Transmammary delivery of firocoxib to piglets reduces stress and improves average daily gain after castration, tail docking, and teeth clipping¹

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ABSTRACT: Painful processing procedures in piglets such as tail docking, castration, and teeth clipping are an emerging animal welfare concern. We hypothesized that transmammary delivery of a nonsteroidal anti-inflammatory drug, firocoxib, would reduce pain associated with processing in piglets. This study compared the pharmacokinetics, efficacy, safety, and tissue residue concentrations of 4 doses of firocoxib (0.5, 1.0, 1.5, or 2.0 mg/kg) administered to sows and delivered to nursing piglets prior to processing. Sixteen sows, 5 ± 2 d postpartum, were randomly assigned to 1 of 4 treatment groups. On day 0, sows received a single intramuscular dose of firocoxib at 7 ± 1 h before piglet surgical castration, tail docking, and teeth clipping (males) or sham handling (females). Firocoxib and cortisol concentrations were determined from selected samples collected from sows and 3 piglets per litter (2 barrows and 1 gilt) at 0, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h after drug administration. On day 21, piglets were weighed and all animals were euthanized and necropsied. Tissues were collected from 3 piglets per litter for histological examination and drug residue analysis. Mean (±SEM) peak plasma firocoxib concentrations (C_{max}) were 107.90 ± 15.18, 157.50 ± 24.91,

343.68 ± 78.89, and 452.83 ± 90.27 ng/mL in sows receiving 0.5, 1.0, 1.5, and 2.0 mg/kg firocoxib, respectively, and 9.53 ± 1.21, 31.04 ± 6.79, 53.30 ± 11.1, and 44.03 ± 7.47 ng/mL in their respective piglets. Mean plasma terminal half-life values ranged from 26 to 31 h in sows and 30 to 48 h in piglets. Barrows nursing sows that received 2.0 mg/kg firocoxib had a lower mean plasma cortisol concentration at 1 ± 1 h after processing compared with barrows nursing sows that received 1.0 mg/kg ($P = 0.0416$) and 0.5 mg/kg of firocoxib ($P = 0.0397$). From processing to weaning, litters of sows receiving 2.0 mg/kg firocoxib gained more weight than litters of sows that received 0.5 mg/kg ($P = 0.008$) or 1.0 mg/kg ($P = 0.005$). No signs of nonsteroidal anti-inflammatory drug toxicity were observed on examination of the kidney, liver, stomach, and small intestine, and concentrations of firocoxib and the descyclopropylmethyl metabolite were below the limit of detection (0.01 µg/g) in all tissues examined from sows and piglets. These findings indicate that maternal delivery of firocoxib to suckling piglets before tail docking and castration may safely reduce processing-induced stress and enhance production by increasing weaning weights.

Key words: firocoxib, transmammary, swine, animal welfare, pain, castration

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INTRODUCTION

Each year, approximately 133 million piglets in the United States undergo painful processing procedures (USDA NASS, 2018). Pain-related behaviors have been found to persist for up to 4 d after castration in piglets (Hay et al., 2003). Consumer concern about the animal welfare implications has resulted in the investigation of methods to provide analgesia and alleviate the distress associated with these practices (Sutherland, 2015). In the European Union (EU Council Directive 2008/120/EC, 2008) and Canada (NFACC, 2014), mandatory use of analgesia and anesthesia is required for castration in piglets older than 7 d. However, in the United States, no drugs are currently labeled by the Food and Drug Administration (FDA) to mitigate pain in swine. Therefore, the development of new pain mitigation strategies would be beneficial.

Firocoxib is a nonsteroidal anti-inflammatory drug (NSAID) with FDA approval in the United States for control of pain and inflammation associated with osteoarthritis in horses and dogs. Firocoxib is 384 times more selective for canine COX-2, the inducible isoform of the enzyme that synthesizes prostaglandins that mediate pain and inflammation, than COX-1, the constitutively induced isoform that produces prostaglandins that maintain normal renal function and gastrointestinal integrity (Vane and Botting, 1995; McCann et al., 2002, 2004).

In the present study, we tested the hypothesis that transmammary delivery of firocoxib, from the lactating sow to the nursing piglets, would achieve safe and effective analgesic drug concentrations in suckling offspring that underwent processing procedures. The specific aims of this study were to 1) describe the pharmacokinetics and transmammary delivery of firocoxib to piglets after intramuscular (IM) administration to sows, 2) investigate the effects of transmammary-delivered firocoxib on the stress response and performance of piglets after castration, tail docking, and teeth clipping; and 3) assess the safety and firocoxib tissue residue concentrations in both sows and piglets at weaning.

MATERIALS AND METHODS

This study was designed as a randomized, blinded, fixed-dose, dose-response trial. Before

initiation of the study, all techniques for animal use, housing, handling, and sampling were approved by the Midwest Veterinary Services (MVS) Institutional Animal Care and Use Committee (Protocol #AC16019P). The study design and sampling time points are outlined in Table 1.

Animals

Twenty-two pregnant Yorkshire × Landrace sows (mean ± SEM bodyweight, 250.3 ± 7.61 kg) that were approximately 1 wk before farrowing were sourced from a commercial swine farm (Table 2). All study animals were bred to the same boar except sow 16149 and sow 16175. The boar line was Maxgro (Hermitage NGT genetics, Gwynne, AB, Canada). Specifically, the male side was Pietrain and the female side was a 5 way cross of Berkshire, Hamp, Duroc, Landrace, and Yorkshire. The sows were Hermitage Lineage-F1. Each sow was examined by a veterinarian to confirm that she was healthy and pregnant. A unique numerical ear tag (Allflex Global Ear Tags, Allflex USA, Inc., DFW Airport, TX) was placed in the right ear of each sow for identification. The sows were housed in a commercial swine operation at MVS (West Point, NE) in accordance with the recommendations in the Guide for the Care and Use of Agricultural Animals in Agricultural Use and Research and Teaching (Federation of Animal Science, 2010). Sows were placed in Quad- or Euro-style farrowing stalls (Thorp Equipment, Thorp, WI), depending on availability. Regardless of stall type, each sow was housed in a farrowing crate measuring 0.6 m × 2.1 m. Quad and Euro crates had piglet creep areas of 7.0 and 6.4 m², respectively. After farrowing, a heat lamp was provided on one side of the creep area for the piglets in each crate. All sows were fed a diet that met or exceeded National Research Council (NRC, 2012) nutrient requirements and water was provided ad libitum. At the time of study commencement, there was no active disease pressure from porcine reproductive and respiratory syndrome, porcine epidemic diarrhea virus, or swine influenza virus.

Animal Phase Study Design

On study day 0, before dosing, 16 sows each nursing 9 piglets [aged 5 (± 2) d] were selected to

Table 1. Outline illustrating the study design and timeline for designated study procedures

Timeline	Time relative to firocoxib dosing (h)	-24	0	2	4	6	8	12	24	48	72	96	120	504
Study day		0	0	0	0	0	0	0	1	2	3	4	5	21
No. of sows ($n = 4/\text{treatment}$)		16	16	16	16	16	16	16	16	16	16	16	16	16
No. of piglets ($n = 36/\text{treatment}$)		144	144	144	144	144	144	144	144	144	144	144	144	144
Sows	Body weight determination	X												
	Randomization	X												
	Treatment		X											
	Firocoxib 0.5 mg/kg		$n = 4$											
	Firocoxib 1.0 mg/kg		$n = 4$											
	Firocoxib 1.5 mg/kg		$n = 4$											
	Firocoxib 2.0 mg/kg		$n = 4$											
	Blood sample for firocoxib	X		X	X	X	X	X	X	X	X	X	X	
	Necropsy and tissue collection													X
Nursing piglets	Time relative to processing (h)	-30	-6	-4	-2	0	2	6	18	42	66	90		
	Body weight determination	X	ADG											X
	Castration, tail docking and teeth clipping (male piglets)					X								
	Blood sample ($n = 3$ piglets/litter)	X		X	X	X	X	X	X	X	X	X	X	
	Firocoxib determination	X		X	X	X	X	X	X	X	X	X	X	
	Cortisol determination					X	X	X	X	X	X	X		
	Necropsy and tissue collection ($n = 3$ piglets/litter)													X

receive clinical examinations that included recording body weights. Each litter provided at least 6 male and 3 female piglets for sampling with the exception of 1 sow, which had 5 males and 4 females in the litter, and 1 sow, which had 3 males and 6 females in the litter. These 2 litters were included in the experiment due to the limited number of litters that were eligible to be enrolled in the animal phase of the study at this commercial swine facility during the designated study period. Furthermore, cross-fostering was not permitted for this experiment. Body weights were used with a random number generator (Microsoft Excel, Redmond, WA) to randomly assign sows to 1 of 4 treatment groups ($n = 4$ sows per group; Table 2). Study personnel were masked to treatment group. A sample size of 4 sows was selected to describe the pharmacokinetics of firocoxib based on principles outlined by Riviere (2011). For the comparison of plasma cortisol concentrations and average daily gain in bodyweight (ADG), a sample size of 36 piglets per treatment was calculated to provide Statistical Power of 80% (0.8), assuming an alpha of 0.05, sigma of 0.54, and delta of 0.20.

