Impact of transdermal flunixin administration on serum prostaglandin E\(_2\) and cortisol concentrations in piglets following castration

Victoria R. Merenda, MS\(^1\); Brooklyn K. Wagner, PhD\(^1\); Andréia G. Arruda, PhD\(^2\); Magdiél Lopez Soriano, MS\(^3\); Shawnee Montgomery, PhD\(^3\); Johann F. Coetzee, PhD\(^3\); Monique D. Pairis-Garcia, PhD\(^1*\)

\(^1\)Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC
\(^2\)Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH
\(^3\)Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS

*Corresponding author: Dr. Pairis-Garcia (pairis-garcia@ncsu.edu)

https://doi.org/10.2460/ajvr.21.12.0201 © 2022 THE AUTHORS. Published by the American Veterinary Medical Association

OBJECTIVE
To assess the effects of transdermal flunixin administration on serum prostaglandin E\(_2\) (PGE\(_2\)) and cortisol concentrations in piglets undergoing castration.

ANIMALS
104 litters with at least 4 male piglets/litter.

PROCEDURES
Litters were randomly assigned to 1 of 4 treatments: transdermal flunixin (3.33 mg/kg) administration followed by surgical castration (CF; n = 28), transdermal flunixin administration followed by sham castration (SF; n = 26), application of physiologic saline solution followed by sham castration (S; n = 26), and application of physiologic saline solution followed by surgical castration (C; n = 24). Blood samples were collected 24 hours before and 1, 4, and 25 hours after castration or sham castration.

RESULTS
Serum PGE\(_2\) concentrations for piglets in the C and CF groups did not differ at any time. Piglets in the S group tended to have higher serum PGE\(_2\) concentrations 1 hour after sham castration compared with piglets in the SF group. One hour after the procedure, piglets that underwent castration had higher serum cortisol concentrations than did piglets that underwent sham castration. Piglets in the CF group had higher serum cortisol concentrations than did piglets in the SF group 4 hours after the procedure, but serum cortisol concentrations did not differ between the C and S groups.

CLINICAL RELEVANCE
Further studies are needed to explore dosing regimens, including effective doses and administration frequencies, and the pharmacokinetics of flunixin following transdermal administration in piglets undergoing castration.

Commercial swine facilities around the globe commonly castrate male piglets to prevent unwanted breeding, reduce aggression, and improve meat quality.\(^1\)\(^-\)\(^3\) Castration is typically performed in the first week of life on nearly all male piglets in the United States\(^3\) and approximately 80% of male piglets in the European Union.\(^4\) However, castration poses an animal welfare challenge as this procedure inflicts tissue damage and inflammation, resulting in pain.\(^5\) Mitigating the pain and inflammation associated with castration can be accomplished by eliminating the procedure entirely, using alternative techniques that minimize pain, or administering pain-relieving agents such as NSAIDs before and after the procedure.\(^6\)\(^-\)\(^8\) NSAIDs are a promising method of controlling pain and inflammation on commercial swine farms because they are relatively easily accessible to livestock veterinarians, can be economically feasible to use on a large scale,\(^7\)\(^,\)\(^10\) and provide flexibility in regards to administration route (eg, oral, IM, or topical administration).

NSAIDs are effective in mitigating inflammation because they inhibit cyclooxygenase 2, decreasing formation of prostaglandin E\(_2\) (PGE\(_2\)).\(^1\)\(^,\)\(^12\) In livestock, serum PGE\(_2\) concentration has been used to quantify the inflammatory response to various disease processes, such as paratuberculosis in cattle,\(^1\)\(^3\) and more recently to quantify efficacy in reducing inflammation associated with common husbandry procedures such as dehorning in cattle\(^1\)\(^,\)\(^14\) and castration in swine.\(^1\)\(^5\),\(^16\)
In addition to mitigating inflammation, NSAIDs are also effective in reducing pain. Physiologic parameters are important when assessing pain in animals, and previous studies have identified serum cortisol concentration as a relevant parameter to evaluate stress and pain in pigs. Previous authors have used serum cortisol concentration to determine drug efficacy in relieving pain in piglets following castration.

