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A study to compare circulating flunixin, meloxicam and gabapentin concentrations with prostaglandin E_2 levels in calves undergoing dehorning

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A B S T R A C T

The purpose of this study was to investigate the pharmacokinetics of intravenous flunixin (2.2 mg/kgb.w.), oral meloxicam (1 mg/kg b.w.), oral gabapentin (15 mg/kg b.w.) alone or co-administrated with meloxicam as well as the effects of these compounds on prostaglandin E_2 (PGE_2) synthesis in calves subjected to surgical dehorning. Plasma samples collected up to 24 h after drug administration were analyzed by liquid chromatography/mass spectrometry, whereas blood PGE_2 levels were measured by immunoenzymatic assay. In plasma, the terminal half-live of flunixin, meloxicam and gabapentin were 6.0 h (range, 3.4-11.0 h), 16.7 h (range, 13.7-21.3 h) and 15.3 h (range, 11-32.9 h), respectively. The co-administration of single doses of gabapentin and meloxicam did not seem to affect the pharmacokinetic profile of the two drugs except for gabapentin that reached significantly ($P < 0.05$) higher maximum serum concentration (C_{max}) when co-administered with meloxicam, than when administered alone. At 5, 360 and 720 min after dehorning, a significant ($P< 0.01$) decrease in PGE_2 concentration was observed in flunixin-treated animals compared with control calves. Moreover, circulating log PGE_2 concentrations were inversely proportional to log flunixin concentrations ($R^2 = 0.75$; $P < 0.0001$). None of the other drugs significantly affected blood PGE_2 levels. Further assessment of oral meloxicam and gabapentin in established pain models is required to formulate science based analgesic recommendations to enhance animal well-being after dehorning.

1. Introduction

Dehorning is one of the most common livestock management procedure performed in the United States with approximately 4 million calves dehorned every year (NASS National Agricultural Statistics Service, 2010). Handling and management of horned animals can negatively impact human and animal safety. Horned dairy cows pose a risk for stockpersons during routine management practices (milking, hoof trimming, calving) and veterinary examinations (Gottardo et al., 2011). Moreover, horned animals can cause injury to herdmates during aggressive interactions and competition during feeding (AVMA, 2006). Therefore, horn buds of young dairy and beef calves are normally removed to reduce the risk of injuries to farm workers or to other cattle. Physiological and behavioral studies indicate that, regardless of the method used, typical procedures used for dehorning are painful for at least 2 h after surgery (Petrie et al. 1996) and potentially as long as 24 h or 44 h (Faulkner and Weary, 2000; Heinrich, 2007). Pain associated with dehorning has been quantified through behavioral measures, such as ear flicks, head rubs, changes in posture (Grandahl-Nielsen et al., 1999; Faulkner and Weary, 2000), and magnitude and duration of physiological stress responses, such as serum cortisol concentration (Petrie et al., 1996; Graf and Senn, 1999; Heinrich, 2007), heart and respiratory rates (Grendahl-Nielsen et al., 1999; Heinrich, 2007; Stewart et al., 2008), corneal temperature (Stewart et al., 2008) and electroencephalogram (Gibson et al., 2007). Despite the fact that this procedure is painful, providing pain relief is uncommon due to a number of factors, including cost, tradition, lack of knowledge, and fear of drug residues in meat (Stafford et al., 2006). Also within Europe, the dehorning of cattle is a very common procedure. The outcomes of a recent study performed by Gottardo et al. (2011) showed that dehorning was carried out on 80% of the 639 surveyed farms. Fifty-two percent of the farmers reported that this practice causes prolonged postoperative pain but pain management is rare. Indeed, in several Member States livestock mutilation such as dehorning is regulated by European Council Directive 98/58/EC (1998). According to this regulation owners and keepers have to take all reasonable steps to ensure that animals are not caused any unnecessary pain, suffering and injury. It has been postulated that the low motivation toward the adoption of practices able to reduce pain might arise from the limited willingness to cover the costs for analgesia or the support of a veterinarian.

In the US, a further barrier is the fact that there are no analgesics specifically approved for pain relief in livestock and this unavailability represents a substantial obstacle in veterinary clinical practice. Under the Animal Medicinal Drug Use Clarification Act (AMDUCA) extra-label drug use (ELDU) is permitted, under the supervision of a veterinarian and only for FDA-approved animal and human drugs, when the health of the animal is threatened. ELDU is not permitted if it results in violative food residues. By contrast, in the European Union flunixin is approved for adjunctive therapy of respiratory disease and pain relief when administered at 2.2 mg/kg IV, and meloxicam is approved for adjunctive therapy of acute respiratory disease, diarrhea, acute mastitis and pain relief at 0.5 mg/kg IV or SC.

In order to facilitate drug approvals, reliable and accurate measures of pain are needed. Additionally, it

is also crucial to understand the relationship between analgesic drug regimens and the suppression or mitigation of pain.

Several studies have demonstrated that the administration of nonsteroidal antiinflammatory drugs (NSAIDs) before dehorning decreases plasma cortisol response (Milligan et al., 2004; Baldridge et al., 2011; Coetzee et al., 2011). Furthermore, NSAID administration while dehorning has been shown to have positive effects on calf behavior (Theurer et al., 2012).

Nonsteroidal anti-inflammatory drugs prevent inflammation by inhibiting cyclooxygenase (COX), the enzyme involved in the synthesis of prostanoids. Prostanoids are synthetized from arachidonic acid *via* the COX pathway, and largely contribute to the outset of inflammatory signs and pain (Atsufumi, 2011). There are two primary isoforms of COX enzymes: COX-1 and COX-2. Prostaglandins associated with the COX-1 isoform mainly regulate processes such as maintenance of the gastrointestinal tract, renal function and other homeostatic processes (Curry et al., 2005). Prostaglandins associated with the COX-2 isoform are mainly associated with pain and inflammation that result from tissue injury, although they are also constitutively expressed in the gastrointestinal tract and kidneys of different animal species (Radi, 2009; Kukanich et al., 2012).

Among prostanoids, prostaglandin E_2 (PGE_2) and, perhaps PGI_2, have the greatest impact on processing of pain signals (Takada et al., 2007). In injured tissues COX-2 expression is increased producing PGE_2 that result in sensitization of peripheral nociceptors coupled with enhanced pain transmission (KuKanich et al., 2012). Nonsteroidal anti-inflammatory drugs reduce production of prostanoids including PGE_2 both in the spinal cord and at the periphery by inhibiting COXs, thereby diminishing inflammatory and/or postoperative pain (Atsufumi, 2011). Flunixin meglumine is the only NSAID approved by the US Food and Drug Administration for use in cattle, and its approval is limited in treating symptoms of fever associated with mastitis and respiratory disease (Coetzee, 2011). Any administration of flunixin meglumine expressly for pain relief in cattle thus represents an *extra-label* use with its concomitant liability. Flunixin meglumine is a nonspecific COX inhibitor with a half-life of approximately 6-7 h in cattle (Landoni et al., 1995). By contrast, meloxicam preferentially inhibits the COX-2 isoform in some species, namely dog and horse (Streppa et al., 2002; Beretta et al., 2005). Little information concerning the inhibitory effects of meloxicam on COX-1 and COX-2 isoforms in cattle is available. In a study performed by Coetzee et al. (2009) it has been observed that, when administered orally, the drug has a half-life of approximately 27 h in cattle. These findings suggest that oral meloxicam administration may provide effective, long-lasting analgesia in ruminants thus representing a practical and cost-effective way for veterinarians to reduce pain associated with dehorning. However, as the drug is not licensed for use in cattle in the United States, meloxicam administration by any route represents an extralabel drug use.