At the time of study commencement (T_0), sows received a single dose of firocoxib (Equioxx Injection, Merial, Duluth, GA; Lot number 4VP07, Expiration Date 11/2017) administered at either 0.5, 1.0, 1.5, or

2.0 mg/kg by IM injection into the right lateral neck using an 18-gauge, 1.5-inch needle attached to a 20-mL syringe (Table 2). In cases where the calculated dose volume exceeded 20 mL, the remaining volume was administered in the left lateral neck muscle.

Blood samples for firocoxib determination were collected from the sows and 3 piglets per litter (2 male and 1 female piglet at each time point) at 0, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h post-drug administration to the sow. For sow 16175 (0.5 mg/kg group) that had 5 male and 4 female piglets, 1 male and 2 females were bled at each time point, and for sow 16149 (1.5 mg/kg group) that had 6 female and 3 male piglets, 2 males and 1 female were bled at two of the time points, whereas 1 male and 2 females were bled at the third time point. No piglet was bled more frequently than any other piglet, and an individual piglet was bled no more than every third time point. The blood sample from each sow (8 mL per sample) was collected via the left or right jugular vein using a 25.4-mm, 16-gauge hypodermic needle (Air-Tite Products, Virginia Beach, VA) attached to a 12-mL Luer-Lok syringe (TycoHealth Care, Mansfield, MA). During blood collection, a pig snare was used for manual restraint of each sow in her crate.

The blood samples (2 mL per sample) from the piglets were collected from the left or right cranial vena cava using a 3.8-cm, 20-gauge hypodermic

Table 2. Study population information and doses of firocoxib administered to lactating sows via intramuscular injection at 0.5, 1.0, 1.5, or 2.0 mg/kg bodyweight

Sow treatment group	Sow ID	Parity	Weight, kg	No. of piglets/litter		Prewaning piglet deaths	Sow dose, mg	Dose volume (mL) administered to sows IM
				Male	Female			
0.5 mg/kg	1427	8	266	6	3	0	133	6.5
	1471	7	255	6	3	1	127.5	6.5
	1578	5	238	6	3	0	119	6.0
	16175	1	179	5	4	1	89.5	4.5
Total				23	13	2		
Mean		5.25	234.5				117.25	5.88
SEM		1.55	19.38				9.69	0.47
1.0 mg/kg	1351	9	211	6	3	0	211	10.5
	1380	9	292	6	3	1	292	14.5
	1448	8	258	6	3	2	258	13.0
	15177	4	241	6	3	0	241	12.0
Total				24	12	3		
Mean		7.5	250.5				250.5	12.5
SEM		1.19	16.90				16.90	0.84
1.5 mg/kg	1441	7	292	6	3	0	438	22.0
	1590	4	248	6	3	2	372	18.5
	14145	6	260	6	3	1	390	19.5
	16149	2	224	3	6	0	336	17.0
Total				21	15	3		
Mean		4.75	256				384	19.25
SEM		1.11	14.14				21.21	1.05
2.0 mg/kg	304	10	262	6	3	0	524	26.0
	1381	8	294	6	3	0	588	29.5
	14156	6	253	6	3	0	506	25.5
	16148	2	231	6	3	0	462	23.0
Total				24	12	0		
Mean		6.5	260				520	26
SEM		1.71	13.07				26.14	1.34

needle (TycoHealth Care, Mansfield, MA) attached to a 3-mL syringe (TycoHealth Care, Mansfield, MA). Physical restraint of the piglet was achieved by placing the animal in a supine position during sample collection. Blood samples were transferred to 6-mL evacuated tubes that contained lithium heparin (Vacuette plasma tubes, Greiner Bio-One, Monroe, NC) that were stored on ice for up to 2 h before processing. The blood samples were centrifuged for 10 min at $1,500 \times g$. The plasma was then removed, placed in cryovials, and frozen at -80°C until analysis.

Castration, teeth clipping, and tail docking were completed without anesthesia for each male piglet between the 6-h and 8-h blood collection time points. Gilts did not undergo any of these procedures and thus served as procedural controls as previously described (Kielly et al., 1999). Castration was performed using a number 10 scalpel blade to making 2 vertical incisions approximately 2 to 3 cm long in the skin covering the testicles. The testicles were then marsupialized, and manual pressure

was applied to the spermatic cord until it separated from the piglet's body. Side-cutter pliers were used to remove the tail at the sixth coccygeal vertebral body, and the canine teeth were filed flush with the gingival tissue.

On day 21 post-drug administration, the body weight of each sow and piglet was recorded before weaning to calculate average daily weight gain. In the absence of tissue residue data that could be used to establish a meat withhold period, each animal was humanely euthanized at the end of the study according to American Veterinary Medical Association guidelines by using a penetrating captive bolt followed by exsanguination in sows and blunt force trauma in the piglets (Leary et al., 2013). Necropsies were performed on all animals to inspect for macroscopic signs of NSAID toxicity. Samples of kidney, liver, small intestine, and stomach were sectioned into 0.5- to 1-cm slices and placed in plastic jars containing 10% buffered formalin in a 10:1 formalin:tissue ratio for histopathology examination. Approximately 200 g of muscle,

liver, kidney, fat, and injection site tissue were also collected from each sow for firocoxib residue determination. The liver, the 2 kidneys, and 50-g muscle and fat were collected from at least 3 piglets per litter (2 barrows and 1 female) for histological and tissue drug residue determination. The tissue samples were stored at -20°C until analysis.

Sample Collection, Processing, and Analysis

All plasma and tissue samples for firocoxib analysis were submitted to the Iowa State University-Pharmacology Analytical Support Team (ISU-PhAST) at the Iowa State University Veterinary Diagnostic Laboratory. All laboratory personnel were masked to treatment group.

Plasma Sample Preparation

Firocoxib analysis was conducted using a previously described method developed in bovine plasma that was adapted and validated for use in swine (Stock et al., 2014). Briefly, frozen samples or standards were thawed at room temperature and rigorously vortexed once completely thawed. Plasma samples, plasma spikes, plasma quality control samples, and blanks (100 μL) were then protein precipitated in 1.5-mL microcentrifuge tubes with 400 μL acetonitrile/0.1% formic acid. A d6-firocoxib internal standard was incorporated into the acetonitrile precipitating agent at a concentration of 200 ng/mL. The samples were vortexed for 5 s after addition of the acetonitrile and centrifuged for 20 min at $3,773 \times g$ to sediment the protein pellet. Following centrifugation, the supernatant was poured into cell culture tubes and evaporated to dryness in a Turbovap concentration evaporator at 48°C . The tube contents were reconstituted with 150 μL 25% acetonitrile and transferred to autosampler vials equipped with 300- μL glass inserts. The samples were centrifuged at $770 \times g$ before liquid chromatography–mass spectrometry (LC–MS) analysis.

Twelve calibration spikes were prepared in blank porcine plasma in the concentration range of 1 to 5,000 ng/mL for the samples from the sows. The samples from the piglets were analyzed using a narrower range of calibration spikes of 1 to 500 ng/mL. A linear (1/X) fit was used for the piglet plasma samples and the narrower 1 to 500 ng/mL concentration range. A quadratic (1/X) fit was used for the sow plasma samples and the 1 to 5,000 ng/mL concentration range.

