Pharmacokinetics and physiologic effects of intramuscularly administered xylazine hydrochloride-ketamine hydrochloride-butorphanol tartrate alone or in combination with orally administered sodium salicylate on biomarkers of pain in Holstein calves following castration and dehorning

Sarah L. Baldridge, DVM, MS; Johann F. Coetzee, BVSc, PhD; Steve S. Dritz, DVM, PhD; James B. Reinbold, DVM, PhD; Ronette Gehring, BVSc, MMedVet (Pharm); James Havel, BS; Butch Kukanich, DVM, PhD

Objective—To determine the pharmacokinetic parameters of xylazine, ketamine, and butorphanol (XKB) administered IM and sodium salicylate (SAL) administered PO to calves and to compare drug effects on biomarkers of pain and distress following sham and actual castration and dehorning.

Animals—40 Holstein bull calves from 3 farms.

Procedures—Calves weighing 108 to 235 kg (n = 10 calves/group) received one of the following treatments prior to sham (period 1) and actual (period 2) castration and dehorning: saline (0.9% NaCl) solution IM (placebo); SAL administered PO through drinking water at concentrations from 2.5 to 5 mg/mL from 24 hours prior to period 1 to 48 hours after period 2; butorphanol (0.025 mg/kg), xylazine (0.05 mg/kg), and ketamine (0.1 mg/kg) coadministered IM immediately prior to both periods; and a combination of SAL and XKB (SAL+XKB). Plasma drug concentrations, average daily gain (ADG), chute exit velocity, serum cortisol concentrations, and electrodermal activity were evaluated.

Results—ADG (days 0 to 13) was significantly greater in the SAL and SAL+XKB groups than in the other 2 groups. Calves receiving XKB had reduced chute exit velocity in both periods. Serum cortisol concentrations increased in all groups from period 1 to period 2. However, XKB attenuated the cortisol response for the first hour after castration and dehorning and oral SAL administration reduced the response from 1 to 6 hours. Administration of XKB decreased electrodermal activity scores in both periods.

Conclusions and Clinical Relevance—SAL administered PO through drinking water decreased cortisol concentrations and reduced the decrease in ADG associated with castration and dehorning in calves. (Am J Vet Res 2011;72:1305–1317)
practices that minimize pain associated with common cattle husbandry practices. Use of analgesia and anesthesia during painful procedures such as castration and dehorning has been suggested by organizations such as the AVMA; however, FDA-approved drugs labeled for the treatment of pain in cattle do not currently exist.\textsuperscript{2,3} To enable the cattle industry to effectively respond to this challenge, research is necessary to evaluate the welfare implications of existing husbandry practices and to identify practical and cost-effective strategies for relieving pain in cattle.

Identification of robust biomarkers for the objective measurement of pain is needed to evaluate analgesic efficacy during procedures such as castration and dehorning. The process of evaluating pain is especially complex in prey species, such as cattle, that inherently conceal pain.\textsuperscript{1} In previous research,\textsuperscript{4-8} biomarkers investigated for associations with the pain and distress of castration and dehorning have included serum cortisol concentration, heart rate, acute phase protein values, and in vitro interferon-\gamma production. Other potential indicators include behavior scores, ADG, feed intake, activity in the cattle chute, and vocalization.\textsuperscript{9,10}

The magnitude of the increase in serum cortisol concentration (as indicated by the change in the height \([C_{max}]\) and duration \([T_{max}]\) of the concentration peak), the integrated response (as indicated by the AUEC), or both reportedly correspond with the predicted noxious stimuli during the procedure.\textsuperscript{3} However, the results of studies in which ADG was used as an indicator for pain have been equivocal. For example, a study\textsuperscript{10} revealed calves undergoing castration have a decrease in ADG, compared with calves not undergoing castration; however, a treatment effect (xylazine and butorphanol vs no treatment) was not observed. Additionally, information is deficient on the use of cattle exit velocity out of a squeeze chute and EDA for the objective measurement of pain in newly castrated and dehorned calves. Because chute exit velocity has been used in temperament and reactivity studies\textsuperscript{11,12} in cattle, exit velocity may be useful in determining the effect of a painful procedure and sedative treatment on calf behavior. Another potential means of pain assessment, EDA, is a measurement of electrical outflow stimuli during the procedure.\textsuperscript{3} However, studies of salicylate administered PO through free-choice water consumption alone or in combination with XKB prior to castration and dehorning are lacking in the published literature. If SAL provided in the drinking water alone or in combination with a parenterally administered sedative and analgesic were to attenuate signs of distress without causing recumbency in calves, this would offer veterinarians and producers a practical and cost-effective way to reduce pain and distress associated with castration and dehorning. The purpose of the study reported here was to evaluate the effects of XKB administered IM alone or in combination with SAL continuously administered PO through free-choice water consumption on ADG, chute exit velocity, EDA, and cortisol response of calves following serial castration and dehorning.

**Materials and Methods**

**Animals**—In June 2008, 40 horned intact male Holstein calves between 2 and 4 months of age and weighing 108 to 235 kg were acquired from 3 farms in Kansas for use in the study. On arrival, the calves were measured for scrotal circumference, horn-base diameter, and horn length. Additionally, all calves
received an SC injection of tulathromycin (2.5 mg/kg) as metaphylactic treatment against bovine respiratory disease, 8-way clostridial vaccine, 4-way modified-live viral respiratory disease vaccine, and pour-on insecticide. For sustained fly control, application of the pour-on solution was repeated every 7 to 10 days for the duration of the study. Five pens were used to house calves (8 calves/pen) in a drylot confinement facility at Kansas State University. Ad libitum access to water was provided to each calf. A ration (3.6 kg/calf/d) from a typical beef feedlot receiving diet was also provided for the duration of the study.