In the United States, flunixin is commonly used on swine farms to control pyrexia associated with swine respiratory disease and has documented success in mitigating pain associated with sow lameness. Most recently, the US FDA has approved the use of flunixin as a topical pour-on for use in cattle to mitigate pain associated with foot rot. Topical administration of products is a unique option for pain mitigation on swine farms, because it provides an attractive option that producers can implement on a large scale. However, to the authors’ knowledge, no studies to date have evaluated the anti-inflammatory and analgesic effects of transdermal flunixin administration in pigs undergoing castration. The objective of the study reported here was to assess the effects of transdermal flunixin administration on inflammation and stress, as determined by measuring serum PGE₂ and cortisol concentrations, in piglets undergoing castration.

Materials and Methods

The study protocol was approved by the Institutional Animal Care and Use Committee of North Carolina State University (protocol No. 20-113-01). Animals were cared for and handled in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching. The study was conducted from January to March 2021 at a commercial swine breeding facility located in the southeastern United States.

Animals, housing, and management

A total of 1,445 Large White × Duroc cross male piglets from 109 litters were used in the study. Sows (first through sixth lactation) and piglets (mean of 11 piglets/litter) were housed in fully slatted and mechanically ventilated farrowing rooms. In each room, sows were housed in individual farrowing crates (2.5 X 1.3 m) with additional space for the piglets (2.5 X 1.3 m) surrounding the crates. Room lights were on between 6:00 PM and 4:30 PM, and sows were provided ad libitum access to water and feed throughout the study. Piglets had access to the sow and a water nipple at all times.

Study design and treatments

Litters were included in the study if the litter consisted of a minimum of 4 male piglets (cross-fostering was permitted prior to study enrollment), piglets were ≥ 8 days old, the piglets had intact tails, both testicles had descended in all piglets, and piglets weighed > 0.5 kg. Litters were excluded from the study if the sow or piglets had any clinical signs of disease, if the sow or piglets had been treated with any antimicrobial, or if the sow received additional treatments from the farm personnel. Litters enrolled in the study did not undergo any additional processing procedures (eg, tail docking, teeth clipping, iron supplementation, or antimicrobial administration) during the time of the study.

Male piglets in enrolled litters were ear tagged (Global piglet ear tags; Allflex Livestock Intelligence) for identification purposes, and litters were randomly assigned to 1 of 4 treatments: transdermal flunixin (Banamine transdermal; 3.33 mg/kg) administration followed by surgical castration (CF), transdermal flunixin administration followed by sham castration (SF), application of physiologic saline solution followed by sham castration (S), and application of physiologic saline solution followed by surgical castration (C). Randomization was done with the RAND function in Excel (Microsoft Corp). Treatments were applied at the litter level to avoid any potential within-litter drug carryover effects.

Drug administration

Twenty-four hours before castration was performed, flunixin (3.33 mg/kg) or physiologic saline solution (equivalent volume) was applied topically by a single individual next to the tail head along the dorsal midline with a disposable syringe (BD disposable syringes; Fisher Scientific). Treatment volumes ranged from 0.1 to 0.3 mL/piglet. Corn starch and a pink nontoxic dye were added to the saline solution to minimize observer bias throughout the study by mimicking the consistency and color of the flunixin solution. To ensure the modified saline solution did not cause local or systemic reactions, a small subset of piglets in which the modified solution had been applied to the skin were observed for any reactions.

Castration procedure

Castration was performed in a routine manner by 2 farm personnel previously trained to perform the procedure and who had > 7 years of experience castrating piglets. Piglets assigned to undergo surgical castration were held in an inverted position, and the testicles were pushed into the scrotum. A vertical incision was made over each testicle with a cutting pliers instrument. The testicle was extruded through the incision and grasped with the thumb and forefinger of the person performing the procedure, and the testicle was removed with the spermatic cord and surrounding tunics. The incisions were sprayed with iodine and left open to heal, and piglets were returned to the farrowing crate.

To account for handling, piglets assigned to the sham castration groups were handled in a manner similar to that for castrated piglets, which included picking up each individual piglet from the crate and holding it in an inverted position against the body of the person performing the procedure. This person then applied pressure to the scrotal area with a thumb and finger but did not incise the skin. Iodine was then applied to the scrotal area, and the piglet was returned to the farrowing crate.
Blood sample collection

Blood samples were collected from a randomly selected subset of 94 piglets 24 hours before and 1 and 25 hours after castration or sham castration for determination of serum PGE$_2$ concentrations. In addition, blood samples were collected from a randomly selected subset of 438 piglets 24 hours before and 1, 4, and 25 hours after castration or sham castration for determination of serum cortisol concentrations.