Tissue Sample Preparation

Samples of muscle, injection site, kidney, liver, and fat tissue were analyzed for firocoxib and the desiccyclopropylmethyl metabolite. Eight calibration spikes were prepared in blank porcine tissue in the concentration range of 0.05 to 10 $\mu\text{g/g}$. The tissue samples were thawed and homogenized in a Waring blender before extraction and analysis. The tissue samples, tissue spikes, blanks, and 1-g tissue homogenate were extracted using 10 mL of a 4:1 mixture of acetonitrile:water in a 50-mL centrifuge tube. An internal standard (d6-firocoxib) of 25 μL of a 100 ng/ μL solution was added to the tissue homogenate before extraction. The solvent extraction was performed on a multitube vortex mixer for 15 min after the addition of the acetonitrile mixture. The extracted samples were then centrifuged for 5 min at $1,000 \times g$ and filtered through glass fiber filters into 15-mL centrifuge tubes. Finally, 1 mL of each extract was pipetted into cell culture tubes and evaporated to dryness at 48°C using a Turbovap concentration evaporator. The tube contents were reconstituted with 150 μL 25% acetonitrile and transferred to autosampler vials equipped with 300- μL glass inserts. The samples were centrifuged at $770 \times g$ before LC–MS analysis.

Firocoxib Plasma and Tissue Analyses

Plasma and tissue firocoxib concentrations were determined using high-pressure liquid chromatography (Agilent 1100 Pump, Column Compartment and Autosampler, Agilent Technologies, Santa Clara, CA) with mass spectrometry detection (LTQ Ion Trap, Thermo Scientific, San Jose, CA). A 25- μL injection volume was used for the LC–MS analysis. The mobile phases were A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile), at flow rates of 0.275 mL/min. The mobile phase began at 25% B with a linear gradient to 95% B in 5 min that was maintained for 1.25 min at 0.325 mL/min, followed by re-equilibration to 25% B. Separation was achieved with a HypersilGoldC18 column (100 mm $\times$ 2.1 mm, 3 μm particles, Thermo Scientific) maintained at 50°C . Firocoxib and d6-firocoxib each eluted at 4.9 min. Full scan MS was used for analyte detection, and 3 fragment ions were used for quantitation of each analyte species. The fragment ions for firocoxib were at 283, 265, and 237 m/z ; fragment ions at 289, 270, and 243 m/z were characteristic of d6-firocoxib fragmentation. The desiccyclopropylmethyl metabolite produced a single fragment ion at 209 m/z . Firocoxib and d6-firocoxib

were analyzed in positive-ion mode. The mass spectrometer was optimized for detection of firocoxib using infusion of a firocoxib solution (10 µg/mL) into the mobile phase of 80% B. Detection of firocoxib was enhanced with a transfer capillary temperature of 350 °C.

The blank (porcine plasma), calibration spike, QC, and porcine samples were batch processed for sequencing using a processing method developed in the Xcalibur software application (Thermo Scientific). The processing method automatically identified and integrated each peak in each sample and calculated the calibration curve based on a weighted (1/X) quadratic or linear fit. Firocoxib concentrations in unknown samples were calculated based on the relevant calibration curve using the Xcalibur software. Results were then viewed in the Quan Browser portion of the Xcalibur software.

For plasma samples, the calibration curves had correlation coefficients (R^2) exceeding 0.995 across the concentration range. The quality control (QC) samples at 7.5, 15, 35, 75, 150, and 1,500 ng/mL were within a tolerance of $\pm 15\%$ of the nominal value. The limit of quantitation of the analysis was 1.0 ng/mL, with a limit of detection of 0.2 ng/mL. For tissue samples, calibration curves had correlation coefficients (R^2) exceeding 0.99 across the concentration range. The limit of quantitation of the analysis for both firocoxib and the despropylmethyl metabolite was 0.05 µg/g; the limit of detection was 0.01 µg/g.

Cortisol Analysis

To accommodate blood volume restrictions, blood samples for cortisol determination were collected from 3 randomly selected piglets per litter (2 male and 1 female piglet at each time point) prior to processing (6 h after firocoxib administration to the sow) and after processing at approximately 8, 12, 24, 48, 72, 96 h after drug administration to the sow. These time points corresponded to approximately 0, 2, 6, 18, 42, 66, and 90 h after processing. The blood for cortisol analysis was collected in 3-mL heparinized blood collection tubes (BD Vacutainer, Franklin Lakes, NJ) and then centrifuged for 10 min at $1,500 \times g$. The plasma was collected, then immediately frozen and stored at -80°C . The analyses for plasma cortisol concentrations were performed within 60 d after sample collection and within 10 consecutive days once the analyses were started. Plasma cortisol concentrations were determined using a commercial radioimmunoassay kit (CortiCote I-125, MP Biomedicals, Santa Ana,

CA). The samples were incubated at 4°C for 2 h to improve assay sensitivity. The samples were processed in duplicates and the processing was repeated if the difference between paired samples in cortisol concentrations were more than 15%. The assay had a detection range of 0.64 to 150 ng/mL. The coefficient of variation for the intra-assay variability was 9.33%. The interassay variability was 10.58%.

Histopathology Examination

Formalin-fixed sections of kidney, liver, small intestine, and stomach from sows and 3 piglets per litter (12 piglets per treatment) were trimmed and positioned in cassettes loaded into an automated tissue processor (Sakura VIP 5, Sakura Finetek, Torrance, CA) for overnight paraffin infiltration. Processed tissues in cassettes were then placed in a paraffin bath (Sakura Tissue-Tek TEC 5, Sakura Finetek) after which they were removed from the cassette and oriented in molds. The paraffin-embedded tissues were then fully exposed through sectioning on a microtome (HM 355S Automatic Microtome, Thermo Fisher, Waltham, MA). Tissue sections were cut at 4 microns from the cooled blocks. Paraffin ribbons with tissue were then laid out on a water bath, and the floating tissue sections were collected onto microscope slides. The unstained tissue sections were then mounted on the slide and dried at 60°C for 20 min. Finally, the tissue was deparaffinized and rehydrated for staining by transfer through xylene and a series of decreasing concentrations of alcohol to hematoxylin on an automated stainer (Sakura Tissue-Tek Prisma, Sakura Finetek). After a tap water rinse, the tissue on the slide was counterstained with eosin, dehydrated in an alcohol series, cleared in xylene and cover slipped (Sakura Tissue-Tek Glas g2) prior to histological examination by a veterinary diagnostician with experience in the histological examination of swine tissues. The number of piglets selected for histological examination and the application of descriptive statistical methods to these data are consistent with the [International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products \(VICH\) GL 43 \(2009\)](#) that describes the target animal safety data required for registration of veterinary products.

Pharmacokinetic Analysis

The firocoxib plasma concentration vs. time profile from each sow from the treatment groups

injected with firocoxib at doses of 2.0, 1.5, 1.0, and 0.5 mg/kg was evaluated using pharmacokinetic analysis software (Phoenix Win-Nonlin 7.0, Certara, Inc. Princeton, NJ). The data were analyzed using noncompartmental methods implemented in the software with Model (Plasma 200 to 202) with uniform weighting. The pharmacokinetic parameters determined were as follows: elimination rate constant (λ_z , slope of the terminal phase), terminal half-life ($T_{1/2\lambda_z}$), maximum plasma concentration ($C_{\max}$), time to achieve peak concentration ($T_{\max}$), area under the concentration–time curve (AUC), area under the first moment of the concentration–time curve (AUMC), apparent volume of distribution during the terminal phase (V_z/F), apparent systemic clearance (CL/F), and mean residence time (MRT). The rate constant (λ_z) associated with the terminal phase was calculated using mean values and linear regression of the terminal part of the log plasma concentration vs. time curve. The linear trapezoidal linear interpolation method was used to determine the AUC and AUMC values. For the calculation of $AUC_{0\text{--last}}$ and $AUMC_{0\text{--last}}$, the time range from the first measurement to the last measurement of the drug concentrations, and the extrapolation to infinity ($AUC_{0\text{--}\infty}$, $AUMC_{0\text{--}\infty}$) were used. AUC and AUMC values were extrapolated to infinity to account for the total sow exposure to firocoxib.

