NPB FINAL RESEARCH GRANT REPORT

Evaluation of transmammary-delivered firocoxib and a vapocoolant spray to alleviate pain in piglets after surgical castration and tail docking (#19-084)

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Industry Summary:

This study aimed to assess a novel strategy for pain management of piglets after routine processing procedures. By providing the sow an analgesic drug that is able to cross the blood-milk barrier in the mammary gland, nursing piglets can be noninvasively provided pain relief transmammary prior to surgical castration and tail docking. This drug delivery option can significantly reduce the time and cost associated with individual administration of an analgesic drug to each piglet prior to processing. Therefore, the main objective of this study was to assess the efficacy of 2.0 mg/kg firocoxib, administered to the sow and delivered transmammary to her nursing piglets, used alone or in combination with a vapocoolant spray (ethyl chloride), to reduce surgical castration and tail docking pain. This study also aimed to determine if cortisol, the primary stress hormone, could be quantified from the saliva and hair of pre-weaned piglets. These noninvasive collection methods provide a more welfare-friendly option to study the piglet's pain response. In broader terms, monitoring the stress level of pigs throughout production using these noninvasive physiologic measures can give pork producers more information about their animal's well-being and result in better decision-making on-farm to improve economic growth.

One hundred and twenty-eight piglets across 16 litters were used. This study was split into a winter cohort (January-February) and a summer cohort (July-August). Seven hours prior to processing piglets, half of the sows received an intramuscular injection of 2.0 mg/kg firocoxib (a nonsteroidal anti-inflammatory drug; NSAID). The following day, piglets were assigned to one of four treatment groups: transmammary-delivered firocoxib + ethyl chloride spray, transmammary-delivered firocoxib only, ethyl chloride spray only, or control/no treatment. The ethyl chloride spray was applied to the tail and scrotum of the piglets immediately before tail docking and surgical castration, respectively, in an attempt to induce cryoanesthesia (freezing or numbing of the skin). The outcome variables in this study included assessment of (1) blood-drug concentration; (2) behavior; (3) cranial and eye temperature; (4) plasma cortisol concentration; (5) hair and salivary cortisol concentration; (6) facial grimacing; (7) gait; (8) vocalization; and (9) weight. Piglet pain measures were taken at baseline (the day before processing) and out to 48h post-castration and tail docking.

The results of this study indicate that firocoxib was successfully transferred from the sow to her nursing piglets. At the time of processing, piglets that were provided firocoxib transmammary did not vocalize as loudly as piglets that were not provided firocoxib. Firocoxib-treated piglets were also observed engaging in more playful behavior and displayed less aggression towards littermates than piglets not provided firocoxib. Unfortunately, there was no significant reduction in piglet pain/stress associated with analgesic drug provision. The addition of the vapocoolant spray also did not lead to a reduction in pain associated with the surgical castration or tail docking procedures.

In examining the blood-drug concentration results, the greatest concentration of firocoxib was found in piglets ~30h post-administration to the sow. This suggests that sows may need to be administered analgesia more than 24h prior to processing piglets, to ensure an adequate blood-drug concentration at the level of the piglet is achieved. Additionally, piglets in the winter cohort had significantly higher levels of firocoxib in their blood compared to piglets in the summer cohort. Seasonal differences in farrowing room temperature also impacted other stress outcome measures, such as plasma cortisol concentration, cranial and eye temperature, average daily gain, vocalization, and gait. Future work will need to account for seasonal differences to determine optimal drug doses and treatment regimens.

Cortisol was successfully extracted and quantified from the saliva and hair of piglets. Piglets had lower salivary cortisol levels pre-procedure compared to post-procedure, which is consistent with plasma cortisol results. Interestingly, female piglets had significantly higher hair cortisol concentrations than male piglets. Piglets in the summer cohort had significantly higher hair cortisol levels than piglets in the winter cohort. Finally, all piglets had significantly higher hair cortisol levels

at baseline (4 days old) than at weaning (21 days old). This is the first time cortisol has been quantified from the hair and saliva of pre-weaned pigs, which may provide new avenues to monitor acute and chronic stress in the swine industry.

In conclusion, the transmammary-delivery of firocoxib from the sow to her nursing piglets was successful and may have provided some benefit to piglets in this study; however, the concentration of firocoxib at the level of the piglet was not sufficient to significantly reduce pain.

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Key Findings:

- Piglets provided firocoxib transmammary engaged in significantly less agonistic behavior and significantly more play behavior throughout the post-processing observation period compared to piglets who were not provided firocoxib
- The concentration of firocoxib at the level of the piglet was not sufficient to significantly reduce post-procedural pain associated with surgical castration and tail docking
- The vapocoolant spray (ethyl chloride) was ineffective at reducing pain associated with surgical castration and tail docking
- Cortisol was successfully extracted and quantified from both hair and saliva that were collected from pre-weaned pigs
- Seasonal temperature differences affected transmammary-drug delivery and stress outcome measures in this study

Keywords: analgesia, animal welfare, castration, pain assessment, piglet, tail docking

Scientific Abstract:

Piglets raised in commercial production systems in the U.S. undergo painful management procedures without analgesia or anesthesia provision. This is a significant animal welfare issue, affecting millions of piglets each year. The objectives of this study were to assess the efficacy of firocoxib, administered to the sow and delivered transmammary to her piglets, and a vapocoolant spray (ethyl chloride), to reduce pain associated with piglet processing procedures. This study also sought to determine if cortisol could be quantified from the hair and saliva of pre-weaned pigs, to provide a more welfare-friendly option to assess piglets' physiological stress response. One hundred and twenty-eight Yorkshire x Landrace piglets (5 days old) across 16 litters were used. The study was split into a winter cohort and a summer cohort, with an equal number of piglets represented in each group. Seven hours prior to processing piglets, half of the sows received an intramuscular injection of 2.0mg/kg firocoxib (for transmammary-delivery to piglets). Piglets were assigned to one of four treatment groups: transmammary-delivered firocoxib + ethyl chloride spray, transmammary-delivered firocoxib only, ethyl chloride spray only, or control/no treatment. The ethyl chloride spray was applied to the tail and scrotum of the piglets immediately before tail docking and surgical castration, respectively. The outcome variables included assessment of blood-drug concentration, behavior, cranial and eye temperature (using infrared thermography), plasma, hair, and salivary cortisol concentration, facial grimacing, gait, vocalization, and weight. Piglet pain measures were taken at baseline (the day before processing) and out to 48h post-castration and tail docking. The addition of the ethyl chloride spray did not lead to significance for any of the outcome measures; therefore, treatments were collapsed into two groups: FIRO piglets who received transmammary-firocoxib prior to processing and CON piglets who were processed without analgesic drug provision. At the time of processing, FIRO piglets emitted calls of lower amplitude than CON piglets ($P<.0001$). FIRO piglets engaged in significantly less agonistic behavior and significantly more play behavior across the observation period compared to CON piglets ($P=0.04$ and $P=0.002$, respectively). There was no significant difference in pain behavior or stress outcome measures in piglets associated with transmammary-delivered firocoxib. The greatest concentration of firocoxib was found in piglets ~30h post-administration to the sow ($P<0.0001$). This suggests that sows may need to be administered the analgesic drug more than 24h prior to processing piglets, to ensure an adequate blood-drug concentration at the level of the piglet is achieved. Additionally, piglets in the winter cohort had

significantly higher levels of firocoxib in their blood compared to piglets in the summer cohort (P=0.0008). Seasonal differences in farrowing room temperature also impacted other stress outcome measures, such as plasma cortisol concentration, cranial and eye temperature, average daily gain, vocalization, and gait (P<0.05). Future work will need to account for seasonal differences to determine optimal drug doses and treatment regimens. Cortisol was successfully extracted and quantified from the saliva and hair of pre-weaned pigs, which may provide new, less invasive options to monitor acute and chronic stress in the swine industry. Overall, transmammary-delivery of firocoxib may have provided some benefit to piglets in this study; however, the concentration of analgesic at the level of the piglet was not sufficient to significantly reduce pain.

Introduction:

Piglets raised in commercial production systems in the U.S. routinely undergo painful management procedures, including surgical castration and tail docking, without the provision of analgesia or anesthesia for pain relief. As more than 100 million pigs are raised in the U.S. each year, this is a significant animal welfare issue and a topic of increasing societal concern (Rollin, 2004). Canada and countries in the European Union have guidelines in their agricultural codes of practice that now require analgesia administration to piglets prior to surgical castration and tail docking for post-procedural pain relief (EU Commission, 2010; NFACC, 2014). To remain competitive with these markets, in terms of pork production and meat exportation, the U.S. will need to satisfy current animal welfare standards of importing countries, and analgesia will need to be provided to piglets at processing for pain management. Therefore, there is an immediate need to identify the most practical and effective analgesic drug option for use on-farm.

A systematic review of the existing literature found large variability in the success of nonsteroidal anti-inflammatory drugs (NSAIDs) at providing pain relief to piglets when they were administered at the time of castration and tail docking, resulting in a weak recommendation for their use from a panel of swine experts (O'Connor et al., 2014). The administration of an intramuscular injection prior to these procedures not only causes acute pain, but requires repeated handling of piglets, which increases animal stress and worker time to process pigs. Bates et al. (2014) demonstrated that an NSAID (meloxicam) can be administered to the sow and delivered to nursing piglets through her milk. This novel approach to drug delivery is ideal for both piglets and producers. Piglets receive the analgesic prior to being castrated and tail docked, ensuring time for appropriate blood-drug levels to be reached and maximizing the pain relief provided by the NSAID. Piglets will only need to be handled once at processing and will not require an intramuscular injection, reducing stress. This method of drug delivery is more cost-effective and will not impact the time to process piglets, which make it an attractive option for producers as well. Recently, Coetzee et al. (2019) demonstrated that an intramuscular injection of firocoxib to the sow is delivered transmammary to her piglets in sufficient quantity to reduce biomarkers of pain in the blood. The ability of firocoxib to reduce other measures of pain, such as behavior, after surgical castration and tail docking have not been assessed.

A topical vapocoolant spray (ethyl chloride) has demonstrated efficacy in reducing pain associated with minor procedures, such as venipuncture in humans and ear notching in piglets (Çelik et al., 2011; Lomax et al., 2018). It induces cryoanesthesia, caused by lowering tissue temperature and inducing cutaneous insensitivity. The desensitization of the surgical site is very rapid (3 seconds) and is unlikely to cause a significant increase in time to process piglets. It's ability to reduce surgical castration and tail docking pain in piglets when used alone, or in combination with an NSAID, has not been assessed.

Cortisol, the primary stress hormone, is a common outcome measure assessed in studies of animal pain (Ison et al., 2016). In pre-weaned piglets, blood is often collected via venipuncture of the jugular vein to facilitate plasma cortisol analysis. This method causes piglets acute pain and distress, and poses more significant risks, such as hematoma development, infection, and even shock/death secondary to the vasovagal response or laceration of the anterior vena cava or jugular vein. Less invasive methods of cortisol extraction, such as from the saliva or from hair samples, have been validated in other species (Casal et al., 2017; Dzviti et al., 2019). The validation of these methods in pigs would have a strong, positive impact on the way we assess pain in research, by eliminating the need for multiple blood draws to obtain cortisol results.