Three days before entering a phase, participating calves were transferred from the drylot facility to the Animal Resource Facility at the university and individually allocated to indoor pens (area, 13.40 m²). Over a 2-day period (trial days –6 to –4), calves were acclimated to housing in individual pens, during which time each calf was restrained with a rope halter within its respective pen for at least 10 to 15 minutes. Each calf was conditioned to walking through an alleyway and restraining in a cattle chute once prior to the start of each phase. Calves were housed in this facility throughout their phase for 10 days.

Study preparation—This study was approved by the Institutional Animal Care and Use Committee at Kansas State University. Because calves in the placebo group were anticipated to have pain as a result of castration and dehorning, all calves were assessed 3 times daily for behavioral signs of excessive pain for a 72-hour period after the procedures. To do this, attitude, gait, appetite, time spent lying down, scrotal swelling, and horn bud assessment were assigned a score from 0 (prestudy levels) to 5 (significantly altered), with a score ≥3 requiring notification of the university veterinarian. A rescue analgesic protocol for administration of flunixin meglumine (2.2 mg/kg, IV, q 12 h) was initiated 24 hours (day –3) prior to period 1 and continued until 48 hours (day 2) after period 2 (SAL); xylazine HCl (0.05 mg/kg), ketamine HCl (0.1 mg/kg), and butorphanol tartrate (0.025 mg/kg) administered IM immediately prior to castration and dehorning in periods 1 and 2 (XKB); and a combination of treatments (SAL+XKB). For SAL administration, four 19-L plastic buckets were weighed. Sodium salicylate powder was added to 10 L of tap water in plastic buckets to achieve a final concentration of 2.5 to 5 mg of SAL/mL. Fifteen to 45 mL of molasses was mixed in with the solution to increase palatability depending on the volume of water consumption. Filled buckets were hung from a chain in each pen for calves assigned to SAL or SAL+XKB treatment.

During each trial, water buckets were inspected 3 times/d. After near depletion of the medicated solution in the bucket, the remaining contents were weighed and the bucket was refilled with a freshly prepared volume of medicated solution. On days –3 and –1, 12 hours prior to sham castration and actual castration, respectively, 2 buckets with differing concentrations of the medicated solution (1.5 and 2.5 mg/mL or 2.5 and 5 mg/mL) were offered to calves to improve the consumption of salicylate and to achieve maximum plasma salicylate concentrations. Calves in the SAL and SAL+XKB groups were offered the medicated solution from 24 hours prior to period 1 to 48 hours after period 2. Forty-eight hours after period 2, calves were offered a final bucket of the medicated solution. Calves were allowed to finish the bucket of medicated solution, and then a bucket of fresh tap water was offered. Calves in the placebo and XKB groups were offered tap water ad libitum via self-filling water units.

The study was completed in five 10-day phases from June 30 to August 11, 2008. Eight calves were assigned to 1 of the 5 phases (2 calves/treatment group/phase for a total of 8 calves/phase). The group with the heaviest calves was assigned to the first phase and the group with the lightest calves was assigned to the last phase to minimize variations in body weight, scrotal circumference, and horn diameter by the time the procedures were performed. Each phase was divided into 2 periods,
with the procedures performed exactly 48 hours from the other: sham castration and sham dehorning on day –2 (period 1) and castration and dehorning on day 0 (period 2). All castration and dehorning procedures were performed by the same veterinarian (JBR).

**Surgical procedures**—On day –2 of each phase, approximately 30 minutes prior to commencement of sham castration and dehorning, calves scheduled for treatment in that phase were fitted with a rope halter and relocated as a group into a holding pen with an adjacent alleyway leading to the squeeze chute. Approximately 2 minutes prior to sham castration, the selected calves were individually led into a squeeze chute with a rope halter and a blood sample was collected for measurement of baseline serum cortisol concentration (all treatment groups) and baseline plasma SAL concentration (SAL and SAL+XKB only). The order of castration and dehorning was predetermined before the start of each trial to maintain consistency between study days with the following order of the treatment groups: placebo, SAL, XKB, SAL+XKB, placebo, SAL, XKB, and SAL+XKB (8 calves total). At time point 0 of day –2 (period 1), a volume of saline solution equivalent to the volume of XKB administered to calves in the XKB group was administered IM to the placebo and SAL groups. Calves in the XKB and SAL+XKB groups were administered butorphanol tartrate (0.025 mg/kg), xylazine (0.05 mg/kg), and ketamine (0.1 mg/kg) at the same time point. Immediately after drug or placebo administration, the scrotum was cleaned with a 0.1% chlorhexidine solution, the apex of the scrotum was manually extended and ventrally elongated, and each testicle was then repeatedly manipulated (4 to 5 times for the left and right testicle) dorsally and ventrally within the scrotum for approximately 20 seconds (sham castration). The calf’s head was then restrained with a halter by extending and flexing the neck laterally to the right, and the hair was trimmed around the base of the left horn (sham dehorning). This process was similarly repeated for the right horn (sham dehorning). The 5-minute blood sample was collected in the chute prior to release of the calf. After release from the chute, the calf exited through another alleyway (set up for measurement of chute exit time) and then restrained prior to each successive sample collection at the intervals described. The process was repeated on each calf in period 1.

During period 2, calves were similarly restrained and blood samples were collected as in period 1. The scrotum was cleaned. Castration was performed by use of a closed surgical castration technique without the provision of local anesthesia. To do so, the apex of the scrotum was secured manually and extended distally, and the distal third of the scrotum was removed with a No. 10 scalpel blade. The right testicle and spermatic cord was exteriorized by blunt dissection of the scrotal fascia. The cremaster muscle and then the testicular artery and vein, epididymis, and vas deferens were stripped ventrally via digital manipulation and traction. The remaining connective tissue was incised with the scalpel blade. The same procedure was used to remove the left testicle. After castration, the calf’s head was restrained as in period 1. The left horn was removed by use of a Barnes dehorning instrument. Hemostasis was achieved through thermocautery by use of a hot iron. The head was released and restrained as in period 1. The right horn was removed with the same procedure for the left horn. The head was released from restraint, the 5-minute blood sample was collected, and the calf was released from the squeeze chute as in period 1. This process was performed on each calf assigned to that particular phase during period 2.