For each treatment group, the plasma firocoxib concentrations vs. time data for the nursing piglets ($n = 36$ per treatment) were evaluated using noncompartment analysis and the sparse data option available in the software (Phoenix Win-Nonlin 7.0, Certara, Inc. Princeton, NJ). The pharmacokinetic parameters λ_z , $T_{1/2\lambda_z}$, $C_{\max}$, $T_{\max}$, AUC, AUMC, and MRT were estimated as described for the sows. The noncompartment analysis (NCA) sparse method calculates pharmacokinetic parameters based on the mean profile for all the subjects in the data set. Therefore, in this analysis, the SEM was calculated only for $T_{\max}$, $C_{\max}$, and $AUC_{0\text{--last}}$.

The relative transfer of firocoxib from medicated sows to piglets was evaluated by comparing the piglet plasma drug concentration as a percentage of the sow plasma drug concentration at each time point. Total piglet exposure to firocoxib via milk consumed from treated dams was evaluated by comparing the firocoxib AUC for the piglets with the drug AUC calculated for the corresponding dams from each treatment. Exposure percentages were determined using the equation:

$$\% \text{ exposure} = 100 \times \frac{AUC(\text{piglet})}{AUC(\text{sow})}$$

Statistical Analysis

Data were entered into a commercial software program for analysis (Microsoft Excel, Redmond, WA). Firocoxib pharmacokinetic parameters were not normally distributed. Therefore, the pharmacokinetic outcomes were compared statistically using Wilcoxon Rank Sum tests (JMP Pro. 13.0, SAS Institute, Cary, NC). Initial analysis examined the impact of piglet gender on plasma firocoxib concentration to determine whether data from male and female piglets could be pooled for the subsequent pharmacokinetic analysis. Thereafter, pharmacokinetic outcomes were compared using nonparametric methods. Dose linearity was investigated by calculating the square of the Pearson correlation coefficient (R^2) for linear regression. Statistical significance for all pharmacokinetic outcomes was set a priori at $P < 0.05$.

Plasma cortisol concentrations and ADG were analyzed using a generalized linear mixed model incorporating both fixed effects and random effects (PROC GLMMIX; SAS university edition v9.04.01, SAS Institute, Cary, NC). The population cortisol concentrations over time best fit a lognormal model. Time, treatment, and their interaction were designated as fixed effects in the model with piglet nested in sow designated as a random effect. The effect of gender across treatments was examined using ANOVA to confirm that data from female piglets could be pooled to serve as a procedural control. Sow parity was also included as a covariate in the model. Where there was evidence of a treatment $\times$ time interaction ($P < 0.1$), simple effect comparisons of least squares means were conducted using the Tukey–Kramer adjustment for multiple comparisons. For ADG, treatment was considered a fixed effect in the statistical model. ADG outcomes best fit a Gaussian distribution. The effect of gender across treatments was also examined by ANOVA to clarify the effect of drug exposure from the effect of processing, and sow parity was included as a covariate in the model. For both outcomes, statistical significance was set a priori at $P < 0.05$.

To account for the fact that not all the piglets were blood sampled at every time point due to blood volume restrictions in piglets, individual piglet peak cortisol concentrations (**CortCmax**) were calculated for each piglet after processing by visual inspection of the data. These data were also compared statistically using the same generalized linear mixed model (PROC GLMMIX; SAS university edition v9.04.01, SAS Institute, Cary, NC).

as previously described. Statistical significance of the C_{ortCmax} data was set a priori at $P < 0.05$.

RESULTS

Firocoxib Pharmacokinetics in Sows

The mean $\pm$ SEM plasma firocoxib concentrations in the sows from the 4 treatment groups that received a single IM dose of firocoxib at 0.5, 1.0, 1.5, or 2.0 mg/kg are presented in Fig. 1. The pharmacokinetic parameters for firocoxib in sows following IM administration are presented in Table 3. Mean $\pm$ SEM firocoxib C_{max} values of 107.90 ± 15.18 , 157.50 ± 24.91 , 343.68 ± 78.89 , and 452.83 ± 90.27 ng/mL were recorded at 3.5, 5.5, 3.0, and 4.5 h, respectively, in sows receiving 0.5, 1.0, 1.5, or 2.0 mg/kg firocoxib. Mean C_{max} was higher in sows that received 2.0 mg/kg compared with sows that received 1.0 and 0.5 mg/kg ($P = 0.03$). Mean AUC was also significantly lower in sows that received 0.5 mg/kg compared with sows that received 1.0, 1.5, and 2.0 mg/kg ($P = 0.0304$) respectively. Firocoxib demonstrated a prolonged plasma elimination half-life ($T_{1/2\lambda_z}$) ranging from 26.71 ± 5.77 h to 31.09 ± 6.73 h.

Dose linearity was investigated by plotting the C_{max} (Fig. 2A) and $\text{AUC}_{0-\text{last}}$ (Fig. 2B) values against the administered doses. The results suggest that for both C_{max} ($R^2 = 0.60$) and $\text{AUC}_{0-\text{last}}$ ($R^2 = 0.55$) the response was linear across the 4 doses that were investigated.

Figure 1. Mean ($\pm$ SEM) plasma firocoxib concentrations (ng/mL) for lactating sows administered firocoxib via the intramuscular route at 0.5, 1.0, 1.5, or 2.0 mg/kg.

Firocoxib Pharmacokinetics in Piglets after Transmammary Delivery

There was no effect of gender on plasma firocoxib concentrations over time ($P = 0.38$); therefore, data from barrows and gilts were combined for subsequent pharmacokinetic analysis. The results for the plasma firocoxib concentrations (mean $\pm$ SEM) in the piglets after the sows were injected with firocoxib (0.5, 1.0, 1.5, or 2.0 mg/kg) are presented in Fig. 3. The results for the pharmacokinetic parameters following transmammary delivery to the piglets are presented in Table 4.

The plasma firocoxib concentration vs. time profiles for the 4 groups of piglets were similar except that there was a more rapid decline in drug concentrations after C_{max} in the piglets that nursed sows that received a 0.5 mg/kg dose. Mean peak plasma concentrations (C_{max}) of 9.53, 31.04, 53.30, and 44.03 ng/mL were found at 24 h after 0.5, 1.0, 1.5, or 2.0 mg/kg firocoxib administration to the sows, respectively. Mean C_{max} was lower in piglets nursing sows that received 0.5 mg/kg compared with piglets from sows that received 1.0 mg/kg ($P = 0.0012$), 1.5 mg/kg ($P < 0.0001$), and 2.0 mg/kg ($P < 0.0001$). Furthermore, C_{max} was significantly higher in piglets from sows that received 1.5 mg/kg compared with piglets from sows that received 1.0 mg/kg ($P = 0.0488$). Mean AUC was also significantly lower in piglets from sows that received 0.5 mg/kg compared with piglets from sows that received 1.0 mg/kg ($P = 0.0003$), 1.5 mg/kg ($P < 0.0001$), and 2.0 mg/kg ($P < 0.0001$), respectively. Firocoxib had a prolonged plasma $T_{1/2\lambda_z}$ between 30.86 and 48.71 h in the piglets after transmammary delivery.

Dose linearity was investigated by plotting the C_{max} (Fig. 2C) and $\text{AUC}_{0-\text{last}}$ (Fig. 2D) values against the administered doses. The results suggest that for both C_{max} ($R^2 = 0.23$) and $\text{AUC}_{0-\text{last}}$ ($R^2 = 0.21$), dose linearity was absent in piglets across the 4 doses that were investigated.