Blood samples were collected by puncture of the orbital sinus with a disposable 20-gauge, 1-inch needle (Vacutainer; Becton, Dickinson and Company) with the rubber sheath removed as previously described. All blood tubes were maintained in a cooler and centrifuged at 2,000 X g for 15 minutes at 4°C within 6 hours after sample collection and serum was harvested. Serum aliquots were stored at −80°C, and assays were performed 2 months later. For determination of serum PGE$_2$ and cortisol concentrations, 375 and 20 μL, respectively, were used.

Determination of serum PGE$_2$ concentration

Prostaglandin E$_2$ concentrations were measured with a commercial enzyme-linked immunosorbent assay (catalog No. 514531; Cayman Chemical) as previously described. Briefly, samples were purified by adding a volume of ice-cold acetone equal to 4 times the volume of the serum sample. Then, samples were incubated at −20°C for 30 minutes and centrifuged at 3,000 X g for 5 minutes. The supernatant was transferred to a 13 X 100-mm glass tube, evaporated with a concentrator (CentriVap; Labconco), and reconstituted to the initial serum volume with kit buffer. An aliquot of the reconstituted sample was derivatized with adjusted kit components; the manufacturer’s protocol was then followed. Samples were analyzed in duplicate. Absorbance was measured at 405 nm following 60 minutes of development (SpectraMax i3; Molecular Devices). Mean PGE$_2$ concentration of a sample used to evaluate repeatability among plates was 12.3 pg/mL. Intra- and interassay coefficients of variation were 19% and 13.2%, respectively.

Determination of serum cortisol concentration

Serum cortisol concentrations were quantified using a commercial enzyme-linked immunosorbent kit (DetectX cortisol EIA kit; Arbor Assays). Upper and lower detection limits of the assay were 50 and 3,200 pg/mL, respectively. Samples were diluted 1:100 with assay buffer and analyzed according to kit directions. All samples were assayed in duplicate. In total, 40 assay kits were used. Mean ± SD intra-assay coefficient of variation for duplicate samples was 6.7 ± 7.5%. Mean interassay coefficient of variation for 2 quality-control samples was 10.0 ± 0.1%.

Statistical analysis

Multivariable mixed-effects linear regression models were separately built at the piglet level for the 2 outcomes of interest: PGE$_2$ concentration and cortisol concentration. Treatment and time were included as main effects. For model building, the first step was assessing linearity of the association between all continuous variables and the outcomes of interest with linear smooth plots. When the linearity assumption was not met, the variable was dichotomized at the median. Second, univariable mixed-effect models were built for each of other captured variables, which included piglet age (continuous, in days), lactation of the sows (continuous, in number), parity of the sows (categorical [primiparous or multiparous]), weekly mortality rate (continuous, in percentage), and body weight of the piglets (continuous, in kg). In addition, cortisol concentration (continuous, in ng/mL) was considered as a fixed effect for the PGE$_2$ model. Variables with a $P$ value ≤ .20 in the univariable analysis were eligible to be tested for inclusion in the final multivariable model. Third, the Spearman correlation method was used to check for collinearity between all independent predictors, with a cutoff of 0.80.

Lastly, final multivariable mixed-effect linear models were constructed with a backwards stepwise approach, starting with all predictors with values of $P$ ≤ .20. To investigate the combined effects of treatment and time on the outcomes, the 3-way interaction between drug, procedure, and time relative was included in all models. In all models, 2 random effects were included: litter, to account for clustering of piglets within litters, and piglet, to account for repeated measurements on individual animals. During construction of the final models, confounders were assessed by removing the variables individually and assessing other variable’s coefficients for a change of ≥ 20%. If this was the case, variables were retained in the models. Adjusted linear predictions for contrasts between castration procedure and drug with 95% CIs were estimated with the delta method. Statistical significance was declared at $P$ ≤ .05. All analyses were performed with standard software (Stata, version 15; StataCorp).