**Outcome measurements**—Upon release from the squeeze chute into the alleyway at the time of catheterization and after processing in period 1 and 2, the calf passed through a series of 2 wireless photo sensors positioned 1.5 and 3 m from the exit of the chute. The time elapsed for each calf to travel the 1.5 m between these 2 sensors (ie, chute exit velocity) was recorded by use of an electronic timer equipped with a printer.

To compute ADG, body weights of calves were determined by use of a squeeze chute with a scale that was used for the entire study. The 8 calves assigned to each phase were weighed the morning of days –3, –2 (the day of sham procedures), 0 (the day of actual procedures), 1, and 2 to determine the mean change in body weight. The calves were then weighed at 4, 6, and 13 days after castration and dehorning.

Blood samples were collected immediately prior to sham castration and sham dehorning and castration and dehorning in periods 1 and 2 (ie, 0 minutes) and at 5, 10, 20, 30, 40, and 50 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours. At approximately 30 seconds prior to each sample collection, 5 mL of blood was drawn from the indwelling catheter of the left jugular vein and directly returned; this process was repeated 3 times so that the third repetition was completed immediately prior to the scheduled sample collection. At the designated time, blood was drawn from the indwelling catheter into 20-mL Luer syringes and transferred to evacuated tubes containing lithium heparin (sample total volume, 6 mL) and evacuated tubes with no additive (sample total volume, 8 mL). Additionally, 5 mL of flush solution was injected into the indwelling catheter after each sample collection to maintain patency of the catheter. The evacuated tubes were immediately stored on ice until centrifugation (10 minutes at 3,000 × g) to separate blood components. Plasma or serum was then transferred into cryovials and frozen at –80°C prior to sample analysis.

Electrodermal activity was measured during phases 3, 4, and 5 of the study (6 calves/treatment) by use of a commercially available pain assessment device. This device was only available for use during the last 3 phases of the study. The device consisted of 2 electrodes that transmit an electric current when touched on a hairless area of an animal’s skin. For the measurement, these electrodes were placed across the nasal planum of each calf, and a numeric score between 0 and 9.9 was digitally displayed on the device (0 = calm or no pain; 9.9 = tense or severe pain). Readings were obtained immediately prior to procedures in periods 1 and 2 and then at 5, 10, 20, 30, 40, and 50 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, and 24 hours after the initial reading for each period. Readings were also obtained during castration and at dehorning.
Serum cortisol concentrations were determined by use of a solid-phase competitive chemiluminescent enzyme immunoassay and an automated analysis system as described. A minimum serum sample volume of 100 μL was used for analysis. The calibration range for the assay was 28 to 1,380 nmol of cortisol/L; analytic sensitivity was 5.5 nmol of cortisol/L. Cortisol samples were analyzed within 3 months after collection. Cortisol reportedly remains stable in human serum after 42 years of storage at –20°C. The laboratory technician performing the analysis was unaware of which treatment the calves had received.

Plasma concentrations of xylazine (H; m/z, 221.2 → 90.1), butorphanol (H; m/z, 328.3 → 157.1), and ketamine (H; m/z, 238.1 → 125.0) were determined via high-pressure liquid chromatography and tandem mass spectrometry. Fifty microliters of an internal standard (ketamine-D4 [100 ng/mL] in a 50:50 mixture of acetonitrile and water; m/z, 242.2 → 129.0) was used for ketamine and xylazine plasma concentration determination. Norketamine-D4 (100 ng/mL) in a 50:50 mixture of acetonitrile and water (m/z, 228.1 → 129.0) was used as an internal standard for butorphanol. The internal standards combined with 400 μL of acetonitrile were added to each 100-μL aliquot of plasma to create standards and quality controls. Each sample was mixed with a vortex machine for approximately 20 seconds to precipitate the proteins and centrifuged for 10 minutes at 6,500 × g.

Approximately 400 μL of supernatant was filtered by use of a 0.45-μm filter. The fluid volume of the filtrate was evaporated under nitrogen at 40°C by use of a dry-down unit. Dried extracts were reconstituted in 100 μL of starting mobile phase (0.2% acetic acid in water and 0.2% acetic acid in acetonitrile [5:95]), mixed with a vortex device, and transferred to autosampler vials for injection. The mobile phase consisted of 0.2% acetic acid in water (starting mobile phase) and 0.2% acetic acid in acetonitrile at a flow rate of 0.4 mL/min (transitioning mobile phase). The mobile phase gradient consisted of 5% of transitioning mobile phase from 0 to 1.0 minutes, a linear gradient to 80% of transitioning mobile phase at 4.5 minutes, and a return to the starting mobile phase. The total runtime of analysis was 7 minutes. Analyte separation was achieved by use of a C18 column maintained at 40°C. The method was accurate and precise across a linear dynamic range of 0.5 to 100.0 ng/mL.

Quality controls of known drug concentrations were analyzed during sample analysis for monitoring of method performance. The coefficient of variation of 45 quality-control samples over 5 analytic runs was ≤ 2.1% and ≤ 4.5% for xylazine, ≤ 9.9% and ≤ 10.7% for butorphanol, and ≤ 8.3% and ≤ 5.8% for ketamine. All samples were analyzed within 6 months after collection. Xylazine and ketamine reportedly remained stable in human serum after 42 years of storage at –20°C. However, the stability of butorphanol has not been reported. The laboratory technician (JH) performing the analysis was unaware of which treatment calves had received (XKB and SAL+XKB).

Pharmacokinetic analysis—The pharmacokinetic properties (Tmax, Cmax, and mean concentration) of salicylate and cortisol were analyzed descriptively via inspection of the time-concentration curve. The AUC for salicylate and the AUEC for cortisol were calculated by use of the trapezoidal rule. The AUC was used to measure the area under the time-concentration curve for the elimination of salicylate (AUCS) and for cortisol (AUCf).