There are currently no pain-relieving compounds in the U.S. specifically approved for use in swine (Coetzee, 2011), making on-farm protocols for pharmaceutical pain management in piglets an even greater challenge. This study sought to evaluate the efficacy of multimodal pain control (analgesia and

cryoanesthesia) to improve the well-being of piglets undergoing surgical castration and tail docking. It also aimed to determine if cortisol could be extracted and quantified from the hair and saliva of pre-weaned pigs.

Objectives

1. To assess the efficacy of 2.0 mg/kg firocoxib, administered to the sow and delivered transmammary to her piglets, to reduce surgical castration and tail docking pain
2. To assess the efficacy of a topical vapocoolant spray (ethyl chloride), used alone or in combination with transmammary-delivered firocoxib, to reduce surgical castration and tail docking pain
3. To determine if cortisol can be quantified from the saliva of < 7 day old piglets
4. To determine if cortisol can be quantified from the hair of 21-28 day old piglets

Materials & Methods

Study 1 (Objectives 1, 2, 4)

One hundred and twenty-eight Yorkshire x Landrace piglets (n=64 male and n=64 female; 5 days old) across 16 litters were used (n=4 male and n=4 female piglets were enrolled per litter). The study was split into a winter cohort (January-February) and a summer cohort (July-August), with an equal number of piglets represented in each cohort.

Seven hours prior to processing piglets, half of the sows (n=8) received an intramuscular injection of 2.0mg/kg firocoxib (Equioxx 20 mg/kg; Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, CT). Firocoxib is able to cross the blood-milk barrier in the mammary gland and be delivered to nursing piglets through the sow's milk. The following day, piglets were assigned to one of four treatment groups: transmammary-delivered firocoxib + ethyl chloride spray, transmammary-delivered firocoxib only, ethyl chloride spray only, or control/no treatment (standard practice in commercial swine production). The ethyl chloride spray was applied to the tail and scrotum of the piglets immediately before tail docking and surgical castration, respectively, in an attempt to induce cryoanesthesia. The outcome variables in this study included assessment of (1) blood-drug concentration; (2) behavior; (3) cranial and eye temperature; (4) plasma cortisol concentration; (5) hair cortisol concentration; (6) facial grimacing; (7) gait; (8) vocalization; and (9) weight.

A high-definition video camera (Sony Handycam HDR-CX405, Sony USA Inc., New York, NY) mounted on a tripod was placed outside of each farrowing crate. Piglet behavior was recorded and scored continuously for 15 min, at baseline (pre-procedure) and 0, 1, 2, 4, 7, 24, 30, 36, and 48h post-procedure. BORIS (Behavioral Observation Research Interactive Software) and a detailed ethogram was used by three observers (blinded to piglet treatment and time point) to score the behavioral data. In total, 19,200min (320h) of behavioral data was scored and analyzed using a generalized linear mixed (GLIMMIX) model with a beta distribution, including treatment, time, litter, and time × treatment interaction in SAS (Statistical Analysis System 9.4, SAS Institute, Inc.). Litter was included as a random effect and time was a repeated measure with piglet as the experimental unit. Post hoc tests were conducted using the Tukey-Kramer adjustment. Statistical significance was set at $P \leq 0.05$.

Approximately 4.0mL of whole blood was collected from the jugular vein of piglets at baseline (pre-procedure) and at 0, 1, and 24h post-procedure. The blood specimen was separated into (1) a sodium heparin tube (BD Vacutainer, Franklin Lakes, NJ) for blood-drug concentration analysis and (2) a serum separator tube (BD Vacutainer, Franklin Lakes, NJ) for cortisol analysis. Blood collection tubes were immediately placed on ice after collection. The samples were then centrifuged at 3000g for 10min, plasma was pipetted into cryovials, and cryovials were stored at -80°C prior to analysis. All samples were analyzed by laboratory personnel at Kansas State University blinded to treatment and time point.

Cranial and eye temperature of piglets were collected at baseline (pre-procedure) and at 1, 2, 4, 7, 24, 30, 36, 48h post-processing, using an infrared thermography (IRT) camera (FLUKE TiX580; FLUKE Corporation, Everett, WA). The average temperature (°F) of the cranium and the eye were recorded and analyzed using research-grade software (SmartView 4.3; FLUKE Corporation, Everett, WA).

Piglet hair samples were collected at baseline (piglets = 4 days old) by using clippers to shave a patch of hair on the left side of each piglet. At weaning (piglets = 20 days old), the hair that had grown

in the spot where baseline samples were taken was shaved and collected. All hair samples were sent to the Iowa State University's SPIT (Stress Physiology Investigative Team) lab, where cortisol was extracted and quantified. Hair cortisol was analyzed statistically using a mixed model in SAS, including treatment, time, and litter. Statistical significance was set at P≤0.05.

In total, 682 still-images of piglet faces were extracted from the recorded behavioral data. We attempted to collect one high-quality image per piglet at each of the following time points: baseline (pre-procedure), and at 0, 1, 2, 4, 7, 24, 30, 36, and 48h post-procedure. Three trained observers, blinded to piglet treatment and time point, used the published Piglet Grimace Scale (PGS; developed by the PI: Viscardi and Turner, 2018a) to score ear position, cheek tightening/nose bulge, and orbital tightening. The PGS score for each image was calculated by summing the scores given to each of the facial action units.

A commercially available pressure/force measurement system (Strideway, Tekscan, Inc., South Boston, MA, USA) was used to record and analyze the steps of each piglet. Piglets were walked across the pressure mat at baseline and at 1, 7, 24, 36, and 48h post-processing. Using research-grade software (Strideway v 7.7, Tekscan, Inc., South Boston, MA, USA), stance time, stride length, contact force, impulse, contact pressure, gait distance, and gait velocity were assessed using previously published methods (Coetzee et al., 2014).

