

Pharmacokinetics and target animal safety of meloxicam and persistence of xylazine and ketamine after rendering in cattle

by

Scott Andrew Fritz

B.A., Hastings College, 2010
D.V.M., Iowa State University, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Abstract

Pharmacologic pain control in food animals undergoing normal management procedures is a recent focus in food production in the United States. Currently, there is only one analgesic drug that is FDA-approved for pain control; however, this drug is limited to control pain only associated with infectious pododermatitis in dairy cattle. Veterinarians can legally prescribe drugs to control pain in food animals for pain associated with other conditions or management procedures under the guidance of the Animal Medicinal Drug Use Clarification Act provided the mandates of that act are met. The lack of FDA-approval for pain control labels on analgesic drugs in food animals can largely be attributed to the difficulty in assessing pain in these species which contributes to variable reports in efficacy studies. Another significant hurdle in achieving regulatory approval is investigations into the safety of these drugs. Meloxicam is a non-steroidal anti-inflammatory drug often selected to control pain associated with painful management procedures in cattle. Adverse effects associated with oral meloxicam administration in other species include damage to the gastric mucosa, hypoxic kidney damage, and potential negative effects on the liver and bone marrow. Oral meloxicam dosed at 1 mg/kg bodyweight is approved to control pain in cattle in Canada.

In this dissertation, the pharmacokinetics, milk concentrations, and safety of a single dose of meloxicam thirty times the magnitude of the Canadian product were evaluated in lactating dairy cattle. Drug concentrations were determined using high-pressure liquid chromatography coupled with mass spectroscopy. Pharmacokinetic parameters were modeled with non-compartmental methods using a commercially-available computational software. The potential adverse effects of this dose were monitored with ante-mortem testing using complete blood counts, serum chemistry analyses, urinalysis, and fecal evaluations. These animals were

euthanized, and post-mortem evaluation included descriptions of gross lesions and histologic evaluation of potential target tissues. There was no impact on absorption of the large dose and maximum concentrations in serum and milk were approximately thirty and eighty times higher than those reported for 1 mg/kg oral doses with similar half-lives, respectively. There were no identifiable changes in blood parameters, urinalysis, or fecal evaluations from baseline. There were also no effects noted at necropsy and the study population tolerated the large dose well for ten days following administration.

A target animal safety study was conducted in Holstein calves between 6 and 10 weeks of age. This study was modeled using FDA guidance documents on study design and outcome measures. The 1 mg/kg dose was used to calculate 3x and 5x doses and these were administered for three consecutive days. Serum drug concentrations were measured daily, and blood parameters were utilized for antemortem organ function monitoring. Calves were euthanized at multiple time points and gross and histologic lesions were described. There was a treatment-associated effect on gastric ulceration with the increasing doses which has not been previously described in cattle. Overall, the effects were clinically minor and unidentifiable via antemortem testing. This work may pave the way for evaluations of targeted analgesic regimens that may prove more effective for controlling pain.

Xylazine and ketamine are two anesthetic drugs that may be used during the euthanasia process of food animals. Sodium pentobarbital was used previously but significant environmental concerns exist regarding its persistence following use. There have also been reports of pentobarbital contaminating pet foods as it survives the rendering process. Xylazine and ketamine were administered to cattle as part of the euthanasia process and samples of liver, kidney, fat, and muscle were collected. Concentrations of both drugs were determined in the raw

tissues. The tissues were then exposed to a simulated rendering process using a commercial autoclave. Drug concentrations were determined in the rendered tissue and both drugs appear to persist through rendering. The concentrations detected after administration were relatively low and unlikely to pose a risk to pet foods.

Future studies related to this work hinge upon the development of validated pain assessment techniques that regulatory bodies will recognize. Meloxicam is well-tolerated by cattle and studies evaluating the timing of different dose magnitudes could demonstrate increased effectiveness compared to the 1 mg/kg dose used in cattle in Canada. There are established limits of xylazine and ketamine in animal products meant for human consumption. Future work should focus on determining oral doses causing sedation and the associated concentration in pet food which may allow animals administered these drugs as part of the euthanasia process to be used for rendering capturing a valuable revenue stream for producers while minimizing the risk for adverse effects in animals consuming these products.

Pharmacokinetics and target animal safety of meloxicam and persistence of xylazine and ketamine after rendering in cattle

by

Scott Andrew Fritz

B.A., Hastings College, 2010
D.V.M., Iowa State University, 2014

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Approved by:

Co-Major Professor
Micheal Kleinhenz

Approved by:

Co-Major Professor (primary)
Steve Ensley

Copyright

© Scott A Fritz 2025.

Abstract

Pharmacologic pain control in food animals undergoing normal management procedures is a recent focus in food production in the United States. Currently, there is only one analgesic drug that is FDA-approved for pain control; however, this drug is limited to control pain only associated with infectious pododermatitis in dairy cattle. Veterinarians can legally prescribe drugs to control pain in food animals for pain associated with other conditions or management procedures under the guidance of the Animal Medicinal Drug Use Clarification Act provided the mandates of that act are met. The lack of FDA-approval for pain control labels on analgesic drugs in food animals can largely be attributed to the difficulty in assessing pain in these species which contributes to variable reports in efficacy studies. Another significant hurdle in achieving regulatory approval is investigations into the safety of these drugs. Meloxicam is a non-steroidal anti-inflammatory drug often selected to control pain associated with painful management procedures in cattle. Adverse effects associated with oral meloxicam administration in other species include damage to the gastric mucosa, hypoxic kidney damage, and potential negative effects on the liver and bone marrow. Oral meloxicam dosed at 1 mg/kg bodyweight is approved to control pain in cattle in Canada.

In this dissertation, the pharmacokinetics, milk concentrations, and safety of a single dose of meloxicam thirty times the magnitude of the Canadian product were evaluated in lactating dairy cattle. Drug concentrations were determined using high-pressure liquid chromatography coupled with mass spectroscopy. Pharmacokinetic parameters were modeled with non-compartmental methods using a commercially-available computational software. The potential adverse effects of this dose were monitored with ante-mortem testing using complete blood counts, serum chemistry analyses, urinalysis, and fecal evaluations. These animals were

euthanized, and post-mortem evaluation included descriptions of gross lesions and histologic evaluation of potential target tissues. There was no impact on absorption of the large dose and maximum concentrations in serum and milk were approximately thirty and eighty times higher than those reported for 1 mg/kg oral doses with similar half-lives, respectively. There were no identifiable changes in blood parameters, urinalysis, or fecal evaluations from baseline. There were also no effects noted at necropsy and the study population tolerated the large dose well for ten days following administration.

A target animal safety study was conducted in Holstein calves between 6 and 10 weeks of age. This study was modeled using FDA guidance documents on study design and outcome measures. The 1 mg/kg dose was used to calculate 3x and 5x doses and these were administered for three consecutive days. Serum drug concentrations were measured daily, and blood parameters were utilized for antemortem organ function monitoring. Calves were euthanized at multiple time points and gross and histologic lesions were described. There was a treatment-associated effect on gastric ulceration with the increasing doses which has not been previously described in cattle. Overall, the effects were clinically minor and unidentifiable via antemortem testing. This work may pave the way for evaluations of targeted analgesic regimens that may prove more effective for controlling pain.

Xylazine and ketamine are two anesthetic drugs that may be used during the euthanasia process of food animals. Sodium pentobarbital was used previously but significant environmental concerns exist regarding its persistence following use. There have also been reports of pentobarbital contaminating pet foods as it survives the rendering process. Xylazine and ketamine were administered to cattle as part of the euthanasia process and samples of liver, kidney, fat, and muscle were collected. Concentrations of both drugs were determined in the raw

tissues. The tissues were then exposed to a simulated rendering process using a commercial autoclave. Drug concentrations were determined in the rendered tissue and both drugs appear to persist through rendering. The concentrations detected after administration were relatively low and unlikely to pose a risk to pet foods.

Future studies related to this work hinge upon the development of validated pain assessment techniques that regulatory bodies will recognize. Meloxicam is well-tolerated by cattle and studies evaluating the timing of different dose magnitudes could demonstrate increased effectiveness compared to the 1 mg/kg dose used in cattle in Canada. There are established limits of xylazine and ketamine in animal products meant for human consumption. Future work should focus on determining oral doses causing sedation and the associated concentration in pet food which may allow animals administered these drugs as part of the euthanasia process to be used for rendering capturing a valuable revenue stream for producers while minimizing the risk for adverse effects in animals consuming these products.

Table of Contents

List of Figures	xiii
List of Tables	xv
List of Abbreviations	xvii
Acknowledgements	xix
Dedication	xx
Chapter 1 - Literature Review	1
Introduction	1
Body	3
Efficacy	5
Safety	10
Pharmacokinetics	13
Xylazine and Ketamine Residues	18
Conclusion	19
References	21
Chapter 2 - Pharmacokinetics, milk residues, and toxicological evaluation of a single high dose of meloxicam administered at 30 mg/kg <i>per os</i> to lactating dairy cattle	32
Abstract:	32
Introduction	33
Materials and Methods	36
Experimental Animals	36
Housing and Husbandry	37
Meloxicam Administration	37
Sample Collection	37
Milk Sample Preparation	39
Plasma Sample Preparation	41
Determination of meloxicam concentrations by LC-MS	41
Noncompartmental pharmacokinetic analysis	42
Statistical analysis	43
Results	43

Milk.....	43
Plasma	45
Complete Blood Counts and Blood Chemistry.....	47
Fecal Occult Blood	52
Urine analysis.....	52
Necropsy Findings	53
Histological Examination.....	53
Discussion.....	54
Conclusions.....	57
References.....	59
Chapter 3 - Evaluation of the Safety of Oral Meloxicam in Young Holstein Calves.....	64
Abstract	64
Introduction.....	65
Materials and Methods.....	67
Experimental Animals	67
Housing and Husbandry	68
Meloxicam Administration	69
Sample Collection	69
Serum Meloxicam Analysis	70
Statistical Analysis.....	72
Results.....	72
Serum Meloxicam	72
Complete Blood Count	74
Serum chemistry	76
Postmortem Lesions.....	79
Histopathology Findings	82
Discussion.....	86
References.....	90
Chapter 4 - Determining the Persistence of Xylazine and Ketamine in Cattle Tissue Following a Simulated Rendering Process	95
Abstract	95

Introduction.....	96
Objectives	99
Materials and Methods.....	99
Rendering.....	101
Drug Analysis	101
Chemicals and Reagents	101
Stock Solutions	101
Standard Working Solutions	102
Internal Standard Working Solutions.....	102
Sample Preparation	102
Instrumentation	103
Method Validation	104
Results.....	105
Tissue Concentrations	105
Raw vs. Rendered Tissue	108
Tissue Concentration Changes.....	110
Moisture Loss.....	111
Discussion	111
References.....	114
Chapter 5 - Overall Conclusions and Future Research	118

List of Figures

Figure 2.1 Mean ($\pm$ SEM) milk production following a single oral dose of meloxicam at 30 mg/kg. ^{a,b,c} Different letters above data bars are statistically significant ($p=0.001$).	39
Figure 2.2 Mean ($\pm$ SEM) milk and plasma concentrations of meloxicam following a single oral dose of 30 mg/kg in adult lactating Holstein cows ($n=5$).	44
Figure 2.3 Individual milk meloxicam concentrations for 5 cows administered meloxicam at 30 mg/kg PO at time zero	44
Figure 2.4 Individual plasma meloxicam concentrations for 5 cows administered meloxicam at 30 mg/kg PO at time zero.	46
Figure 2.5 Summary mean ($\pm$ SEM) WBC counts (A), Neutrophil counts (B), total protein (C), and BUN (D) prior to, and for 10 days following administration of 30 mg/kg meloxicam PO to lactating dairy cows ($n=5$). Dotted lines denote provided upper and lower reference limits.	49
Figure 3.1 Mean($\pm$ SD) serum meloxicam concentrations ($n=8$ for days 0-4, on days 5-7, $n=5$ for control and 1mg/kg groups, $n=2$ for 3 mg/kg group, and $n=4$ for 5 mg/kg group).	73
Figure 3.2 A. Abomasal pylorus (p) and duodenum (d). The torus pyloricus (*) and mucosa is normal. B. Abomasal pylorus (p) and duodenum (d). Multifocal and random pinpoint to 2mm diameter irregular depressed areas (arrows) are evident on the pyloric and duodenal mucosa, including the torus pyloricus (*). C. Abomasal pylorus (p) and duodenum (d). Multifocal and random pinpoint to 1cm diameter irregular depressed areas (arrows) are evident on the pyloric mucosa with the largest on the torus pyloricus (*). D. Abomasal pylorus (p) and duodenum (d). Multifocal and random pinpoint to 3cm diameter irregular depressed areas (arrows) are evident on the pyloric mucosa. The largest and most severe is on the torus pyloricus (*); this ulcer extends full thickness through the mucosal surface, the submucosa is exposed and hemorrhagic, and the underlying submucosa is markedly thickened due to dense white firm connective tissue (arrowhead).	81
Figure 3.3. A. Abomasal pylorus (torus) grade 1A (10x): Abomasal pylorus (torus). There is superficial small intestinal mucosal injury extending from the surface epithelium to the superficial mucosa (erosion), with loss of surface epithelium, superficial pyloric glands, and replacement by karyorrhectic and inflammatory debris. The mid to deeper pyloric glands are	

intact. Hematoxylin and eosin. B. Abomasal pylorus (torus) grade 1B (10x): Abomasal pylorus (torus). There is superficial small intestinal mucosal injury extending from the surface epithelium to the mid-mucosa (erosion), with loss of surface epithelium, superficial pyloric glands, and replacement by karyorrhectic debris and inflammatory cells. The deeper pyloric glands are intact. Hematoxylin and eosin. C. Abomasal pylorus grade 1C (10x): Abomasal pylorus (torus). There is full thickness small intestinal mucosal injury extending from the surface epithelium to the submucosa (ulceration), with loss of pyloric glands and replacement by immature fibrous and granulation tissue, karyorrhectic debris, fibrin, and inflammatory cells. Hematoxylin and eosin.	83
Figure 3.4 Number of calves with Type 1c ulcers on the abomasal torus within 8 days of meloxicam administration (n=8/treatment group).	85
Figure 4.1. Individual Group 1 xylazine concentrations in raw and rendered bovine tissue (n=6). ● = Raw Tissue Δ = Rendered Tissue.....	108
Figure 4.2 Individual Group 2 xylazine concentrations in raw and rendered bovine tissue (n=6). ● = Raw Tissue Δ = Rendered Tissue.....	109
Figure 4.3. Individual Group 2 ketamine concentrations in raw and rendered bovine tissue (n=6). ● = Raw Tissue Δ = Rendered Tissue.....	110

List of Tables

Table 1.1. Summary of research evaluating the efficacy of meloxicam use in cattle for multiple purposes.	8
Table 1.2. Summary of research evaluating the safety of meloxicam in multiple species with multiple routes of administration.	12
Table 1.3. Summary of basic pharmacokinetic parameters in multiple age classes of cattle receiving different doses of meloxicam by different routes of administration.	16
Table 2.1 Graphical representation of the study design calendar.	36
Table 2.2 Geometric mean milk pharmacokinetic parameters of meloxicam following single oral administration at the dose rate of 30 mg/kg body weight in lactating dairy cows (n=5).	45
Table 2.3 Geometric mean plasma pharmacokinetic parameters of meloxicam following single oral administration at 30 mg/kg in lactating dairy cows (n=5).	46
Table 2.4 Summary mean ($\pm$ SEM) CBC parameters prior to meloxicam treatment and for 10 days post-treatment for cows administered 30 mg/kg per os (n=5).	48
Table 2.5 Summary of mean ($\pm$ SEM) serum blood chemistry parameters prior to meloxicam treatment and for 10 days post-treatment for cows administered 30 mg/kg per os (n=5). ...	51
Table 2.6 Individual cow urine analysis prior to meloxicam treatment and for 10 days post-treatment for cows administered 30 mg/kg meloxicam per os.	53
Table 3.1 Mean($\pm$ SD) serum meloxicam concentrations expressed as $\mu\text{g/mL}$ (n=8 for days 0-4, on days 5-7, n=5 for control and 1mg/kg groups, n=2 for 3 mg/kg group, and n=4 for 5 mg/kg group.	72
Table 3.2. Mean ($\pm$ SD) complete blood count results for Holstein calves administered 0, 1, 3, and 5mg/kg meloxicam per os for three consecutive days (n=8 per group for days -3 to 3, on day 7 n=5 for control and 1mg/kg groups, n=2 for 3 mg/kg group, and n=4 for 5 mg/kg group).	75
Table 3.3. Mean ($\pm$ SD) serum chemistry results for Holstein calves administered 0, 1, 3, and 5mg/kg meloxicam per os for three consecutive days (n=8 per group for days -3 to 3, on day 7 n=5 for control and 1mg/kg groups, n=2 for 3 mg/kg group, and n=4 for 5 mg/kg group).	78

Table 3.4 Contingency table of Type 1 abomasal torus pyloricus ulcer subtypes by treatment group and postmortem examination day. Percentages were calculated as number of calves with lesion type out of possible number of calves.	85
Table 4.1. Individual and Mean($\pm$ SD) xylazine and ketamine concentrations (ng/g) from individual and composite raw and rendered bovine tissue samples (n=6/treatment group).	107
Table 4.2. Mean($\pm$ SD) percent change in individual xylazine and ketamine concentrations between raw and rendered cattle tissue samples (n=6/treatment group).	110
Table 4.3. Mean (SD) percent change in sample weight attributed to moisture loss during the simulated rendering process (n=12).	111

List of Abbreviations

FARM	National Dairy Farmers Assuring Responsible Management Program
AVMA	American Veterinary Medical Association
AABP	American Association of Bovine Practitioners
FDA	United States Food and Drug Administration
AMDUCA	Animal Medicinal Drug Use Clarification Act
USDA	United States Department of Agriculture
FSIS	Food Safety and Inspection Service
ELDU	Extra Label Drug Use
VCPR	Veterinary Client Patient Relationship
COX	Cyclooxygenase
NSAID	Non-Steroidal Anti-Inflammatory Drug
LD50	Dose Lethal to 50% of a Test Population
NOEL	No Observed Effect Level
IM	Intramuscular
IV	Intravenous
SQ	Subcutaneous
IACUC	Institutional Animal Care and Use Committee
H&E	Hematoxylin and Eosin Stain
QC	Quality Control
SPE	Solid Phase Extraction
UPLC-MS/MS	Ultra Performance Liquid Chromatography Tandem Mass Spectrometry
HPLC-MS	High Performance Liquid Chromatography with Mass Spectroscopy
CBC	Complete Blood Count
HCT	Hematocrit
PCV	Packed Cell Volume
RBC	Red Blood Cells
PLT	Platelets
Na	Sodium
K	Potassium

Cl	Chloride
P	Phosphorus
Mg	Magnesium
BUN	Blood Urea Nitrogen
AST	Aspartate Aminotransferase
GLU	Glucose
GGT	Gamma Glutamyl Transpeptidase
CK	Creatinine Kinase
SDH	Sorbitol Dehydrogenase
ALP	Alkaline Phosphatase
PGE ₂	Prostaglandin E ₂
C _{max}	Maximum Concentration
T _{max}	Time to Maximum Concentration
T _{1/2}	Terminal Half-life
FARAD	Food Animal Residue Avoidance Databank
US	United States
EU	European Union
GI	Gastrointestinal
LOD	Limit of Detection
LOQ	Limit of Quantification

Acknowledgements

A sincere and heartfelt thanks goes to my mentor and friend Dr. Steve Ensley for his patience and genuine interest in my training as a toxicologist. I also want to thank my co-major professor Dr. Mike Kleinhenz and the remaining members of my committee: Drs. Mike Apley, Hans Coetzee, and Bob Larson for their guidance not only during my academic advancement but also for serving as moral compasses in life. Surrounding myself with these people is the most intelligent thing I have ever done.