Transmammary Delivery of Firocoxib

The results for the firocoxib concentrations in piglet plasma as a percentage of the plasma firocoxib concentrations in the medicated sows at each time point are presented in Fig. 4. There was evidence of a significant effect of treatment ($P = 0.0005$), time ($P < 0.0001$), and a time $\times$ treatment interaction ($P < 0.0001$). Specifically, at 48 h post-injection, the concentrations of firocoxib in the piglets as percentages of the concentrations in the sows were significantly greater for the 0.5 and 1.5

Table 3. Summary of pharmacokinetic parameters (mean and SEM) for lactating sows ($n = 4/\text{treatment}$) administered firocoxib via intramuscular injection at 0.5, 1.0, 1.5, or 2.0 mg/kg

Dose		0.5 mg/kg		1.0 mg/kg		1.5 mg/kg		2.0 mg/kg	
Parameter ¹	Units	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
λ_z	1/h	0.03	0.006	0.03	0.002	0.02	0.003	0.02	0.002
$T_{1/2\lambda z}$	h	28.87	6.92	26.70	2.88	29.89	4.84	31.09	3.36
$T_{\max}$	h	3.50	0.96	5.50	0.96	3.00	0.58	4.50	1.26
$C_{\max}$	ng/mL	107.90 ^b	15.18	157.50 ^b	24.91	343.68 ^{ab}	78.89	452.83 ^a	90.27
CL/F	L/h/kg	0.33	0.04	0.21	0.05	0.19	0.03	0.18	0.04
AUC _{0-24h}	h × ng/mL	1,030 ^c	62	2,673 ^b	0.486	4,841 ^{ab}	961	6,639 ^a	1,421
AUC _{0-last}	h × ng/mL	1,534 ^b	182	5,331 ^a	1.357	8,323 ^a	1,814	12,721 ^a	3,548
AUC _{INF}	h × ng/mL	1,586 ^b	184	5,624 ^a	1.534	8,656 ^a	1,760	13,651 ^a	4,045
AUC_Exp	%	3.39	0.66	4.15	1.50	4.98	2.92	5.61	1.83
AUMC	h ² × ng/mL	31,765 ^b	8,530	164,892 ^a	58,409	215,365 ^a	56,451	392,753 ^a	142,492
MRT _{0-last}	h	27.16	7.64	29.38	2.89	26.35	3.66	29.07	2.86
MRT _{INF}	h	29.36	9.93	34.80	4.87	33.48	7.73	36.80	5.44
V_z/F	L/kg	13.82	4.25	7.82	1.44	9.12	3.24	7.75	1.55

^{a-c}Columns with different superscripts are significantly different ($P < 0.05$).

¹ λ_z = slope of the terminal phase; $T_{1/2\lambda z}$ = terminal half-life; $T_{\max}$ = time to achieve peak concentration; $C_{\max}$ = maximum plasma concentration; CL/F = apparent systemic clearance; AUC_{0-24h} = area under the concentration–time curve for the first 24 h after administration AUC_{0-last} = area under the curve to the last measured time point; AUC_{INF} = area under the curve extrapolated to infinity; AUC_Exp = % of the AUC extrapolated to infinity; AUMC = area under the first moment of the concentration–time curve; MRT_{0-last} = mean residence time to the last time point; MRT_{INF} = mean residence time extrapolated to infinity; V_z/F = apparent volume of distribution during the terminal phase.

Figure 2. Comparison of the dose response between mean ($\pm$ SEM) peak plasma firocoxib concentrations ($C_{\max}$) in sows (A); area under the plasma concentration vs. time curve (AUC) in sows (B); peak plasma firocoxib concentrations ($C_{\max}$) in nursing piglets (C); and area under the plasma concentration vs. time curve (AUC) in nursing piglets (D) after administration of firocoxib via the intramuscular route to lactating sows at 0.5, 1.0, 1.5, or 2.0 mg/kg (a–b: $P < 0.05$).

mg/kg groups compared with the other 2 groups ($P \leq 0.05$). After 48 h, there was a plateau in the piglets who nursed on the sows that received the 2.0

mg/kg dose. At 120 h after drug administration, the concentrations in the piglet plasma samples as a percentage of the concentrations in the sow plasma

samples were significantly greater in the 1.0 and 1.5 mg/kg groups, compared with the 0.5 and 2.0 mg/kg groups.

Comparison of the AUC for firocoxib in sows with the AUC for firocoxib in piglets is presented in Table 5. The total drug exposure following transmammary delivery of firocoxib from medicated sows to piglets across the 4 treatment groups ranged from 25.32% in piglets from sows that received 2.0 mg/kg to 46.83% in piglets from sows treated with 1.5 mg/kg.

Figure 3. Mean ($\pm$ SEM) plasma firocoxib concentration (ng/mL) for piglets nursing lactating sows administered firocoxib at 0.5, 1.0, 1.5, or 2.0 mg/kg via intramuscular injection ($n = 12$ piglets/treatment/time point).

Plasma Cortisol Concentrations

The plasma cortisol concentrations in the piglets were determined approximately 6 h after firocoxib was administered to the sows (Fig. 5). This time point occurred immediately before castration, tail docking, and teeth clipping was performed in barrows and was designated as T_0 relative to processing. Tail docking and teeth clipping were not performed on the gilts. Therefore, cortisol concentrations from gilts across treatment groups were not significantly different ($P = 0.36$). Accordingly, these data were pooled across the 4 treatment groups to comprise a procedural control group.

The subsequent statistical analysis indicated that there were effects of treatment group ($P = 0.0003$), time ($P < 0.0001$), and a time $\times$ treatment interaction ($P < 0.0001$) on plasma cortisol concentrations after castration, tail docking, and teeth clipping.

Prior to processing (T_0), there was no difference in cortisol concentration between treatment groups. However, at 2 ± 1 h after processing, plasma cortisol concentrations in processed male piglets was higher than female, procedural control, piglets ($P < 0.0002$). Furthermore, at 2 ± 1 h after processing, barrows nursing sows that received 2.0 mg/kg firocoxib had lower mean plasma cortisol concentration compared with barrows nursing sows that received 0.5 mg/kg ($P = 0.0397$) and 1.0 mg/kg ($P = 0.0416$) firocoxib. At 6 ± 1 h after processing, higher plasma cortisol concentrations were recorded in barrows from sows treated with 0.5 mg/

Table 4. Summary of pharmacokinetic parameters (mean $\pm$ SEM)¹ for piglets ($n = 36$ /treatment) nursing lactating sows administered firocoxib via intramuscular injection at 0.5, 1.0, 1.5, or 2.0 mg/kg bodyweight

Sow dose		0.5 mg/kg		1.0 mg/kg		1.5 mg/kg		2.0 mg/kg	
Parameter ²	Units	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
λ_z	1/h	0.02		0.01		0.02		0.02	
$T_{1/2\lambda_z}$	h	30.86		48.71		37.91		32.42	
T_{max}	h	24.00	12.56	24.00	14.83	24.00	14.17	24.00	12.21
C_{max}	ng/mL	9.53 ^a	1.21	31.04 ^b	6.79	53.30 ^c	11.10	44.03 ^{bc}	7.47
AUC_{0-24h}	h $\times$ ng/mL	175.98		406.67		662.37		608.69	
AUC_{0-last}	h $\times$ ng/mL	635.36 ^a	55.82	2,468.00 ^b	315.10	3,897.55 ^b	459.80	3,220.90 ^b	279.30
AUC_{INF}	h $\times$ ng/mL	690.47		3,178.00		4,615.17		3,652.32	
AUC_{Exp}	%	0.08		0.22		0.16		0.12	
AUMC	h ² $\times$ ng/mL	28,433.00		136,805.00		204,246.00		166,016.00	
MRT_{0-last}	h	44.76		55.42		52.41		51.54	

^{a-c}Columns with different superscripts are significantly different at $P < 0.05$.

¹The noncompartment analysis sparse method was used to calculate pharmacokinetic parameters based on the mean profile for all the subjects in the data set. Therefore, in this analysis, SEM was only calculated for T_{max} , C_{max} , and AUC_{0-last} .

² λ_z = slope of the terminal phase; $T_{1/2\lambda_z}$ = terminal half-life; T_{max} = time to achieve peak concentration; C_{max} = maximum plasma concentration; AUC_{0-24h} = area under the concentration–time curve for the first 24 h after administration; AUC_{0-last} = area under the curve to the last measured time point; AUC_{INF} = area under the curve extrapolated to infinity; AUC_{Exp} = % of the AUC extrapolated to infinity; AUMC = area under the first moment of the concentration–time curve; MRT_{0-last} = mean residence time to the last time point.

kg ($P = 0.0017$), 1.0 mg/kg ($P = 0.0078$), and 1.5 mg/kg ($P = 0.0597$) compared with gilts that served as procedural controls.