Noncompartmental pharmacokinetic analysis of XKB time-concentration data was performed (RG) by use of a commercially available software program. Pharmacokinetic parameters determined were AUC (first to last measured concentration) by use of the trapezoidal rule, slope of the terminal portion of the time-concentration curve (λz), terminal elimination half-life time (t1/2el), Tmax, Cmax, total body clearance per fraction of drug absorbed (Cl/F), volume of distribution per fraction of drug absorbed (Vz/F), and MRT. These parameters are represented in the following equations:

\[
\begin{align*}
  t_{1/2el} & = \frac{0.693}{\lambda_z} \\
  Cl/F & = D/AUC \\
  Vz/F & = Dose/(AUC \times \lambda_z) \\
  MRT & = AUMC/AUC \\
  AUC_{0-\infty} & = AUC + C_{last}/\lambda_z
\end{align*}
\]

where D is the dose, AUMC is the area under the moment curve, AUC0-∞ is the AUC from 0 to infinity, and Clast is the last measured concentration.

Statistical analysis—Individual and combined effects of XKB and SAL were analyzed statistically. All calves that received XKB (XKB and SAL+XKB groups) were compared with those that did not receive XKB (placebo and SAL groups). The same comparison was performed for calves receiving salicylate. The effect of study day was determined by evaluating the interaction between phase and treatment. Additionally, study outcomes were compared among treatment groups. The cortisol data within each period were evaluated for evidence of departure from normality by use of statistical software. There was significant evidence of departure from normality for several of the cortisol parameters; therefore, data were ranked by use of the statistical software. An ANOVA was conducted on unranked and ranked data with fixed effects of period, salicylate treatment, and combined XKB treatment and the interactions of these 3 effects. Means and SEs reported are LSMeans and pooled SEMs. For the unranked data, LSM and SEM are reported. The P values reported to assess significance among the LSMs are those derived from the analysis of the ranked data. Data for ADG, chute exit velocity, and EDA were analyzed by use of a commercial software program. Values of P < 0.05 were considered significant.
Results

Animals—Rescue analgesia was not administered during this study because no overt signs of pain were evident in calves after castration and dehorning. Scrotal circumference ranged from 12.5 to 23.5 cm, horn-base diameter ranged from 22.3 to 50.9 mm, and horn length ranged from 23.4 to 73.4 mm. Two calves from the placebo group and 2 calves in the SAL+XKB group developed thrombophlebitis during different phases of the study and therefore were not included in all statistical analyses. No evidence of a treatment day (phase) X treatment interaction was detected for cortisol response (P = 0.160), weight gain (P = 0.24), chute exit velocity (P = 0.41), or EDA (P = 0.67). Therefore, data were pooled across study days for all analyses.

ADG—The ADG ± SEM for the first 13 days after castration and dehorning were summarized (Figure 1). Castration and dehorning had a significant (P = 0.043) impact on ADG among all treatment groups. Calves in the SAL and SAL+XKB treatment groups had a significantly (P = 0.029) higher ADG for the first 13 days after castration and dehorning than did calves in the placebo and XKB groups. A large scrotal circumference was associated with a decrease in ADG following castration and dehorning (P = 0.004).

Chute exit velocity—The chute exit velocity for 1 calf in the placebo group and 1 calf in the SAL+XKB groups during period 1 was not recorded because of a failure to reset the timer and was not included in the statistical analysis. Another calf in the SAL+XKB group became sternally recumbent between the sensors and therefore an accurate velocity could not be determined and its data were not included in the analysis. One calf in the placebo group and 1 calf in the SAL group became sternally recumbent in the squeeze chute during the dehorning procedure in period 2; however, this did not influence the chute exit velocity. No evidence of an effect of castration and dehorning on exit velocity was detected across treatment groups (P = 0.65). Exit velocity was greater after catheterization (1.53 ± 0.09 m/s) than in period 1 (0.99 ± 0.09 m/s) and period 2 (0.99 ± 0.09 m/s; P < 0.001). Compared with placebo-treated calves (1.69 ± 0.15 m/s), calves that received XKB alone (0.85 ± 0.14 m/s; P = 0.002) or SAL+XKB (0.86 ± 0.15 m/s; P = 0.002) took longer to exit the chute. However, there was no evidence of a difference in exit velocity between SAL (1.29 ± 0.15 m/s) and placebo-treated calves (P = 0.23).

EDA—The EDAs of the 4 treatment groups over time were summarized (Figure 2). A treatment effect...
(P = 0.017) was detected; specifically, the EDA of calves treated with XKB (from 10 to 50 minutes and 1.5 hours after actual castration and dehorning) and SAL+XKB (10 minutes to 1.5 hours) were significantly (P < 0.050) lower, compared with EDAs in the other treatment groups. There was also a significant (P < 0.001) difference in EDA depending on the point measured after treatment. A significant (P = 0.001) difference existed between phase number and time of EDA recording. However, there was a significant (P < 0.001) difference between treatment group and time of EDA recording. Because of the large variability in individual serum cortisol concentrations in calves receiving XKB in period 1, compared with concentrations in calves not receiving XKB, the difference in mean serum cortisol concentrations between these 2 groups was nonsignificant (P = 0.384). However, the cortisol T<sub>max</sub> for calves in the SAL+XKB group was significantly less than that in the placebo (P = 0.015) and XKB (P = 0.006) groups during period 2 (Figure 5). The difference in cortisol C<sub>max</sub> between calves treated with or without XKB during period 2 was nonsignificant (P = 0.254; Figure 6), as was the difference between compared in the following manner: all calves receiving XKB (XKB vs SAL+XKB), no XKB (placebo vs SAL), all calves receiving SAL (SAL vs SAL+XKB), and no SAL (placebo vs XKB). Compared with values in period 1, cortisol T<sub>max</sub> was significantly (P < 0.001) shorter and cortisol C<sub>max</sub>, AUEC<sub>0–1 h</sub>, AUEC<sub>1–6 h</sub>, and AUEC<sub>6–24 h</sub> were significantly (P < 0.001) greater in period 2.