Results

One-hundred nine litters were enrolled in the study, but 5 litters were removed because the sows received additional treatment by farm personnel. Therefore, 104 litters were used in the analyses. Mean age of piglets in these 104 litters was 3 days (SD, 0.9 days; range, 2 to 8 days) and mean body weight was 2.1 kg (SD, 0.5 kg; range, 1.03 to 3.40 kg) at the time of enrollment. Of the 104 litters, 28 were assigned to the CF group, 26 were assigned to the SF group, 26 were assigned to the S group, and 24 were assigned to the C group.

Effect of drug and procedure on serum PGE$_2$ concentration

The final multivariate model for serum PGE$_2$ concentration retained weekly mortality rate ($P$ = .005) and body weight of the piglets ($P$ = .002) as predictors. Serum PGE$_2$ concentration of piglets in the C and CF groups did not differ significantly ($P$ ≥ .26) at any time (Figure 1). Similarly, serum PGE$_2$ concentration of piglets in the SF group did not differ from
concentration for piglets in the S group at baseline \((P = .7)\) or 25 hours after sham castrations \((P = .3)\).

Piglets in the S group tended to have \((P = .09)\) higher serum \(PGE_2\) concentrations 1 hour after sham castration than did piglets in the SF group.

**Effect of drug and procedure on serum cortisol concentration**

Body weight of the piglets \((P < .001)\) was retained in the final multivariable model for serum cortisol concentration. For consistency, weekly mortality rate was retained in the multivariate model even though it was not statistically significant \((P = .85)\). The conclusions did not change regardless of whether mortality rate was or was not retained in the model.

Serum cortisol concentrations were highest 1 hour after castration or sham castration for piglets in the CF, SF, and C groups and 4 hours after sham castration for piglets in the S group (Figure 2). Maximum serum cortisol concentrations were 54.2, 34.1, 72.8, and 34.5 ng/mL for the CF, SF, C, and S groups, respectively.

At baseline, the serum cortisol concentration did not differ significantly \((P > .83)\) among treatment groups (Figure 2). However, 1 hour after the procedure, piglets that underwent castration had significantly higher serum cortisol concentrations than did piglets that underwent sham castration (ie, group C vs group S and group CF vs group SF; \(P \leq .02\)). Four hours after the procedure, piglets in the CF group had significantly \((P = .03)\) higher serum cortisol concentrations than did piglets in the SF group, but cortisol concentration did not differ significantly \((P = .32)\) between piglets in the C and S groups. At 25 hours after the procedure, serum cortisol concentration did not differ significantly \((P > .21)\) among treatment groups.

Compared with baseline concentrations, mean serum cortisol concentration was 61% higher for piglets in the CF group 1 hour after castration (Figure 3). In contrast, for piglets in the C group,
mean serum cortisol concentration was 96% higher 1 hour after castration, compared with the baseline concentration.

**Discussion**

Transdermal flunixin application is an attractive option to swine producers, given that it is a needleless method that can be applied prior to castration. However, results of the present study suggested that transdermal flunixin administration at a dose of 3.33 mg/kg was not effective in decreasing serum PGE2 and cortisol concentrations in piglets undergoing castration. Further studies are needed to explore dosing regimens, including effective doses and administration frequencies, and the pharmacokinetics of flunixin following transdermal administration in piglets undergoing castration.

In the present study, serum PGE2 concentrations did not differ between castrated piglets that received transdermal flunixin and those that received saline solution. These results contrast with findings of Bates et al., who demonstrated that meloxicam administered to the sow was effective at decreasing PGE2 concentrations in piglets for up to 90 hours after drug administration. However, results from Bates et al may not be directly comparable, given that the NSAID evaluated and the administration route were different from those in the present study. Unlike flunixin, meloxicam is considered a cyclooxygenase 2-selective inhibitor, and Bates et al administered the drug to the piglets via the transmammary route of the sow. Given this administration route, sows were given a substantially higher dose than label recommendations (30 mg/kg) for 3 consecutive days. Therefore, these studies cannot be directly compared, and the lack of significant differences in our study may have been influenced by drug type, concentration, and administration frequency.