I want to thank Drs. Brandon Plattner, Yuntao Zhang, Savannah Charnas, Jamie Retallick, and the Kansas Veterinary Diagnostic Laboratory for supporting this work. The entire laboratory personnel are a sensational group of people who work every day to safeguard human and animal health in Kansas and beyond and their dedication is inspiring.

I would not be drafting this dissertation if it were not for my student colleagues Mikaela Weeder, Ally Nelson, Charles Wassberg, and Jacob Schumacher for their selfless help in carrying out the work contained herein. A special thank you is due to Misty Bear for obtaining supplies and the fiscal management of this work.

Lastly, I want to thank the Kansas State University College of Veterinary Medicine Intramural Grant Program, the Kansas Veterinary Diagnostic Laboratory, the Fats and Proteins Research Foundation, and the Marguarite Brockmeyer Foundation for providing the financial support for this work.

Dedication

I dedicate this dissertation to my daughters Jensyn, Dottie, and Saylor, may you develop a lifelong love of learning and recognize you can do anything you give sincere effort to. To my wife Carly, who suffered most throughout this process, thank you for your unending grace, patience, and love. To my parents, Duane, and Marcia Fritz, for always being available for anything anyone anywhere could ever need, I am sure better people do not exist on this planet.

Chapter 1 - Literature Review

Introduction

Pain control in production animals is a relatively recent area of research focus. Greater recognition of benefits by veterinarians and researchers, requests for transparency by consumers, and a desire to better care for animals by producers have driven considerable research into pain control over the last fifteen years. Production animals experience pain associated with disease, husbandry procedures, and normal life occurrences like parturition[1]. It follows that the caretakers of animals can improve the quality of life of these animals if analgesic protocols prove safe and effective. The greatest challenge of providing analgesia to production animals is production animals inherently disguise pain due to their historical existence as prey species. Pain assessment techniques are imperative to research pain control in order to show that analgesic drugs are effective. The lack of repeatable methods of pain assessment has been a large hurdle in the regulatory approval of safe and effective analgesic regimens for food animals[2-4]. Research in this area has focused on multiple different assessment techniques including endocrinology, behavior, and physiology[5]. Specific biomarker changes used as an indicator of pain or stress in cattle include substance P, and cortisol[6,7]. Behavioral changes associated with painful stimuli are vast and a detailed description of the various behaviors employed to evaluate pain is outside the scope of this review. Many of the changes are associated with decreased movement, mental activity, and postural changes[5]. Physiological responses to pain include alterations in heart and respiratory rate, and changes in pupil size [5]. According to the United States Food and Drug Administration (FDA), outcomes for pain assessment related to drug approvals must be objective, repeatable, and clearly defined[8].

Efficacy of analgesic drugs is often evaluated using these outcomes of pain assessment as well as improvements in production parameters including increases in feed intake and average daily gain, and increased carcass quality - dependent upon the class of animals in question. Age-associated differences in husbandry practices, performance metrics, and disease susceptibility reduces the capacity to make blanket statements regarding safety and efficacy of therapeutic drugs. Due to the challenges outlined above, there is only a single anti-inflammatory drug with FDA approval for use in food animals to alleviate pain in the United States. Specifically, this single compound is approved to control pain associated with one clinical syndrome (foot rot). There are also no anesthetic or sedative drugs approved for use in food animals.

Due to the lack of FDA-approved analgesic and anesthetic/sedative drugs for use in food animals, veterinarians are forced to use these drugs in an extra-label manner. This drug use scenario is defined as Extra Label Drug Use (ELDU) and is legally allowable under the Animal Medicinal Drug Use Clarification Act (AMDUCA)[9]. Part of the AMDUCA requirements regarding ELDU in food animals mandate that no approved products exist for the specific use desired and the drug in question must be approved for use in other animal species or humans. Veterinarians are also charged with establishing extended withdrawal times to ensure that residues of these drugs do not enter the human food chain via meat, milk, or eggs. As such, veterinarians are forced to extrapolate pharmacokinetic, pharmacodynamic, and safety data from other species to make recommendations for food animals. This can be problematic as significant differences in drug metabolism can exist between and within species[10]. As ruminants, cattle can differ significantly in their ability to absorb a compound compared to monogastric species for which the data may be available. This is an important consideration for evaluating the safety, effectiveness, and the potential residue concerns for food animals.

Humane euthanasia is a potential outcome for animals succumbing to disease or injury. The American Veterinary Medical Association (AVMA) has provided guidelines for the euthanasia of animals for veterinarians[11]. While there are pharmacologic options for euthanasia like sodium pentobarbital, there exist some environmental concerns regarding the fate of carcasses euthanized with this compound[12]. Sodium pentobarbital is also known to survive the rendering process and has caused clinical disease in animals consuming rendered products[13,14]. Physical methods of euthanasia are recommended for food animals including gunshot or penetrating captive bolt, often in conjunction with the use of an anesthetic or sedative drug like xylazine or ketamine[11]. The drug deposition in tissues of animals administered these drugs for euthanasia is unknown as is their fate following rendering. Considering the above, this review will consider the challenges associated with using meloxicam as an analgesic drug and xylazine and ketamine as chemical restraint drugs for euthanasia in cattle.

Body

Commonly-performed management procedures recognized as painful for cattle in the United States include hot-iron branding, castration of male animals, and dehorning[5,15-18]. Currently, there are no FDA-approved analgesic drugs labelled to control pain during these common procedures. Veterinarians are forced to use analgesic drugs in an extra-label manner in order to provide pain control during these procedures. Non-steroidal anti-inflammatory drugs (NSAIDs) are often the analgesic drugs of choice and can be used in conjunction with a local anesthetic[4,16,17,19]. Various NSAIDs are currently used including flunixin meglumine, ketoprofen, and meloxicam among others[18,20]. A recent review of the efficacy of various strategies of pain control suggested that oral meloxicam in combination with a local anesthetic is the preferred method of providing analgesia during common management procedures[20].

Meloxicam is an NSAID which acts by inhibiting cyclooxygenase (COX) enzymes that synthesize prostaglandins from arachidonic acid[21] There are multiple COX isozymes that have various functions and many are inducible[21-23]. In general, COX-1 is responsible for maintaining homeostatic processes including kidney perfusion and maintenance of the gastrointestinal mucosa[21]. COX-2 is recognized as an inducible isoenzyme in the face of pain and inflammation; preferential inhibition of COX-2 is a desirable characteristic in therapeutic preparations in order to maintain the normal processes of COX-1 to avoid adverse effects[22,23]. Adverse effects of NSAID use are attributable to the inhibition of the COX-1 isozyme and include the following: gastrointestinal irritation and ulceration with associated hemorrhage, hypoxic kidney damage, and hepatocyte degeneration[24]. Dose-related injection site lesions have also been observed with injectable preparations [25]. It is commonly accepted that NSAIDs interact with the mucosa of the gastrointestinal tract eventually reducing its hydrophobicity and exposing the mucosa to gastric acids[24]. Further, it has been shown that inhibition of both COX-1 and COX-2 is necessary to cause gastric ulceration in a rat model[26]. NSAIDs have been shown to uncouple oxidative phosphorylation and are known to have vascular effects indicating that multiple pathways are likely involved in the gastric effects of NSAID overdose scenarios[27,28]. Prostaglandin production is critical to maintaining kidney function and prostaglandin inhibition by NSAIDs causes the renal damage associated with overdose[24,28-30]. Prostaglandin inhibition ultimately results in reduced blood flow, slower glomerular filtration rate, and disturbances in salt and water elimination by the kidney[29,30]. Papillary necrosis is a common lesion described in NSAID toxicosis and is caused primarily by ischemia related to the vasoactive properties of NSAIDs[30,31]. The mechanism by which NSAIDs cause liver damage has not been fully elucidated. Current theories revolve around reduced ATP production from

oxidative phosphorylation in hepatocytes, negative effects on hepatocyte mitochondrial membranes, and lipid peroxidation from metabolic products[24].

Meloxicam is considered a COX-2-selective NSAID but has been shown to lose this selectivity in overdose situations[26,32,33]. Meloxicam COX-2 selectivity has been demonstrated in humans and some veterinary species but has not been demonstrated in cattle. Characterization of the dosage needed to result in adverse effects in multiple age classes of cattle is necessary for judicious use of this compound by veterinarians and is currently unknown - representing a significant data gap.

Efficacy

Efficacy is an important consideration of pain control regimens, and a significant body of research has evaluated the efficacy of meloxicam for controlling pain associated with multiple procedures and diseases in different classes of cattle[2,3]. A summary of this research is presented in Table 1.1. Hirsch et. al. (2009) gave 1.5 mg/kg meloxicam intravenously and did not demonstrate an effect on reproductive outcomes[34]. Heinrich et. al. (2010) reported a reduction in pain-associated behavior in Holstein calves administered 0.5 mg/kg IM meloxicam at the time of cautery dehorning compared to controls[35]. Todd et. al. (2010) administered 0.5 mg/kg meloxicam subcutaneously to neonatal Holstein calves affected with neonatal diarrhea and demonstrated an increase in average daily gain but no difference in weaning weights compared to control animals[36]. Fitzpatrick et. al. (2012) utilized an induced mastitis model in lactating dairy cows and showed that 0.5 mg/kg meloxicam administered subcutaneously decreased udder edema and body temperature compared to untreated controls[37]. Theurer et. al. (2012) utilized an oral dose of 0.5 mg/kg in 10-week-old calves at the time of cautery dehorning and reported treated calves spent more time laying down or at the feed bunk than untreated

controls[38]. Offinger et. al. (2013) evaluated 0.5 mg/kg meloxicam administered IV at the time of resection of the interphalangeal joint in adult dairy cows and reported decreased cortisol, decreased lameness scores, and decreased body temperature compared to controls[39]. Allen et. al. (2013) demonstrated decreased cortisol, substance P, and prostaglandin E₂ (PGE₂) in 8-10 week old Holstein calves administered 1 mg/kg meloxicam orally (given either 12 hours prior or at the time of cautery dehorning)[40]. There were also notable differences in PGE₂ associated with timing of administration[40]. Glynn et. al. (2013) utilized 1 mg/kg oral meloxicam in Holstein steers at the time of dehorning and reported a decrease in substance P and increased average daily gain compared to controls but no effect on cortisol concentrations[41]. Barrier et. al. (2014) administered 0.5 mg/kg meloxicam subcutaneously to adult beef cows at the time of cesarean section and documented that treated cows spent more time lying after surgery than untreated controls[42]. The increased lying time was interpreted as increased cow comfort. Newby et. al. (2014) were unable to demonstrate an effect of 0.5 mg/kg subcutaneous meloxicam administration on retained fetal membranes in adult dairy cows[43]. Nagel et. al. (2016) evaluated the effect of an oral dose of 1 mg/kg meloxicam on lameness in cattle weighing 309-954 kg and noted a 93% reduction in lameness scores[44]. Olson et. al. (2016) evaluated the effects of 1 mg/kg oral meloxicam on calf pain markers following band and surgical castration and noted that treated calves maintained a lower heart rate, lower cortisol, and lower substance P than controls[45]. Swartz et. al.(2018) showed oral meloxicam at 1mg/kg increased milk yield and altered behavior in adult dairy cows experiencing dystocia compared to dystocia cows not administered meloxicam [46]. In two studies, Melendez et. al. (both 2018) demonstrated a decrease in painful behaviors and a reduction in physiological and behavioral pain indicators in 2- and 7-month-old beef calves given 0.5 mg/kg subcutaneous meloxicam at the time of

castration in one study and castration and branding in the other (2-month-old)[47,48]. Ede et. al. (2019) utilized spatial recognition to demonstrate that calves receiving 0.5 mg/kg subcutaneous meloxicam and a local block at the time of hot iron disbudding spent more time in a pen where they were disbudded compared to disbudded calves that only received a local block[49]. Clark et. al. (2020) evaluated 1mg/kg oral meloxicam on pre-weaning performance of dairy calves and noted an increase in calf starter intake but no difference in IgG uptake[50]. While recent meta-analyses have indicated that NSAIDs appear to positively affect pain markers, the body of research into pain assessment and control is variable between studies and a more standardized approach should be used to make larger comparisons valid[2,3]. The unreliability of pain assessment combined with relatively large variability in study designs to evaluate interventions remains a significant hurdle in the recommendation of pain control regimens.

Table 1.1. Summary of research evaluating the efficacy of meloxicam use in cattle for multiple purposes.

Procedure/Disease	Dose Route	Effect	Animal Type	Citation
Reproduction	1.5 mg/kg IV	No effect	Adult Dairy Cows	Hirsch 2009[34]
Neonatal diarrhea	0.5 mg/kg SQ	Treated calves had higher ADG over 8 weeks, no difference in WW	Neonatal Holstein Calves	Todd 2010[36]
Cautery Dehorning	0.5 mg/kg IM	Reduced pain-associated behavior and activity	Holstein Calves	Heinrich 2010[35]
Induced Mastitis	0.5 mg/kg SQ	Decreased udder edema and body temperature	Lactating dairy cows	Fitzpatrick 2012[37]
Cautery Dehorning	0.5 mg/kg PO	More time laying, more time at feed bunk	10-week-old calves	Theurer 2012[38]
Distal interphalangeal joint resection	0.5 mg/kg IV	Decreased cortisol, lameness scores, and body temperature	Adult Holstein cows	Offinger 2013[39]
Cautery Dehorning	1mg/kg 12 hours prior or at time	Decreased cortisol, substance P, and PGE2 with timing effect	8–10-week-old Holstein calves	Allen 2013[40]
Dehorning	1 mg/kg	Decreased Sub-P Increased MNT and ADG	Holstein Steers	Glynn, 2013[41]
Cesarean Section	0.5 mg/kg SQ	Treated cows spent more time lying	Adult Beef Cows	Barrier 2014[42]
Retained Fetal Membranes	0.5 mg/kg SQ	No effect	Adult dairy cows	Newby 2014[43]
Musculoskeletal Lameness	1 mg/kg PO	93% reduction in lameness scores	309-954kg cattle	Nagel 2016[44]

Band and Surgical Castration	1 mg/kg PO	Lower HR, Sub-P, Cortisol Increased movement Less inflammation	4–5-month-old Holstein Calves	Olson 2016[45]
Parturition	1 mg/kg PO	Increased milk yield, altered behavior in dystocia	Adult Dairy Cows	Swartz 2018[46]
Castration and branding	0.5 mg/kg SQ	Decreased pain- associated behaviors	2-month-old beef calves	Melendez 2018[47]
Surgical Castration	0.5 mg/kg SQ	Reduced physiological and behavioral pain indicators	7–8-month-old beef calves	Melendez 2018[48]
Hot iron disbudding	0.5 mg/kg SQ	Spent more time in the pen where they received meloxicam	1-month-old dairy calves	Ede 2019[49]
Neonatal IgG uptake, performance	1 mg/kg PO Bolus and mixed in CR	Positive effects on starter intake	Pre-weaned dairy calves	Clark 2020[50]

Safety

Adverse effects associated with NSAID administration are generally attributed to NSAID action on the COX-1 isozyme. These effects include the following: gastrointestinal erosion and ulceration, hypoxic damage to the kidneys, and hepatocyte degeneration[24]. Various methods have been used to identify and quantify these effects. Evaluation criteria for determination of negative effects on the GI tract include presence of fecal occult blood, decreased PCV and hematocrit, abdominal pain characterized by kicking and rolling, and gross and histologic observation of erosions, ulcers, or perforations. Kidney function is often evaluated using serum chemistry analysis - specifically blood urea nitrogen (BUN) and creatinine, as well as urinalysis, and gross and histologic examination of the kidneys themselves. Liver function is evaluated using liver-specific enzyme assays in serum, and post-mortem examination. Injectable preparations may cause injection site lesions that can be evaluated for heat and swelling ante-mortem and characterized microscopically post-mortem. The safety of meloxicam and the determination of lethal dose fifty (LD50) dosages as well as determination of “no observed adverse effect level” have been performed in multiple species. This work is summarized in Table 1.2. Raidal et. al. (2012) noted damage to the gastrointestinal and renal systems, effects on the bone marrow, and a reduction in serum protein in adult horses given 3.0 mg/kg meloxicam orally every day for two weeks[51]. Raidal et. al. (2013) administered oral meloxicam at 1.8 mg/kg for seven days, and 0.6 mg/kg for three weeks to foals and did not report any effects[52]. Summa et. al. (2017) evaluated the effects of oral meloxicam at 20 mg/kg every twelve hours for seven consecutive days in American Kestrels and did not report any effects[53]. Hare et. al. (2018) utilized a mucosal spray of meloxicam in dogs up to 0.6 mg/kg once daily for six months and did not report any adverse effects[54]. Sarfaty et. al. (2019)

administered subcutaneous meloxicam at 20 mg/kg in mice and described some injection site pathology but no systemic toxic effects[25]. Bharani et. al. (2020) administered oral meloxicam at 4 mg/kg and 8 mg/kg to rats for 28 consecutive days and reported damage to the liver, kidney, and gastrointestinal tract and a decrease in packed cell volume and erythrocyte count[55]. Kendall et. al. (2021) administered subcutaneous meloxicam at 20 mg/kg to female mice for 3-7 days and reported fecal blood and acute gastritis[56]. There is a lack of data characterizing the dosage and time of administration necessary to cause adverse effects in cattle of various ages. The safety of meloxicam administered as a single large dose, and as multiple small doses should be evaluated in order to generate data that may allow for the development of more targeted and flexible dosing regimens.