Serum cortisol concentration—Mean ± SEM serum cortisol concentrations measured during periods 1 and 2 were graphically displayed (Figures 3 and 4). All parameters (C<sub>max</sub>, T<sub>max</sub>, AUEC<sub>0–1 h</sub>, AUEC<sub>1–6 h</sub>, and AUEC<sub>6–24 h</sub>) for serum cortisol concentration were significantly (P < 0.001) different in period 2 versus period 1. Unless otherwise indicated, for cortisol analysis, groups were

Figure 6—Mean ± SEM C<sub>max</sub> for serum cortisol concentrations in calves treated with 0.05 mg of xylazine/kg, 0.1 mg of ketamine/kg, and 0.025 mg of butorphanol/kg administered IM (XKB; n = 20); not treated with XKB (20); treated with 2.5 to 5 mg of SAL/mL administered PO through free-choice water consumption (SAL; 20); and not treated with SAL (20) after sham castration and sham dehorning (period 2). The T<sub>max</sub> values of serum cortisol concentrations with different letters differ significantly (P < 0.05).

Figure 7—Mean AUECs for serum cortisol concentrations in calves treated with 0.05 mg of xylazine/kg, 0.1 mg of ketamine/kg, and 0.025 mg of butorphanol/kg administered IM (XKB; n = 20); not treated with XKB (20); treated with 2.5 to 5 mg of SAL/mL administered PO through free-choice water consumption (SAL; 20); and not treated with SAL (20) during the first hour (black portion of bars), 1 through 6 hours (gray portion), and 6 through 24 hours (hatched portion) after sham castration and sham dehorning (period 1) and actual castration and dehorning (period 2). The AUECs with different letters within the same time period differ significantly (P < 0.001).

Table 1—Mean AUEC for serum cortisol concentrations at various intervals in calves treated with saline (0.9% NaCl) solution administered IM (placebo; n = 10); 2.5 to 5 mg of SAL/mL administered PO through free-choice water consumption (SAL; 20); 0.05 mg of xylazine/kg, 0.1 mg of ketamine/kg, and 0.025 mg of butorphanol/kg administered IM (XKB; 10); or a combination of SAL and XKB (SAL+XKB; 10) during sham castration and sham dehorning (period 1) and actual castration and dehorning (period 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>AUEC&lt;sub&gt;0–1 h&lt;/sub&gt; (nmol/L)</th>
<th>AUEC&lt;sub&gt;1–6 h&lt;/sub&gt; (nmol/L)</th>
<th>AUEC&lt;sub&gt;6–24 h&lt;/sub&gt; (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>1</td>
<td>92.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>152.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>597.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>132.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>342.90&lt;sup&gt;d&lt;/sup&gt;</td>
<td>756.28&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAL</td>
<td>1</td>
<td>84.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>119.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>434.29&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>119.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>216.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>583.64&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>XKB</td>
<td>1</td>
<td>42.10&lt;sup&gt;e&lt;/sup&gt;</td>
<td>123.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>574.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>111.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>216.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>583.64&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAL+XKB</td>
<td>1</td>
<td>46.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>131.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>455.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>104.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>259.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>637.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Within columns, means with different superscript letters differ significantly (P < 0.05).
calves that did or did not receive SAL during period 2 (P = 0.343).

The AUEC estimates for serum cortisol concentration in calves that received XKB and calves that received SAL were compared at 3 distinct intervals (ie, from 0 to 1 hours after sham or actual surgery [AUEC0–1h], 1 to 6 hours afterward [AUEC1–6h], and 6 to 24 hours afterward [AUEC6–24h]; Figure 7; Table 1). A period effect was detected between periods 1 and 2 for all 3 measurement intervals. For AUEC0–1h, the value was significantly (P = 0.007) less during period 2 in all calves that received XKB than in all those that did not receive XKB.

Furthermore, the AUEC0–1h of the XKB-only group was significantly lower than that of the placebo (P = 0.016) or SAL groups (P = 0.042) during period 2. A significant difference was not detected for AUEC1–6h (P = 0.389) and AUEC6–24h (P = 0.208) between all calves that received XKB and those that did not. The difference in AUEC0–1h between calves that did and did not receive SAL was nonsignificant (P = 0.872) during period 2; however, the AUEC1–6h was significantly (P = 0.024) less during period 2 for all calves that received SAL. Additionally, AUEC6–24h was significantly less in the SAL-only group than in the placebo (P = 0.030) and XKB groups (P = 0.028) during period 2. There was also a lower AUEC6–24h for the combined SAL groups, compared with value for the combined XKB groups in period 2; however, the difference was not significant (P = 0.064).

XKB pharmacokinetic parameter estimates—Pharmacokinetic parameter estimates (Tmax, Cmax, AUC, volume of distribution per fraction of drug absorbed, total body clearance per fraction of drug absorbed, MRT, and terminal elimination half-life time) for XKB were determined by noncompartmental analysis and summarized (Table 2). Additionally, the plasma pharmacokinetic profiles were summarized (Figures 8 and 9). The volume of distribution per fraction of drug absorbed was significantly (P = 0.045) greater in the SAL+XKB group, compared with that in the XKB group.

SAL pharmacokinetic parameter estimates—The Tmax, Cmax, AUC, and mean plasma drug concentration were determined for SAL and SAL+XKB and summarized (Table 3). Plasma salicylate concentration was graphically displayed (Figure 10). Calves in the SAL and SAL+XKB group received doses of SAL that ranged from 13.62 to 151.99 mg of salicylate/kg from 24 hours prior to period 1 to 48 hours after period 2.