Vocalizations were recorded during the castration and tail docking procedures using a video camera placed as close to the focal piglet's face as possible. Vocalizations from the recorded videos were analyzed using Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, NY) by an individual blinded to piglet treatment. From the spectrograms, maximum frequency (Hz), maximum amplitude (μ), and energy (dB) of each call was determined.

Finally, piglets were weighed at baseline (piglets = 4 days old) and at weaning (piglets = 20 days old) to calculate average daily gain.

Blood cortisol, blood-drug concentration, cranial and eye temperature, facial grimacing, gait, average daily gain, and vocalizations were analyzed using a mixed model in SAS, including treatment, time, litter, cohort, and time × treatment interaction. Litter was included as a random effect and time was a repeated measure with piglet as the experimental unit. Post hoc tests were conducted using the Tukey-Kramer adjustment. Statistical significance was set at P≤0.05.

Study 2 (Objective 3)

Thirty Yorkshire x Landrace piglets (male and female; 2 days old) across 3 litters were used. Piglets were randomly allocated to one of three treatment groups (n=10 piglets/treatment): tail docking using side pliers, tail docking using a CO_2 surgical laser (VetScalpel; Aesculight, LLC, Bothell, WA), and sham tail docking (undocked control). This study was part of a larger USDA, NIFA, AFRI funded project (#2020-67015-31540); however, we used these study animals to determine if salivary cortisol could be quantified and used to assess pain associated with common processing procedures. Therefore, the Materials & Methods, Results, and, Discussion of this report will only focus on the salivary cortisol outcome measure of this study.

Salivette® Cortisol tubes (SARSTEDT Ag & Co., Germany) equipped with a synthetic cotton swab designed for cortisol determination were used to collect salivary samples at baseline (pre-procedure) and at 0.5 and 8h post-procedure. To stimulate salivation, 2-3 drops of a citric acid solution (Citric acid anhydrous crystalline; Fisher Bioreagents, Ottawa, ON) were placed on the tongue of each piglet. Approximately 2-3min after the citric acid drops, the individual handling the piglets inserted a cotton swab into the mouth of the animal and gently held it closed for 2min. Then the cotton swab with the absorbed saliva was removed and returned to the tube. Saliva was then centrifuged at 1500g for 15min and pipetted into wells. Saliva samples were stored at -20°C prior to analysis. Saliva cortisol concentrations were determined using a commercially available enzyme immunoassay (EIA) kit (Salimetrics, State College, PA) following manufacturer specifications. A mixed model was used to analyze salivary cortisol. The mixed model included litter, time, treatment, and time × treatment interaction. Litter was included as a random effect and time was a repeated measure with piglet as the experimental unit. Post hoc tests were conducted using the Tukey-Kramer adjustment. Statistical significance was set at P≤0.05.

Results

The addition of the ethyl chloride spray did not lead to significance for any of the outcome measures; therefore, treatments were collapsed into two groups: **FIRO** piglets who received transmammary-

firocoxib prior to processing (n=32 male and n=32 female); **CON** piglets who were processed without analgesic drug provision (n=32 male and n=32 female).

Objective 1: To assess the efficacy of 2.0 mg/kg firocoxib, administered to the sow and delivered transmammary to her piglets, to reduce surgical castration and tail docking pain

Behavior: There was no significant difference in proportion of time piglets displayed pain behavior between treatment groups (P=0.26); however, FIRO piglets engaged in significantly less agonistic behavior and significantly more play behavior across the observation period compared to CON piglets (P=0.04 and P=0.002, respectively). Irrespective of treatment, piglets were observed displaying significantly more pain behavior (stiffness, tail wagging, spasms, rump rubbing) at 24h and 30h post-processing compared to all other time points (P<0.0001; **Figure 1**). This increase in piglet pain behavior at 24h post-processing is consistent with previous work conducted by the PI (Viscardi and Turner, 2018a; Viscardi and Turner, 2018b).

Plasma cortisol: Cortisol concentrations were significantly higher in the winter cohort (mean: 189.5±6.9ng/mL) compared to the summer cohort (mean: 134.2±7.4ng/mL; P<0.0001). Male piglets had significantly higher cortisol concentrations across the observation period (mean: 182.3±7.1ng/mL) compared to female piglets (mean: 141.5±7.2ng/mL; P<0.0001). This sex difference is likely due to male piglets being in a more painful state, having undergone surgical castration and tail docking, where female piglets were only tail docked. Cortisol concentrations peaked 1h post-processing (P<0.0001; **Figure 2**). FIRO piglets had significantly higher cortisol concentrations across the observation period (mean: 173.1±7.2ng/mL) compared to CON piglets (mean: 150.6±7.1ng/mL), which suggests firocoxib administration did not reduce piglet stress

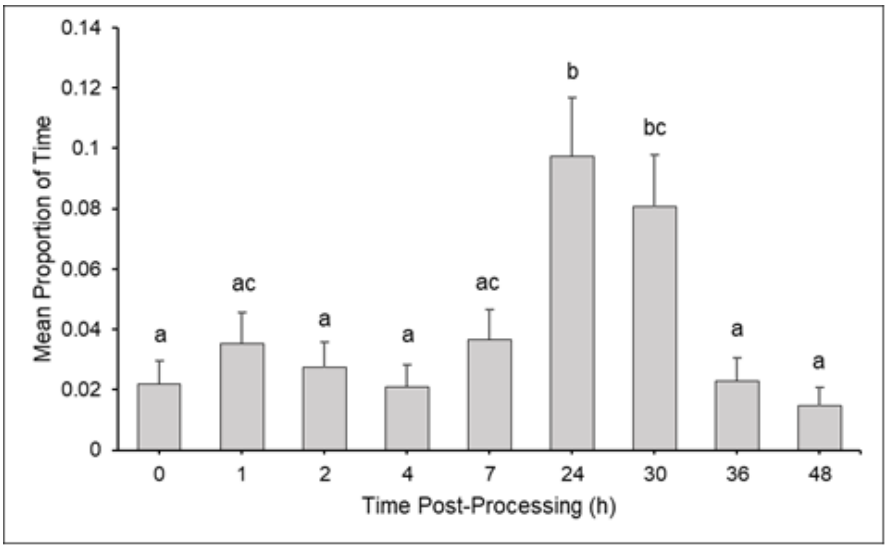


Figure 1: The effect of time on the amount of pain-related behaviors displayed by piglets post-processing (mean ± SE)