Table 1.2. Summary of research evaluating the safety of meloxicam in multiple species with multiple routes of administration.

Species	Dose – Route - Duration	Effect	Citation
Adult Horses	3.0 mg/kg PO daily for 2 weeks	Reduction in serum protein, GI and renal damage, bone marrow effects	Raidal 2012[51]
Foals	1.8 mg/kg PO 7 days 0.6 mg/kg PO 3 weeks	None	Raidal 2013[52]
Kestrels	20 mg/kg PO every 12 hours for 7 days	None	Summa 2017[53]
Dogs	0.6 mg/kg mucosal, daily for 188 days	None	Hare 2018[54]
Mice	20 mg/kg SQ 6 days	Injection site pathology, not toxic effects	Sarfaty 2019[25]
Rats	4 mg/kg and 8 mg/kg PO for 28 days	Liver, kidney, GI, decreased PCV and erythrocyte count	Bharani 2020[55]
Female Mice	20 mg/kg SQ 3-7 days	Fecal blood, gastritis	Kendall 2021[56]

Pharmacokinetics

The safety profile for meloxicam in cattle has not been evaluated and significant differences in pharmacokinetics and pharmacodynamics of meloxicam exist between species - especially considering ruminant versus monogastric species. A summary of basic pharmacokinetic parameters identified in cattle to date is presented in Table 1.3. Coetzee et al. (2009) described a maximum concentration ($C_{\max}$) of 3.1 ug/mL in plasma 11.6 hours ($T_{\max}$) following oral administration of 1 mg/kg in calves[57]. Coetzee et. al. (2011) demonstrated a maximum plasma concentration of 2.11 ug/mL 11.67 hours following an oral dose of 0.5 mg/kg concurrently with gabapentin in calves[58]. Mosher et. al. (2012) administered oral dosages of 0.5 mg/kg to pre-ruminant and ruminant calves as a bolus or dissolved in colostrum replacer. Maximum plasma concentrations ranged from 1.27-2.20 ug/mL at 14.3-17.3 hour post administration[59]. Malreddy et. al. (2012) gave adult cows an oral dose of 1.0 mg/kg and described maximum plasma concentrations of 2.89 ug/mL at 11.33 hours[60]. Allen et. al. (2013) administered 1.0 mg/kg orally to calves 12 hours before or at the time of cautery dehorning in 8–10-week-old Holstein calves. Maximum plasma concentrations were similar at 3.61 ug/mL and 3.27 ug/mL for the 12 hours pre, and at the time of dehorning, respectively. These concentrations were achieved at 13.95- and 15-hours post-administration respectively, and the half-lives were similar at 38.62 and 35.81 hours[40]. Glynn et. al. (2013) administered meloxicam at 1.0 mg/kg alone and in conjunction with gabapentin to dairy calves and observed maximum concentrations in plasma of 1.9 and 2.3 ug/mL at 18-24 hours[41]. Coetzee et. al. (2015) gave repeated doses of 0.5 mg/kg orally every 24 hours for 4 days and described a maximum plasma concentration of 4.52 ug/mL at 88.5 hours[61]. This work demonstrated higher concentrations than single

administration trials as well as a delay to maximum concentration. Gorden et. al. (2018) administered oral doses of 1 mg/kg to post-partum and mid-lactation cows and identified a maximum plasma concentration of 2.92 and 1.82 ug/mL for each respective group. The time to maximum concentration was 17.6 hours for the post-partum group and 11.62 hours for the mid-lactation group showing differences in physiological state[62]. Shock et. al. (2019) gave 0.5 mg/kg subcutaneously and 1 mg/kg orally to adult cows and found maximum plasma concentrations of 1.22 and 1.68 ug/mL at 5.3 and 13.3 hours respectively[63]. Melendez et. al. (2019) did similar work in beef calves giving 0.5 mg/kg meloxicam subcutaneously with C_{max} of 2.37 ug/mL and T_{max} of 3.7 hours compared to 1.0 mg/kg orally with C_{max} of 2.33 ug/mL at 24 hours[64]. Warner et. al. (2020) used mid-lactation and post-partum cows and intravenous (0.2 mg/kg) and oral (1.0 mg/kg) doses to demonstrate differing C_{max} and T_{max} for each group and route. Post-partum cows had a C_{max} of 1.06 ug/mL at 0.23 hours following IV administration and a C_{max} of 2.61 ug/mL at 16.75 hours after oral administration. Mid-lactation cows had a C_{max} of 1.22 ug/mL at 0.13 hours following IV administration and 1.45 ug/mL at 10.48 hours following oral administration which are similar to the Gorden study. Jokela et. al. (2024) gave 0.5 mg/kg intravenously, subcutaneously, and orally to pre-ruminant calves with associated C_{max} of 1.91 and 1.77 for the SQ and PO routes at 7.6 and 10 hours respectively[65].

Although expanded understanding of the pharmacodynamics of meloxicam is needed, it is clear that maintaining longer-term analgesia with a single administration in cattle is desirable given the challenges of handling this species in many production systems. Longer-term analgesia would need to be achieved with either repeated dosing or administration of a larger dose. Multiple administrations appear to maintain drug concentrations for a longer period of time but the risk of adverse effects of multiple doses has not been investigated in cattle. Larger doses

generally achieve larger maximum concentrations; thus, a single large dose may result in longer durations of plasma concentrations above the concentrations reported in these studies, resulting in a longer duration of analgesia with a single administration. Pharmacokinetic and toxicologic evaluation of a single large dose and multiple smaller doses in cattle should be evaluated for their utility in safely providing longer duration analgesia.

Table 1.3. Summary of basic pharmacokinetic parameters in multiple age classes of cattle receiving different doses of meloxicam by different routes of administration.

Animal Type	Dose & Route	Cmax (ug/mL)	Tmax (h)	T ½ (h)	Citation
Ruminant Calves	1 mg/kg PO	3.1	24	16.7	Coetzee 2009[57]
Beef Calves	0.5 mg/kg PO	2.11	11.67	20.5	Coetzee 2011[58]
Pre-ruminant and Ruminant calves	0.5 mg/kg PO	1.27 2.20 1.95	14.3 17.0 17.3	36 40 29.9	Mosher 2012[59]
Adult Cows	1 mg/kg PO	2.89	11.33	14.58	Malreddy 2012[60]
Dairy Calves	1.0 mg/kg PO 12 h pre or at time of dehorning	3.61 3.27	13.95 15.0	38.62 35.81	Allen 2013[40]
Dairy Calves	1 mg/kg PO	1.9	24	16.7	Glynn 2013[41]
Beef Calves	0.5 mg/kg q24 for 4 days	4.52	88.5	25.66	Coetzee 2015[61]
Post-partum and mid-lactation cows	1 mg/kg PO	2.92 1.82	17.6 11.6	13 11.9	Gorden 2018[62]
Adult Cows	0.5 mg/kg SQ 1 mg/kg PO	1.22 1.68	5.3 13.33	8.95 25.71	Shock 2019[63]
Beef Calves	1.0 mg/kg PO 0.5 mg/kg SQ	2.33 2.37	24 3.7	15.6 16.2	Melendez 2019[64]
Post-partum and mid-lactation cows	0.2 mg/kg IV 1 mg/kg PO	1.22 1.45 1.06	0.13 10.48 0.23	8.23 9.55 17.31	Warner 2020[66]

		2.61	16.75	12.28	
Pre-ruminants	0.5 mg/kg IV,	0	0	79	Jokela
	SQ, and PO	1.91	7.6	84.6	2024[65]
		1.77	10	84.8	

Xylazine and Ketamine Residues

According to AMDUCA regulations, veterinarians are legally allowed to use certain drugs in ways not indicated on the drug's label (with certain restrictions). One of the restrictions is ensuring that drug residues are not present in marketable products through the establishment of extended withdrawal periods supported by scientific literature. As there are no anesthetic or sedative drugs approved for use in food animals, veterinarians are forced to use these drugs approved for use in other veterinary species or humans. Xylazine and ketamine can be used to chemically restrain food animals prior to physical euthanasia via gunshot or penetrating captive bolt[11]. The Food Animal Residue Avoidance Databank (FARAD) suggests meat withdrawal times of four and three days for xylazine and ketamine for human consumption, respectively[67,68]. When used for euthanasia, these anesthetics are administered a few minutes before death, thus, euthanized animals administered these drugs cannot legally enter the human food chain. Rendering is an option for deceased animals to utilize this resource in the manufacturing of multiple products including base ingredients for pet food[69]. Sodium pentobarbital is approved for euthanasia alone and situations have happened where treated carcasses used either fresh or rendered into dog food resulted in clinical disease in animals consuming these tissues[12,13,70]. It has also been shown that pentobarbital survives the rendering process[14]. Xylazine and ketamine are potential alternatives to sodium pentobarbital but their distribution in edible tissue immediately following administration as well as their persistence through the rendering process are unknown. This represents a significant data gap that could potentially influence the recovery of a revenue stream for producers - provided animals euthanized using these drugs could safely be rendered.

Conclusion

Significant hurdles exist in providing adequate pain control for food animals during common management procedures. One such hurdle in the US is the lack of FDA-approved products labelled for controlling pain encountered in various scenarios beyond foot rot. The lack of approved products in the US is multi-factorial and can be attributed partially to the lack of objective and repeatable pain assessment techniques, variable efficacy for certain conditions, and the lack of safety information regarding commonly-used analgesic drugs in ruminant species. Meloxicam is a commonly-used NSAID in cattle production in many countries and has been suggested to be the preferred method of providing analgesia to cattle given its efficacy, ease of use, and extended elimination kinetics compared to other NSAIDs. The safety of meloxicam use in ruminants is a significant data gap in building the information necessary to potentially secure FDA approval and to guide dosing decisions by veterinarians. The safety profile of meloxicam in cattle is further compounded by significant differences in age classes given the transition from pre-ruminant calves to ruminating adults. The pharmacokinetics and toxicological effects of a single large dose and multiple small doses of meloxicam should be evaluated in cattle.

In certain disease states and following injuries to food animals, analgesic therapy is not intended to be curative, but rather, to provide welfare benefits prior to and during euthanasia. On-farm humane euthanasia of food animals is unfortunately a necessity of production. Various methods are approved by the AVMA including pharmacologic options like sodium pentobarbital alone or physical methods like gun shot or penetrating captive bolt combined with secondary options including exsanguination, pithing, or intravenous potassium chloride. Sodium pentobarbital is effective but has significant long-term environmental concerns. Animals euthanized by this method are unable to enter the rendering process due to its ability to survive

rendering which eliminates a possible revenue stream for producers[14]. There have been instances where carcasses euthanized with pentobarbital have served as sources of exposure when fresh (un-rendered) tissue was fed to dogs and tigers or where rendered carcasses of cattle euthanized with pentobarbital were used in the production of dog food and resulted in clinically poisoned dogs and dog food recalls[12,13,70]. Animals can be chemically restrained prior to utilizing the physical methods of euthanasia with anesthetic or sedative drugs. The American Veterinary Medical Association (AVMA) recommends several drugs for this purpose including xylazine and ketamine[11]. The fate of these drugs through the rendering process is unknown. The potential use of these drugs for euthanasia presents a possible opportunity to allow treated animals to be rendered and the resulting products used for various purposes, including dog food. The tissue distribution and persistence through rendering of xylazine and ketamine should be evaluated to guide their use for euthanasia in food animals and the fate of the carcasses of animals administered these drugs.

References

1. Weary, D.M.; Niel, L.; Flower, F.C.; Fraser, D. Identifying and preventing pain in animals. *Applied Animal Behaviour Science* **2006**, *100*, 64-76, doi:<https://doi.org/10.1016/j.applanim.2006.04.013>.
2. Wagner, B.K.; Nixon, E.; Robles, I.; Baynes, R.E.; Coetzee, J.F.; Pairis-Garcia, M.D. Non-Steroidal Anti-Inflammatory Drugs: Pharmacokinetics and Mitigation of Procedural-Pain in Cattle. *Animals (Basel)* **2021**, *11*, doi:10.3390/ani11020282.
3. Winder, C.B.; Miltenburg, C.L.; Sargeant, J.M.; LeBlanc, S.J.; Haley, D.B.; Lissemore, K.D.; Godkin, M.A.; Duffield, T.F. Effects of local anesthetic or systemic analgesia on pain associated with cautery disbudding in calves: A systematic review and meta-analysis. *J Dairy Sci* **2018**, *101*, 5411-5427, doi:10.3168/jds.2017-14092.
4. Steagall, P.V.; Bustamante, H.; Johnson, C.B.; Turner, P.V. Pain Management in Farm Animals: Focus on Cattle, Sheep and Pigs. *Animals (Basel)* **2021**, *11*, doi:10.3390/ani11061483.
5. Hudson, C.; Whay, H.; Huxley, J. Recognition and management of pain in cattle. *In Practice* **2008**, *30*, 126-134, doi:<https://doi.org/10.1136/inpract.30.3.126>.
6. Tschoner, T.; Feist, M. Substance P concentrations in the blood plasma and serum of adult cattle and calves during different painful procedures and conditions – a systematic review. *BMC Veterinary Research* **2022**, *18*, 232, doi:10.1186/s12917-022-03304-6.
7. Barragan, A.A.; Piñeiro, J.M.; Schuenemann, G.M.; Rajala-Schultz, P.J.; Sanders, D.E.; Lakritz, J.; Bas, S. Assessment of daily activity patterns and biomarkers of pain, inflammation, and stress in lactating dairy cows diagnosed with clinical metritis. *Journal of Dairy Science* **2018**, *101*, 8248-8258, doi:<https://doi.org/10.3168/jds.2018-14510>.

8. United States Food and Drug Administration. Guidance for Industry #123 Development of data supporting approval of NSAIDs for use in animals. Available online: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-123-development-data-supporting-approval-nsaids-use-animals> (accessed on 04/21).
9. United States Food and Drug Administration. Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). Available online: <https://www.fda.gov/animal-veterinary/guidance-regulations/animal-medicinal-drug-use-clarification-act-1994-amduca#animals> (accessed on 5/25/2025).
10. Packiasabapathy, S.; Sadhasivam, S. Gender, genetics, and analgesia: understanding the differences in response to pain relief. *J Pain Res* **2018**, *11*, 2729-2739, doi:10.2147/jpr.S94650.
11. AVMA. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Available online: <https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf> (accessed on 5/7/2025).
12. Kaiser, A.M.; McFarland, W.; Siemion, R.S.; Raisbeck, M.F. Secondary pentobarbital poisoning in two dogs: a cautionary tale. *Journal of Veterinary Diagnostic Investigation* **2010**, *22*, 632-634, doi:10.1177/104063871002200423.
13. Buchweitz, J.P.; Johnson, M.; Jones, J.L.; Lehner, A.F. Development of a Quantitative Gas Chromatography-Tandem Mass Spectrometry Method for the Determination of Pentobarbital in Dog Food. *Journal of Agricultural and Food Chemistry* **2018**, *66*, 11166-11169, doi:10.1021/acs.jafc.8b04178.

14. O'Connor, J.J., Stowe, Clarence M., Robinson, Robert R. Fate of Sodium Pentobarbital in Rendered Products. *American Journal of Veterinary Research* **1985**, 46, 1721-1724, doi:<https://doi.org/10.2460/ajvr.1985.46.08.1721>.
15. Coetzee, J.F.; Nutsch, A.L.; Barbur, L.A.; Bradburn, R.M. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. *BMC Veterinary Research* **2010**, 6, 12, doi:10.1186/1746-6148-6-12.
16. Fajt, V.R.; Wagner, S.A.; Norby, B. Analgesic drug administration and attitudes about analgesia in cattle among bovine practitioners in the United States. *J Am Vet Med Assoc* **2011**, 238, 755-767, doi:10.2460/javma.238.6.755.
17. Johnstone, E.C.S.; Coetzee, J.F.; Pinedo, P.J.; Edwards-Callaway, L. Current attitudes of veterinarians and producers regarding the use of local and systemic analgesia in beef and dairy cattle in the United States. *J Am Vet Med Assoc* **2021**, 258, 197-209, doi:10.2460/javma.258.2.197.
18. Robles, I.; Arruda, A.G.; Nixon, E.; Johnstone, E.; Wagner, B.; Edwards-Callaway, L.; Baynes, R.; Coetzee, J.; Pairis-Garcia, M. Producer and Veterinarian Perspectives towards Pain Management Practices in the US Cattle Industry. *Animals (Basel)* **2021**, 11, doi:10.3390/ani11010209.
19. Robles, I.; Arruda, A.G.; Nixon, E.; Johnstone, E.; Wagner, B.; Edwards-Callaway, L.; Baynes, R.; Coetzee, J.; Pairis-Garcia, M. Producer and Veterinarian Perspectives towards Pain Management Practices in the US Cattle Industry. *Animals* **2021**, 11, 209.