Discussion
As concern for improving the welfare of livestock increases, the need for pain management research in cattle becomes more necessary. The objective of the study reported here was to determine the pharmacokinetic parameters of XKB administered IM and SAL administered PO and to compare their effect on bio-

Table 2—Mean ± SEM pharmacokinetic estimates derived from noncompartmental analysis of data from calves treated with 0.05 mg of xylazine/kg, 0.1 mg of ketamine/kg, and 0.025 mg of butorphanol/kg administered IM (XKB; n = 10) immediately prior to castration and dehorning.

<table>
<thead>
<tr>
<th>Variable</th>
<th>XKB</th>
<th>SAL+XKB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmax (min)</td>
<td>96.40±20.3a</td>
<td>122.47±24.90a</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>20.95±1.68a</td>
<td>19.50±2.07a</td>
</tr>
<tr>
<td>AUC0–∞ (µg·min/mL)</td>
<td>16.68±1.44a</td>
<td>17.48±1.29a</td>
</tr>
<tr>
<td>Vz/F (L/kg)</td>
<td>6.70±1.09a</td>
<td>8.27±1.54a</td>
</tr>
<tr>
<td>CL/F (mL/min/kg)</td>
<td>52.68±4.88a</td>
<td>49.30±2.72a</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>99.31±18.15a</td>
<td>120.03±22.48a</td>
</tr>
</tbody>
</table>

AUC0–∞ = Area under the curve from 0 to infinity, CL/F = Total body clearance per fraction of drug absorbed, Tmax = Terminal elimination half-life, Vz/F = Volume of distribution per fraction of dose absorbed.

Within a row, estimates with different superscript letters differ significantly (P < 0.05).
markers of pain and distress following sham (period 1) and actual (period 2) castration and dehorning. Results indicated that the treatment of cattle prior to castration and dehorning with SAL alone or in combination with XKB increased ADG and decreased circulating cortisol concentrations. Currently, protocols for the provision of analgesic treatment are not routinely used during most routine animal husbandry procedures. In a survey of bovine practitioners, 21% of US veterinarians reported using analgesia at the time of castration. In a similar Canadian survey, 6.9% of beef calves and 18.7% of dairy calves (both < 6 months old) reportedly received treatments to provide pain relief during castration and dehorning with SAL alone or in combination with XKB.

In a survey of dairy practices in the Northeastern and Central United States, 12.4% of dairy personnel administered an anesthetic at the time of dehorning and 1.8% provided analgesic treatment. This may be due to the absence of FDA-approved, long-acting, cost-effective analgesic drugs with established withdrawal times.

Studies designed to examine the combined effect of castration and dehorning are scarce, although 90% of veterinarians responding to a survey reported castrating and dehorning calves concurrently. Several studies have measured short-term changes in serum cortisol concentration as a method to determine the extent and duration of distress associated with castration or dehorning in cattle. In a previous study involving 2- to 4-month-old untreated bull calves, a mean peak serum cortisol concentration of 68 nmol/L was reported within 30 minutes after surgical castration and the duration of increase in serum cortisol concentration from the pretreatment value was > 4 hours. During a study involving 3-month-old calves dehorned with a Barnes dehorner, the mean serum cortisol concentration increased to 76 nmol/L within 0.5 hours after dehorning, decreased to 45 nmol/L between 1.5 to 2.5 hours after dehorning, and decreased further to pretreatment concentrations within 4.5 to 8 hours after dehorning.

In the present study, the mean serum cortisol concentration of calves in the placebo group ranged from 141.46 to 34.94 nmol/L at 20 and 360 minutes after castration and dehorning, respectively. These values are higher than those reported for some studies in which castration or dehorning was performed alone. The increase in serum cortisol likely reflects the cumulative stress of performing castration and dehorning procedures in series; however, this difference could also be attributed to differences in trial design or variability in cortisol response between animals.

The development of a drug regimen to reduce the decrease in ADG after painful management procedures would make such practices practical and desirable for cattle producers. Furthermore, possible production benefits resulting from that increase in ADG would likely make the addition of analgesic treatments to castration and dehorning protocols more cost-effective. Research reveals that use of analgesics and anesthetics influences feed intake and weight gain after painful procedures. For example, investigators found calves treated with local anesthesia during surgical castration, but not burdizzo castration, had a greater ADG than cattle castrated without a local anesthetic. Another study revealed that calves treated with ketoprofen prior to and 2 to 7 hours after dehorning, in addition to treatment with xylazine and lidocaine (administered as a local anesthetic at the time of the procedure), gained more weight (1.2 ± 0.4 kg) than control calves that received only a local anesthetic or xylazine and lidocaine during the first 24 hours after dehorning.

The period effect on serum cortisol concentration could be attributed to pain associated with castration and dehorning, which caused a greater physiologic rise in concentrations during period 2 than in period 1. It should be considered that an increase in serum cortisol concentration is not necessarily attributable to painful stimuli but may also increase when an animal is distressed. This was demonstrated in period 1 as an increase in cortisol values at the time of sham castration.
and dehorning; however, that increase was not as great as the increase in period 2. In a previous dehorning study, serum cortisol concentrations were reportedly increased 2-fold in response to distress caused by handling and peak 4- to 5-fold in response to dehorning with rechargeable or conventional electric dehorners. In the present study, cortisol concentrations increased 3-fold from time 0 to reach the C\text{max} in period 1 in response to sham castration and dehorning across all treatment groups and approximately 4-fold in period 2 in response to castration and dehorning.

Investigations of the effect of extended administration of an analgesic and anti-inflammatory compound on ADG in livestock undergoing painful procedures are lacking. The results of the study reported here support the hypothesis that extended exposure to an NSAID in painful situations may be beneficial because ADG was significantly greater for 13 days after castration and dehorning in calves receiving XKB in drinking water compared with ad libitum. This effect may in part be attributable to prolonged analgesic effects by the drug but may also be due to anti-inflammatory effects. Additional research on the effectiveness of analgesics on feed intake and ADG over a prolonged period after castration and dehorning would be beneficial. This research could determine whether analgesia impacts final market weight or cost in feed to compensate for loss in ADG after painful procedures.