Analysis of the CortCmax data revealed a significant treatment effect ($P < 0.0001$; Fig. 6). Specifically, maximum cortisol concentrations were greater in individual barrows from sows in the 0.5 mg/kg firocoxib treatment group compared with barrows from sows that received 2.0 mg/kg firocoxib ($P = 0.014$). Similarly, CortCmax concentrations were higher in barrows from sows administered 1.5 mg/kg firocoxib compared with barrows from sows that received 2.0 mg/kg firocoxib ($P = 0.05$). Barrows across all treatment groups had significantly greater CortCmax concentrations compared with gilts that served as procedural controls ($P < 0.044$).

Piglet ADG

Eight piglets died between processing and weaning (Table 6). Average daily gain in body

Figure 4. Comparison of the plasma firocoxib concentration of nursing piglets as a percentage of the plasma firocoxib concentration in lactating sows administered firocoxib via the intramuscular route at 0.5, 1.0, 1.5, or 2.0 mg/kg (a–b: $P < 0.05$).

weight of the surviving piglets over the 21 d from processing to weaning was calculated by subtracting the body weight at processing from the body weight at weaning and dividing the result by the days from processing to weaning (Table 7 and Fig. 7). Average daily gain between barrows and gilts was not significantly different ($P = 0.53$); therefore, these data were pooled for analysis. Sow parity had a significant effect on ADG ($P = 0.0478$) and was therefore retained as a covariate in the model. The results suggest that there was an effect of treatment on ADG over the 21 d from processing to weaning ($P = 0.0157$). Specifically, ADG increased with the increasing doses of firocoxib administered to the lactating sows. Piglets that consumed milk from sows that received 2.0 mg/kg firocoxib at 6 h before processing gained more weight than piglets that consumed milk from sows that received 0.5 mg/kg ($P = 0.0076$) or 1.0 mg/kg ($P = 0.0047$) firocoxib.

Histopathology Examination of Tissues

No macroscopic lesions were evident on postmortem examination of the kidney, liver, stomach, and small intestines at 21 d after processing. Upon histological examination, all sections of liver and small intestine from lactating sows and nursing piglets across all 4 treatment groups were within normal limits. Tubular ectasia, which is considered a congenital anomaly in swine, was observed in 5 sow and 10 piglet kidneys (Table 8). These findings were not associated with higher firocoxib doses. Mild gastritis was observed in 19 sections of the stomach lining of the piglets, but this finding is considered to be not specific for a singular etiology. No macroscopic or histological evidence of NSAID intoxication was observed in any planes of the sections of sow and piglet tissues examined.

Firocoxib and Descyclopropylmethyl Metabolite Concentrations in Tissues

At 21 d after IM administration, no detectable concentrations of firocoxib or its

Table 5. Total firocoxib exposure as a percentage in piglets nursing lactating sows administered firocoxib via the intramuscular route at 0.5, 1.0, 1.5, or 2.0 mg/kg

Sow dose	Parameter ¹	Sow, h × ng/mL	Piglet, h × ng/mL	Piglet exposure, %
0.5 mg/kg	AUC _{0-last}	1,534.00	635.36	41.42
1.0 mg/kg	AUC _{0-last}	5,332.00	2,468.00	46.29
1.5 mg/kg	AUC _{0-last}	8,323.00	3,897.50	46.83
2.0 mg/kg	AUC _{0-last}	12,722.00	3,220.90	25.32

¹AUC_{0-last} = area under the curve to the last measured time point.

desacyclopropylmethyl metabolite were found in concentrations above the limit of quantitation (0.05 µg/g) in any of the muscle, liver, kidney, fat, or

injection site tissue samples in sows. Similarly, there were no detectable concentrations of firocoxib in the tissues harvested from the sampled piglets at weaning.

DISCUSSION

To the best of our knowledge, this is the first report examining the pharmacokinetics and effectiveness of firocoxib in swine. NSAIDs are the most commonly administered class of analgesic drugs in swine production systems in the United States due to their effectiveness, availability, and relatively low cost. However, there are currently no analgesic drugs that have FDA-approved label indications for pain relief in pigs. Consumer concern about the welfare of farm animals experiencing pain during routine management procedures has increased efforts to develop effective, safe, and practical analgesic protocols for use in piglets (Sutherland, 2015). Specifically, the “European Declaration on alternatives to surgical castration of pigs” required that from 1 January 2012, surgical castration of pigs would only be performed with prolonged analgesia and/or anesthesia in piglets over 7 d of age in all EU countries with the intent of phasing out the procedure by 2018 (European Commission, 2008). However, a 2015 survey of swine producers in 24 European countries found that only 5% of piglets received both anesthesia and analgesia and 41% of piglets received only analgesia at the time of surgical castration (De Briyne et al., 2016). In over 50% of the countries surveyed, 1) increased production costs; 2) the need for additional labor; and 3) the lack of practical and effective analgesic/anesthetic protocols were identified as the primary factors that reduced compliance with the EU Declaration. The results of the present study suggest that a single injection of firocoxib administered to sows resulted in successful transmammary delivery of analgesia to nursing piglets prior to processing. This finding could potentially address many of the current impediments to routine analgesic drug use in piglets at

Figure 5. Mean ($\pm$ SEM) plasma cortisol concentrations (ng/mL) following castration, tail docking, and teeth clipping in barrows nursing lactating sows administered firocoxib at 0.5, 1.0, 1.5, or 2.0 mg/kg via intramuscular injection ($n = 8$ /time point/treatment). Gilts served as procedure controls and were not subject to processing (teeth clipping or tail docking; $n = 4$ time point/treatment; a-c: $P < 0.05$).

Figure 6. Dose-response curve of mean ($\pm$ SEM) peak plasma cortisol concentrations from individual piglets (CortCmax) after castration, tail docking, and teeth clipping of barrows nursing sows administered firocoxib at 0.5, 1.0, 1.5, or 2.0 mg/kg via intramuscular injection. Gilts served as procedure controls and were not subject to processing (teeth clipping or tail docking; a-c: $P < 0.05$).

Table 6. Summary of age, treatment group, and cause of death of the piglets that died between processing (T_0) and weaning (day 21)

Piglet ID	Sow of origin	Sow treatment group	Piglet age	Cause of death
22	1590	1.5 mg/kg	4 d	Post-bleeding stress
10	16175	0.5 mg/kg	7 d	Colibacillosis
23	1590	1.5 mg/kg	7 d	Colibacillosis
50	14145	1.5 mg/kg	9 d	Trauma
76	1380	1.0 mg/kg	12 d	Trauma
119	1448	1.0 mg/kg	13 d	Failure to thrive
122	1448	1.0 mg/kg	19 d	Colibacillosis
114	1471	0.5 mg/kg	23 d	Trauma

Table 7. Mean ($\pm$ SEM) piglet bodyweights at T_0 and differences in mean average daily weight gain (ADG) (g) at 21 d after castration, tail docking, and teeth clipping of barrows nursing sows administered firocoxib at 0.5, 1.0, 1.5, or 2.0 mg/kg via intramuscular injection¹

Piglet weight for each treatment group				
Treatment group	Mean $\pm$ SEM initial weight, kg (no. of piglets)	Mean $\pm$ SEM final weight, kg (no. of piglets)		
Gilts	2.15 $\pm$ 0.08 (52)	6.98 $\pm$ 0.21 (51)		
0.5 mg/kg	2.22 $\pm$ 0.14 (23)	6.65 $\pm$ 0.34 (22)		
1 mg/kg	2.11 $\pm$ 0.08 (24)	6.44 $\pm$ 0.33 (21)		
1.5 mg/kg	1.80 $\pm$ 0.12 (21)	6.69 $\pm$ 0.40 (18)		
2.0 mg/kg	2.45 $\pm$ 0.43 (24)	7.80 $\pm$ 0.96 (24)		
Differences of treatment group least squares means				
Treatment		ADG		
Treatment group	Treatment group	Mean difference, g	SEM	P-value
0.5 mg/kg	1.0 mg/kg	2.56	14.54	0.860
0.5 mg/kg	1.5 mg/kg	−17.98	14.29	0.211
0.5 mg/kg	2.0 mg/kg	−39.01	14.37	0.008
1.0 mg/kg	1.5 mg/kg	−20.54	14.35	0.155
1.0 mg/kg	2.0 mg/kg	−41.57	14.43	0.005
1.5 mg/kg	2.0 mg/kg	−21.03	14.18	0.141

¹Gilts served as procedure controls and were not subject to processing (teeth clipping or tail docking). Treatment was designated as a fixed effect in the model. Sow parity had a significant effect on ADG ($P = 0.0478$) and was therefore retained as a covariate in the model. ADG between barrows and gilts was not significantly different ($P = 0.53$); therefore, these data were pooled for analysis.