Gabapentin [1-(aminomethyl) cyclohexane acetic acid] is a novel anti-epileptic agent, originally developed as a gamma-amino-butyric acid (GABA)-mimetic compound to treat spasticity, and it has been shown to have anticonvulsive effects not only in humans (Taylor, 1993; Satzinger, 1994) but also in dogs

(Govendir et al., 2005), rats (Mares and Haugvicova, 1997) and mice (Akula et al., 2009). Subsequent studies have established that gabapentin is also effective for the management of chronic pain of inflammatory or neuropathic origin (Hurley et al., 2002). It has also been reported that gabapentin can interact synergistically with NSAIDs to produce anti-hyperalgesic effects (Hurley et al., 2002; Picazo et al., 2006). Although gabapentin is commonly prescribed for the treatment of chronic pain in humans, there is a paucity of trials investigating its pharmacokinetics and effects in cattle (Vinuela-Fernandeza et al., 2007; Coetzee et al., 2011; Malreddy et al. 2012). Pharmacokinetics information of flunixin meglumine, meloxicam and gabapentin associated with painful procedures such as dehorning are needed to provide useful information regarding the potential use of these drugs to relieve pain in ruminants. Furthermore, the use of prostaglandin levels as bio-markers for NSAID-induced COX-inhibition (Landoni et al., 1995; Königsson et al., 2003; Lees et al., 2004; Takada et al., 2007) may provide useful information about the pharmacodynamic effects of these drugs.

The aim of the present study was twofold: (1) to assess any potential effects of the above mentioned drug treatment on PGE_2 blood levels in cattle undergoing dehorning; (2) to investigate the pharmacokinetic profile of flunixin meglumine, meloxicam and gabapentin (alone or in combination with meloxicam) in cattle undergoing dehorning.

2. Materials and methods

All experimental procedures were performed with the permission of the Institutional Animal Care and Use Committee (IACUC) at the Kansas State University.

2.1. Animals

All animals were housed at the Kansas State University Beef Cattle Research Center, Manhattan, KS. Thirty-five Holstein steer calves aged 6 months, with an average weight of 169.5 kg, were enrolled in the study. All calves received a single subcutaneous dose (30 mg/kg) of oxytetracycline 300 mg/mL (Noromycin™ 300 LA, Norbrook Inc., Lenexa, KS), a single subcutaneous dose (5 mL) of an eight-way clostridial vaccine (Covexin® 8, Intervet/Scher-ing-Plough Animal Health, Summit, NJ), and a single subcutaneous dose (2 mL) of a bovine rhinopneumonitis preventative (Bovi-Shield GOLD® 5, Pfizer Animal Health, New York, NY). A topical pour-on comprised of 5% permethrin and 5% piperonylbutoxide (Ultra Boss® Pour-On Insecticide, Intervet/Schering-Plough Animal Health, Summit, NJ) was applied to all calves upon arrival and repeated as needed for fly control. Calves were acclimated for 19 days. Animals were considered healthy based on a physical examination performed by a veterinarian. Horn circumference and length were recorded for each calf.

2.2. Housing and husbandry

Calves were housed in identical 36.52 m^2 pens with concrete floors in groups of five. One half of each

pen was shaded by mesh shade cover. Calves were fed 10.10 kg of a growth diet at 1:00 PM each day. The diet was composed of 47.15% brome hay, 18.61% dried distillers grains with solids, 13.37% steep-monocalcium phosphate, 9.42% soybean hull, 7.98% dry rolled corn, 2.10% vitamin premix, and 1.38% mineral supplement. Water was offered *ad libitum* via an automatic waterer.

2.3. Jugular catheterization

Approximately 24 h before the beginning of the study, each calf was restrained for catheter placement. The area over the jugular vein was clipped and disinfected using 70% isopropyl alcohol and povidone iodine swabs. A 14G x 140 mm jugular catheters (Abbo-cath-T (305 mL/min), Abbott Ireland, Sligo, Republic of Ireland) with 76 cm extension sets (Extension Set with Slip Luer Slide Clamp, Abbott Ireland, Sligo, Republic of Ireland) were placed on the shaved left side of the neck of all calves. Prior to catheter placement, 0.5 mL of 2% lidocaine hydrochloride (Lidocaine HCl 2% (20 mg/mL), Hospira Inc., Lake Forest, IL) were injected into the neck to provide analgesia before stab incision with a #22 scalpel blade and catheter insertion. Catheter patency was maintained using a heparinized saline (0.9% NaCl) flush solution (3 U of heparin sodium/mL of saline solution). Thereafter, calves were catheter-ized one day prior to their assigned study days following the above outlined procedure.

2.4. Assignment to groups and drug administration

Thirty-five steers were randomly assigned to one of five treatment groups (n = 7): control (CONT; saline); meloxicam (MEL; 1mg/kgb.w.); gabapentin (GABA; 15 mg/kgb.w.); meloxicam and gabapentin (MELGABA; 1 mg/kg, and 15 mg/kgb.w., respectively); flunixin (FLU; 2.2 mg/kgb.w.). All steers were then given a random number using Excel random number generator so as to determine the order in which they would be dehorned. The dehorning procedure was recorded as d0. Weights were obtained one day (d-1) prior to dehorning in order to calculate individual dosages. Meloxicam and gabapentin were administered *per os* (PO) while flunixin was given IV (average 7.55 mL). Each steer received one oral dose and one intravenous (IV) dose, thus each received either a placebo bolus and IV drug or a drug bolus and IV placebo. Boluses (Porcine Hard Gelatin Capsules, Torpac, Inc., Fairfield, NJ) were prepared manually by opening a capsule and filling it full with whey (Spray Dried Pasteurized Whey, Kraft, Hartford, CA). If the bolus was to contain meloxicam and/or gabapentin, the whey and pills were placed in layers in the bolus so as to completely mask the pills in the bolus. The IV syringes (Monoject™ 12 mL syringes, Covidien, Mansfield, MA) were prepared with either the flunixin dosage or a volume of saline equivalent to the volume of flunixin when administered at 2.2 mg/kg. Steers were subjected to the same handling procedures including an intravenous injection on the right side of the neck and oral dosage of the bolus using a balling gun. Calves in the MEL group were given meloxicam tablets (Meloxicam Tablets USP 15 mg [NDC 29300-125-01], Unichem Pharmaceuticals USA Inc. Rochelle Park, NJ; Lot # GMMH10078) at 1 mg meloxicam/kg b.w. *per os* and given saline IV. For the GABA group, gabapentin capsules (Gabapentin

Capsules 100 mg, Amneal Pharmaceuticals, Lot #HA39811; Gabapentin Capsules 300 mg, Greenstone Brand, Lot #V110200; Gabapentin Capsules Tablets 600 mg, Greenstone Brand, Lot #V110370) were administered at 15 mg gabapentin/kg b.w. *per os* as well as a dose of saline IV. Flunixin (Banamine - Flunixin Meglumine Injectable Solution, Schering-Plough Animal Health, Lot #0681105) was administered 2.2 mg flunixin/kg b.w. IV and a whey only bolus was administered to the FLU group *per os*. Steers placed in the control group received a bolus containing whey only *per os* and injection of saline IV. Drugs were administered by oral or IV routes immediately prior to dehorning.