20. Kleinhenz, M.D.; Viscardi, A.V.; Coetzee, J.F. Invited Review: On-farm pain management of food production animals. *Applied Animal Science* **2021**, *37*, 77-87, doi:<https://doi.org/10.15232/aas.2020-02106>.
21. Morita, I. Distinct functions of COX-1 and COX-2. *Prostaglandins & Other Lipid Mediators* **2002**, 68-69, 165-175, doi:[https://doi.org/10.1016/S0090-6980\(02\)00029-1](https://doi.org/10.1016/S0090-6980(02)00029-1).
22. Lehmann, H.A.; Baumeister, M.; Lützen, L.; Wiegler, J. Meloxicam: A toxicology overview. *InflammoPharmacology* **1996**, *4*, 105-123, doi:10.1007/BF02735465.
23. Warner, T.D.; Mitchell, J.A. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. *The FASEB Journal* **2004**, *18*, 790-804, doi:<https://doi.org/10.1096/fj.03-0645rev>.
24. Panchal, N.K.; Prince Sabina, E. Non-steroidal anti-inflammatory drugs (NSAIDs): A current insight into its molecular mechanism eliciting organ toxicities. *Food and Chemical Toxicology* **2023**, *172*, 113598, doi:<https://doi.org/10.1016/j.fct.2022.113598>.
25. Sarfaty, A.E.; Zeiss, C.J.; Willis, A.D.; Harris, J.M.; Smith, P.C. Concentration-dependent Toxicity after Subcutaneous Administration of Meloxicam to C57BL/6N Mice (Mus musculus). *J Am Assoc Lab Anim Sci* **2019**, *58*, 802-809, doi:10.30802/aalas-jaalas-19-000037.
26. Wallace, J.L.; McKnight, W.; Reuter, B.K.; Vergnolle, N. NSAID-induced gastric damage in rats: Requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology* **2000**, *119*, 706-714, doi:<https://doi.org/10.1053/gast.2000.16510>.
27. Díaz-González, F.; Sánchez-Madrid, F. NSAIDs: learning new tricks from old drugs. *Eur J Immunol* **2015**, *45*, 679-686, doi:10.1002/eji.201445222.

28. Bindu, S.; Mazumder, S.; Bandyopadhyay, U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical Pharmacology* **2020**, *180*, 114147, doi:<https://doi.org/10.1016/j.bcp.2020.114147>.
 29. Ejaz, P.; Bhojani, K.; Joshi, V.R. NSAIDs and kidney. *Journal of Association of Physicians of India* **2004**, *52*, 632-640.
 30. Saad, J.; Mathew, D. Nonsteroidal Anti-Inflammatory Drugs Toxicity. In *StatPearls*; StatPearls Publishing
- Copyright © 2025, StatPearls Publishing LLC.: Treasure Island (FL) 2025.
31. Rahman, S.; Malcoun, A. Nonsteroidal Antiinflammatory Drugs, Cyclooxygenase-2, and the Kidneys. *Primary Care: Clinics in Office Practice* **2014**, *41*, 803-821, doi:<https://doi.org/10.1016/j.pop.2014.09.001>.
 32. Del Tacca, M.; Colucci, R.; Fornai, M.; Blandizzi, C. Efficacy and Tolerability of Meloxicam, a COX-2 Preferential Nonsteroidal Anti-Inflammatory Drug. *Clinical Drug Investigation* **2002**, *22*, 799-818, doi:10.2165/00044011-200222120-00001.
 33. C. M. Modi, S.K.M., H.B. Patel, G.B. Dudhatra, Avinash Kumar, Madhavi Avale. *Toxicopathological overview of analgesic and anti-inflammatory drugs in animals*; Issue : 1: Volume Volume : 2, pp. 149-157.
 34. Hirsch, A.C.; Philipp, H. Effects of meloxicam on reproduction parameters in dairy cattle. *J Vet Pharmacol Ther* **2009**, *32*, 566-570, doi:10.1111/j.1365-2885.2009.01109.x.
 35. Heinrich, A.; Duffield, T.F.; Lissemore, K.D.; Millman, S.T. The effect of meloxicam on behavior and pain sensitivity of dairy calves following cautery dehorning with a local anesthetic. *Journal of Dairy Science* **2010**, *93*, 2450-2457, doi:<https://doi.org/10.3168/jds.2009-2813>.

36. Todd, C.G.; Millman, S.T.; McKnight, D.R.; Duffield, T.F.; Leslie, K.E. Nonsteroidal anti-inflammatory drug therapy for neonatal calf diarrhea complex: Effects on calf performance1. *Journal of Animal Science* **2010**, *88*, 2019-2028, doi:10.2527/jas.2009-2340.
37. Fitzpatrick, C.E.; Chapinal, N.; Petersson-Wolfe, C.S.; DeVries, T.J.; Kelton, D.F.; Duffield, T.F.; Leslie, K.E. The effect of meloxicam on pain sensitivity, rumination time, and clinical signs in dairy cows with endotoxin-induced clinical mastitis. *J Dairy Sci* **2013**, *96*, 2847-2856, doi:10.3168/jds.2012-5855.
38. Theurer, M.E.; White, B.J.; Coetzee, J.F.; Edwards, L.N.; Mosher, R.A.; Cull, C.A. Assessment of behavioral changes associated with oral meloxicam administration at time of dehorning in calves using a remote triangulation device and accelerometers. *BMC Vet Res* **2012**, *8*, 48, doi:10.1186/1746-6148-8-48.
39. Offinger, J.; Herdtweck, S.; Rizk, A.; Starke, A.; Heppelmann, M.; Meyer, H.; Janssen, S.; Beyerbach, M.; Rehage, J. Postoperative analgesic efficacy of meloxicam in lame dairy cows undergoing resection of the distal interphalangeal joint. *J Dairy Sci* **2013**, *96*, 866-876, doi:10.3168/jds.2011-4930.
40. Allen, K.A.; Coetzee, J.F.; Edwards-Callaway, L.N.; Glynn, H.; Dockweiler, J.; KuKanich, B.; Lin, H.; Wang, C.; Fraccaro, E.; Jones, M.; et al. The effect of timing of oral meloxicam administration on physiological responses in calves after cautery dehorning with local anesthesia. *J Dairy Sci* **2013**, *96*, 5194-5205, doi:10.3168/jds.2012-6251.
41. Glynn, H.D.; Coetzee, J.F.; Edwards-Callaway, L.N.; Dockweiler, J.C.; Allen, K.A.; Lubbers, B.; Jones, M.; Fraccaro, E.; Bergamasco, L.L.; KuKanich, B. The

- pharmacokinetics and effects of meloxicam, gabapentin, and flunixin in postweaning dairy calves following dehorning with local anesthesia. *J Vet Pharmacol Ther* **2013**, *36*, 550-561, doi:10.1111/jvp.12042.
42. Barrier, A.C.; Coombs, T.M.; Dwyer, C.M.; Haskell, M.J.; Goby, L. Administration of a NSAID (meloxicam) affects lying behaviour after caesarean section in beef cows. *Applied Animal Behaviour Science* **2014**, *155*, 28-33, doi:<https://doi.org/10.1016/j.applanim.2014.02.015>.
43. Newby, N.C.; Renaud, D.; Tremblay, R.; Duffield, T.F. Evaluation of the effects of treating dairy cows with meloxicam at calving on retained fetal membranes risk. *Can Vet J* **2014**, *55*, 1196-1199.
44. Nagel, D.; Wieringa, R.; Ireland, J.; Olson, M.E. The use of meloxicam oral suspension to treat musculoskeletal lameness in cattle. *Vet Med (Auckl)* **2016**, *7*, 149-155, doi:10.2147/vmrr.S112200.
45. Olson, M.E.; Ralston, B.; Burwash, L.; Matheson-Bird, H.; Allan, N.D. Efficacy of oral meloxicam suspension for prevention of pain and inflammation following band and surgical castration in calves. *BMC Vet Res* **2016**, *12*, 102, doi:10.1186/s12917-016-0735-3.
46. Swartz, T.H.; Schramm, H.H.; Bewley, J.M.; Wood, C.M.; Leslie, K.E.; Petersson-Wolfe, C.S. Meloxicam administration either prior to or after parturition: Effects on behavior, health, and production in dairy cows. *Journal of Dairy Science* **2018**, *101*, 10151-10167, doi:<https://doi.org/10.3168/jds.2018-14657>.
47. Meléndez, D.M.; Marti, S.; Pajor, E.A.; Moya, D.; Gellatly, D.; Janzen, E.D.; Schwartzkopf-Genswein, K.S. Effect of subcutaneous meloxicam on indicators of acute

- pain and distress after castration and branding in 2-mo-old beef calves^{1,2}. *Journal of Animal Science* **2018**, 96, 3606-3621, doi:10.1093/jas/sky245.
48. Meléndez, D.M.; Marti, S.; Pajor, E.A.; Sidhu, P.K.; Gellatly, D.; Moya, D.; Janzen, E.D.; Coetzee, J.F.; Schwartzkopf-Genswein, K.S. Effect of meloxicam and lidocaine administered alone or in combination on indicators of pain and distress during and after knife castration in weaned beef calves. *PLOS ONE* **2018**, 13, e0207289, doi:10.1371/journal.pone.0207289.
 49. Ede, T.; von Keyserlingk, M.A.G.; Weary, D.M. Assessing the affective component of pain, and the efficacy of pain control, using conditioned place aversion in calves. *Biology Letters* **2019**, 15, 20190642, doi:doi:10.1098/rsbl.2019.0642.
 50. Clark, M.O.C.; Stahl, T.C.; Erickson, P.S. The effect of meloxicam on neonatal dairy calves: Immunoglobulin G uptake and preweaning performance. *Journal of dairy science*. **2020**, doi:10.3168/jds.2020-18501.
 51. Raidal, S.; Schneiders, F.; Pippia, J.; Noble, G. *Safety evaluation of repeated doses of meloxicam and phenylbutazone in adult horses*; publisher location: Artarmon
publisher name: Australian Veterinary Association (N.S.W) Division: Volume 28, p. 65.
 52. Raidal, S.; Pippia, J.; Noble, G. *Pharmacokinetics and safety of oral doses of meloxicam in foals less than six weeks of age*; publisher location: Lexington
publisher name: American Association of Equine Practitioners (AAEP): p. 33.
 53. Summa, N.M.; Guzman, D.S.; Larrat, S.; Troncy, E.; Bird, D.M.; Lair, S.; Fitzgerald, G. Evaluation of High Dosages of Oral Meloxicam in American Kestrels (*Falco sparverius*). *J Avian Med Surg* **2017**, 31, 108-116, doi:10.1647/2015-150.

54. Hare, J.E.; Niemuller, C.A.; Petrick, D.M. Target animal safety study of meloxicam administered via transmucosal oral spray (Promist® technology) for 6 months in dogs. *Journal of Veterinary Pharmacology and Therapeutics* **2013**, *36*, 412-416, doi:<https://doi.org/10.1111/jvp.12018>.
55. Bharani, S.P., Naik, A.K., Parija, S.C.; and Panda, S.K. Meloxicam induced toxicopathology studies in Wistar rats. . *Indian Journal of Animal Research* **2020**, *54*, 363-366, doi:10.18805/ijar.B-3766.
56. Kendall, L.V.; Bailey, A.L.; Singh, B.; McGee, W. Toxic Effects of High-dose Meloxicam and Carprofen on Female CD1 Mice. *J Am Assoc Lab Anim Sci* **2022**, *61*, 75-80, doi:10.30802/aalas-jaalas-21-000071.
57. Coetzee, J.F.; KuKanich, B.; Mosher, R.; Allen, P.S. Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. *Vet Ther* **2009**, *10*, E1-8.
58. Coetzee, J.F.; Mosher, R.A.; Kohake, L.E.; Cull, C.A.; Kelly, L.L.; Mueting, S.L.; KuKanich, B. Pharmacokinetics of oral gabapentin alone or co-administered with meloxicam in ruminant beef calves. *The Veterinary Journal* **2011**, *190*, 98-102, doi:<https://doi.org/10.1016/j.tvjl.2010.08.008>.
59. Mosher, R.A.; Coetzee, J.F.; Cull, C.A.; Gehring, R.; KuKanich, B. Pharmacokinetics of oral meloxicam in ruminant and preruminant calves. *J Vet Pharmacol Ther* **2012**, *35*, 373-381, doi:10.1111/j.1365-2885.2011.01331.x.
60. Malreddy, P.R.; Coetzee, J.F.; Kukanich, B.; Gehring, R. Pharmacokinetics and milk secretion of gabapentin and meloxicam co-administered orally in Holstein-Friesian cows. *J Vet Pharmacol Ther* **2013**, *36*, 14-20, doi:10.1111/j.1365-2885.2012.01384.x.

61. Coetzee, J.F.; Mosher, R.A.; Griffith, G.R.; Gehring, R.; Anderson, D.E.; KuKanich, B.; Miesner, M. Pharmacokinetics and tissue disposition of meloxicam in beef calves after repeated oral administration. *Journal of veterinary pharmacology and therapeutics*. **2015**, doi:10.1111/jvp.12215.
62. Gorden, P.J.; Burchard, M.; Ydstie, J.A.; Kleinhenz, M.D.; Wulf, L.W.; Rajewski, S.J.; Wang, C.; Gehring, R.; Mochel, J.P.; Coetzee, J.F. Comparison of milk and plasma pharmacokinetics of meloxicam in postpartum versus mid-lactation Holstein cows. *J Vet Pharmacol Ther* **2018**, *41*, 463-468, doi:10.1111/jvp.12488.
63. Shock, D.; Roche, S.; Olson, M. A Comparative Pharmacokinetic Analysis of Oral and Subcutaneous Meloxicam Administered to Postpartum Dairy Cows. *Vet Sci* **2019**, *6*, doi:10.3390/vetsci6030073.
64. Meléndez, D.M.; Marti, S.; Pajor, E.A.; Sidhu, P.K.; Gellatly, D.; Janzen, E.D.; Schwinghamer, T.D.; Coetzee, J.F.; Schwartzkopf-Genswein, K.S. Pharmacokinetics of oral and subcutaneous meloxicam: Effect on indicators of pain and inflammation after knife castration in weaned beef calves. *PLoS One* **2019**, *14*, e0217518, doi:10.1371/journal.pone.0217518.
65. Jokela, A.; Nyrhilä, A.; Adam, M.; Salla, K.; Raekallio, M.; Aho, R.; Norring, M.; Hokkanen, A.-H. Pharmacokinetics of meloxicam in pre-ruminant calves after intravenous, oral, and subcutaneous administration. *Journal of Veterinary Pharmacology and Therapeutics* **2024**, *47*, 143-149, doi:<https://doi.org/10.1111/jvp.13412>.
66. Warner, R.; Ydstie, J.A.; Wulf, L.W.; Gehring, R.; Coetzee, J.F.; Mochel, J.P.; Gorden, P.J. Comparative Pharmacokinetics of Meloxicam Between Healthy Post-partum vs. Mid-lactation Dairy Cattle. *Front Vet Sci* **2020**, *7*, 548, doi:10.3389/fvets.2020.00548.

67. Craigmill, A.L.; Rangel-Lugo, M.; Damian, P.; Riviere, J.E. Extralabel use of tranquilizers and general anesthetics. *Journal of the American Veterinary Medical Association* **1997**, *211*, 302-304.
68. Smith, G. Extralabel Use of Anesthetic and Analgesic Compounds in Cattle. *Veterinary Clinics: Food Animal Practice* **2013**, *29*, 29-45, doi:10.1016/j.cvfa.2012.11.003.
69. USDA. Carcass Management Course: Rendering Module. Available online: <https://www.aphis.usda.gov/sites/default/files/7-rendering.pdf> (accessed on 05/27/2025).
70. Jurczynski, K.; Zittlau, E. Pentobarbital poisoning in Sumatran tigers (*Panthera tigris sumatrae*). *Journal of Zoo and Wildlife Medicine* **2007**, *38*, 583-584, doi:10.1638/2006-0051r.1.

Chapter 2 - Pharmacokinetics, milk residues, and toxicological evaluation of a single high dose of meloxicam administered at 30 mg/kg *per os* to lactating dairy cattle

Original Citation: *Veterinary Sciences*. April 2023 doi: 10.3390/vetsci10040301

© 2023 by the authors

Scott A. Fritz¹, Steve M. Ensley¹, Jay R. Lawrence², Nicholas Van Engen², Zhoumeng Lin³, Michael D. Kleinhenz⁴, Larry W. Wulf², Somchai Rice², Patrick J. Gorden², Jackie Peterson² and Johann F. Coetzee¹

¹Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506, USA

² Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011, USA

³ Department of Environmental and Global Health, University of Florida, Gainesville, FL 32610, USA

⁴ Department of Clinical Sciences, Kansas State University, Manhattan, KS 66506, USA

Abstract:

Adverse effects associated with overdose of NSAIDs are rarely reported in cattle and the risk level is unknown. If high doses of NSAIDs can be safely administered to cattle, this may

provide a longer duration of analgesia than using current doses where repeated administration is not practical. Meloxicam was administered to 5 mid-lactation Holstein dairy cows orally at 30 mg/kg, which is 30 times higher than the recommended 1 mg/kg oral dose. Plasma and milk meloxicam concentrations were determined using high-pressure liquid chromatography with mass spectroscopy (HPLC-MS). Pharmacokinetic analysis was performed on using non-compartmental analysis. The geometric mean maximum plasma concentration ($C_{\max}$) was 91.06 $\mu\text{g/mL}$ at 19.71 hours ($T_{\max}$), terminal elimination half-life ($T_{1/2}$) was 13.79 hours. The geometric mean maximum milk concentration was 33.43 $\mu\text{g/mL}$ at 23.74 hours, with terminal elimination half-life of 12.23 hours. A thorough investigation into potential adverse effects of a meloxicam overdose was performed with no significant abnormalities reported. The cows were humanely euthanized at 10 d after the treatment and no gross or histologic lesions were identified. As expected, significantly higher plasma and milk concentrations were attained after administration of 30 mg/kg meloxicam with similar half-lives to previously published reports. However, no identifiable adverse effects were observed with a drug dose 30 times greater than the industry uses within 10 days of treatment. More research is needed to determine a tissue withdrawal period, safety, and efficacy of meloxicam after a dose of this magnitude in dairy cattle.

Introduction

Adequate pain management in veterinary medicine is an important consideration when elective invasive procedures are performed or when animals experience pain associated with disease. Veterinarians should be aware of the compounds available for use, their efficacy in abating pain, and any undesirable effects they may have on the animals being treated. In cattle, non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat pain. Meloxicam is

an NSAID used in the EU and Canada for alleviating pain associated with disbudding, and as adjunctive therapy when treating a variety of infectious diseases[1]. Meloxicam has been shown to be efficacious in improving treatment outcomes in cattle associated with neonatal diarrhea, varying forms of castration, as well as cesarean section surgeries[2-4]. Meloxicam is not currently approved for use in food animals in the United States. However, in a recent survey 16.1% of producers and 80.5% of veterinarian respondents indicated that they had knowledge and felt comfortable using oral meloxicam in an extralabel manner in cattle to alleviate pain[5].