Figure 7. Dose-response curve of mean ($\pm$ SEM) ADG (g) at 21 d after castration, tail docking, and teeth clipping of barrows nursing sows administered firocoxib at 0.5 mg/kg ($n = 22$), 1.0 mg/kg ($n = 21$), 1.5 mg/kg ($n = 18$), or 2.0 mg/kg ($n = 24$) via intramuscular injection. Gilts ($n = 51$) served as procedure controls and were not subject to processing (teeth clipping or tail docking; a–b: $P < 0.05$).

the time of processing by reducing labor costs and improving piglet welfare through reduced stress. Furthermore, the cost of analgesia may be offset by enhanced production through increases in piglet weaning weights.

Plasma elimination half-life is the pharmacokinetic parameter that describes the time taken for the plasma drug concentrations to decrease by half. The long plasma elimination half-life of firocoxib reported in sows in the present study (26.7 to 31.1 h) was similar to the results obtained in horses

(29.6 to 31.1 h) and calves (31.8 h; Kvaternick et al., 2007; Stock et al., 2014; Holland et al., 2015). A long terminal half-life is desirable from a clinical perspective because this may result in a longer duration of analgesia following a single dose that could reduce dosing frequency. In contrast, a short half-life of 5.9 ± 1.1 h has been reported for firocoxib in dogs and 5.75 h in camels in previous studies (McCann et al., 2004; Wasfi et al., 2015). In comparison to other commonly used NSAIDs in pigs, the elimination half-life of firocoxib in sows was approximately 10-fold longer than ketoprofen (3 h; Raekallio et al., 2008), 5-fold longer than meloxicam (6 h; Pairis-Garcia et al., 2015), and 4-fold longer than flunixin (7.5 h; Pairis-Garcia et al., 2013). These data support the hypothesis that firocoxib is a suitable analgesic for single dose administration in swine resulting in reduced labor costs and stress associated with frequent injections.

Volume of distribution is the pharmacokinetic measurement that describes the tendency of a drug to move from the blood into the tissues. A large volume of distribution (7.75 to 13.8 L/kg) for firocoxib in sows in the present study was similar to calves (6.54 L/kg; Stock et al., 2014) but greater than previously reported results in camels (2.34 L/kg; Wasfi et al., 2015), horses (1.81 L/kg; Holland et al., 2015), and dogs (2.9 L/kg; McCann et al., 2004). A large volume of distribution is associated

Table 8. Histopathological findings in nursing piglets ($n = 48$) at 21 d after intramuscular administration of firocoxib to lactating sows at 0.5, 1.0, 1.5, or 2.0 mg/kg

Tissue	Histopathology findings	Treatment groups			
		0.5 mg/kg	1.0 mg/kg	1.5 mg/kg	2.0 mg/kg
Liver	Within normal limits	12	12	12	12
Small intestine	Within normal limits	12	12	12	12
Kidney	Within normal limits	1	2	0	3
	Rare tubular ectasia	1	1	0	0
	Mild tubular ectasia	5	9	9	5
	Moderate ectasia	0	0	0	1
	Prominent ectasia	5	0	3	3
	Within normal limits	5	4	3	9
Stomach	Mild vasculitis, muscle layer	2	2	3	1
	Mild gastritis	5	6	6	2

with high lipophilicity leading to greater distribution of a drug to tissues and body fluids. This property of firocoxib has been previously demonstrated in a radioresidue study that reported penetration of up to 30% of plasma concentrations of firocoxib into equine synovial fluid (Kvaternick et al., 2007). In comparison to other commonly used NSAIDs in pigs, the volume of distribution of firocoxib in sows was approximately 26-fold larger than flunixin (0.30 L/kg; Pairis-Garcia et al., 2013), 22-fold greater than ketoprofen (0.35 L/kg; Raekallio et al., 2008), and 18-fold greater than meloxicam (0.42 L/kg; Pairis-Garcia et al., 2015). These data suggest that firocoxib could be expected to have a greater tendency to distribute into the mammary gland and milk compared with other NSAIDs that demonstrate a smaller volume of distribution.

Transmammary transfer of NSAIDs has been demonstrated in lactating females of other mammalian species (Jacqz-Aigrain et al., 2007; Malreddy et al., 2013). Previously, our group described the successful transmammary delivery of the NSAID, meloxicam, from medicated sows to 5-d-old piglets before processing (Bates et al., 2014). Piglets nursing sows that received oral meloxicam at 30 mg/kg for 3 consecutive days demonstrated a significant reduction in plasma cortisol concentrations over 10 h after processing compared with piglets nursing unmedicated sows. The results of the present study advance our understanding of transmammary delivery of analgesic compounds to manage pain in the offspring by demonstrating that this can be accomplished with a single injection using a dose volume that is attainable in a swine production environment.

The results of the pharmacokinetic analysis of the plasma firocoxib concentrations in the piglets indicate that the passage of firocoxib from the sow plasma into the milk was not linear. This suggests

that transport across the blood-milk barrier may be a saturable process. Therefore, an increase in the sow dose above 1.5 mg/kg may not result in higher firocoxib concentrations in the milk. Furthermore, the AUC values represented total firocoxib exposure over time. Expressing the AUC values for firocoxib in piglets as a percentage of the AUC for firocoxib in sows is an alternative approach to investigation of the extent of the transmammary delivery of firocoxib from sows to piglets. Based on the AUC values calculated from 0 h to the last time point, piglets nursing on sows administered 0.5 to 1.5 mg/kg firocoxib as a single IM injection received between 41% and 46% of the total sow firocoxib exposure. In contrast, the piglets nursing on sows that received 2.0 mg/kg firocoxib were exposed to 25% of the sow exposure. These results suggest that firocoxib doses above 2 mg/kg IM will probably not be associated with a proportional increase in drug transfer to nursing piglets.

Piglet behavior after castration, tail docking, and teeth clipping were not assessed in the present study due to the likelihood that the frequency of animal handling for blood sample collection after processing would have had a negative impact on the expression of pain-related behaviors in the barrows thus confounding the experiment. The results of this study suggest that a dose of 2 mg firocoxib/kg reduced stress and improved the average daily gain in barrows. Therefore, this would be a candidate dose for a subsequent experiment focused on assessing behavior in medicated piglets after processing.

Increased plasma cortisol concentrations are associated with stressful events such as those performed during processing. Specifically, assessment of the stress response using cortisol has been used as a proxy for measuring pain in livestock (Carroll et al., 2006). However, an increase in plasma cortisol is

not specific to any type of physical or mental stress. Routine animal handling procedures have been found to increase plasma cortisol concentrations in piglets (Moya et al., 2008). However, a study comparing plasma cortisol concentrations of surgically castrated animals to sham-castrated animals found that animals that did not experience castration pain had lower peak cortisol concentrations and returned to baseline concentrations faster than surgically castrated animals (Prunier et al., 2005). Persistent elevated plasma cortisol concentrations in the surgically castrated group could be a result of tissue damage or procedural pain (Tenbergen et al., 2014a). Until a pain-specific biomarker is identified and validated, the use of cortisol (with its limitations) as proxy measure for assessing pain in livestock will remain widespread in studies assessing the impact of production procedures and analgesic drugs on animal welfare.