2.5. Dehorning

On the day of the procedure calves were restrained in a chute with a head gate. 6 mL of a 2% lidocaine hydrochloride solution was administered around both corneal nerves 10 min before dehorning, prior to entering the chute. All animals were dehorned in a hydraulic, double-alley squeeze chute with a belly bar to prevent collapse (Daniels Manufacturing Co., Ainsworth, NE). Ten minutes after the application of local anaesthetic, both horns were removed with a Barnes-type scoop dehorner followed by cautery of the blood vessels via hot iron. Dehorning was performed by the same technician for all animals.

2.6. Collection of blood samples

Five milliliter blood samples were collected through a pre-placed jugular catheter (Abbocath-T (305 mL/min), Abbott Ireland, Sligo, Republic of Ireland) at baseline (12 min before drugs administration), 5 min, 360 min, 720 min and 24 h after drug administration for PGE_2 determination. Further 5 mL aliquots samples were taken at baseline (before drugs administration), 5 min, 30 min, 60 min, 120 min, 240 min, 360 min, 480 min, 720 min and 24 h and once a day for 7 days after treatment for pharmacokinetic parameter analysis. Samples were placed in sodium-heparinised and lithium-heparinised tubes (Vacuette 6 mL LH Tubes, Greiner Bio-One, Kremsmünster, Austria) for pharmacodynamic and pharmacokinetic assays, respectively. Blood samples collected into lithium heparin tubes were stored on ice for no longer than 30 min before incubation and analysis for PGE_2 concentration.

2.7. PGE_2 analysis

For each time point, 3 mL of whole blood were incubated in glass tubes for 24 h at 37 °C. At the end of incubation, all samples were centrifuged at 400g for 10 min at room temperature. The resulting supernatant was stored at —80 °C until the determination of plasma PGE_2 levels using a commercially available standard ELI-SA kit (Prostaglandin E_2 Kit, Cayman Chemical Company, Ann Arbor, MI) (Fuchs et al., 2002).

This assay is based on the competition between PGE_2 and a PGE_2-acetylcholinesterase (AChE) conjugate (PGE Tracer) for a limited amount of PGE Monoclonal Antibody. Because the concentration of the PGE_2 Tracer is held constant while the concentration of PGE_2 varies, the amount of PGE_2 Tracer that is able to

bind to the PGE_2 Monoclonal Antibody will be inversely proportional to the concentration of PGE_2 in the well. This antibody-PGE complex binds to goat polyclonal anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PGE_2 Tracer bound to the well, which is inversely proportional to the amount of free PGE_2 present in the well during the incubation. (Prostaglandin E_2 EIA Kit - Monoclonal. Cayman Chemical Item No. 514010. Kit booklet)

2.8. Plasma drug analysis

Plasma concentrations of meloxicam *(m/z* 352.09?1 14.90) and gabapentin (m/z 172.1?154.1) were determined with high-pressure liquid chromatography (Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, MD, USA) and mass spectrometry (API 2000, Applied Biosystems, Foster City, CA, USA). Plasma samples or standards (50 lL) were added to 200 lL of internal standard (piroxicam 0.5 lg/mL in methanol, *m/z* 332.12?95.10 and pregab-alin 5 lg/mL, *m/z* 160.00?142.00) in methanol with 0.1% formic acid to precipitate the proteins. The samples were vortexed for 5 s and centrifuged for 10min at 10,000xg. The supernatant, 200 lL, was transferred to an injection vial with the injection volume set to 10 lL. The mass spectrometer was set to multiple reaction mode (MRM), source temperature was 350 °C, spray voltage was +5000 V, curtain gas 10a.u., and the collision associated gas 12 a.u. The collision energy was 20,17,33, and 35 V for gabapentin, pregabalin, meloxicam, and piroxicam, respectively. The mobile phase consisted of A: acetonitrile and B: 0.1% formic acid at a flow rate of 0.4 mL/min. The mobile phase consisted of 100% B from 0 to 0.5 min with a linear gradient to 50% B at 2.5 min which was maintained until 5 min, followed by a linear gradient to 100% B at 5.5 min with a total run time of 8 min. Separation was achieved with a C1 8 column (ACE C18AR, 150 mm x 2.1 mm x 5 lm, MAC-MOD Analytical, Chadd's Ford, PA, USA) maintained at 40 °C. The standard curve was linear from 0.01 to 5 lg/mL for meloxicam and from 0.25 to 10 lg/mL for gabapentin. The standard curves were accepted if the correlation coefficient exceeded 0.99 and predicted values were within 15% of the actual values. The accuracy of the assay was 98 ± 10% of the actual value and the coefficient of variation was 6% determined on replicates of 5 each at 0.01, 1, and 5 lg/mL for meloxicam. The accuracy of the assay was 98 ± 5% of the actual value and the coefficient of variation was 5% determined on replicates of 5 each at 0.25, 2.5, and 10 lg/mL for gabapentin.

Plasma concentrations of flunixin *(m/z* 297.14?264.00) were also determined with high-pressure liquid chromatography and mass spectrometry. Plasma samples or standards (50 lL) were added to 200 lL of internal standard (piroxicam 0.5 lg/mL in methanol, *m/z* 332.12?95.10) in methanol with 0.1% formic acid to precipitate the proteins. The samples were vortexed for 5 s and centrifuged for 10 min at 10,000 xg. The supernatant, 200 lL, was transferred to an injection vial with the injection volume set to

10 lL. The mass spectrometer was set to MRM, source temperature was 400 °C, spray voltage was +5000 V, curtain gas 20 a.u., and the collision associated gas 12 a.u. The collision energy was 53 and 35 for flunixin and piroxicam, respectively. The mobile phase consisted of A: acetonitrile and B: 0.1% formic acid at a flow rate of 0.4 mL/min. The mobile phase consisted of 85% B from 0 to 0.5 min with a linear gradient to 50% B at 2.5 min which was maintained until 5 min, followed by a linear gradient to 85% B at 5.5 min with a total run time of 8 min. Separation was achieved with a C1 8 column (Supelco Discovery 50 mm x 4.6 mm x 5 uM, St. Louis, MO, USA) maintained at 40 °C. The standard curve was linear from 0.01 to 55 lg/mL. The standard curves were accepted if the correlation coefficient exceeded 0.99 and predicted values were within 15% of the actual values. The accuracy of the assay was 100 ± 10% of the actual value and the coefficient of variation was 6% determined on replicates of 5 each at 0.01, 1, and 25 lg/mL.