Meloxicam acts by inhibiting the synthesis of prostaglandin and has anti-inflammatory, anti-exudative, analgesic, and antipyretic activities[6]. Meloxicam is recommended to be administered extra-label to cattle orally at 1 mg/kg of body weight in the US and in accordance with the labeled product in Canada (Meloxicam Oral Suspension USP, Solvet, Calgary, Canada). However, despite widespread familiarity with the extralabel use of meloxicam in the United States, there is no established risk level for adverse effects related to such use in cattle. Moreover, characterizing the margin of safety between efficacy and toxicity could lead to the safe use of higher doses of meloxicam that produce higher plasma drug concentrations for longer and thus provide pain management over an extended period of time after a single dose. Protocols involving higher than currently recommended doses of oral meloxicam could thus advance the welfare of treated animals, especially in situations where repeated therapy is not practical.

The acute oral toxicity for meloxicam has been investigated in other species and ranges from 83.5 mg/kg in rats to >1600 mg/kg in mini-pigs[1]. However, the margin of safety between effectiveness and toxicity has not been well characterized[7]. There is a growing body of work suggesting higher and more frequent dosing of NSAIDs may be needed to achieve adequate analgesia in laboratory rodents[7-9]. An oral dose of 8 mg/kg was delivered to Wistar rats for 28

continuous days, which resulted in severe ulcerative lesions and hemorrhage in the gastrointestinal tract[10]. However, a single oral dose of 20 mg/kg meloxicam in mice was well tolerated[11]. Furthermore, while meloxicam administered subcutaneously at 20 mg/kg caused injection site lesions, there were no systemic signs of toxicity[9]. Another group used subcutaneous injections of 20 mg/kg meloxicam in mice for 3 or 7 days continuously[8]. Fecal occult blood was associated with treatment, gastritis was not associated with treatment and no other lesions were detected. A subset of the different treatment groups was allowed 7 days to recover, and no differences could be identified from baseline at that time, suggesting the lesions are clinically insignificant and recovery is likely. American kestrels were given oral doses of 20 mg/kg meloxicam every 12 hours for 7 days with no evidence of toxicity[12]. The use of NSAIDs at calving has been associated with retained fetal membranes, yet other studies have been unable to detect a difference between NSAID-treated cows and non-NSAID treated cows[13-15]. Effects on re-production may be as economically significant as individual toxicosis. Taken together, multiple high doses of meloxicam should be used with caution, but a single high dose of meloxicam appears to be well tolerated in multiple species and could result in longer duration of analgesia without the need for repeated dosing.

Sows were administered 30 mg/kg meloxicam per os for 3 consecutive days in an attempt to deliver adequate meloxicam for analgesia in milk to their offspring[16]. Minor lesions in the gastrointestinal tract were noted at necropsy in some animals, but this dose was well-tolerated clinically. The sow study serves as the basis for the selected dose in the present study of 30 mg/kg per os due to its lack of clinical toxicity even when administered for three consecutive days. Adverse effects of NSAID overdoses in other species have been reported and ruminants are considered to be at risk for similar adverse effects[17]. The present report investigated the

pharmacokinetics, milk residue depletion characteristics, and toxicologic response to a single, large oral dose approximately 30 times the recommended dose used for therapy in lactating cows. If a single high dose of meloxicam in place of repeated dosing is well tolerated in cattle, a longer duration of analgesia may be achievable.

Materials and Methods

This protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Iowa State University (ISU), protocol 5-12-7363B.

Table 2.1 Graphical representation of the study design calendar.

Study Day	-1	0	1	2	3	4	5	6	7	8	9	10
Catheter	X											
Drug administration		X										
CBC/Chem	X	X	X	X	X	X	X	X	X	X	X	X
Plasma Drug		X	X	X	X	X	X	X	X	X	X	X
Milk Drug		X	X	X	X	X	X	X	X	X	X	X
Physical Exam	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis	X	X	X	X	X	X	X	X	X	X	X	X
Fecal blood	X	X	X	X	X	X	X	X	X	X	X	X
Euthanasia												X

Experimental Animals

Five clinically healthy adult Holstein-Friesian cows weighing between 600-800 kg were acquired from the ISU Dairy Farm. All animals were currently in lactation producing between 12.5-21.5 kg milk per day. Prior to the study, each cow's health was screened utilizing a physical exam, serum chemistry, and complete blood count.

Housing and Husbandry

Phase I was conducted in the ISU Lloyd Veterinary Medical Center with a single animal. The cow was housed in the large animal medicine facilities in a 12.6 m² individual stall bedded with rubber mats and pine wood shavings. After the successful completion of phase I, the four remaining animals were housed at the ISU Dairy Farm in individual 28 m² pens bedded with sand and straw. For the duration of the study, all animals were maintained on a total mixed ration consisting of corn silage, hay, cotton, fine ground corn, corn gluten, soy plus, soybean meal, and grain mix. This ration was composed of 16.4% crude protein and provided a net energy of 35.72 megacalories per kilogram. Water and feed were offered ad libitum.

Meloxicam Administration

A single oral dose of meloxicam (Mylan Pharmaceuticals Inc., Morgantown, WV; NDC 0378-1089-01; Lot 3032625) was administered to each cow at time zero. The 30 mg/kg dose was administered via balling gun in the form of 1 ounce gelatin capsules (Torpac, Inc., Fairfield, NJ) containing 15 mg meloxicam tablets. Individual doses ranged from 29.98 to 30.02 mg/kg as tablets were left whole to preserve the integrity of the drug.

Sample Collection

Intravenous jugular catheters (Abbocath-T, 14G x 140 mm) were aseptically placed in each animal and sutured into the skin using 3-0 nylon suture (Ethicon, Inc.) 16 hours prior to the study's start. Throughout the study, catheter patency was maintained using a heparin saline flush containing 2 USP units heparin sodium/mL saline (Heparin Sodium Injection, Baxter Healthcare, Deerfield, IL, USA). Blood samples were collected once daily for complete blood counts and

serum chemistry analysis. Approximately 15 mL of whole blood was drawn from the indwelling jugular catheter and immediately transferred into both an 8.5 mL serum separator vacutainer tube (BD Diagnostics, Franklin Lakes, NJ, USA) and a 5 mL vacutainer tube containing 15% EDTA (BD Diagnostics). The vacutainer tubes were refrigerated until they were submitted for analysis. Samples were submitted to the ISU Clinical Pathology Laboratory for serum chemistry analysis and complete blood counts with manual differential. Blood samples for plasma drug analysis were collected at each time point daily (05:00, 10:00, 15:00, 19:00, 24:00). Whole blood was drawn from the jugular catheter and placed into a 10 mL lithium heparin vacutainer tube (BD Diagnostics). The samples were immediately centrifuged for 10 minutes at 1600 x g. Plasma was then pipetted to 2 mL cryovials (Fisher Scientific International, Inc., Hanover Park, IL, USA) and frozen at -70 °C prior to analysis.

Physiological monitoring included daily temperature, heart rate, respiratory rate, urinalysis, and fecal occult blood test. Vital signs (heart rate, respiratory rate, temperature) were assessed daily. Urine was collected in a polycarbonate vial (Fischer Scientific, Hampton, NH, USA) once daily via free catch. Urine analyses were performed immediately following the free catch and consisted of a specific gravity via refractometer and a urine reagent dipstick analysis (Multistix 10 SG, Siemens Healthcare Diagnostics, Inc., Tarrytown, NY, USA). Assessment of fecal occult blood was performed daily using a fresh stool sample and a Seracult (Propper Manufacturing Co, Inc., Long Island City, NY, USA) rapid card test.

Milk collection for milk drug analysis was performed at each time point (05:00, 10:00, 15:00, 19:00, 24:00) for the duration of study. Prior to collection, each teat was pre-dipped using a hydrogen peroxide-based dip and fore-stripped to check for signs of mastitis. Milk weights are presented in Figure 2.1. Following the milking procedure, a 45 mL sample was then taken from

the collection vessel and placed in a polycarbonate vial (Fisher Scientific). Samples were immediately taken to the laboratory and frozen at -70 °C prior to analysis. After each collection, the teats were treated with iodine-based post-milking dip. All residual milk collected during the study was discarded due to potential drug residues.

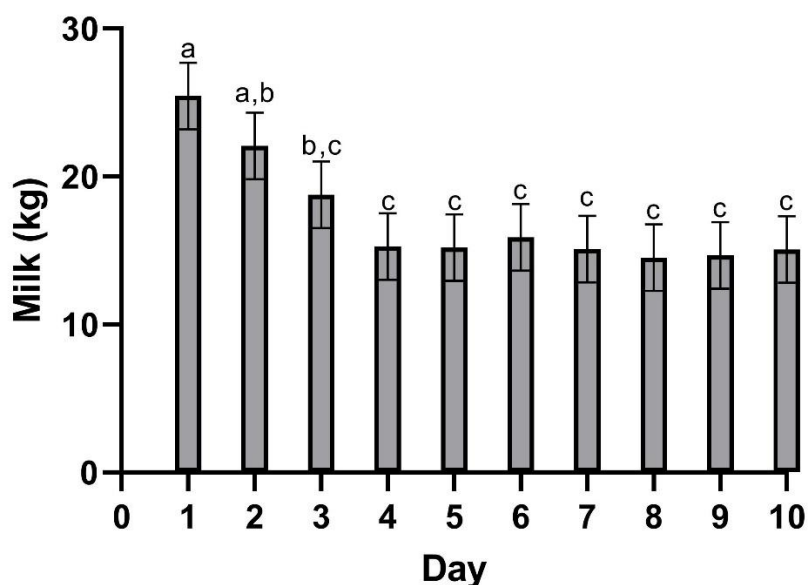


Figure 2.1 Mean ($\pm$ SEM) milk production following a single oral dose of meloxicam at 30 mg/kg. ^{a,b,c} Different letters above data bars are statistically significant ($p=0.001$).

At the end of the study, 10 days after treatment, all cows were humanely euthanized via captive bolt and exsanguination, and necropsy was performed by a trained diagnostician (SE). Gross lesions were noted, and histopathologic evaluation was performed on selected tissues expected to be impacted by NSAID toxicity.

Milk Sample Preparation

After thawing at room temperature, milk samples, milk spikes and milk QC samples (1.0 mL in 16 x 150 mm glass screw-top tubes), were fortified with the internal standard piroxicam

(10 mL of 10 ng/mL). An equivalent volume of ultra-pure water was added to each sample and vortexed. Then, 0.2 M Disodium EDTA (0.5 mL) was added to each sample and vortexed. Finally, 0.5% acetic acid in acetonitrile (5 mL) was added to each sample and vortexed. Additionally, 0.5 g $\pm$ 0.1g of sodium sulfate fractions were premeasured and added to each sample simultaneously to vigorous vortexing to avoid clumping during salt-out. Tubes were capped tightly and mixed by inversion for 5 minutes at 300 rpm, then centrifuged at 2000 x g for 10 minutes. The top organic layer was transferred to a clean 16 x 100 mm glass tube and was evaporated under a stream of nitrogen at 48° C to approximately 0.5 mL total volume. One mL of 0.1 ammonium acetate buffer, pH 4.5, was added to each reduced sample.

A solid phase extraction (SPE) clean-up followed using a positive pressure manifold (UCT, Inc., Bristol, PA, USA). Strata-X SPE cartridges (60 mg/3 mL, Phenomenex, Torrance, CA, USA) were conditioned and equilibrated sequentially with methanol (2 mL), ultra-pure water (2 mL), then 0.1M ammonium acetate buffer, pH 4.5 (1 mL). The buffered samples were loaded onto the cartridges under gravitational flow. The cartridges were washed with 5% acetonitrile in 1% formic acid (0.5 mL) and dried under full vacuum for 5 minutes. A hexane wash (0.5 mL) followed, with an additional 5-minute dry under full vacuum. Target analytes were eluted with 2 x 1 mL fractions of 70:30 acetonitrile to methanol. Eluent was reduced to dryness under a stream of nitrogen at 48° C. 150 μ L of 25% acetonitrile in water was added to reconstitute the sample residues and vortexed briefly. Then, 50 μ L of ultra-pure water was added and vortexed briefly. The entire reconstituted samples were transferred to labelled auto-sampler vials equipped with glass inserts for analysis by LC-MS.

Plasma Sample Preparation

After thawing at room temperature, plasma samples, plasma spikes, and plasma QC samples (0.2 mL), were treated with 30% perchloric acid (20 mL) after addition of the internal standard piroxicam (10 mL of 10 ng/mL). The samples were vortexed for 5 seconds and centrifuged for 20 minutes at 2,000 x g to sediment the precipitate. A portion of supernatant, 100 μ L, was transferred to an injection vial fitted with a glass insert containing 100 μ L of 1.5% ammonium hydroxide in 25% aqueous acetonitrile for analysis by LC-MS.

Determination of meloxicam concentrations by LC-MS

Plasma and milk concentrations of meloxicam were determined using high-pressure liquid chromatography (Surveyor MS Pump and Autosampler, Thermo Scientific, San Jose, CA, USA)-tandem mass spectrometry (TSQ Quantum Discovery MAX, Thermo Scientific, San Jose, CA, USA). The sample injection volume was set to 12.5 μ L. The mobile phases consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile at a flow rate of 0.280 mL/min. The mobile phase began at 40% B with a linear gradient to 95% B at 4 minutes, which was maintained for 1.5 minutes, followed by re-equilibration to 40% B. Separation was achieved with a solid-core C18 column (KinetexXB -C18, 100 mm x 2.1 mm, 2.6 μ m particles, Phenomenex, Torrance, CA, USA) maintained at 40 °C. Piroxicam eluted at 2.96 minutes and meloxicam at 4.31 minutes. Four SRM transitions were monitored for meloxicam and three SRM transitions were used with the internal standard, piroxicam. The quantifying ions for meloxicam were 72.95, 87.94, 114.91, and 140.89 m/z and 77.97, 94.98, and 120.98 m/z for piroxicam. Sequences consisting of milk and plasma blanks, calibration spikes, QC samples, and bovine milk and plasma samples were batch processed with a processing method developed in the

Xcalibur software (Thermo Scientific, San Jose, CA, USA). The processing method automatically identified and integrated each peak in each sample and calculated the calibration curve based on a weighted ($1/X$) linear fit. Plasma concentrations of meloxicam in unknown samples were calculated by the Xcalibur software based on the calibration curve. Results were then viewed in the Quan Browser portion of the Xcalibur software. Twelve calibration spikes were prepared in bovine milk and plasma covering the concentration range of 5 to 20,000 ng/mL. QC samples were prepared at concentrations of 15, 150, and 1500 ng/mL. QC samples were prepared in duplicate with each set of samples. Calibration curves exhibited a correlation coefficient (r^2) exceeding 0.998 across the entire concentration range. The QC samples were accepted as passing within 15 % of their nominal values.

Noncompartmental pharmacokinetic analysis

The plasma and milk time-concentration data were analyzed with a noncompartmental method using a commercial software Phoenix® (Version 8.3, Certara, Inc., Princeton, NJ, USA). The following pharmacokinetic parameters were calculated, including AUC_{0-last} (area under the curve from the time of dosing to the last measurable concentration), $AUC_{0-\infty}$ (AUC from dosing time extrapolated to infinity based on the last observed concentration), AUC_{Extrap} (percentage of $AUC_{0-\infty}$ due to extrapolation from T_{last} to infinity), $AUMC_{0-\infty}$ (area under the first moment curve extrapolated to infinity based on the last observed concentration), C_{max} (maximum observed concentration occurring at T_{max}), CL/F (total body clearance per fraction of dose absorbed), λ_z -HL (terminal elimination half-life), λ_z (terminal elimination rate constant, calculated using log-linear regression of the terminal portions of the concentration-versus-time curves), $MRT_{0-\infty}$

(mean residence time extrapolated to infinity), $T_{\max}$ (time of maximum observed concentration), and V_z/F (volume of distribution based on the terminal phase per fraction of dose absorbed).

Statistical analysis

Hematological and serum biochemistry outcomes were entered into spreadsheets prior to statistical analysis (Excel, Microsoft Corporation, Redmond, WA, USA). Data distributions were visually examined to confirm that data were normally distributed. Changes in each of the hematology and serum chemistry variables over time were assessed using One-way Analysis of Variance (ANOVA) and a commercially available statistical software package (JMP® Pro 16.1.0, SAS Institute, Cary, NC, USA). Means and standard errors were extracted from the statistical reports and tabulated. Statistical significance was designated a priori as $p < 0.05$. Where a significant effect of time on the outcome was reported, the effect was clarified graphically in Excel.

Results

Milk

Table 2.2 summarizes pharmacokinetic parameters of meloxicam in milk. The geometric mean peak milk concentration was 33.43 (17.70-41.39) $\mu\text{g/mL}$. This occurred at 23.74 (15-34) hours. The geometric mean terminal elimination half-life in milk was 12.23 (10.59-14.4) hours and geometric mean $\text{AUC}_{0-\infty}$ was 1360.77 $\text{h} \times \mu\text{g/mL}$. Mean daily milk production is displayed in Figure 2.1, there was a slight decrease in milk production over the course of the study. Mean ($\pm$ SEM) plasma and milk concentrations of meloxicam are presented in Figure 2.2. Individual milk concentrations of meloxicam are presented in Figure 2.3.