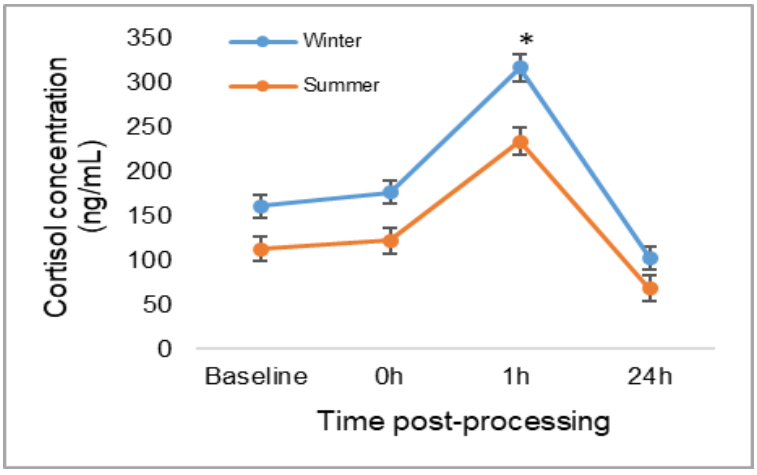


Figure 2: Mean cortisol concentration (±SE) of piglets in each season over time

Cranial and eye temperature: Piglets in the summer cohort had significantly higher cranial and eye temperatures than piglets in the winter cohort (P<0.0001 for both). Female piglets had significantly higher cranial temperatures across the observation period compared to male piglets (P=0.0001). There was no effect of treatment on cranial temperature of piglets (P=0.13); however, FIRO piglets had significantly higher eyes temperature across the observation period compared to CON piglets (P=0.02). This suggests that firocoxib administration may have influenced sympathetic nervous system activation in response to pain in piglets.

Blood-drug concentration: Transmammary-delivery of firocoxib to piglets were significantly higher in the winter cohort (mean: 2.39±0.33ng/mL) compared to the summer cohort (mean: 1.53±0.33ng/mL; P=0.0008). There was also a significant effect of time, with the greatest concentration of firocoxib found in piglets ~30h post-administration to the sow (P<0.0001; **Figure 3**).

Average daily gain (ADG): There were no differences found in ADG between male and female piglets (P=0.51) or FIRO piglets and CON piglets (P=0.36) in this study. There was a significant seasonal effect of ADG, with piglets in the summer cohort having a higher ADG (0.24 kg/day) than piglets in the winter cohort (0.21 kg/day; P=0.0043).

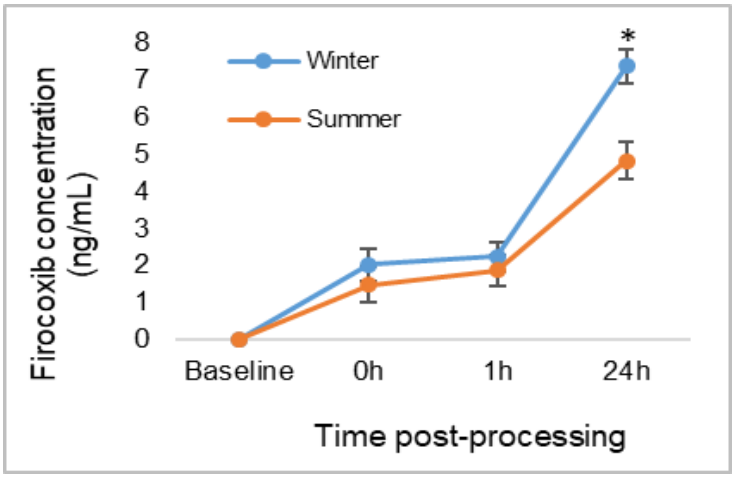


Figure 3: Mean firocoxib concentration (±SE) of piglets in each season over time

Facial grimacing: There were no sex, treatment, or seasonal differences found in piglet facial grimacing; however, there was a trend in time (P=0.054), with the highest amount of facial grimacing observed at 1 and 7h post-processing than at all other time points.

Gait analysis: Pressure mat gait analysis results are summarized in **Table 1**.

Stance time: There were no treatment, time, or sex differences in stance time of piglets throughout this study (P>0.1); however, piglets in the summer cohort had a significantly longer stance time of their hind limbs (0.70±0.05 s) compared to piglets in the winter cohort (0.48±0.05 s; P=0.0029)

Stride length: This refers to the distance measured between the posterior heel of two consecutive foot falls. There were no significant treatment or cohort differences in stride length of the front or hind limbs, nor were their time or sex differences found in stride length of the hind limbs (P>0.1). Female piglets took significantly longer strides using their front limbs (16.31±0.18 cm) compared to male piglets (15.62±0.17 cm; P=0.006). Piglets also took significantly shorter strides using their front limbs at baseline (15.11±0.30 cm) than at 36h (16.40±0.30 cm; P=0.03) and 48h (16.40±0.29 cm; P=0.026) post-procedure.