2.9. Pharmacokinetic analysis

Pharmacokinetic analyses were performed with computer software (WinNonlin 5.2, Pharsight Corporation, Mountain View, CA, USA) using noncompartmental methods. The variables calculated included the area under the curve from time 0 to infinity (AUC 0-INF) using the linear trapezoidal rule, area under the first moment curve from time 0 to infinity (AUMC 0-INF), plasma clearance (Cl), plasma clearance per fraction of the dose absorbed (Cl/F), apparent volume of distribution (area method) (Vz), Vz per fraction of the dose absorbed (Vz/F), terminal half-life ($7>_2$), and mean residence time extrapolated to infinity (MRT 0-INF). The percent of the AUC extrapolated to infinity (AUC extrapolated) was determined. The maximum serum concentration (C_{max}) and time to maximum serum concentration (T_{max}) were determined directly from the data. The concentration extrapolated to time 0 (C0) was determined by log-linear regression of the first two time points after IV drug administration.

2.10. Statistical analysis

Differences in the plasma PGE_2 concentrations between treated animals and control group were determined using Kruskal-Wallis rank sum test at each sampling time point. In the case of significant differences among all groups a Wilcoxon rank sum test was used for post-hoc comparisons between each treatment group and the controls, at each sampling time. Correlation between PGE_2 and drug concentrations was tested using Spearman's rank correlation

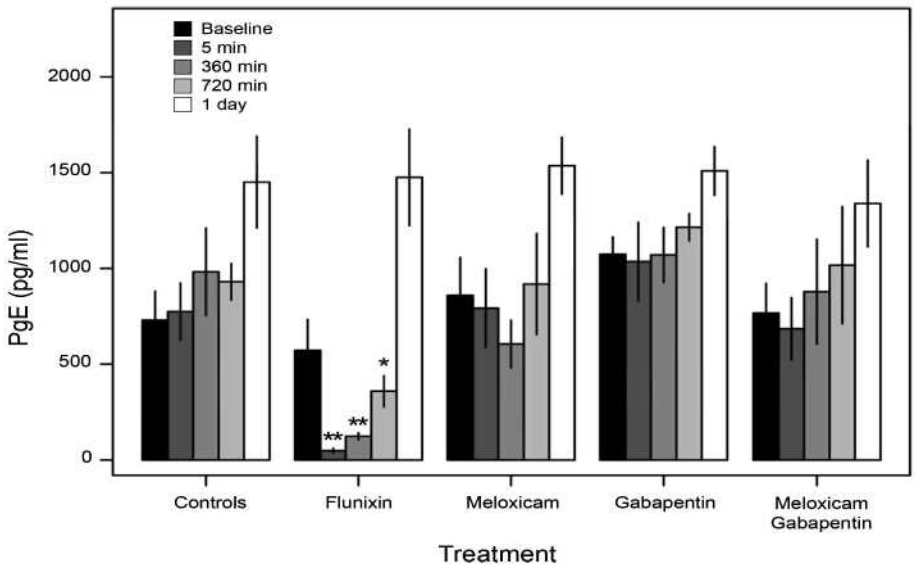

Fig. 1. Time course of plasma prostaglandin E_2 (PGE_2) synthesis in control and treated calves (2.2 mg/kg b.w IV flunixin, 1 mg/kg b.w. meloxicam per os, 15 mg/kg b.w. per os gabapentin, 1 mg/kg b.w. per os meloxicam and 15 mg/kg b.w per os gabapentin). Values are mean ± SEM (n = 7). *P < 0.01 control vs treated calves; *"P* < 0.001 control vs treated calves.

test, assuming a directional alternative hypothesis (H1: Rho<0). All analyses were performed using R software (R Development Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/).

Pharmacokinetic parameters (AUC 0-INF, $C_{MA}X$, *T^*, MRT 0-INF, and TMAX) for meloxicam and meloxicam with gabapentin and gabapentin and gabapentin with meloxicam were assessed for differences using the Mann-Whitney Rank Sum Test (Sigma Plot 12, SyStat Software). The significance level was *P* < 0.05.

3. Results

3.1. Plasma PGE_2 concentration

Plasma PGE_2 concentrations measured at each time point in the different treatment groups are shown in Fig. 1. A significant decrease in plasma PGE_2 concentrations was observed at 5 (P = 0.0003), 360 (P = 0.0003) and 720 min (P = 0.008) after treatment in the flunixin group compared with controls. Even though a slight decrease in PGE_2 concentration was found also in the meloxicam treated group at 360 min, no significant differences were recorded by comparing each time point with control group values (Wilcoxon rank sum test P > 0.05 in all cases).

3.2. Pharmacokinetic parameter estimates

Fig. 2A-E shows plasma concentrations relative to time profile of flunixin, meloxicam, gabapentin and

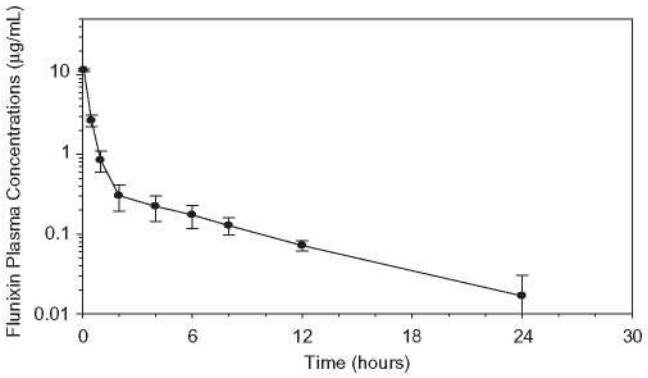

Fig. 2A. Plasma flunixin concentrations (mean ± SEM)

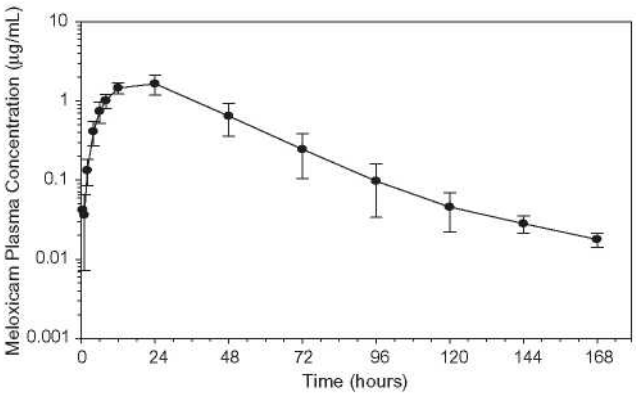

Fig. 2B. Plasma meloxicam concentration (mean ±

meloxicam with gabapentin treatments in calves. Pharmacokinetic parameter estimates

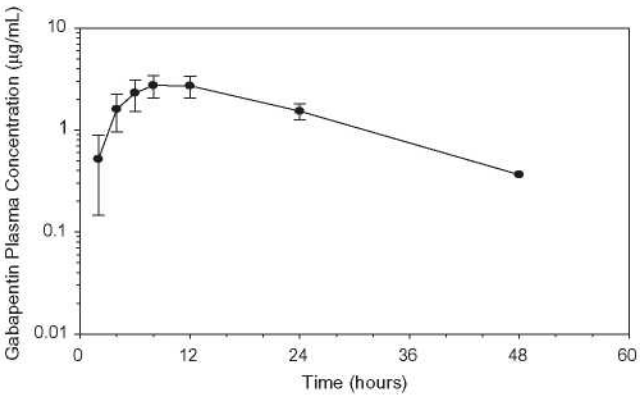

Fig. 2C. Mean values ± SEM of the plasma gabapentin concentrations after oral administration of the drug at 15 mg/kg per os in calves.