On the other hand, results of the present study are similar to those reported by Viscardi et al. Although not specific to pharmaceutical drug efficacy, Viscardi et al measured PGE2 metabolites to quantify differences in inflammatory response between 2 surgical castration methods (CO2 surgical laser or scalpel vs sexually intact male control group). That study found no difference in PGE2 metabolites between groups, and the authors suggested that the lack of differences may have been associated with the role of cortisol inhibition on the PGE2 pathway. The synthesis of glucocorticoids (eg, cortisol) is a classic endocrine response to stress, and glucocorticoid production is part of an orchestrated biological mechanism that promotes gluconeogenesis, amino acid mobilization, and stimulation of fat breakdown to maintain the fight-or-flight response. Prostaglandin is a byproduct produced by this stress cascade, and cortisol concentrations can interrupt PGE2 production.

This concept fits with the hypothesis postulated by Viscardi et al and may have influenced the results of our study. In the current study, piglets administered flunixin prior to sham castration had the lowest PGE2 concentrations 1 hour after the procedure, compared with all other treatments. This suggests that transdermal flunixin has the properties to mitigate PGE2 concentrations, but perhaps only in situations when stress is controlled. Therefore, castration may induce enough of a stress response that NSAIDs cannot effectively mitigate inflammation unless given at a higher dose or greater frequency, such as in the study by Bates et al. Future work evaluating transdermal flunixin should use a higher drug dose or consider multiple administration to achieve concentrations great enough to compensate for the stress response.

An additional factor that may have influenced the lack of differences in PGE2 concentrations between treatment groups in this study is sampling site collection. Blood samples in this study were collected via orbital bleeding, and physiologic changes to the orbital sinus represent physiologic changes occurring on a systemic level. Previous work by Thiry et al demonstrated that transdermal flunixin was effective in reducing PGE2 concentrations in bovine exudate following induced inflammation. However, that study used chambers to induce the local inflammation, and samples were collected directly from those chambers. Given that flunixin has a high degree of protein binding, distributes effectively throughout inflammatory exudate, and has a slow clearance rate, drug concentrations at the site of inflammation may exceed concentrations found in plasma. Therefore, in our study, PGE2 concentrations may have been lower at the site of injury than in serum, and future work including interstitial probes as described by Nixon et al may allow for a more accurate assessment of inflammatory mediation via PGE2 in castrated piglets.

Analysis of cortisol concentrations in the present study demonstrated that surgical castration increased systemic cortisol concentrations, compared with sham concentration, and showed that cortisol remains an effective biomarker in assessing stress responses. Coetzee et al previously reported that male piglets nursing sows that received 2.0 mg of firocoxib/kg had a significantly lower mean serum cortisol concentration at 1 ± 1 hour after processing, compared with piglets nursing sows that received firocoxib at doses of 1.0 mg/kg or 0.5 mg/kg. The dose-dependent reduction in peak cortisol concentration after NSAID administration at the time of castration reported previously supports the assessment of cortisol as a surrogate marker of pain in piglets.

To the author’s knowledge, this was the first study to evaluate transdermal flunixin administration in piglets. Previous work in other species demonstrates mixed results in the efficacy of transdermal...
flunixin for mitigating pain and inflammation. A recent study evaluating transdermal flunixin administration in castrated bucklings demonstrated no effect on PGE_2 or cortisol concentrations, whereas another study showed that flunixin-treated calves undergoing sham dehorning had higher PGE_2 concentrations than did flunixin-treated calves that were surgically dehorned. Given that this was the first study to evaluate transdermal administration of flunixin in piglets and the drug dose was chosen on the basis of label recommendations for cattle, an appropriate and effective dose for piglet use has yet to be determined. Future studies should consider applying transdermal flunixin at various time points and potentially reapplying the drug to maximize concentrations. Given the unique qualities of transdermal flunixin, further research to identify an appropriate dosing regimen is critical given that with transdermal administration, piglets need to be handled only minimally and additional needle injections are not required.

Finally, given that flunixin is not label approved for use in swine, any use of flunixin in swine must be performed in compliance with the Animal Medicinal Drug Use Clarification Act.

Acknowledgments

This research was funded by a Veterinary Pharmacology Research Foundation Grant.

The authors declare that there were no conflicts of interest.

The authors thank Dr. Mary Battrell, Mark Underwood, Marco Lopez, and farm staff at farm S2 for allowing access to farms and support during data collection. In addition, the authors thank Matthew Browning and Jasmine Olivares for assisting with data organization and blood sample preparation.