Assessment of chute exit velocity has typically been used in studies of temperament in cattle. A study of the effect of injections and handler visibility on chute exit velocity found no correlation between the 2 events. The hypothesis that painful procedures, such as castration and dehorning, are associated with faster chute exit velocity has not been tested. However, chute activity during castration appears to decrease after the administration of butorphanol and xylazine. Results of the present study indicated that chute exit velocity was indeed reduced in calves receiving XKB, particularly during period 1. This can most likely be attributed to the sedative effects of XKB resulting in a slower reaction time exiting the chute than in the SAL and placebo groups. However, there was no significant difference between period 1 and 2 in any treatment group. This suggests that chute exit velocity may not be a specific indicator of pain and distress, particularly in acclimated Holstein calves.

Electrodermal activity is the measurement of the electrical resistance between 2 electrodes applied to the skin. It can be influenced by changes in resistance as a result of changes in sympathetic outflow. Devices are available to measure EDA, although there is a paucity of data to support this use in livestock species. In rats, EDA measurement provides an inaccurate assessment of postoperative pain because pain scores do not decrease with increasing doses of analgesics. In the present study, a significant decrease in EDA values coinciding with the presence of quantifiable plasma drug concentrations was observed in calves that received XKB. After 90 minutes, EDA increased and was not significantly different from values in other treatment groups. It is noteworthy that a difference in EDA between study periods was not observed. Therefore, EDA measurement was not a reliable indicator of pain associated with dehorning and castration in the calves in our study.

The observed differences in EDA in the XKB-treated calves were likely due to the \( \alpha_2 \)-adrenergic receptor agonist effect of xylazine on eccrine sweat gland output and to the sedative effect of this combination. The nasal planum of calves in which the EDA measurements were obtained contains a dense population of serous nasolabial glands, or eccrine glands. Unmyelinated post-ganglionic sympathetic axons surround eccrine sweat glands, which secrete water, electrolytes, and mucin when stimulated. Therefore, these alterations in electrolyte secretion likely changed the conductivity of the skin in XKB-treated calves and thus the EDA measurements. Similarly, differences between phases during recording times were likely due to fluctuations in ambient temperature or humidity between days of the study or individual variation among calves; however, this was not investigated.

In the present study, XKB, SAL, or both were used. Butorphanol is an opioid drug that has partial receptor agonist-antagonist effects. Butorphanol provides analgesia by binding to \( \kappa \) (partial agonist) and \( \mu \) (agonist) receptors. When combined with xylazine, butorphanol lowers the dose required to provide analgesia and enhances the sedative effect. The combined effect of xylazine and butorphanol on pain associated with dehorning was investigated in a dehorning study, revealing that coadministration of the drugs alone or in combination with a cornual nerve block significantly decreased the change in mean circulating cortisol concentration immediately after dehorning, compared with the change in untreated calves. Xylazine has sedative and analgesic effects when administered to cattle at doses ranging from 0.05 to 0.3 mg/kg. Ketamine is an \( N \)-methyl-D-aspartate receptor antagonist that can yield analgesic and dissociative effects when administered IV to calves at doses ranging from 2 to 4 mg/kg. A combination of low-dose xylazine (0.02 to 0.05 mg/kg), ketamine (0.04 to 0.1 mg/kg), and butorphanol (0.02 to 0.05 mg/kg) administered IV or IM in cattle is reported to provide mild sedation without the adverse effect of recumbency.

Plasma cortisol concentrations reach a peak within 30 minutes after dehorning, after which values decrease to a plateau concentration that persists for 5 to 6 hours. Therefore, we chose to examine cortisol concentrations from 0 minutes to 1 hour because this coincided with peak cortisol concentrations and peak XKB concentrations. In the present study, XKB was rapidly absorbed following IM administration and achieved a peak concentration approximately 10 minutes after administration. The administration of this drug combination appeared to attenuate the increase in cortisol concentration during castration and dehorning from 0 minutes to 1 hour after treatment. Therefore, treatment with XKB is likely to be more effective than placebo or SAL alone for controlling short-term distress associated with castration and dehorning.

The effects of XKB are short-lived; therefore, it was not surprising that the effects of the coadministration of XKB on serum cortisol concentration did not persist > 1 hour. An IV dose of 0.2 mg of xylazine/kg
was associated with a peak plasma xylazine concentration of 1.050 µg/mL, an absorption t<sub>1/2</sub> of 36.48 minutes, and a total body clearance of 42 mL/min/kg. Ketamine administered IV in calves had a t<sub>1/2</sub> of 60.3 ± 5.4 minutes and a total body clearance of 40.39 ± 6.6 mL/min/kg. In addition, IV administration of ketamine in mature Holstein cows at a dose of 5 mg/kg results in the following pharmacokinetic parameters: C<sub>max</sub> of 18.13 ± 22.720 ng/mL, T<sub>max</sub> of 0.083 hours, AUC of 4.848 ± 1,398 ng·h/mL, and elimination t<sub>1/2</sub> of 1.80 ± 0.0 hours. In a previous study, dairy cows that received 0.25 mg of butorphanol/kg IV had a t<sub>1/2</sub> of 82.0 minutes, total body clearance of 34.6 ± 77 mL/kg/min, and mean AUC of 7,567 ± 54 ng·min/mL. In the present study, the t<sub>1/2</sub> was 109.4 ± 22.62 minutes for xylazine, 81.45 ± 10.44 minutes for ketamine, and 71.28 ± 7.64 minutes for butorphanol. Differences between the present and previous studies include lower drug dosages and a longer t<sub>1/2</sub> of drugs used (with the exception of butorphanol, which had a shorter t<sub>1/2</sub>). Total body clearance for all 3 drugs was also found to be greater than in previous studies. The T<sub>max</sub> for ketamine in the present study was also longer than values reported previously.