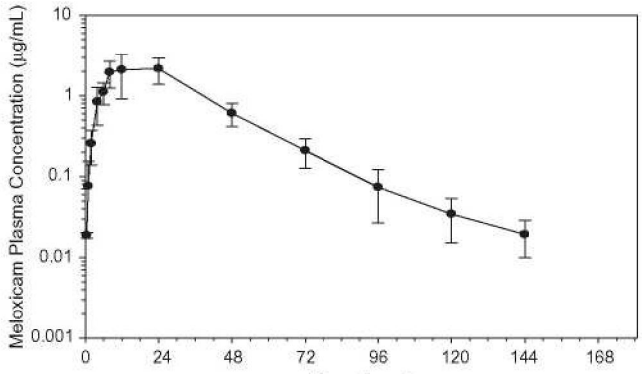

Fig. 2D. Plasma meloxicam concentration (mean ± SEM) following the administration of 1 mg/kgb.w. per os meloxicam with 15 mg/kgb.w. per os gabapentin in calves.

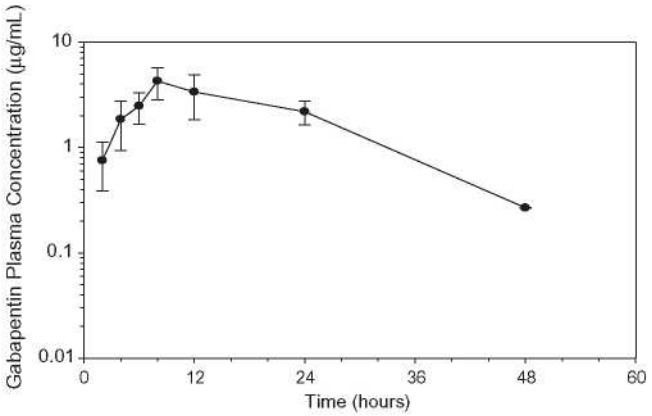

Fig. 2E. Plasma gabapentin concentration (mean ± SEM) following the administration of 1 mg/kg b.w. per os meloxicam with 15 mg/kgb.w. per os gabapentin in calves.

are summarized in Table 1 and include the median and range. One animal was excluded from the flunixin pharmacokinetic analysis due to a sample labelling error. In plasma, the terminal half-lives of flunixin, meloxicam and gabapentin were 6.0 h (3.4-11.0 h), 16.7 h (13.7-21.3 h) and 15.3 h (11-32.9 h), respectively. The median and range volume of distribution (Vz) for flunixin (2.28 L/kg; 1.27-4.46 L/kg).The Vz/F for meloxicam was 0.338 (0.285-0.479) L/kg and was 4.45 (2.94-6.79 L/kg for gabapentin).

Table 1

Plasma median (minimum-maximum) pharmacokinetic variables of IV flunixin (2.2 mg/kg b.w), oral meloxicam (1 mg/kg b.w.), oral gabapentin alone (15 mg/kg b.w.), and oral meloxicam with gabapentin (1 mg/kg b.w. and 15 mg/kg b.w, respectively) in calves. AUC0-1 $^{=}$ Area under the curve from 0 to 1. Cl/F = Total body clearance per fraction of drug absorbed. T^ = terminal elimination half-life. Vz/F = Volume of distribution per fraction of dose absorbed. MRTo_oo $^{=}$ mean residence time extrapolated to infinity. The P-value, if included, compared the median meloxicam and gabapentin pharmacokinetic parameter values with those derived from the individual meloxicam or gabapentin treatment group, and was determined using the Mann-Whitney Rank Sum test.

Parameter	Flunixin	Meloxicam	Gabapentin	Meloxicam (Meloxicam + Gabapentin)	Gabapentin (Meloxicam + Gabapentin)
$AUC_{0-\infty}$ (h μg/mL) [*P*-value]	7.9 (6.5–9.0)	78 (41.3–93.4)	87.2 (59.2–134.9)	91.4 (48.1–146.2) [0.279]	122. 8 (61.9–142.3) [0.105]
Cl (mL/min/kg)	4.7 (4.1–5.7)	–	–	–	–
Cl/F (mL/min/kg)	-	0.218 (0.177–0.403)	2.87 (1.82–4.24)	0.18 (0.11–0.33)	2.06 (1.73–4.06)
C0 (μg/mL)	15.3 (14.5–16.2)	-	-	-	-
C_{max} (μg/mL) [*P*-value]	-	1.9 (1.3–2.1)	2.7 (2.2–4)	2.3 (1.4–4.6) [0.130]	4.1 (2.3–6.5) [0.038]
$T_{½}z$ (h) [*P*-value]	6.0 (3.4–11)	16.7 (13.7–21.3)	15.3 (11–32.9)	14.6 (11.9–24.5) [0.328]	13.2 (10.4–23.3) [0.721]
$MRT_{0-\infty}$ (h) [*P*-value]	3.2 (2.0–5.9)	34.2 (26.2–42.3)	26.6 (19.8–51.2)	29.4 (23.9–33.4) [0.161]	23.7 (18.3–37.1) [0.645]
T_{max} (h) [*P*-value]	-	24 (12–24)	8 (6–12)	18 (8–24) [0.574]	8 (8–12) [0.645]
Vz (L/kg)	2.28 (1.27–4.46)	-	-	-	-
Vz/F (L/kg)	-	0.338 (0.285–0.479)	4.45 (2.94–6.79)	0.25 (0.13–0.40)	3.4 (1.6–3.7)

The area under the plasma-concentration time curve (AUC) was 7.9h1g/mL (6.5-9.0 h lg/mL), 78 h lg/mL (41.3-93.4 h lg/mL) and 87.2 h lg/mL (59.2-134.9 h lg/mL) for flunixin, meloxicam and gabapentin, respectively. When co-administered with meloxicam, gabapentin showed significantly ($P < 0.05$) higher C_{max} values (4.1 lg/mL; 2.3-6.5 lg/mL) than those relating to the drug given alone (2.7 lg/mL; 2.2-4.0 lg/mL. The only significant correlation between blood drug and PGE_2 levels was the negative correlation observed between blood PGE_2 and flunixin concentrations (Rho: -0.707, $P<0.0001$).

4. Discussion

In the present study the pharmacokinetics of flunixin, meloxicam, and gabapentin and their capability to inhibit prostaglandin E_2 synthesis in calves undergoing dehorning were investigated.

The pharmacokinetic properties of intravenous flunixin and oral meloxicam had already been studied in calves (Landoni et al., 1995; Coetzee et al., 2009; Mosher et al., 2011). Flunixin pharmacokinetic parameters in our study are in agreement with those reported by Landoni and colleagues (1995). Flunixin elimination half-life in calves is longer than that reported for cats (Lees and Taylor, 1991), dogs (Hardie et al., 1985) and horses (Lees et al., 1987).