References

1. Guay K, Salgado G, Thompson G, et al. Behavior and handling of physically and immunologically castrated market pigs on farm and going to market. J Anim Sci. 2013;91(11):5410–5417.
2. Andreassen OØ. Boartaint related compounds: androsteneone/skatole/other substances. Acta Vet Scand. 2006;48:5.
3. Wagner B, Royal K, Park R, Pairs-Garcia MD. Identifying barriers to implementing pain management for piglet castration: a focus group of swine veterinarians. Animals. 2020;10(7):1202.
4. De Bryne N, Berg C, Blaha T, Tempel D. Pig castration: will the EU manage to ban pig castration by 2018? Porcine Health Manag. 2016;20(2):29.
5. Shill M, Polkinghorne A. Optimal methods of documenting analgesic efficacy in neonatal piglets undergoing castration. Animals. 2020;10(9):1450.
6. Guattoo R, Levionnois O, Fournier D, et al. Minimizing pain in farm animals: the 3S approach—"suppress, substitute, soothe". Animal. 2012;6(8):1261–1274.
7. Reiner G, Schollasch F, Hillen S, Willems H, Piechotta K. Effects of meloxicam and flunixin on pain, stress and discomfort in male piglets during and after surgical castration. Berl Munch Tierarztl Wochenschr. 2012;125(7–8):305–314.
8. Kluivers-Poodt M, Zonderland JJ, Verbraak J, Lambooij E, Hellebrekers LJ. Pain behaviour after castration of piglets; effect of pain relief with lidocaine and/or meloxicam. Animal. 2013;7(7):1158–1162.
9. Tenbergen R, Friendship R, Cassar G, Amezcu a MR, Haley D. Investigation of the use of meloxicam for reducing pain associated with castration and tail docking and improving performance in piglets. J Swine Health Prod. 2014;22(2):64–70.
10. Robles I, Arruda AG, Nixon E, et al. Producer and veterinarian perspectives towards pain management practices in the US cattle industry. Animal. 2021;11(1):209.
11. Yuan CJ, Mandal AK, Zhang Z, Mukherjee AB. Transcriptional regulation of cyclooxygenase-2 gene expression: novel effects of nonsteroidal anti-inflammatory drugs. Cancer Res. 2000;60(4):1084–1091.
12. Allen KA, Coetzee JF, Edwards-Callaway LN, et al. The effect of timing of oral meloxicam administration on physiological responses in calves after cautery dehorning with local anesthesia. J Anim Sci. 2013;96:5194–5205.
13. Sajiki Y, Konnai S, Okagawa T, et al. Prostaglandin E2 induction suppresses the th1 immune responses in cattle with Johne’s disease. Infect Immun. 2018;86(5):e00910–e00917.
14. Fracar o E, Coetzee JF, Odore R, et al. A study to compare circulating flunixin, meloxicam and gabapentin concentrations with prostaglandin E2 levels in calves undergoing dehorning. Res Vet Sci. 2013;95:204–221.
15. Bates JL, Karriker LA, Stock ML, et al. Impact of transmammary-delivered meloxicam on biomarkers of pain and distress in piglets after castration and tail docking. Plos One. 2014;9(12):e113678.
16. Viscardi AV, Cull CA, Kleinhenz MD, et al. Using a CO2 surgical laser for piglet castration to reduce pain and inflammation, and to improve wound healing. Kans Agric Exp Stn Res Rep. 2020;6:10. doi: 10.4148/2277-5978.2016.
17. Cashman JN. The mechanisms of action of NSAIDs in analgesia. Drugs. 1996;52(5):15–23. doi:10.2165/00003495-199605025-00004.
18. Dzikamunhenga, RS, Anthony R, Coetzee J, et al. Pain management in the neonatal piglet during routine management procedures. Part 1: A systematic review of randomized and non-randomized intervention studies. Anim Health Res Rev. 2014;15(1):14–38.
19. Ison SH, Clutton RE, Di Giminiani P, Rutherford KM. A review of pain assessment in pigs. Front Vet Sci. 2016;3:108. doi:10.3389/fvets.2016.00108.
20. Sutherland MA, Davis BL, Brooks TA, Coetzee JF. The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia. J Anim Sci. 2012;90(7):2211–2221.
21. Kluivers-Poodt M, Houx BB, Robben SRM, Koop G, Lambooij E, Hellebrekers LJ. Effects of a local anesthetic and NSAID in castration of piglets, on the acute pain responses, growth and mortality. Animal. 2012;6:1469–1475.
22. Pairs-Garcia MD, Johnson AK, Abell CA, et al. Measuring the efficacy of flunixin meglumine and meloxicam for lame sows using a GAITFour pressure mat and an embedded microcomputer-based force plate system. Anim Sci J. 2015;93(5):2100–2110.
23. U.S. Food and Drug Administration. 2017. FDA approves first medication for pain control in a food-producing animal (NADA 141-450). Accessed February 14, 2021. www.fda.gov/animal-veterinary/cvm-updates/fda-approves-first-medication-pain-control-food-producing-animal.
24. Vaughn SE. Review of the third edition of the Guide to the Care and Use of Agricultural Animals in Agricultural Research and Teaching. J Am Assoc Lab Anim Sci. 2012;51(3):298–300.
25. Dove CR, Alworth LC. Blood collection from the orbital sinus of swine. Lab Anim. 2015;44:383–384.
26. Giorgi M, Curiberti M, Ye G, Barbero R, Sgorbini M. Oral administration of tepoxalin in the horse: a PK/ PD study. Vet J. 2011;190(1):143–149.
27. Schattenkirchner M. Meloxicam: a selective COX-2 inhibitor non-steroidal anti-inflammatory drug. Expert Opin Investig Drugs. 1997;6(3):321–334.
28. Whirledge S, Cidlowski JA. Glucocorticoids, stress, and fertility. *Minerva Endocrinol.* 2010;35(2):109–125.