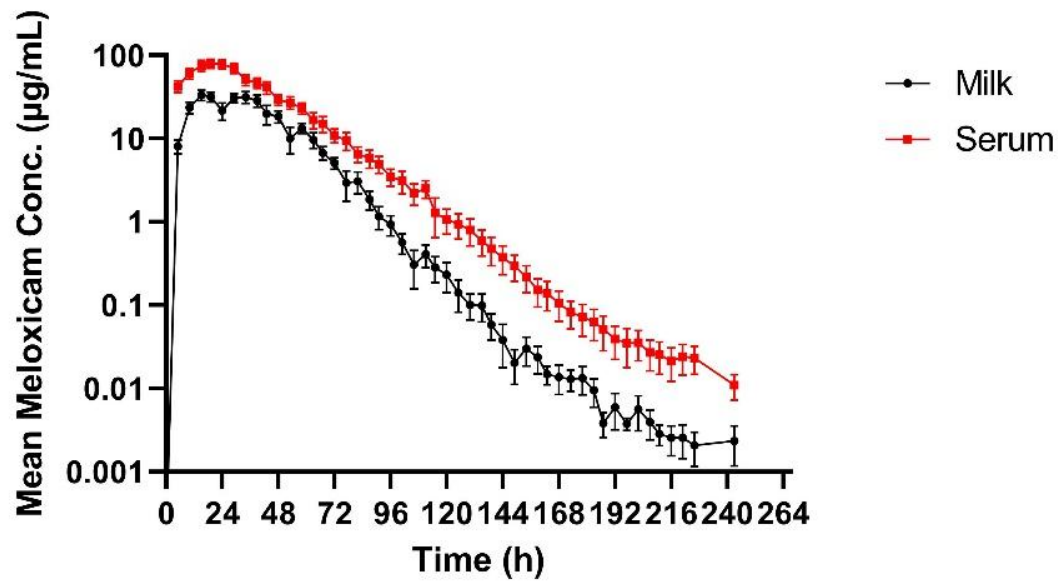


Figure 2.2 Mean ($\pm$ SEM) milk and plasma concentrations of meloxicam following a single oral dose of 30 mg/kg in adult lactating Holstein cows (n=5).

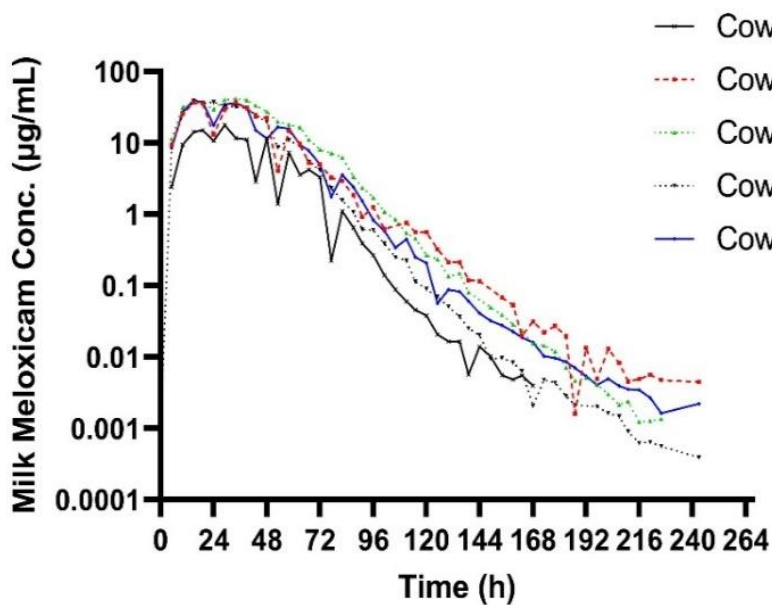


Figure 2.3 Individual milk meloxicam concentrations for 5 cows administered meloxicam at 30 mg/kg PO at time zero

Table 2.2 Geometric mean milk pharmacokinetic parameters of meloxicam following single oral administration at the dose rate of 30 mg/kg body weight in lactating dairy cows (n=5).

Parameter	Unit	Mean	SD	Median	Range
λ_z	1/h	0.060	1.130	0.060	0.05-0.07
λ_z -HL	h	12.230	1.130	11.600	10.59-14.40
AUC _{0-last}	h×µg/ml	1360.71	1.580	1553.23	621.102-2035.769
AUC _{0-∞}	h×µg/ml	1360.77	1.580	1553.28	621.162-2035.791
AUC _{extrap}	%	0.002	3.590	0.003	0.0004-0.01
C _{max}	ug/ml	33.4	1.43	39.0	17.704-41.393
T _{max}	h	23.740	1.530	29.000	15-34

Plasma

Summary plasma pharmacokinetic parameters for meloxicam are displayed in Table 2.3. The geometric mean peak plasma concentration was 91.06 (63.13-110.58) µg/mL at 19.71 (15-29) hours. The geometric mean terminal elimination half-life was 13.79 (11.33-17.42) hours, oral clearance was 9.09 mL/h/kg, and the bioavailability was 180.77 mL/kg. Individual plasma concentrations of meloxicam are presented in Figure 2.4.

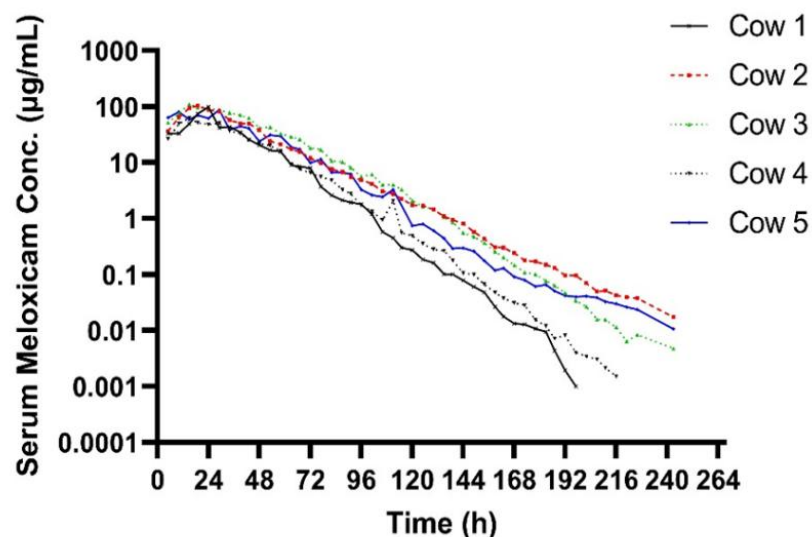


Figure 2.4 Individual plasma meloxicam concentrations for 5 cows administered meloxicam at 30 mg/kg PO at time zero.

Table 2.3 Geometric mean plasma pharmacokinetic parameters of meloxicam following single oral administration at 30 mg/kg in lactating dairy cows (n=5).

Parameter	Unit	Mean	SD	Median	Range
λ_z	1/h	0.050	1.204	0.052	0.04-.061
λ_z -HL	h	13.79	1.20	13.41	11.328-17.422
T_{max}	h	19.71	1.34	19.00	15-29
C_{max}	µg/ml	91.061	1.248	98.164	63.10-110.578
CL/F	ml/h/kg	9.09	1.3	8.5	6.283-12.716
$AUC_{0-\infty}$	h×µg/ml	3301.30	1.342	3509.40	2359.279-4775.071
AUC_{last}	h*µg/ml	3301.16	1.342	3509.16	2359.278-4774.979
AUC extrap	%	0.001	8.646	0.002	0-0.011
$AUMC_{0-\infty}$	h ² ×µg/ml	109842.0	1.4	117505	75845-172426
$MRT_{0-\infty}$	h	33.27	1.07	33.48	30.515-36.11
Vz/F	ml/kg	181	1	194	121.542-217.102

Complete Blood Counts and Blood Chemistry

Summary of mean ($\pm$ SEM) CBC parameters are presented in Table 2.4. CBC parameters that showed significant changes include WBCs, MCHC, neutrophils, basophils, and plasma protein. WBC counts and neutrophils increased throughout the study with neutrophils being above the reference ranges for the last three measures. Mean WBC and neutrophil data are presented in Figures 2.5(A) and 2.5(B), respectively. WBC counts were within the normal range for the entirety of the study. Hematocrit and red blood cell analyses were normal across all individuals for the entirety of the study. Plasma protein had significant day-to-day variation and was above the reported reference range at most time points, including baseline.

Table 2.4 Summary mean ($\pm$ SEM) CBC parameters prior to meloxicam treatment and for 10 days post-treatment for cows administered 30 mg/kg per os (n=5).

Parameter (Ref Range)	Days after meloxicam administration at 30 mg/kg PO								p-value
	Baseline	1	2	3	4	6	8	10	
WBC $\times 10^3/\mu\text{L}$ (4-12)	6.20 ^c (0.90)	6.29 ^c (0.81)	5.9 ^c (0.89)	5.62 ^c (0.89)	7.39 ^{bc} (0.89)	8.77 ^{ab} (0.89)	10.03 ^a (0.89)	10.16 ^a (0.90)	<.001
RBC $\times 10^6/\mu\text{L}$ (5-10)	6.40 ^a (0.25)	5.57 ^b (0.24)	5.34 ^b (0.25)	5.48 ^b (0.25)	5.66 ^b (0.25)	5.55 ^b (0.25)	5.6 ^b (0.25)	5.49 ^b (0.24)	.26
Hemoglobin g/dL (5-15)	11.98 ^a (0.61)	9.97 ^b (0.58)	9.66 ^b (0.61)	9.8 ^b (0.61)	10.2 ^b (0.61)	10.02 ^b (0.61)	10.24 ^b (0.61)	9.73 ^b (0.58)	.30
Hematocrit % (24-46)	32.9 ^a (1.64)	27.33 ^b (1.57)	26.34 ^b (1.64)	27.12 ^b (1.64)	27.88 ^b (1.64)	27.40 ^b (1.64)	27.64 ^b (1.64)	27.01 ^b (1.57)	.29
MCV fl (40-60)	51.20 ^a (1.62)	49.04 ^a (1.56)	49.26 ^a (1.62)	49.36 ^a (1.62)	49.16 ^a (1.62)	49.3 ^a (1.62)	49.28 ^a (1.62)	49.26 ^a (1.56)	.99
MCH pg (11-17)	*18.60 ^a (0.61)	*17.91 ^a (0.58)	*18.06 ^a (0.61)	*17.88 ^a (0.61)	*18.00 ^a (0.61)	*18.02 ^a (0.61)	*18.26 ^a (0.61)	*17.75 ^a (0.58)	.99
MCHC g/dL (30-36)	*36.38 ^{ab} (0.28)	*36.56 ^{ab} (0.23)	*36.68 ^{ab} (0.28)	*36.22 ^{ab} (0.28)	*36.58 ^{ab} (0.28)	*36.58 ^{ab} (0.28)	*37.04 ^a (0.28)	*36.02 ^b (0.23)	.04
Platelets $\times 10^3/\mu\text{L}$ (100-800)	472.4 ^{ab} (44.34)	450.9 ^{ab} (41.99)	419.6 ^b (44.34)	435.8 ^{ab} (44.34)	492.8 ^{ab} (44.34)	452.2 ^{ab} (44.34)	494.4 ^{ab} (44.34)	511.4 ^a (41.99)	.78
Neutrophils $\times 10^3/\mu\text{L}$ (0.6-4.0)	2.69 ^{bc} (0.50)	2.5 ^{bc} (0.37)	2.37 ^{bc} (0.50)	1.79 ^c (0.50)	2.85 ^{bc} (0.50)	*4.34 ^{ab} (0.50)	*5.20 ^a (0.50)	*5.85 ^a (0.37)	<.001
Lymphocytes $\times 10^3/\mu\text{L}$ (2.5-7.5)	2.74 ^b (0.75)	3.22 ^{ab} (0.73)	2.81 ^b (0.75)	3.12 ^{ab} (0.75)	3.84 ^{ab} (0.75)	3.49 ^{ab} (0.75)	4.00 ^a (0.75)	3.49 ^{ab} (0.73)	.98
Monocytes $\times 10^3/\mu\text{L}$ (0.03-0.85)	0.348 ^a (0.07)	0.165 ^a (0.05)	0.156 ^a (0.07)	0.218 ^a (0.07)	0.216 ^a (0.07)	0.308 ^a (0.07)	0.368 ^a (0.07)	0.294 ^a (0.05)	.24
Eosinophils $\times 10^3/\mu\text{L}$ (0.0-2.4)	0.338 ^a (0.18)	0.374 ^a (0.16)	0.506 ^a (0.18)	0.298 ^a (0.18)	0.420 ^a (0.18)	0.598 ^a (0.18)	0.418 ^a (0.18)	0.388 ^a (0.16)	.96
Basophils $\times 10^3/\mu\text{L}$ (0.0-0.2)	0.082 ^a (0.07)	0.021 ^a (0.05)	0.050 ^a (0.07)	0.024 ^a (0.07)	0.058 ^a (0.07)	0.012 ^a (0.07)	0.054 ^a (0.07)	*0.221 ^a (0.05)	.02
RDW % (8-15)	*17.78 ^a (0.47)	*17.63 ^a (0.46)	*17.52 ^a (0.47)	*17.44 ^a (0.47)	*17.34 ^a (0.47)	*17.5 ^a (0.47)	*17.52 ^a (0.47)	*17.46 ^a (0.46)	.99

Plasma Protein g/dL (6.9-7.7)	*8.76 ^a (0.22)	*8.33 ^{abc} (0.17)	7.64 ^c (0.22)	*7.86 ^{bc} (0.22)	*8.48 ^{abc} (0.22)	*8.32 ^{abc} (0.22)	*8.6 ^{ab} (0.22)	*8.63 ^{ab} (0.17)	.02
Fibrinogen mg/dL (100-500)	*540 ^{ab} (77.82)	490 ^b (64.60)	440 ^b (77.82)	460 ^b (77.82)	*520 ^{ab} (77.82)	*560 ^{ab} (77.82)	*620 ^{ab} (77.82)	*720 ^a (64.60)	.12

^{a,b,c} Different letters within rows are statistically significant ($p=0.05$). * Indicates values that are outside the reference range provided by the analyzing laboratory. WBC, white blood cells; RBC, red blood cells; MCV, mean corpuscular volume' MCH, mean cell hemoglobin' MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width

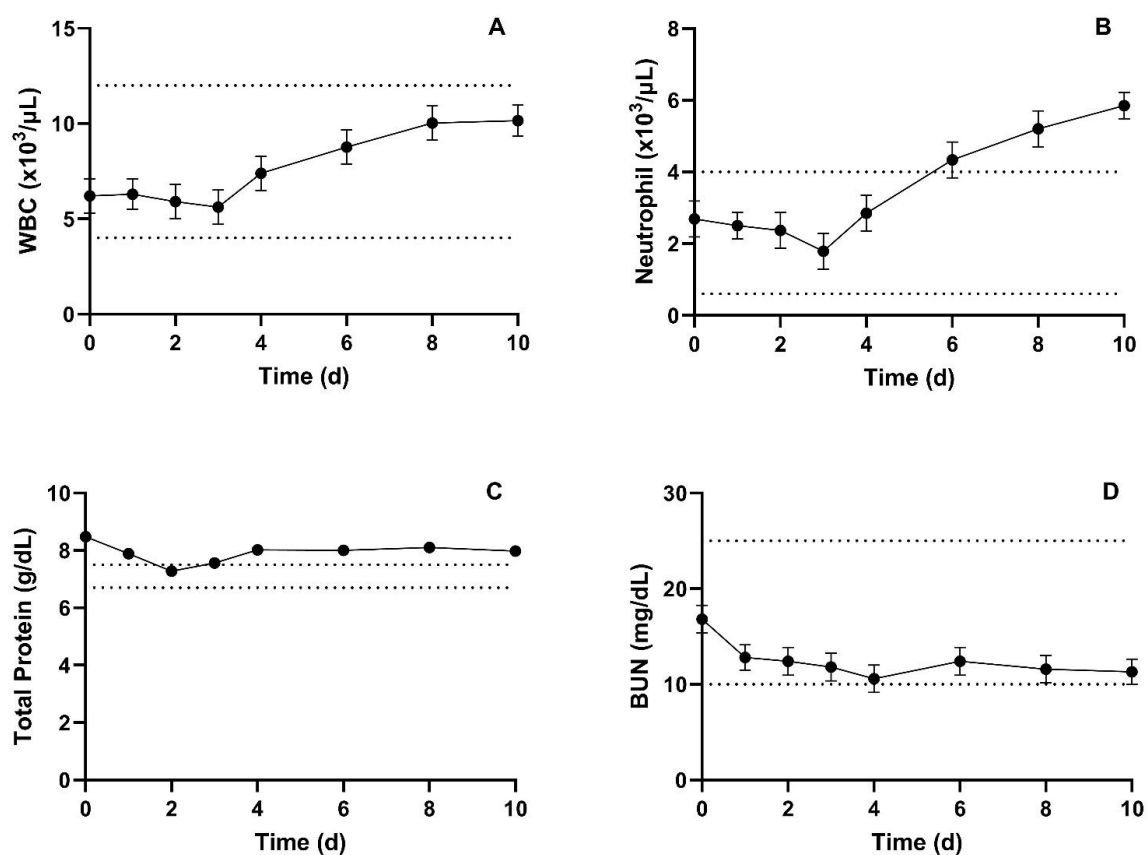


Figure 2.5 Summary mean ($\pm$ SEM) WBC counts (A), Neutrophil counts (B), total protein (C), and BUN (D) prior to, and for 10 days following administration of 30 mg/kg meloxicam PO to lactating dairy cows (n=5). Dotted lines denote provided upper and lower reference limits.

Summary of mean ($\pm$ SEM) serum blood chemistry parameters are presented in Table 2.5. Serum blood chemistry parameters that showed significant differences include potassium, total protein, albumin, total bilirubin, and anion gap. While potassium, albumin, and total bilirubin had significant day-to-day variation, the measured values were still within the reported normal ranges at all time points except for potassium 2 days post-administration. Total protein had significant day-to-day variation and was above the reference range at all but one time point. However, the baseline measure was numerically higher than all other measures and there was a slight return towards the normal range over time. Mean total protein and BUN are presented in Figures 2.5(C) and 2.5(D), respectively. The anion gap was significantly lower post-administration compared to baseline.

Table 2.5 Summary of mean ($\pm$ SEM) serum blood chemistry parameters prior to meloxicam treatment and for 10 days post-treatment for cows administered 30 mg/kg per os (n=5).