Our results indicate that a median C_{max} of 1.9 lg/mL (range 1.3-2.1 lg/mL) occurred approximately 24 h (range 12-24 h) after oral meloxicam administration. The mean T_{max} value in our study is longer than those previously observed in calves treated with oral meloxicam (Coetzee et al., 2009, 2011; Mosher et al., 2011). By contrast, lower mean AUC values were found. The discrepancies could be ascribed to the different oral formulations used to perform the studies. In the studies performed by Coetzee and others (2009 and 2011) and by Mosher and others (2011) tablets were crushed and mixed with water to make a suspension, whereas in our study meloxicam tablets were administered in a bolus mixed with whey to completely mask the tablets.

The pharmacokinetic estimates for gabapentin alone are similar to those found by Coetzee and colleagues (2011), whereas some differences have been found when considering the co-administration with meloxicam. It is noteworthy that the plasma elimination half-life of gabapentin in calves is considerably longer than previously reported in horses (Dirikolu et al., 2008) and dogs (Kukanich and Cohen, 2011). Moreover, in humans and horses the drug peak plasma concentration is achieved much sooner than reported in our study. The differences can probably be attributed to a decreased rate of absorption due to dilution and retention of the drug in the forestomachs (Coetzee et al., 2011).

When considering the co-administration of gabapentin and meloxicam, gabapentin elimination half-life in the present study was longer than 8.12 h reported previously (Coetzee et al., 2011). The reason for the difference is not known, but may be due random individual variability, differences in formulation, or true pharmacokinetic differences in these animals.

Although the C_{max} of gabapentin co-administered with meloxicam was higher compared with gabapentin alone, the co-administration of single doses of gabapentin and meloxicam does not seem to affect any other

pharmacokinetic parameters. Therefore, as with respect to pharmacodynamics any synergistic effect was observed, the therapeutic impact of higher C_{max} should be further investigated.

Our data indicate that flunixin administered intravenously at a dose of 2.2 mg/kg significantly suppresses the synthesis of PGE_2 at 5, 360 and 720 min post-treatment as confirmed by the negative correlation between PGE_2 concentrations and blood flunixin levels. Königsson and colleagues (2003) found that in dairy goats plasma concentrations of 15 - ketodihydroPGF_{2a}, a PG-metabolite, significantly declined 15 min after flunixin irrespective of route of administration. Furthermore, Landoni and colleagues (1995) demonstrated that the intravenous administration of flunixin exerts inhibitory effects on serum thromboxane B_2 and exudate PGE_2 concentrations in calves using a model of acute inflammation. As observed for thromboxane B_2 by Landoni and colleagues, in the present study flunixin inhibited blood PGE_2 levels up to 12 h, although maximal inhibition was reached at early sample collection time. In beef calves, the intravenous administration of flunixin has been shown to provide visible pain relief up to 8 h postcastra-tion (Currah et al., 2009). On the other hand, prolonged PGE_2 inhibition is more likely to induce NSAID adverse effects (KuKanich et al., 2012). From this point of view it has been suggested that recently licensed more selective NSAIDs show a decreased frequency of adverse effects compared to drugs such as flunixin. Wallace et al. (2000) have found that selective inhibition of either COX-1 or COX-2 does not elicit gastric damage in the rat, whereas inhibition of both isoforms of COX is required for the development of gastric erosions after NSAIDs administration.

Indeed, it is of interest that meloxicam did not significantly affect blood PGE_2 levels, although a slight decrease was observed at 360 min after treatment. This could in part be due to the time of treatment, immediately prior to dehorning. According to Coetzee et al., (2009) and to our data, in fact, the T_{max} for oral meloxicam ranges from 12 to 24 h depending on drug formulation used. However, when considering plasma PGE_2 levels at 720 min and 1 day post-treatment, PGE_2 concentration was even higher than at baseline suggesting no effect of the drug on PGE_2 synthesis. Some studies investigating the effects of meloxicam on pain sensitivity of calves following dehorning have reported changes in behaviour and decrease of physiological response (e.g. heart and respiratory rate, serum cortisol levels) during the first 6-9 h post-surgery (Heinrich et al., 2009, 2010). Recently, Theurer et al. (2012) have demonstrated that the administration of oral meloxicam shows potential positive effects on calf behavioral changes post-dehorning. This finding seems partially in disagreement with our results. However, it has been suggested that NSAIDs can exert actions other than COX inhibition (McCormack and Brune, 1991; Landoni et al., 1995). Some NSAIDs such as carprofen, for example, inhibit the activation of Nuclear Factor Kappa-B which in turn regulates proinflammatory enzymes and cytokines (Tegeder et al., 2001). As such, the correlation of PGE_2 levels with other indicators of pain and/or pain relief (e.g. behavioral and clinical observations) could provide more precise information about meloxicam analgesic effect in cattle undergoing dehorning. It has been demonstrated that in piglets the analgesic effects of meloxicam should be tested by an experimental method in which drug effect is assessed for responses directly related to its

clinical use (e.g. local temperature) as the inhibition of PGE_2 levels is limited (Fosse et al., 2008, 2011). Furthermore, it is important to note that compounds that are highly selective COX-2 inhibitors in one species may not necessarily exert the same selectivity in other species (Beretta et al., 2005). From this point of view there is little information concerning the inhibitory activity of meloxicam on COX-1 and COX-2 isoforms in cattle. The evaluation of blood thromboxane levels following meloxicam treatment could probably provide more information about the pharmacodynamic behavior of the drug in this species.

As expected gabapentin alone did not significantly affect blood PGE_2 levels. The slight effect observed when co-administered with meloxicam can probably be ascribed to the latter. Nevertheless the present research is part of a larger study investigating the effects of selected analgesic drugs including gabapentin on different outcome measures. From this point of view, further steps will be the correlation of such indicators with gabapentin pharmacokinetic parameters in order provide new insights into gabapentin analgesic effects in cattle undergoing dehorning. Although gabapentin mechanism of action has not been fully elucidated, the drug is thought to bind to voltage gated calcium channels acting presinap-tically to decrease the release of excitatory neurotransmitters (Taylor, 2009). It has been suggested that gabapentin is effective against hypersensitivity induced by tissue injury and neuropathy but not in acute pain models (Cheng and Chiou, 2006). Thus, as mentioned above, the focus of future research will be to investigate the analgesic effect of the drug by means of physiological and behavioural reactions.

Together with other invasive husbandry procedures that have become common practice in modern animal husbandry, dehorning has recently been under the scrutiny of public opinion and nongovernmental organizations. Livestock producers and consumers have indicated that they need scientific measures of animal well-being to be used to determine how farm animals should be treated (Lusk et al., 2007). The FDA Center for Veterinary Medicine guidance for the development of effectiveness data for NSAIDs indicates that validated methods of pain assessment must be used for a drug to be indicated for pain relief in the target species (Coetzee et al., 2009). The need to provide pain relief and to identify reproducible markers of pain is also demonstrated by the huge amount of literature concerning this topic (Stafford and Mellor, 2005; Duffield etal.,2010; Baldridge et al., 2011; Gottardo etal., 2011). Therefore, the development of science-based dehorning guidelines, using validated measures of well-being, are urgently needed to assist production agriculture in addressing these concerns. Moreover, veterinarians need effective and convenient means of providing analgesia to calves undergoing dehorning. Within the EU, meloxicam is approved for IV and SC injection at a dose rate of 0.5 mg/kgb.w. with a withdrawal time of 15 days for meat and 5 days for milk (Smith et al., 2008). According to our pharmacokinetic data and to those by Coetzee and colleagues (2009), the drug administered by oral route shows longer plasma half-life than previously reported for other NSAIDs and therefore it might be an effective long-acting practical alternative to injectable analgesia By contrast, given the time to maximum plasma drug concentration, oral preemptive analgesia should be administered several hours before surgery so that surgery coincides with peak drug concentrations. Neverthless, considering the mild effects induced by the drug on blood PGE_2

levels, the pharmacodynamic behavior of oral meloxicam in cattle undergoing dehorning should be further investigated before formulating analgesic recommendation in such cattle.