29. Gryglewski RJ, Panczenko B, Korbut R, Grodzińska L, Oczkiewicz A. Corticosteroids inhibit prostaglandin release from perfused mesenteric blood vessels of rabbit and from perfused lungs of sensitized guinea pig. *Prostaglandins.* 1975;10(4):343–355.

30. Thiry J, Fournier R, Roy O, et al. Evaluation of flunixin meglumine pour-on administration on prostaglandin E2 concentration in inflammatory exudate after induction of inflammation in cattle. *Res Vet Sci.* 2017;114:294–296. doi:10.1016/j.rvsc.2017.04.010.

31. Galbraith EA, McKellar QA. Protein binding and in vitro serum thromboxane B2 inhibition by flunixin meglumine and meclofenamic acid in dog, goat and horse blood. *Vet Sci Res J.* 1996;61:78–81.

32. Espinasse J, Thouvenot JP, Dalle S, et al. Comparative study of the action of flunixin meglumine and tolfenamic acid on prostaglandin E2 synthesis in bovine inflammatory exudate. *J Vet Pharmacol Ther.* 1994;17:271–274.

33. Nixon E, Almond GW, Baynes RE, Messenger KM. Comparative plasma and interstitial fluid pharmacokinetics of meloxicam, flunixin, and ketoprofen in neonatal piglets. *Front Vet Sci.* 2020;7:82. doi:10.3389/fvets.2020.00082.

34. Prunier A, Mounier AM, Hay M. Effects of castration, tooth resection, or tail docking on plasma metabolites and stress hormones in young pigs. *J Anim Sci.* 2005;83(1):216–222.

35. Coetzee JF, Sidhu PK, Seagen J, et al. Transmammary delivery of firocoxib to piglets reduces stress and improves average daily gain after castration, tail docking, and teeth clipping. *J Anim Sci.* 2019;97(7):2750–2768.

36. Graves MT, Schneider L, Cox S, et al. Evaluation of the pharmacokinetics and efficacy of transdermal flunixin for pain mitigation following castration in goats. *Transl Anim Sci.* 2020;4(4):198. doi:10.1093/tas/txaa198.

37. Kleinhenz MD, Van Engen NK, Gorden PJ, et al. The impact of pain on the pharmacokinetics of transdermal flunixin meglumine administered at the time of cauterization in Holstein calves. *Vet Anaesth Analg.* 2018;45(6):849–857.

38. AMDUCA. Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). Accessed November 3, 2021. https://www.fda.gov/animal-veterinary/acts-rules-regulations/animal-medicinal-drug-use-clarification-act-1994-amduca.