Force: This refers to the maximum force applied to the mat for each step. There were significant time and cohort differences found in the mean force applied by the front limbs of piglets. Piglets in the winter cohort had significantly higher measured force in their front limbs (1.30±0.02 kg) compared to piglets in the summer cohort (1.19±0.02 kg; P<.0001). Across all treatment groups, there was significantly less force applied by the front limbs of piglets at baseline (1.18±0.03 kg), and at 1h

(1.24±0.03 kg) and 7h (1.25±0.03 kg) post-castration and tail docking compared to 36h (1.40±0.03 kg) and 48h (1.38±0.03 kg) post-processing (P<.0001).

Male piglets had significantly higher measured force in their hind limbs (0.67±0.01 kg) compared to female piglets (0.63±0.01 kg; P=0.03). Piglets in the winter cohort also had significantly higher measured force in their hind limbs (0.69±0.01 kg) compared to piglets in the summer cohort (0.62±0.01 kg; P<.0001).

Impulse: This refers to the maximum force applied per unit time. There was a significant cohort effect found in the mean impulse in the front limbs of piglets (P=0.02). Piglets in the winter cohort had a significantly higher measured impulse in their front limbs (0.76±0.03 kg × s) compared to piglets in the summer cohort (0.67±0.03 kg × s).

Male piglets had significantly higher measured impulse in their hind limbs (0.28±0.02 kg × s) compared to female piglets (0.20±0.02 kg × s; P=0.003). FIRO piglets had significantly lower measured impulse in their hind limbs (0.21±0.02 kg × s) compared to CON piglets (0.27±0.02 kg × s; P=0.02).

Contact pressure: This refers to the peak amount of pressure applied by each foot fall on the mat. There were significant time and cohort differences found in the mean pressure applied by the front limbs of piglets. Piglets in the winter cohort had significantly higher measured pressure in their front limbs (1.19±0.01 kg/cm^2) compared to piglets in the summer cohort (0.96±0.01 kg/cm^2; P<.0001). Across all treatment groups, there was significantly less pressure applied by the front limbs of piglets at baseline (1.00±0.02 kg/cm^2) compared to 24h (1.10±0.02 kg/cm^2; P=0.05), 36h (1.13±0.02 kg/cm^2; P=0.001), and 48h (1.10±0.02 kg/cm^2; P=0.03) post-processing.

There were no treatment, time, sex, or cohort differences in mean pressure applied by the hind limbs throughout the study period.

Table 1: Mean (±SEM) outcome measures from the pressure mat gait analysis (n=64 piglets per treatment group)

Parameter	Treatment [1]		P-values			
	FIRO	CON	Treatment	Time	Sex	Cohort
Front limbs						
Stance time (s)	0.85 ± 0.03	0.83 ± 0.04	0.77	0.93	0.63	0.51
Stride length (cm)	16.1 ± 0.17	15.8 ± 0.19	0.29	*0.03*	*0.006*	0.63
Force (kg)	1.28 ± 0.02	1.31 ± 0.02	0.20	*<.0001*	0.12	*<.0001*
Impulse (kg × s)	0.70 ± 0.03	0.72 ± 0.03	0.61	0.45	0.52	*0.02*
Contact Pressure (kg/cm^2)	1.06 ± 0.01	1.09 ± 0.01	0.12	*0.001*	0.34	*<.0001*
Hind limbs						
Stance time (s)	0.55 ± 0.05	0.64 ± 0.05	0.19	0.19	0.20	*0.003*
Stride length (cm)	19.0 ± 0.68	17.7 ± 0.71	0.17	0.95	0.13	0.17
Force (kg)	0.64 ± 0.01	0.67 ± 0.01	0.12	0.20	*0.03*	*<.0001*
Impulse (kg × s)	0.21 ± 0.02 [a]	0.27 ± 0.02 [b]	*0.02*	0.78	*0.003*	0.14
Contact Pressure (kg/cm^2)	0.79 ± 0.04	0.90 ± 0.04	0.08	0.15	0.51	0.07

[1] FIRO = transmammary-delivered firocoxib; CON = no analgesic drug

[a,b] Values within a row with different superscripts differ significantly (P≤0.05)

Vocalization: FIRO piglets emitted calls of lower amplitude at the time of surgical castration and tail docking (mean: 16,463±122.02 µ) compared to CON piglets (mean: 17,186±120.04 µ;). Piglets in the winter cohort emitted calls of lower amplitude and higher frequency than piglets in the summer cohort (P<.0001 and P=0.01, respectively). Irrespective of treatment, the castration and tail docking procedures caused piglets to emit calls of significantly higher amplitude (means: 17,374±177.32 µ and 17,396±192.31 µ, respectively) compared to handling piglets (mean: 16,766±221.92 µ), spraying the tail and scrotum with ethyl chloride (mean: 16,416±178.07 µ), and disinfecting the surgical site pre-procedure with an alcohol wipe (mean: 16,282±296.68 µ; P<.0001).