5. Conclusion

Although NSAIDs are recognized as having analgesic properties, there currently are no analgesic drugs specifically approved in the US for alleviating pain in livestock. Our results suggest that administration of flunixin at a dose of 2.2 mg/kg intravenously, suppresses blood prostaglandin E_2 synthesis in calves up to 12 h. Conversely, oral administration of meloxicam, gabapentin, meloxicam with gabapentin had no significant effect on PGE_2 blood concentration. However, other mechanisms could be involved in the analgesic action of these drugs. Further assessment of oral meloxicam and gabapentin in established pain models is required to formulate science based analgesic recommendations to enhance animal well-being after dehorning.

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References

Akula, K.K., Dhir, A., Kulkarni, S.K., 2009. Effect of various antiepileptic drugs in a pentylenetetrazol-induced seizure model in mice. Methods & Findings in Experimental & Clinical Pharmacology 31, 423-432.

American Veterinary Medical Association, 2006. Backgrounder: welfare implications of the dehorning and disbudding of cattle. Available at: http:// www.avma.org/ reference/backgrounders/dehorning_cattle_bgnd.asp.

Atsufumi, K., 2011. Prostaglandin E_2 and Pain-An Update. Biological and Pharmaceutical Bulletin 34,1170-1173.

Baldridge, S.L., Coetzee, J.F., Dritz, S.S., Reinbold,J.B., Gehring, R., Havel, J., Kukanich, B., 2011. 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. American Journal of Veterinary Research 72,1305-1317. Banamine (flunixin meglumine) Injectable Solution 50 mg/mL Veterinary (NADA #101-479) (product information). Union, NJ: Intervet/Schering-Plough Animal Health. Available at: http://intervetus.naccvp.com/?m=product_view&u=intervetus&p=intervetus&id=1047018. Beretta, C., Garavaglia, G., Cavalli, M., 2005. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: an *in vitro* analysis. Pharmacological Research 52, 302-306. Cheng, J.K., Chiou, L.C., 2006. Mechanisms of the antinociceptive action of gabapentin. Journal of Pharmacological Sciences 100, 471-486. Coetzee, J.F., KuKanich, B., Mosher, R., Allen, P.S., 2009. Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. Veterinary Therapeutics 10, E1-E8. Coetzee, J.F., Mosher, R.A., Kohake, L.E., Cull, C.A., Kelly, L.L., Mueting, S.L., KuKanich, B., 2011. Pharmacokinetics of oral gabapentin alone or co-administered with meloxicam in ruminant beef calves. The Veterinary Journal 190, 98-102. Coetzee, J.F., 2011. A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration in the United States. Invited Review. Applied Animal Behavioral Science 135,192-213. Currah, J.M., Hendrick, S.H., Stookey, J.M., 2009. The behavioral assessment and alleviation of pain associated with castration in beef calves treated with flunixin meglumine and caudal lidocaine epidural anesthesia with epinephrine. Canadian Veterinary Journal 50, 375-382. Curry, S.L., Cogar, S.M., Cook, J.L., 2005. Non-steroidal antiinflammatory drugs: a review. Journal of the American Animal Hospital Association 41, 298-309. Dirikolu, L., Dafalla, A., Ely, K.J., Connerly, A.L., Jones, C.N., ElkHoly, H., Lehner, A.F., Thompson, K., Tobin, T., 2008. Pharmacokinetics of gabapentin in horses. Journal of Veterinary Pharmacology and Therapeutics 31,175-177. Duffield, T.F., Heinrich, A., Millman, S.T., DeHaan, A., James, S., Lissemore, K., 2010. Reduction in pain responses by combined use of local lidocaine anesthesia and systemic ketoprofen in dairy calves dehorned by heat cauterization. Canadian Veterinary Journal 51, 283-288. Faulkner, P.M., Weary, D.M., 2000. Reducing pain after dehorning in dairy calves. Journal of Dairy Science 83, 2037-2041. Fosse, T.K., Haga, G.A., Hormazabal, V., Haugejorden, G.,

Horsberg, T.E., Ranheim, B.,
2008. Journal of Veterinary Pharmacology and Therapeutics 31, 246-252. Fosse, T.K., Toutain, P.L., Spadavecchia, C., Haga, H.A., Horsberg, T.E., Ranheim, B.,
2011. Ketoprofen in piglets: enantioselective pharmacokinetics, pharmacodynamics and PK/PD modelling. Journal of Veterinary Pharmacology and Therapeutics 34, 338-349. Fuchs, A.R., Graddy, L.G., Kowalski, A.A., Fields, M.J., 2002. Oxytocin induces PGE_2 release from bovine cervical mucosa *in vivo*. Prostaglandins and Other Lipid Mediators 70,119-129. Gibson, T.J., Johnson, C.B., Stafford, K.J., Mitchinson, S.L., Mellor, D.J., 2007.
Validation of the acute electroencephalographic response of calves to noxious stimulus with scoop dehorning. New Zealand Veterinary Journal 55,152-157. Gottardo, F., Nalon, E., Contiero, B., Normando, S., Dalvit, P., Cozzi, G., 2011. The dehorning of dairy calves: practices and opinions of 639 farmers. Journal of Dairy Science 94, 5724-5734. Govendir, M., Perkins, M., Malik, R., 2005. Improving seizure control in dogs with refractory epilepsy using gabapentin as an adjunctive agent. Australian Veterinary Journal 83, 602-608. Graf, B., Senn, M., 1999. Behavioural and physiological responses of calves to dehorning by heat cauterisation with or without local anesthesia. Applied Animal Behaviour Science 62, 153-171. Grendahl-Nielsen, C., Simonsen, H.B., Damkjer Lund, J., Hesselholt, H., 1999.
Behavioural, endocrine and cardiac responses in young calves undergoing dehorning without and with the use of sedation and analgesia. Veterinary Journal 158,14-20. Hardie, E.M., Hardee, G.E., Rawlings, C.A., 1985. Pharmacokinetics of flunixin meglumine in dogs. American Journal of Veterinary Research 46, 235-237. Heinrich, A., 2007. An investigation of meloxicam for relief of pain associated with dehorning of dairy calves (MSc thesis). Guelph, Ontario: University of Guelph. Heinrich, A., Duffield, T.F., Lissemore, K.D., Squires, E.J., Millman, S.T., 2009. The impact of meloxicam on post surgical stress associated with cautery dehorning. Journal of Veterinary Science 92, 540-547. Heinrich, A., Duffield, T.F., Lissemore, K.D., Millman, S.T., 2010. The effect of meloxicam on behaviour and pain sensitivity on dairy calves following cautery dehorning with a local anesthetic. Journal of Dairy Science 93, 2450-2457. Hurley, R.W., Chatterjea, D., Rose Feng, M., Taylor, C.P., Hammond, D.L., 2002.

