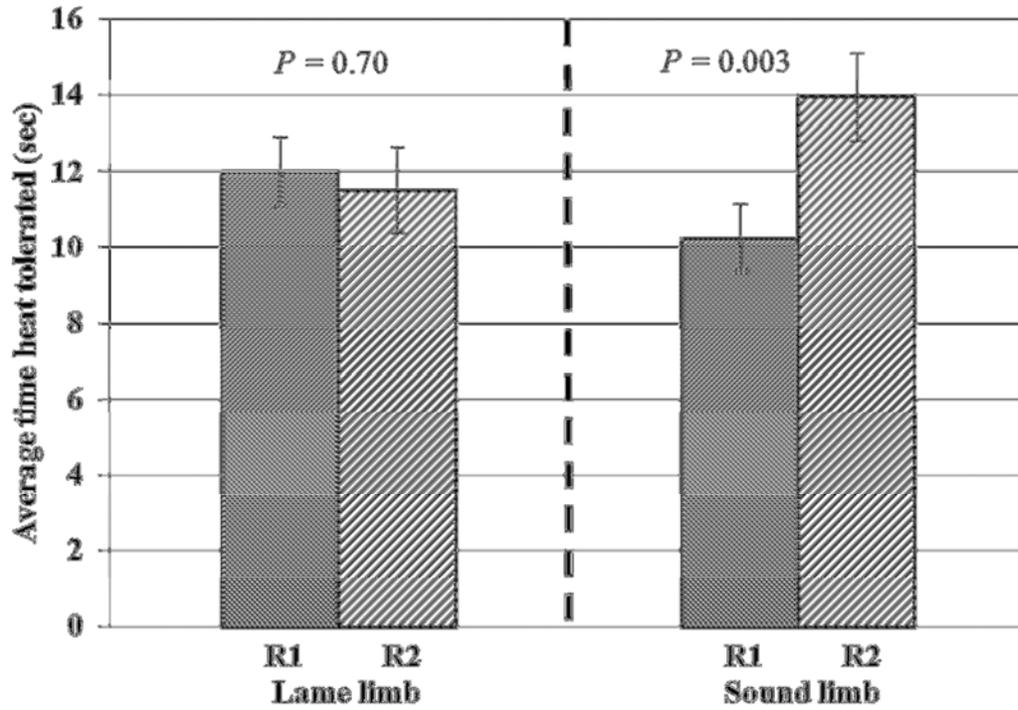
<sup>3</sup>P-values ( $P < 0.05$ ) represent differences between rounds (R1 and R2) within a landmark.



<sup>1</sup>Mechanical nociception threshold (MNT) test used to quantify mechanical nociceptive thresholds (MNTs) in kilograms of force tolerated by the sow on the lame and sound limb (kgf).

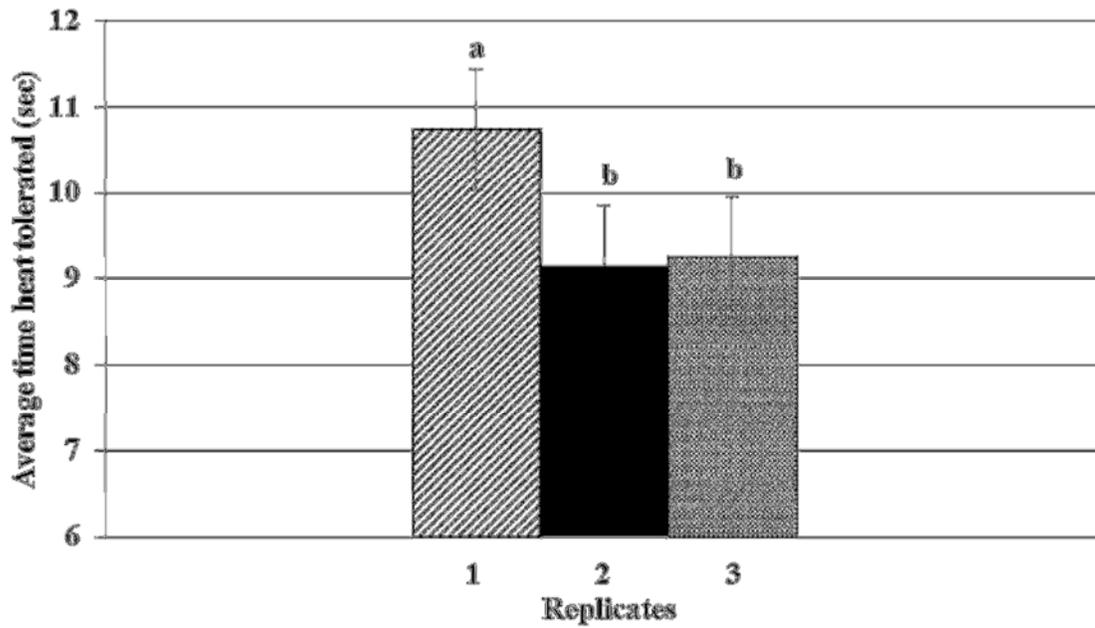
<sup>2</sup>Three landmarks used for MNT test: Cannon landmark defined as middle of cannon on the hind limb, Medial claw defined as 1 cm above the coronary band on the medial hind claw, Lateral claw defined as 1 cm above the coronary band on the lateral hind claw.

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



<sup>1</sup>Thermal nociception threshold (TNT) test used to quantify the latency in seconds for a sow to withdraw her hind limb in response to radiant heat stimulation (sec). TNT test measurement on lateral claw of the hind limb 1 cm above the coronary band.

<sup>2</sup>P-values ( $P < 0.05$ ) represent differences between rounds (R1 and R2) within the lame or sound limb.



<sup>1</sup>Thermal nociception threshold (TNT) test used to quantify the latency in seconds for a sow to withdraw her hind limb in response to radiant heat stimulation (sec). TNT test measurement on lateral claw of the hind limb 1 cm above the coronary band.

<sup>ab</sup>Means without a common superscript differ ( $P < 0.05$ ).