Parameter (Ref. Range)	Days after meloxicam administration at 30 mg/kg PO								p-value
	Baseline	1	2	3	4	6	8	10	
Sodium mEq/L (132-152)	142 ^a (0.92)	140 ^a (0.75)	140.2 ^a (0.92)	139.6 ^a (0.92)	141.4 ^a (0.92)	140.6 ^a (0.92)	140.6 ^a (0.92)	140.5 ^a (0.75)	0.21
Potassium mEq/L (3.9-5.8)	4.5 ^a (0.12)	3.96 ^{bc} (0.09)	*3.8 ^c (0.12)	3.94 ^{bc} (0.12)	4.2 ^{abc} (0.12)	4.22 ^{abc} (0.12)	4.36 ^{ab} (0.12)	4.28 ^{ab} (0.09)	0.01
Chloride mEq/L (100-115)	100.6 ^b (1.40)	102.8 ^{ab} (1.22)	105.2 ^a (1.40)	103.8 ^{ab} (1.40)	102 ^{ab} (1.40)	102.2 ^{ab} (1.40)	101.2 ^{ab} (1.40)	102.8 ^{ab} (1.22)	0.60
Bicarbonate mEq/L (21-31)	29 ^a (1.13)	28.5 ^a (0.87)	26.8 ^a (1.13)	27.2 ^a (1.13)	*31.2 ^a (1.13)	30 ^a (1.13)	29.4 ^a (1.13)	29.5 ^a (0.87)	0.18
Calcium mg/dL (8.0-11.4)	9.14 ^{ab} (0.27)	8.98 ^{ab} (0.22)	8.44 ^b (0.27)	8.78 ^{ab} (0.27)	9.64 ^a (0.27)	9.06 ^{ab} (0.27)	9.4 ^{ab} (0.27)	9.52 ^a (0.22)	0.06
Phosphorus mg/dL (5.6-8.0)	6.88 ^a (0.68)	7.21 ^a (0.47)	7.66 ^a (0.68)	6.18 ^a (0.68)	6.38 ^a (0.68)	6.34 ^a (0.68)	5.82 ^a (0.68)	5.75 ^a (0.47)	0.15
BUN mg/dL (10-25)	16.8 ^a (1.45)	12.8 ^b (1.33)	12.4 ^b (1.45)	11.8 ^b (1.45)	10.6 ^b (1.45)	12.4 ^b (1.45)	11.6 ^b (1.45)	11.3 ^b (1.33)	0.19
Creatinine mg/dL (0.1-1.8)	1.38 ^a (0.14)	1.21 ^b (0.13)	1.14 ^b (0.14)	1.12 ^b (0.14)	1.14 ^b (0.14)	1.14 ^b (0.14)	1.2 ^{ab} (0.14)	1.16 ^b (0.13)	0.97
Glucose md/dL (40-100)	70.4 ^{ab} (3.95)	72.2 ^a (3.48)	63.6 ^{ab} (3.95)	65.4 ^{ab} (3.95)	64 ^{ab} (3.95)	62.4 ^{ab} (3.95)	59.6 ^b (3.95)	67.6 ^{ab} (3.48)	0.30
Total Protein g/dL (6.7-7.5)	*8.48 ^a (0.16)	*7.89 ^{ab} (0.12)	7.28 ^c (0.16)	*7.56 ^{bc} (0.16)	*8.02 ^{ab} (0.16)	*8.00 ^{ab} (0.16)	*8.10 ^{ab} (0.16)	*7.98 ^{ab} (0.12)	0.002
Albumin g/dL (2.5-3.8)	3.38 ^a (0.10)	2.93 ^{bc} (0.09)	2.66 ^c (0.10)	2.72 ^{bc} (0.10)	2.94 ^{bc} (0.10)	2.9 ^{bc} (0.10)	2.98 ^b (0.10)	2.9 ^{bc} (0.09)	0.003
AST U/L (55-125)	98.8 ^a (11.74)	95.3 ^a (10.53)	76.2 ^a (11.74)	83.8 ^a (11.74)	90.6 ^a (11.74)	84.6 ^a (11.74)	82.8 ^a (11.74)	74.6 ^a (10.53)	0.71
CK U/L (1-350)	265.8 ^a (121)	327.7 ^a (99)	258.8 ^a (121)	248 ^a (121)	98 ^a (121)	91.6 ^a (121)	81.4 ^a (121)	64.7 ^a (99)	0.55
ALP U/L (25-250)	104.4 ^a (16.95)	78 ^{ab} (15.86)	63.8 ^b (16.95)	65 ^b (16.95)	72 ^{ab} (16.95)	64.6 ^b (16.95)	72.8 ^{ab} (16.95)	60.3 ^b (15.86)	0.7
GGT U/L (1-50)	*57.4 ^{ab} (8.59)	*58.1 ^a (8.46)	*50.6 ^{ab} (8.59)	49.4 ^b (8.59)	*55.8 ^{ab} (8.59)	*54.4 ^{ab} (8.59)	*56 ^{ab} (8.59)	*52.4 ^{ab} (8.46)	0.99
Total Bilirubin mg/dL (0.1-1.6)	0.42 ^a (0.04)	0.338 ^{ab} (0.03)	0.146 ^c (0.04)	0.186 ^c (0.04)	0.222 ^{bc} (0.04)	0.274 ^{abc} (0.04)	0.284 ^{abc} (0.04)	0.291 ^{abc} (0.03)	0.001

Anion Gap (14-21)	17 ^a (0.96)	*12.7 ^b (0.71)	*12.2 ^b (0.96)	*12.6 ^b (0.96)	*12.4 ^b (0.96)	*12.6 ^b (0.96)	14.4 ^{ab} (0.96)	*12.4 ^b (0.71)	0.01
Lipemic Indice	20 ^a (0)	20 ^a (0)	20 ^a (0)	20 ^a (0)	20 ^a (0)	20 ^a (0)	20 ^a (0)	20 ^a (0)	
Hemolytic Indice	32 ^a (3.94)	22.3 ^{ab} (2.72)	18.2 ^{ab} (3.94)	15 ^{ab} (3.94)	18.6 ^{ab} (3.94)	15.8 ^{ab} (3.94)	17.8 ^{ab} (3.94)	15.1 ^b (2.72)	0.01
Icteric Indice	3.8 ^a (0.15)	3.5 ^{ab} (0.12)	2.8 ^c (0.15)	2.8 ^c (0.15)	3 ^{bc} (0.15)	3 ^{bc} (0.15)	3 ^{bc} (0.15)	3 ^{bc} (0.12)	

a,b,c Different letters within rows are statistically significant (p=0.05). * Indicates values that are outside the reference range provided by the analyzing laboratory. BUN, blood urea nitrogen; AST, aspartate transferase; CK, creatinine kinase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase.

Fecal Occult Blood

Fecal occult blood analyses were negative for all individuals at all time points.

Urine analysis

Individual cow urinalyses are presented in Table 2.6. Urine specific gravity was evaluated daily and remained normal for four animals at all time points, the fifth animal had lower USG at the time of baseline measurement and throughout the study.

Table 2.6 Individual cow urine analysis prior to meloxicam treatment and for 10 days post-treatment for cows administered 30 mg/kg meloxicam per os.

Animal ID	Parameter	Baseline	1	2	3	4	6	8	10
1	Sp. Gravity	1.034	1.04	1.046	1.047	1.042	1.036	1.038	1.032
	pH	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
	Protein	3	3	3	3	1	1	2	2
	Leukocytes	1	1	1	0.5	1	0.5	0.5	1
2	Sp. Gravity	1.013	1.021	N/A	1.023	1.02	1.016	1.015	1.015
	pH	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
	Protein	1	1	0.5	0.5	0.5	0.5	0.5	0.5
	Leukocytes	0	0.5	0.5	0.5	0	0	0	0
3	Sp. Gravity	1.035	1.03	1.044	1.05	1.046	1.031	1.027	1.025
	pH	8.5	8	8.5	8.5	8.5	8.5	8.5	8.5
	Protein	0.5	0.5	1	0.5	1	2	0.5	0.5
	Leukocytes	0.5	0.5	0.5	0.5	0.5	0	0	0
4	Sp. Gravity	1.026	1.045	1.043	1.037	1.036	1.027	1.029	1.028
	pH	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
	Protein	1	1	0.5	3	1	1	1	1
	Leukocytes	1	1	0.5	0.5	0.5	0	0	0
5	Sp. Gravity	1.039	1.03	1.039	1.04	1.03	1.037	1.03	1.027
	pH	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
	Protein	0	0	0	0	0	0	0	0
	Leukocytes	1	0.5	0.5	0.5	0.5	0	0	0

Necropsy Findings

Gross lesions included mild, non-suppurative superficial abomasitis in 4 of 5 cows and were considered mild and incidental findings by the pathologist along with notable phlebitis around the indwelling catheter in one animal.

Histological Examination

Gross and histologic examination of the kidneys on all animals revealed no evidence of nephritis. NSAID-induced changes in the liver can be both cholestatic and parenchymal in nature

[3]. Gross and microscopic evaluation of all livers was unremarkable. Histologically, no lesions were appreciated in the gastrointestinal tract or liver in any of the five animals. One cow had minute foci of lymphoplasmacytic inflammatory aggregates in renal cortical and medullary interstitium.

Discussion

Milk and plasma pharmacokinetic parameters reported in this study were different when compared to other published data. The $C_{\max}$ in the present study was similar to sows dosed with the same dose (30 mg/kg meloxicam PO) approaching 100 ug/mL[18]. Plasma $C_{\max}$ was approximately 30 times higher than reported $C_{\max}$ for studies using 1 mg/kg dose in cattle that reported plasma $C_{\max}$ ranging from 1.45-3.10 $\mu\text{g/mL}$ [19-23]. The much higher $C_{\max}$ and longer $T_{\max}$ were likely directly due to the delivered dose being 30 times higher. The $T_{\max}$ in these studies in cattle were shorter than the present study ranging from 10.48-16.75 hours compared to 19.7 hours. The terminal elimination half-life presented here was similar to other data in cattle using a much smaller dose. This also suggests that excretion is not impacted with the increased dose. Oral clearance was also similar to cattle studies using a 1 mg/kg PO dosing regimen [19-22]. Plasma concentrations following 30 mg/kg meloxicam administrations at 120th hour were similar to the $C_{\max}$ reported in 1 mg/kg dose studies in cattle. This suggests that a 30 mg/kg oral dose could provide prolonged plasma concentrations and possibly prolonged analgesia. The terminal elimination half-life in the present study in cows was shorter than reported half-lives in calves[21]. Multiple reports using various NSAIDs have shown decreased clearance in young animals compared to older animals[24-26]. The maximum milk concentration and time to maximum concentration in the present study are much higher than data reported from 1 mg/kg

oral dose in cattle[19,20]. In those studies, $C_{\max}$ in milk ranged from 0.388-0.421 $\mu\text{g/mL}$ compared to the present study ranging from 17.7-41.39 $\mu\text{g/mL}$, $T_{\max}$ in milk was 9.33 hours in one study in cattle and while not specified in the text, 8-16 hours in the other compared to a milk $T_{\max}$ of nearly 24 hours in the present study. Mean milk terminal elimination half-life in the present study was 12.23 hours compared to the reported a milk terminal elimination half-life of 10.38 hours in cows receiving 1 mg/kg per os[20]. The data presented here are insufficient to establish a withdrawal period for a single extreme over-dose because the plasma and milk concentrations were never below the detection limit of the assay. In the event that extreme doses of meloxicam are accidentally administered in the field, more work is needed to identify the post-treatment interval required to clear meloxicam from the plasma and milk. Previous research has shown significant differences in meloxicam depletion in post-partum and mid-lactation cows[19]. The data suggest that post-partum cows will retain violative drug residues longer than mid-lactation cows similar to the ones used in our study. In the present study, the slight reduction in milk yield over time is most likely due to frequent handling.

Inhibition of prostaglandin production is the basis for both the adverse and therapeutic effects of NSAIDs. The most common target organs for NSAID-induced lesions are the gastrointestinal tract, kidney, and liver[27]. Expected lesions in the gastrointestinal tract are irritation, erosion, and ulceration[17]. These effects can lead to anemia via blood loss, protein-losing enteropathy, and melena. NSAID nephropathy in other species is characterized by oliguria, azotemia, hyperkalemia, and nephritis[28]. Thus, these biochemical changes were evaluated to identify any abnormalities related to the toxic potential of a single large oral dose in cattle. BUN and creatinine remained within the reference ranges at all time points. A slight

hypokalemia was identified on day 2 and nephritis was not identified in the post-mortem exam or histopathology.

The toxicologic evaluation of a single oral dose of 30 mg/kg meloxicam was largely unremarkable. Expected target organ function was thoroughly evaluated ante-mortem, and no significant abnormalities were identified. Approximate oral LD50 dosages have been reported in a number of laboratory species ranging from 83.5 mg/kg to >1600 mg/kg with a maximum non-lethal oral dose in rats of 50 mg/kg[29]. Data evaluating the toxic potential of using large doses in cattle are lacking. Multiple dosing regimens have been used to evaluate the toxic effects of high doses of meloxicam in laboratory animals with most utilizing daily doses over time. There is one report of a dog accidentally receiving 1 mg/kg IV (intravenously) meloxicam where therapeutic plasma exchange was successfully employed to circumvent any toxicologic effects, so it is unknown if this dose would have resulted in any adverse effects in a dog[30]. Severe clinical signs and lesions occurred in a study using Wistar rats where the rats were orally gavaged with 8 mg/kg meloxicam for 28 days. In the rat study, gross lesions included hemorrhage and congestion of the GI tract, liver, and kidney. These organs also showed histopathologic changes including vacuolar degeneration of hepatocytes with hemorrhage, hyperplasia of intestinal villi projecting into the lumen, sloughing of the glandular epithelium of the stomach and gastric erosions, as well as tubular degeneration and hemorrhage in the kidneys. Blood parameters were also evaluated in the rat study with the most relevant changes related to the hemorrhage appreciated on gross exam (decreases in packed cell volume, total erythrocyte count, and hemoglobin)[10]. A single oral dose of 20 mg/kg meloxicam did not result in any toxicologic effects in one study in mice[11], while a separate study in mice receiving 20 mg/kg meloxicam administered subcutaneously resulted in concentration-dependent lesions at the

injection site with no evidence of gastric ulceration or kidney or liver lesions[9]. Meloxicam was administered at 20 mg/kg PO to American Kestrels every 12 hours for 7 days with no clinical signs or abnormalities appreciated in PCV and biochemistry and no evidence of renal toxicity was identified[12]. Llamas were administered 1 mg/kg meloxicam PO once and no adverse effects were observed[31]. Sows were administered 30 mg/kg meloxicam orally for three days to evaluate whether analgesic concentrations could be delivered to their offspring via milk. Button ulcers and gastritis were identified in 2/5 sows and their litters[16].

Blood parameters were evaluated in the llama study and the most significant change was an increase in mean BUN; however, pre- and post-treatment values were still within the utilized reference range and deemed not clinically relevant[31]. CBC changes were reported in Wistar rats given 8 mg/kg meloxicam per os for 28 days, the abnormalities were directly related to the post-mortem findings of hemorrhage in multiple tissues[10]. The neutrophil count in the present study was the only parameter with a statistical difference from baseline to the end of the study and with values outside of expected reference ranges. The elevation in neutrophil count correlated clinically with the confirmed phlebitis. Post-mortem evaluations were also unremarkable from gross observations to the lack of histologic lesions. The inflammatory lesions in the kidney of one cow were categorized as mild and incidental by the pathologist. The post-mortem findings are similar to multiple reports utilizing a relatively comparable dose for a brief time period in other species suggesting that multiple doses over time are needed to cause appreciable changes.

Conclusions

Administration of meloxicam at 30 mg/kg per os did not result in any significant, clinically relevant changes that would be considered indicative of NSAIDs toxicity in five lactating dairy cattle within 10 days of treatment. These results suggest a single large oral dose is

well-tolerated by lactating dairy cows. This work also suggests a wide safety margin between analgesic effectiveness and clinical toxicity of a single dose of meloxicam. Further work is needed to evaluate a dose of this magnitude in other age classes and multiple doses of this magnitude. Additionally, further work is needed to determine the toxic dose of meloxicam in cattle and the dose, if any, that results in negative impacts on reproduction.

References

1. EMEA. *Committee for Veterinary Medical Products: Meloxicam Summary Report (2)*; The European Agency for the Evaluation of Medicinal Products Website, 1999.
2. Todd, C.G.; Millman, S.T.; McKnight, D.R.; Duffield, T.F.; Leslie, K.E. Nonsteroidal anti-inflammatory drug therapy for neonatal calf diarrhea complex: Effects on calf performance¹. *Journal of Animal Science* **2010**, *88*, 2019-2028, doi:10.2527/jas.2009-2340.
3. Olson, M.E.; Ralston, B.; Burwash, L.; Matheson-Bird, H.; Allan, N.D. Efficacy of oral meloxicam suspension for prevention of pain and inflammation following band and surgical castration in calves. *BMC Vet Res* **2016**, *12*, 102, doi:10.1186/s12917-016-0735-3.
4. Barrier, A.C.; Coombs, T.M.; Dwyer, C.M.; Haskell, M.J.; Goby, L. Administration of a NSAID (meloxicam) affects lying behaviour after caesarean section in beef cows. *Applied Animal Behaviour Science* **2014**, *155*, 28-33, doi:<https://doi.org/10.1016/j.applanim.2014.02.015>.
5. Robles, I.; Arruda, A.G.; Nixon, E.; Johnstone, E.; Wagner, B.; Edwards-Callaway, L.; Baynes, R.; Coetzee, J.; Pairis-Garcia, M. Producer and Veterinarian Perspectives towards Pain Management Practices in the US Cattle Industry. *Animals (Basel)* **2021**, *11*, doi:10.3390/ani11010209.
6. Lees, P. Analgesic, Antiinflammatory, Antipyretic Drugs. In *Veterinary Pharmacology and Therapeutics*, 9th ed.; Jim E. Riviere, M.G.P., Ed.; Blackwell Publishing: 2009; pp. 457-492.