Gabapentin and pregabalin can interact synergistically with naproxen to produce antihyperalgesia. Anesthesiology 97,1263-1273. Königsson, K., Törneke, K., Engeland, I.V., Odensvik, K., Kindahl, H., 2003. Pharmacokinetics and pharmacodynamic effect of flunixin after intravenous, intramuscular and oral administration to dairy goats. Acta Veterinaria Scandinavica 44,153-159. KuKanich, B., Cohen, R.L., 2011. Pharmacokinetics of oral gabapentin in greyhound dogs. The Veterinary Journal 187, 133-135. KuKanich, B., Bidgood, T., Knesl, O., 2012. Clinical pharmacology of non-steroidal antinflammatory drugs in dogs. Veterinary Anaesthesia and Analgesia 39, 69-**90.**

Landoni, M.F., Cunningham, F.M., Lees, P., 1995. Determination of pharmacokinetics and pharmacodynamics of flunixin in calves by use of pharmacokinetic/ pharmacodynamics modeling. American Journal of Veterinary Research 56, 786-794. Lees, P., Ewins, C.P., Taylor, J.B., Sedgwick, A.D., 1987. Serum thromboxane in the horse and its inhibition by aspirin, phenylbutazone and flunixin. British Veterinary Journal 143, 462-476. Lees, P., Taylor, P.M., 1991. Pharmacodynamics and pharmacokinetics of flunixin in the cat. British Veterinary Journal 147, 298-305. Lees, P., Landoni, M.F., Giraudel, J., Toutain, P.L., 2004. Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. Journal of Veterinary Pharmacology and Therapeutics 27, 479-490. Lusk, J.L., Norwood, F.B., Prickett, R.W., 2007. Consumer Preferences for Farm Animal Welfare: results of a nationwide telephone survey. Animal Welfare 19, 335-347. Malreddy, P.R., Coetzee, J.F., Kukanich, B., Gehring, R., 2012. Pharmacokinetics and milk secretion of gabapentin and meloxicam co-administered orally in Holstein-Friesian cows. Journal of Veterinary Pharmacology and Therapeutics. http://dx.doi.org/10.1111/j.1365-2885.2012.01384.x. Mares, P., Haugvicova, R., 1997. Anticonvulsant action of gabapentin during postnatal development in rats. Epilepsia 38, 893-896.

McCormack, K., Brune, K., 1991. Dissociation between the antinociceptive and antiinflammatory effects of the nonsteroidal antiinflammatory drugs. A survey of their analgesic efficacy. Drugs 41, 533-547. Milligan, B.N., Duffield, T., Lissemore, K., 2004. The utility of ketoprofen for alleviating pain following dehorning in young dairy calves. Canadian Veterinary

Journal 45,140-143. Mosher, R.A., Coetzee, J.F., Cull, C.A., Gehring, R., KuKanich, B., 2011.
Pharmacokinetics of oral meloxicam in ruminant and preruminant calves.
Journal Of Veterinary Pharmacology And Therapeuthic. http://dx.doi.org/
10.1111/j.1365-2885.2011.01331.x. NASS, 2010. US Department of Agriculture National Agricultural Statistics Service.
Agricultural Statistics 2009. Available at http://www.usda.mannlib.cornell.edu/
usda/current/Catt/Catt-07-24-2009.pdf. Accessed Aug 19, 2009. Petrie, N.J., Mellor, D.J., Stafford, K.J., Bruce, R.A., Ward, R.N., 1996. Cortisol responses
of calves to two methods of disbudding used with or without local anaesthetic.
New Zealand Veterinary Journal 44, 9-14. Picazo, A., Castaneda-Hernandez, G., Ortiz, M.I., 2006. Examination of the
interaction between peripheral diclofenac and gabapentin on the 5% formalin
test in rats. Life Sciences 79, 2283-2287. Prostaglandin E_2 EIA Kit -Monoclonal. Cayman Chemical Item Number 514010. Kit
booklet. http://www.caymanchem.com/app/template/Product.vm/catalog/
514010. Radi, Z.A., 2009. Pathophysiology of cyclooxygenase inhibition in animal models.
Toxicologic Pathology 37, 34-46. Satzinger, G., 1994. Antiepileptics from gamma-aminobutyric acid.
Arzneimittelforschung 44, 261-266. Smith, G.W., Davis, J.L., Tell, L.A., Webb, A.I., Riviere, J.E., 2008. Extralabel use of
nonsteroidal anti-inflammatory drugs in cattle. Journal of the American
Veterinary Medical Association 232, 697-701. Stafford, K.J., Mellor, D.J., 2005. Dehorning and disbudding distress and its
alleviation in calves. The Veterinary Journal 169, 337-349. Stafford, K.J., Chambers, J.P., Mellor, D.J., 2006. The alleviation of pain in cattle: a
review. CAB reviews: perspectives in agriculture, veterinary science. Nutrition
and Natural Resources 1 (032), 1-7. Stewart, M., Stafford, K.J., Dowling, S.K., Schaefer, A.L., Webster, J.R., 2008. Eye
temperature and heard rate variability of calves disbudded with or without
local anaesthetic. Physiology and Behaviour 93, 789-797. Streppa, H.K., Jones, C.J., Budsberg, C., 2002. Cyclooxygenase selectivity of
nonsteroidal anti-inflammatory drugs in canine blood. American Journal of
Veterinary Research 63, 91-94. Takada, M., Fukusaki, M., Terao, Y., Yamashita, K., Inadomi, C., Takada, M.,

Sumikawa, K., 2007. Preadministration of flurbiprofen suppresses prostaglandin production and postoperative pain in orthopedic patients undergoing tourniquet inflation. Journal of Clinical Anhestesia 19, 97-100. Taylor, C.P., 1993. Mechanisms of Action of New Antiepileptic Drugs. In: Chadwick, D. (Ed.), New Trends in Epilepsy Management: The Role of Gabapentin. Royal Society of Medicine, London, pp. 13-40. Taylor, C.P., 2009. Mechanisms of analgesia by gabapentin and pregabalin - calcium channel a2-d[Cava2-d] ligands. Pain 142,13-16. Tegeder, I., Pfeilschifter, J., Geisslinger, G., 2001. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. Faseb Journal 15, 2057-2072. Theurer, M.E., White, B.J., Coetzee, J.F., Edwards, L.N., Mosher, R.A., Cull, C.A., 2012. Assessment of behavioral changes associated with oral meloxicam administration at time of dehorning in calves using a remote triangulation device and accelerometers. BioMed Central Veterinary Research. http://dx.doi.org/10.1186/1746-6148-8-48. Vinuela-Fernandeza, I., Jones, E., Welsh, E.M., Fleetwood-Walker, S.M., 2007. Pain mechanisms and their implication for the management of pain in farm and companion animals. The Veterinary Journal 147, 227-239. Wallace, J.L., Mcknight, W., Reuter, B.K., Vergnolle, N., 2000. NSAID induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2.