In the present study, plasma cortisol concentrations reached a peak at approximately 30 to 60 min after the processing procedures in the piglets sampled at that time point. The time to peak plasma cortisol concentration and the magnitude of the response following processing procedures reported herein was similar to that reported in other studies (Prunier et al., 2005; Carroll et al., 2006; Marchant-Forde et al., 2009; Reiner et al., 2012; Tenbergen et al., 2014a). Furthermore, the results of the present study suggest that plasma cortisol concentrations in male piglets nursing sows that received the higher doses of firocoxib (1.5 or 2.0 mg/kg IM), at 6 to 8 h before processing, were lower compared with plasma cortisol concentrations in piglets nursing sows that received lower doses of firocoxib (0.5 mg/kg IM and 1.0 mg/kg IM). To account for the fact that not all piglets were sampled at this time point CortCmax concentrations were compared. These further support the conclusion that piglets from sows that received 2.0 mg/kg of firocoxib tended to have a lower observed peak cortisol concentration. However, the observation of a dose-dependent reduction in peak cortisol concentrations was less conclusive in this analysis because the actual $C_{\max}$ may have occurred before or after the sparse sampling time point. To the best of our knowledge, this is the first published report demonstrating that NSAID administration reduces plasma cortisol concentrations after processing in a dose-dependent manner. This finding supports the use of plasma cortisol as a surrogate biomarker of pain in dose-titration studies in swine.

Several studies have reported that NSAIDs, including meloxicam, ketoprofen, and flunixin,

reduce plasma cortisol concentrations when administered prior to processing (Reiner et al., 2012; Schwab et al., 2012; Bates et al., 2014; Tenbergen et al., 2014a). Specifically, a recent meta-analysis of 14 studies involving 634 animals found that mean cortisol concentrations within 60 min of castration in piglets were 93.59 units lower (range: 48.74 to 138.44 units lower) in piglets receiving an NSAID compared with control animals (O'Connor et al., 2014). Although it is recognized that NSAIDs do not mitigate the acute, incisional pain associated with castration, these results suggest that transmammary delivery of firocoxib administered to sows at 1.5 and 2.0 mg/kg reduces cortisol and therefore processing stress in piglets.

A potential criticism of the present study was to use of gilts as a procedural control for the castration and tail docking procedures. Data describing a sex difference in the stress response in young piglets are deficient in the published literature. However, Zupan and Zanella (2017) reported that cortisol response was similar between barrows and gilts exposed to various stressors including a human test, transportation, a novel object test, and a novel arena test ($P > 0.10$). Based on these data, it would be reasonable to assume that response to stress between 6-d-old barrows and gilts would be similar, thus justifying their use as procedural controls. Furthermore, based on salivary cortisol concentrations, Gallagher et al. (2002) observed that circadian rhythms only became established after day 6 in piglets. This suggests that circadian fluctuations in plasma cortisol would be expected to have a minimal impact on the results of the present study.

Previous studies examining the impact of meloxicam or ketoprofen administered immediately before castration on growth rates in piglets found no effects of NSAID administration on piglet average daily gain (Hansson et al., 2011; Kluivers-Poodt et al., 2012; Cassar et al., 2014; Tenbergen et al., 2014a; Bonastre et al., 2016). However, these studies focused on the administration of the NSAID individually to each piglet at the time of processing. Therefore, one explanation for the beneficial effect of transmammary-delivered firocoxib on piglet performance reported herein was that the NSAID had a positive effect on material milk production or sow welfare. This hypothesis is supported by the observation that ADG increased in both barrows and gilts in the present study regardless of processing status.

Specifically, parturition is associated with weight loss, reduced feed intake, and an increase in stress, acute phase proteins, and pain-related behaviors in

sows (Mainau and Manteca, 2011). The negative impacts of parturition on sows may be mitigated by postpartum administration of an NSAID resulting in reduced weight loss, reduced lying times, and improved growth rates in piglets (Mainau et al., 2012; Tenbergen et al., 2014b; Viitasaari et al., 2014). Furthermore, oral meloxicam administration to sows at the start of farrowing has been found to increase piglet serum IgG concentrations, weaning weights, and average daily gain (Mainau et al., 2016). Recently, several investigators have reported that NSAID therapy immediately after calving resulted in a significant increase in milk production and milk fat and protein composition over the course of lactation (Farney et al., 2013; Carpenter et al., 2016; Shock et al., 2018; Swartz et al., 2018). Taken together, these findings support the hypothesis that the dose-dependent increase in ADG observed in the present study may have resulted from the beneficial effects of the NSAID, firocoxib, on postpartum physiology and behavior in the sows. Further large-scale studies focusing on changes in feed intake, bodyweight, and milk composition of sows medicated with firocoxib are needed to elucidate whether the NSAID improves the welfare of the sows in addition to impacting the welfare of the nursing piglets.

NSAID toxicity causes renal papillary necrosis and gastric ulceration, which is considered a pathognomonic lesion for this condition (Black, 1986). No evidence of NSAID toxicity was found on postmortem examination of the kidney, liver, stomach, and small intestines of the sows and piglets enrolled in the present study at 21 d after treatment. Ecstatic tubules are considered a congenital anomaly in pigs (Wells et al., 1980; Jansen and Nordstoga, 1992). Gastric changes observed in the present study were considered mild and not specific for a singular etiology. Interstitial nephritis lesions in sow kidneys are considered an incidental finding (Kongsted and Sorensen, 2017). None of the histological changes that were reported were over-represented in any of the 4 treatment groups, suggesting that these observations were not dose dependent. Therefore, it is reasonable to conclude that firocoxib was safe for transmammary delivery from medicated sows to piglets at the doses that were tested.

Firocoxib administered to swine by any dose, route, for any duration or frequency constitutes extralabel drug use (ELDU) because currently there are no analgesic drugs specifically approved for pain management in pigs in the United States (Smith et al., 2008). Under the Animal Medicinal Drug

Use Clarification Act (AMDUCA), ELDU is permitted for relief of suffering in pigs provided specific conditions are met (AMDUCA, 1994). These conditions include that 1) ELDU is permitted only by or under the supervision of a veterinarian, 2) ELDU is allowed only for FDA-approved animal and human drugs, 3) ELDU is permitted only when the health of the animal is threatened and not for production purposes, 4) ELDU in feed is prohibited, and 5) ELDU is not permitted if this results in a violative food residue. In the present study, there were no detectable concentrations of firocoxib or its desacyclopropylmethyl metabolite detected above the limit of quantitation (0.05 µg/g) for the assay in both sow and piglet tissues at 21 d after IM injection. In the EU, a maximum residue limit (MRL) of 10 µg/kg has been established in muscle and kidney, 15 µg/kg in fat, and 60 µg/kg in the liver of horses (EMEA, 2006). Based on these data, tissue concentrations in the present study were below the MRL for liver at 21 d after administration but the assay was not sensitive enough to quantify concentrations below the MRL for the other tissues, although none of these concentrations were above the limit of detection (0.01 µg/g) for the assay. Based on these data, additional studies conducted in accordance with FDA Guidance for Industry (GFI) #207 (FDA, 2015) (Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs In Food Producing Animals: Marker Residue Depletion Studies to Establish Product Withdrawal Periods) and GFI #3 (FDA, 2018) (General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food Producing Animals) are needed to characterize the tissue depletion of firocoxib after IM administration in sows.

The results of this study suggest that IM administration of firocoxib to sows at 7 ± 1 h before performing piglet processing procedures resulted in successful transmammary drug delivery to the nursing piglets. Transmammary delivery of firocoxib resulted in a dose-dependent reduction of plasma cortisol concentrations after processing with barrows nursing sows that received 1.0 and 2.0 mg/kg IM recording lower plasma cortisol concentrations than barrows nursing sows that received 1.5 and 1.0 mg/kg IM. Furthermore, a dose-dependent increase in average daily gain was observed at 21 d after processing. Drug concentrations in tissue samples taken 21 d post-maternal administration were below the level of detection of the assay. When given via the transmammary route, firocoxib has potential as a therapeutic drug used for analgesia, to reduce processing-induced stress, improve piglet

welfare, and enhance production through increases in weaning weights.

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