More variability between period 1 and period 2 was evident in the present study for the T<sub>max</sub> of serum cortisol concentration. This variability was most likely the result of individual calf variability in response to treatment with XKB. A previous study involving 4- to 6-month-old bull calves found no significant difference in T<sub>max</sub> for serum cortisol concentration between calves surgically castrated versus those undergoing simulated castration. Another study found a significantly longer T<sub>max</sub> in calves blocked with 11 mL of lidocaine in the spermatic cord or a caudally administered epidural injection with xylazine (0.05 mg/kg) and lidocaine HCl (0.4 mg/kg), compared with burdizzo castration without analgesia and burdizzo castration following administration of ketoprofen (3 mg/kg, IV). The T<sub>max</sub> might be shorter during painful procedures because a painful stimulus would quickly increase cortisol secretion, and the T<sub>max</sub> was shorter in period 2 versus period 1 for calves that received SAL but not for any other treatment group. Research into the effects of salicylic acid derivatives (ie, salicylate) on the change in biomarkers of pain after castration and dehorning is deficient in the literature. The only study to date that involved SAL administration during castration found a bolus of SAL (50 mg/kg, IV) administered to calves prior to castration reduced the cortisol C<sub>max</sub>, compared with the value in calves receiving aspirin (acetylsalicylic acid) PO immediately prior or with calves left untreated before castration. The efficacy of other NSAIDs (eg, carprofen) in minimizing increases in serum cortisol concentration that are caused by castration and dehorning has not been established. Various concentrations of ketoprofen administered IV to cattle prior to castration fail to reduce the initial peak in serum cortisol concentration that is associated with castration; however, serum cortisol concentrations from 2 to 6 hours after castration were significantly reduced. Treatment with SAL (SAL and SAL+XKB groups) in our study resulted in a decrease in serum cortisol concentrations from 1 to 6 hours after castration and dehorning. However, we could not establish whether the same effect would have been observed following oral administration of a single dose of SAL as opposed to multiple dosing during the sham and castration and dehorning periods. The AUEC for serum cortisol concentration was examined from 1 to 6 hours after the sham and actual procedures because this coincided with a previously described plateau phase in which the effect of SAL should predominate. This decrease in concentration supports salicylate as having analgesic and anti-inflammatory properties. It can be concluded that although SAL may not provide immediate analgesia at the time of a painful procedure, at the dosing regimen described here, it may provide analgesia and reduce inflammation for several hours after painful procedures. Furthermore, this effect could have future implications for the use of SAL in chronic pain management. Research will be necessary to determine the duration of treatment to minimize the cost and maximize the efficiency of treatment with SAL in drinking water.

Limited research has been conducted to establish estimates of the pharmacokinetic parameters of salicylate administered PO in cattle. The bioavailability of salicylate when administered PO in cattle is reportedly 61.05%. One study found that SAL administered IV at 50 mg/kg at the time of castration in calves attenuated peak cortisol response when plasma drug concentrations were > 25 µg/mL. In the present study, mean plasma salicylate concentrations at the time of castration and dehorning were > 25 µg/mL (SAL, 40.36 µg of SAL/mL; SAL-XKB, 53.11 µg of SAL/mL). Therefore, the observed attenuation of the cortisol response in the present study was in agreement with results of previous studies. The consumption of SAL-treated water by calves in the SAL and SAL+XKB groups after castration and dehorning on day 0 (period 2) at 72 hours after initiation of SAL treatment decreased markedly. However, the mean plasma drug concentration of salicylate remained > 25 µg/mL in most calves until treatment with SAL ceased on day 2. This was likely due to constant access to medicated water as well as dose accumulation resulting from the plasma elimination t<sub>1/2</sub> of 4.31 ± 0.42 hours as previously reported for SAL administered PO.

Compounded drugs used in studies must have documented tissue residue information, including withdrawal times as well as concentration, carrier, and stability data. Under the AMDUCA, ELDU is permitted for relief of suffering in cattle, provided specific conditions are met. These conditions include that ELDU is permitted only by or under the supervision of a veterinarian, such use is allowed only for FDA-approved animal and human drugs and is only permitted when the health of the animal is threatened and not for production purposes, ELDU in feed is prohibited, and ELDU in general is not permitted if it results in a violative food residue. Although salicylic acid derivatives are marketed for use in cattle and swine in the United States, the use of salicylate in the manner conducted in the present study would be considered compounding because there are no FDA-approved aspirin or SAL formulations available for use in animals. Aspirin has a recommended meat and milk withdrawal time of 24 hours. Further studies are needed to evaluate tissue residues when SAL is used as described for the present
study. Xylazine administered at a dose of 0.05 to 0.30 mg/kg IM has a recommended withdrawal time of 4 days in meat and 24 hours in milk.51 The Food Animal Residue Avoidance Databank has suggested that withdrawal times for ketamine at dosages up to 10 mg/kg IM be 3 days for meat and 48 hours for milk.52 Butorphanol has a suggested withdrawal time of 48 hours.57

In the study reported here, castration and dehorning in series was associated with an increase in plasma cortisol concentration in excess of values previously13,24 reported for either castration or dehorning in Holstein calves. Co-administration of XKB alone or in combination with salicylate in drinking water attenuated the cortisol response after castration and dehorning. Furthermore, ADG in calves that received free-choice salicylate was significantly greater than in calves in the placebo and XKB groups, suggesting NSAID treatment in water may mitigate negative performance effects associated with castration and dehorning in calves. Chute exit velocity was not a specific indicator of pain and distress associated with castration and dehorning; however, administration of XKB significantly decreased chute exit velocity. Electrodermal activity measurement was not a specific indicator of pain associated with dehorning and castration, but EDA measurement may be influenced by pharmacological effects that were unrelated to analgesic activity in calves. These findings suggest that administration of SAL through drinking water may provide long-term performance benefits.

References