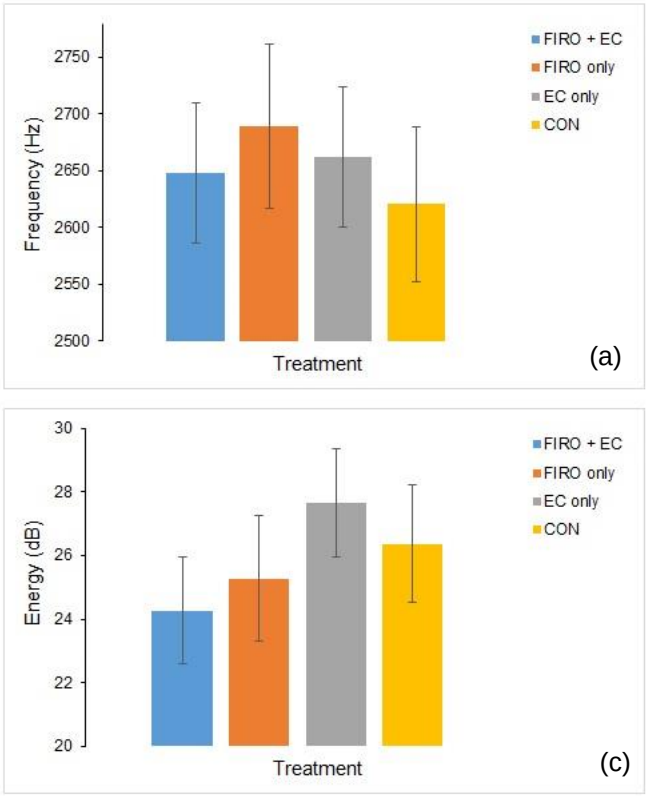
Objective 2: To assess the efficacy of a topical vapocoolant spray (ethyl chloride), used alone or in combination with transmammary-delivered firocoxib, to reduce surgical castration and tail docking pain

As mentioned above, the addition of the ethyl chloride spray did not provide any significant benefit to the piglets in terms of pain relief based on the measured outcomes. We anticipated that we would observe treatment differences in frequency, amplitude, and energy of vocalizations at the time of surgical castration and tail docking; however, these were not detected (see **Figure 4**).

Objective 3: To determine if cortisol can be quantified from the saliva of < 7 day old piglets

Saliva was successfully quantified from the 90 samples collected from 2-3 day old piglets (n=30 piglets). There were no treatment or treatment × time interactions for salivary cortisol concentrations. However, irrespective of treatment, at 8h post-procedure, piglets had higher salivary cortisol concentrations compared to baseline (P=0.02; **Table 2**).

Complete study has been published: Lou ME, Kleinhenz MD, Schroeder R, Lechtenberg K, Montgomery S, Coetzee JF, Viscardi AV. 2022. Evaluating the utility of a CO_2 surgical laser for piglet tail docking to reduce behavioral and physiological indicators of pain and to improve wound healing: a pilot study. Appl Anim Behav Sci 254:105720. doi:10.1016/j.applanim.2022.105720.



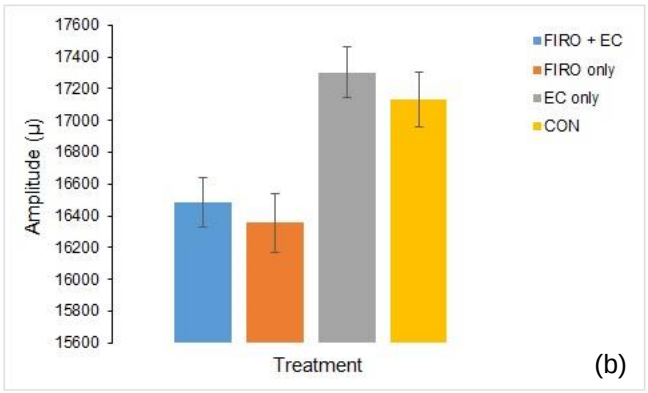


Figure 4: Vocalization (**a**) frequency, (**b**) amplitude, and (**c**) energy (± SEM) of piglets in each treatment group undergoing surgical castration and tail docking. FIRO + EC = transmammary-delivered firocoxib + ethyl chloride spray; FIRO only = transmammary-delivered firocoxib only; EC only = ethyl chloride spray only; CON = no treatment. Different letters indicate significance

Table 2: Mean (+ 95% confidence interval) concentration of salivary cortisol analyzed for piglets undergoing tail docking using a CO_2 surgical laser, side pliers, or sham (no procedure)

Salivary cortisol (ng/mL)	Treatment			P-value		
	CO_2 laser	Side pliers	Sham	Treatment	Time	Treatment × Time
				0.14	*0.02*	0.22
Baseline	6.09	9.72	11.02			
	2.08 – 13.44	1.72 – 9.72	3.86 – 19.53			
0.5h post-procedure	6.11	7.80	8.11			
	1.80 – 8.80	3.47 – 16.76	1.23 – 12.52			
8h post-procedure	6.70	4.70	6.29			
	0.98 – 25.27	1.55 – 8.04	0.87 – 12.60			

Objective 4: To determine if cortisol can be quantified from the hair of 21-28 day old piglets

Cortisol was successfully extracted and quantified from 256 samples of 4 day old (baseline; n=128) and 20 day old (weaning; n=128) piglets. There were no treatment differences in hair cortisol concentration; however, there were significant time, sex, and cohort differences. Irrespective of treatment, female piglets had significantly higher hair cortisol (mean: 0.41±0.03 ug/dL) than male piglets (mean: 0.33±0.03 ug/dL; P=0.04). Piglets in the summer cohort had significantly higher hair cortisol levels (mean: 0.49±0.03 ug/dL) than piglets in the winter cohort (mean: 0.25±0.03 ug/dL; P<.0001). Finally, all piglets had significantly higher hair cortisol levels at baseline (mean: 0.56±0.03 ug/dL) than at weaning (mean: 0.18±0.03 ug/dL; P<.0001).

Discussion

Firocoxib, a nonsteroidal anti-inflammatory drug, has demonstrated success in pain management for various species and contexts, including musculoskeletal pain and osteoarthritis in horses (Orsini et al., 2012) and pain associated with soft tissue surgery in dogs (Kondo et al., 2012). It has previously been shown to reduce stress biomarkers (e.g., cortisol) in piglets after surgical castration and tail docking (Coetzee et al., 2019). Ethyl chloride, a topical vapocoolant spray, has demonstrated efficacy in reducing pain associated with minor procedures, such as venipuncture in humans (Çelik et al., 2011) and ear notching in piglets (Lomax et al., 2018). It causes cryoanesthesia, by lowering tissue temperature and inducing cutaneous insensitivity. Tissue desensitization is rapid (~3 sec), making it a practical option for on-farm use. This is the first study that assessed the ability of firocoxib, provided to the sow and delivered transmammary to her nursing piglets, with or without an ethyl chloride spray, to alleviate pain in piglets after routine husbandry procedures.

Pain behavior associated with surgical castration and tail docking of piglets has been well-described in the literature and includes an increase in tail wagging, scooting or rubbing the rump, and body spasms (Hay et al., 2003). Neither firocoxib nor the topical vapocoolant spray were able to significantly reduce these behavioral indicators of pain across the study period. The greatest amount of pain-associated behavior in piglets was observed at 24h post-processing, which is consistent with previous work (Viscardi and Turner, 2018a; Viscardi and Turner, 2018b). This may be due to progression of the inflammatory process, which was not sufficiently controlled by firocoxib, causing an increase in pain (Kumar et al., 2015). Interestingly, piglets who were provided firocoxib transmammary were observed engaging in more play and had fewer aggressive interactions with littermates. Negative-valence emotions can lead to aggressive behavior (Crump et al., 2020) whereas play and affiliative behaviors are associated with positive experiences and emotions (Boissy et al., 2007). This suggests that there may have been some benefit to providing firocoxib to piglets, in that they were in a more positive emotional state than their untreated counterparts.

Many outcome variables in this study were impacted by season, or, more accurately, the temperature of the farrowing barn. The farrowing barn where this study was conducted (Kansas State University's Swine Teaching and Research Center, Manhattan, KS) was poorly ventilated and the ambient temperature differed drastically between the winter and summer cohorts. Unfortunately, we did not

record the temperature and humidity of the farrowing barn throughout this study, which would have certainly provided us with more information. Irrespective of treatment group, piglets in the summer cohort had significantly higher cranial (cutaneous) temperature than piglets in the winter cohort. This finding suggests that the environmental temperature of the farrowing house in the summer impacted the body temperature of piglets. Blood-drug concentration at the level of the piglet was significantly higher in the winter cohort compared to the summer cohort. Body temperature in humans and animals has been shown to impact drug metabolism and drug response (Zhou and Poloyac, 2012). For example, piglets in the early stages of hypothermia who were given fentanyl (an opioid) had higher plasma-drug concentrations, with the drug having an increased half-life and slower clearance rate than piglets with normal body temperature (Koren et al., 1987). High ambient temperature has also been associated with decreased feed intake and reduced milk production in lactating sows (Choi et al., 2019), which may have provided less opportunity to nursing piglets to acquire firocoxib in the milk. However, we would expect in this case that average daily gain of piglets in the summer cohort would be lower than piglets in the winter cohort, when the opposite occurred. This is an unexpected outcome from this study that warrants further analysis, as drug doses may need to be altered based on environmental temperature to obtain therapeutic effects. Piglets in the winter cohort also had higher plasma cortisol concentrations and emitted quieter vocalizations of a higher pitch than piglets in the summer cohort, suggesting that stress may have been higher when the ambient temperature was cooler.

Cortisol, the stress hormone, is a validated measure that can be extracted from blood (swine: Newman et al., 2014), urine (dog: Moya et al., 2022), feces (deer: Gholib et al., 2021), saliva (dog: Bergamasco et al., 2010), and hair (cattle: Heimbürge et al., 2020), each providing a different timeline of stress. For example, an elevation of cortisol can be detected in the blood and saliva within minutes of a stressful event, and are ideal to measure the acute stress response. Hair provides a much longer window of time for cortisol assessment (e.g., in humans, a 3-cm hair segment reflects cortisol secretion over a three-month period; Wenning, 2000) and can provide important information on chronic stress, a state that is very difficult to assess in livestock species. This study provides the first indication that cortisol can be extracted and assessed from the saliva and hair of pre-weaned pigs, and has utility as non-invasive measures of physiologic stress. We anticipate these tools to be utilized more in a research setting while also providing new avenues to monitor acute and chronic stress within the swine industry. This can give us more information on the animal welfare implications of current industry practices.

It is anticipated that analgesia will eventually be required for piglets undergoing surgical castration and tail docking in the U.S., as societal pressure mounts for improved food animal welfare. As well, many countries, including Canada and countries in the European Union (EU), have guidelines in their agricultural codes of practice that now require analgesia administration to piglets prior to surgical castration and tail docking for post-procedural pain relief (EU Commission, 2010; NFACC, 2014). Therefore, it is beneficial to explore current options for pain relief to determine the most practical and efficacious for on-farm use. While the dose and method of administration of firocoxib in this study (2.0 mg/kg, intramuscular to the sow) was not found to have been delivered transmammary in a sufficient quantity to manage pain at the level of the piglet, this method of drug delivery has promise for reducing time, effort, and stress associated with administering a drug to each individual piglet prior to processing. Future studies on transmammary drug delivery should explore different doses of firocoxib and different modes of delivery (e.g., oral), to try and optimize pain relief. Another analgesic drug may be worth exploring; for example, meloxicam has been previously been shown to cross the blood-milk barrier in sows (Bates et al., 2014) and is approved for use in swine in Canada and the EU. The role of body temperature on drug absorption and drug metabolism would allow us to better define pain management protocols based on ambient barn temperature. All of this would lead to improved on-farm animal welfare within the swine industry.