7. Kim, J.; Cannon, B.A.; Freeman, L.E.; Tan, S.; Knych, H.K.; Kendall, L.V. High-dose Meloxicam Provides Improved Analgesia in Female CD1 Mice: A Pharmacokinetic and Efficacy Study. *J Am Assoc Lab Anim Sci* **2023**, *62*, 74-80, doi:10.30802/aalas-jaalas-22-000064.
8. Kendall, L.V.; Bailey, A.L.; Singh, B.; McGee, W. Toxic Effects of High-dose Meloxicam and Carprofen on Female CD1 Mice. *J Am Assoc Lab Anim Sci* **2022**, *61*, 75-80, doi:10.30802/aalas-jaalas-21-000071.
9. Sarfaty, A.E.; Zeiss, C.J.; Willis, A.D.; Harris, J.M.; Smith, P.C. Concentration-dependent Toxicity after Subcutaneous Administration of Meloxicam to C57BL/6N Mice (*Mus musculus*). *J Am Assoc Lab Anim Sci* **2019**, *58*, 802-809, doi:10.30802/aalas-jaalas-19-000037.
10. Bharani, S.P., Naik, A.K., Parija, S.C.; and Panda, S.K. Meloxicam induced toxicopathology studies in Wistar rats. . *Indian Journal of Animal Research* **2020**, *54*, 363-366, doi:10.18805/ijar.B-3766.
11. Ingrao, J.C.; Johnson, R.; Tor, E.; Gu, Y.; Litman, M.; Turner, P.V. Aqueous stability and oral pharmacokinetics of meloxicam and carprofen in male C57BL/6 mice. *J Am Assoc Lab Anim Sci* **2013**, *52*, 553-559.
12. Summa, N.M.; Guzman, D.S.; Larrat, S.; Troncy, E.; Bird, D.M.; Lair, S.; Fitzgerald, G. Evaluation of High Dosages of Oral Meloxicam in American Kestrels (*Falco sparverius*). *J Avian Med Surg* **2017**, *31*, 108-116, doi:10.1647/2015-150.
13. Waelchli, R.O.; Thun, R.; Stocker, H. Effect of flunixin meglumine on placental expulsion in dairy cattle after a caesarean. *Vet Rec* **1999**, *144*, 702-703, doi:10.1136/vr.144.25.702.

14. Newby, N.C.; Renaud, D.; Tremblay, R.; Duffield, T.F. Evaluation of the effects of treating dairy cows with meloxicam at calving on retained fetal membranes risk. *Can Vet J* **2014**, *55*, 1196-1199.
15. Hirsch, A.C.; Philipp, H. Effects of meloxicam on reproduction parameters in dairy cattle. *J Vet Pharmacol Ther* **2009**, *32*, 566-570, doi:10.1111/j.1365-2885.2009.01109.x.
16. Bates, J.L. Transmammary-delivered meloxicam in piglets undergoing castration and tail docking: Impact on pharmacokinetics and pain biomarkers. Iowa State University, 2015.
17. McLean, M.K.; Khan, S.A. Toxicology of Frequently Encountered Nonsteroidal Anti-inflammatory Drugs in Dogs and Cats: An Update. *Vet Clin North Am Small Anim Pract* **2018**, *48*, 969-984, doi:10.1016/j.cvsm.2018.06.003.
18. Bates, J.L.; Karriker, L.A.; Stock, M.L.; Pertzborn, K.M.; Baldwin, L.G.; Wulf, L.W.; Lee, C.J.; Wang, C.; Coetzee, J.F. Impact of transmammary-delivered meloxicam on biomarkers of pain and distress in piglets after castration and tail docking. *PLoS One* **2014**, *9*, e113678, doi:10.1371/journal.pone.0113678.
19. Gorden, P.J.; Burchard, M.; Ydstie, J.A.; Kleinhenz, M.D.; Wulf, L.W.; Rajewski, S.J.; Wang, C.; Gehring, R.; Mochel, J.P.; Coetzee, J.F. Comparison of milk and plasma pharmacokinetics of meloxicam in postpartum versus mid-lactation Holstein cows. *J Vet Pharmacol Ther* **2018**, *41*, 463-468, doi:10.1111/jvp.12488.
20. Malreddy, P.R.; Coetzee, J.F.; Kukanich, B.; Gehring, R. Pharmacokinetics and milk secretion of gabapentin and meloxicam co-administered orally in Holstein-Friesian cows. *J Vet Pharmacol Ther* **2013**, *36*, 14-20, doi:10.1111/j.1365-2885.2012.01384.x.
21. Coetzee, J.F.; KuKanich, B.; Mosher, R.; Allen, P.S. Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. *Vet Ther* **2009**, *10*, E1-8.

22. Warner, R.; Ydstie, J.A.; Wulf, L.W.; Gehring, R.; Coetzee, J.F.; Mochel, J.P.; Gorden, P.J. Comparative Pharmacokinetics of Meloxicam Between Healthy Post-partum vs. Mid-lactation Dairy Cattle. *Front Vet Sci* **2020**, *7*, 548, doi:10.3389/fvets.2020.00548.
23. Shock, D.; Roche, S.; Olson, M. A Comparative Pharmacokinetic Analysis of Oral and Subcutaneous Meloxicam Administered to Postpartum Dairy Cows. *Vet Sci* **2019**, *6*, doi:10.3390/vetsci6030073.
24. Igarza, L.; Soraci, A.; Auza, N.; Zeballos, H. Some Pharmacokinetic Parameters of R-(-)- and S-(+)-Ketoprofen: The Influence of Age and Differing Physiological Status in Dairy Cattle. *Veterinary Research Communications* **2004**, *28*, 81-87.
25. Volner, Z.; Nouws, J.F.; Kozjek, F.; van Ginneken, C.A.; Primožic, S. Age-dependent pharmacokinetics of phenylbutazone in calves. *Vet Q* **1990**, *12*, 98-102, doi:10.1080/01652176.1990.9694251.
26. Kissell, L.W.; Brinson, P.D.; Gehring, R.; Tell, L.A.; Wetzlich, S.E.; Baynes, R.E.; Riviere, J.E.; Smith, G.W. Pharmacokinetics and tissue elimination of flunixin in veal calves. *Am J Vet Res* **2016**, *77*, 634-640, doi:10.2460/ajvr.77.6.634.
27. Roder, J.D. Analgesics. In *Clinical Veterinary Toxicology*, Plumlee, K.H., Ed.; Mosby: 2004; pp. 282-284.
28. Beasley, V. Analgesic Nephropathy in Nephrotoxic Organic Compounds. In *Veterinary Toxicology*; International Veterinary Information Service: 1999.
29. Lehmann, H.A.; Baumeister, M.; Lützen, L.; Wiegler, J. Meloxicam: A toxicology overview. *InflammoPharmacology* **1996**, *4*, 105-123, doi:10.1007/BF02735465.

30. Walton, S.; Ryan, K.A.; Davis, J.L.; Acierno, M. Treatment of meloxicam overdose in a dog via therapeutic plasma exchange. *J Vet Emerg Crit Care (San Antonio)* **2017**, *27*, 444-450, doi:10.1111/vec.12607.
31. Kreuder, A.J.; Coetzee, J.F.; Wulf, L.W.; Schleining, J.A.; KuKanich, B.; Layman, L.L.; Plummer, P.J. Bioavailability and pharmacokinetics of oral meloxicam in llamas. *BMC Veterinary Research* **2012**, *8*, 85, doi:10.1186/1746-6148-8-85.

Chapter 3 - Evaluation of the Safety of Oral Meloxicam in Young Holstein Calves

Abstract

Meloxicam can be used in an extra-label manner in the United States to alleviate pain associated with castration and dehorning in cattle; however, its safety in young calves has not been thoroughly evaluated. This study assessed the safety of oral meloxicam administered at doses of 1, 3, and 5 mg/kg once daily for three consecutive days, alongside a placebo control. Thirty-two Holstein calves were blocked by sex and randomly allocated to one of four treatment groups, with eight calves per group. Peak serum meloxicam concentrations were detected 24 hours after the final dose and demonstrated dose-dependent increases across treatment groups. Complete blood counts and serum chemistry analyses revealed no significant differences between groups. Post-mortem examinations focused on the gastrointestinal, hepatic, and renal systems. Calves receiving the 5 mg/kg dose developed significantly more severe ulceration at the torus pyloricus compared to other groups ($p=0.0145$), while no treatment-related lesions were identified in the other organs examined. The gastrointestinal lesions were characterized as minor, with evidence of healing observed within five days after cessation of exposure. These findings suggest that three-day oral administration of high doses of meloxicam (5 mg/kg) may induce mild, yet reversible, mucosal damage in the abomasum. Balancing analgesic efficacy with safety remains critical for dose optimization, and the results of this study provide valuable guidance for dose selection in young Holstein calves.

Introduction

Consumers of animal products in the United States (US) have developed an increasing interest in the welfare of the animals from which these products are derived. A recent survey showed 81% of respondents found it important that pain relief be provided when removing horns or horn buds from cattle, and 83% found it important that pain relief be provided when castrating cattle[1]. The National Dairy Farmers Assuring Responsible Management (FARM) Program is committed to demonstrating that “farmers are doing what’s right for cows, customers, and consumers,” and therefore requires producers to provide pain relief to calves for procedures like castration and dehorning/disbudding as part of its program mandates. The American Veterinary Medical Association (AVMA) and the American Association of Bovine Practitioners (AABP) have also developed position statements supporting pain relief measures for management procedures like castration and dehorning. There are currently no therapeutic drugs approved for the control of pain following castration and dehorning by the United States Food and Drug Administration (US FDA). The FDA has provided guidance on assessing pain in target species for drugs to be considered for approval, and requires that pain assessment outcomes be objective, repeatable, and clearly defined[2]. Reliably assessing pain in traditional prey species has been a significant hurdle in securing FDA approval for analgesic drugs in food animals. The Animal Medicinal Drug Use Clarification Act (AMDUCA) allows analgesic drugs to be used in an extra-label manner provided the stipulations within AMDUCA are met which includes: the existence of a veterinary-client-patient relationship (VCPR), the extra-label drug used is FDA-approved for use in animals and/or humans, the extra-label drug use is not for production purposes, the extra-label drug is not administered through feed, and the extra-label drug use does not result in any

violative residues in food for human consumption[3]. Thus, all drugs used by veterinarians to provide pain relief for castration and or dehorning at this time constitute extra-label drug use.

Meloxicam is approved in Canada for the alleviation of pain associated with castration and dehorning in young calves and has been shown to reduce pain markers associated with castration and dehorning[4-9]. A recent review suggested that meloxicam administered orally at 1 mg/kg or 0.5 mg/kg subcutaneously combined with a local anesthetic is the current preferred method for providing analgesia to calves undergoing castration, dehorning, or both[10]. Another important aspect of approval for animal drugs in the US is the safety of the drug in the target animal. The FDA has several guidance documents addressing target animal safety studies for new animal drugs[11]. The safety of oral meloxicam in young calves (approximately 6 weeks of age) which is the typical age for dehorning and castration has not been investigated. One study evaluated the safety of the one-time oral administration of 30 mg/kg meloxicam to lactating dairy cows and was unable to demonstrate any significant negative effects[12]. However, differences in physiology may contribute to a possible increased sensitivity of calves compared to adult cows. Thus, if pain relief is mandated by consumers, producer certification programs, and veterinary interest groups, an evaluation of the safety of proposed treatment regimens in cattle is critical.

Multiple efficacy studies have been performed to evaluate how meloxicam affects pain response in calves using intramuscular (IM) intravenous (IV), and oral dosing. An oral dose of 1 mg/kg reduced pain response variables associated with cautery dehorning while neonatal dairy calves had a similar positive response to 1 mg/kg oral doses of meloxicam given with colostrum[6,13]. Oral doses of 1 mg/kg in conjunction with other analgesics improved average daily gain and reduced pain biomarkers in calves at the time of dehorning, and the same oral

administration of 1 mg/kg meloxicam provided to calves at the time of band or surgical castration also showed positive effects such as reduced painful behaviors, pain responses, and inflammation[5,7]. A study evaluating a lower oral dose of 0.5 mg/kg administered before cautery dehorning resulted in 10-week-old calves spending more time either laying down or at the feed bunk[14]. An intramuscularly administered meloxicam dose of 0.5 mg/kg resulted in reduced painful behavior following cautery dehorning in calves[4]. The same dose administered subcutaneously at the time of castration and branding also decreased painful behaviors in 2-month-old and 7-8-month-old beef calves[8,9]. An oral suspension of meloxicam is approved for the alleviation of pain and inflammation following surgical and band castration in cattle using a dose of 1 mg/kg in Canada. Consequently, in the present study, 1 mg/kg *per os* was considered the recommended dose on which the dosage selections were based.

Materials and Methods

This protocol (#4836) was approved by the Institutional Animal Care and Use Committee (IACUC) at Kansas State University (KSU). All study personnel responsible for data collection, drug administration, postmortem investigation, histologic examination, and drug analysis were blinded to treatment groups.

Experimental Animals

Thirty-nine Holstein calves (20 female, 19 male) weighing between 46.3 kg and 87.1kg were purchased from commercial dairy operations and were acclimated for 14 days prior to drug administration. During the acclimation phase, several animals developed respiratory disease, and the study population was administered tildipirosin (Zuprevo™, Merck Animal Health, Rahway,

NJ, USA) following label instructions. 32 animals (16 male, 16 female) were ultimately enrolled, blocked by sex, and randomly allocated to one of four treatment groups for a total of eight animals (4 male, 4 female) per treatment. Treatment groups included:

- A. Negative control whey placebo (n=8)
- B. 1 mg/kg meloxicam *per os* for three consecutive days (n=8)
- C. 3 mg/kg meloxicam *per os* for three consecutive days (n=8)
- D. 5 mg/kg meloxicam *per os* for three consecutive days (n=8)

Housing and Husbandry

Each calf was housed in a calf hutch with a 12 ft² area pen at the Kansas State University Sheep and Meat Goat Center. Pens initially had grass and were subsequently bedded with straw. Calves were fed 5 quarts of a commercial calf milk replacer (Land O Lakes Herd Maker®PB, Arden Hills, MN, USA) twice daily. The milk replacer offered at least 22% crude protein, 15% crude fat, and 72 g/ton of lasalocid. Calves were also provided a commercial calf starter composed of 14% crude protein and 2% crude fat (Calf Grower, Mid Kansas COOP, Manhattan, KS, USA) which was refreshed twice daily along with *ad libitum* fresh water. Due to variable starter intake across the study population, milk replacer intake was used to evaluate appetite. This project took place in June and July with ambient temperatures ranging from 55 to 101° F (12.8 to 38.3° C).

Meloxicam Administration

Calf starter was removed the evening prior to meloxicam administration, at which point calves were fed milk. The following morning, meloxicam was administered to fasted calves and milk was distributed 1 hour after the last calf received treatment. Meloxicam was administered once daily for three consecutive days via balling gun using 1-ounce gelatin capsules (Torpac, Inc., Fairfield, NJ, USA) filled with 15 mg meloxicam tablets (MF: Cadila healthcare, Dist: Zydus Pharmaceuticals Inc., Pennington, NJ, USA). Doses were rounded up to the nearest whole tablet to maintain the integrity of the formulation. Mean meloxicam doses for each treatment group were 0 (control), 1.10 mg/kg, 3.07 mg/kg, and 5.08 mg/kg, respectively.

Sample Collection

Intravenous jugular catheters (MILAcath®-Extended Use, 16ga. X 130 mm, MILA International, Inc., Florence, KY, USA) were placed using aseptic techniques and sutured to the skin 24 hours before meloxicam administration. Catheter patency was maintained using a heparin flush solution containing 10 USP/mL in 0.9% sodium chloride (Medefil, Inc., Glendale Heights, IL, USA). Blood samples were collected prior to administration via catheters or venipuncture if catheters failed, and immediately transferred to a 6 mL serum tube (BD Diagnostics, Franklin Lakes, NJ, USA) and a 6 mL tube containing EDTA (BD Diagnostics) for complete blood counts, serum chemistries, and serum drug analysis. All tubes were refrigerated prior to processing. The serum tubes were centrifuged at 1600 x g for 10 minutes, with half of the serum subsequently transferred to a 2.0 mL cryovial and frozen at -80°C until analysis. The remaining serum and whole blood were submitted to the Kansas Veterinary Diagnostic Laboratory for serum chemistry and complete blood count analysis, respectively.

On Study Days four and eight, half of the calves of each sex from each treatment group were intended to be humanely euthanized via captive bolt and exsanguination. These times were selected to coincide with the anticipated maximum concentration following the last dose (Day 4) and the anticipated day where drug would be eliminated (Day 8) based on previous pharmacokinetic data. On Day four, 3 calves from both the control and 1 mg/kg groups, 6 calves from the 3 mg/kg group, and 4 calves from the 5 mg/kg group were euthanized due to an error with postmortem examination day randomization. Full postmortem examinations were performed on each individual animal by a board-certified pathologist (BLP) with all abnormalities recorded. Lesions were identified, recorded, and digital photographs were taken; tissues were collected for histopathologic evaluation using standard techniques of formalin fixation, paraffin embedding, sectioning at 5 μ m, followed by hematoxylin and eosin (H&E) staining. Organs evaluated microscopically included the liver, kidney, abomasum, torus pyloricus, and duodenum. Gross and histologic grading schemes for abomasal ulceration were adapted from previously published work [15,16]. Gross lesions in the gastrointestinal tract were macroscopically characterized as Types 1-4 as previously described[16]. Only type 1 lesions were identified at postmortem examination; these tissues were then graded histologically and assigned a sub-type of 1a, 1b, 1c, or 1d. These lesions were characterized by superficial inflammation (1a), erosion and associated hemorrhage (1b), ulcers with or without granulation (1c), and perforation (1d), as previously described[15].

Serum Meloxicam Analysis

A standard curve was built by removing standard stock solutions from a -80° C freezer to allow them to warm to room temperature before use (20 min). For daily analysis,

the meloxicam stock solution (100 µg/mL) was diluted with negative bovine plasma to achieve meloxicam concentrations of 10 µg/mL and 1 µg/mL. Subsequently, the 1 µg/mL meloxicam solution was further diluted to obtain a set of concentrations at 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 25, 50, and 100 ng/mL in negative bovine plasma. Meloxicam-d3 was used as an internal standard for meloxicam. The meloxicam-d3 stock solution (100 µg/mL) was diluted in methanol to get concentrations at 10 µg/mL and 1 µg/mL. This was further diluted in 4% phosphoric acid to 100 ng/mL. Quality controls (QC) were prepared daily and spiked in negative bovine plasma at 5, 10, 50, and 100 ng/mL. The calibrates, blank (negative bovine plasma), QC, and the bovine plasma samples were extracted and cleaned by solid-phase extraction (SPE) using an Oasis HLB Prime µElution 96-well plate. Briefly, 100 µL of the bovine plasma sample, 50 µL of the internal standard solution (meloxicam-d3), and 150 µL of 4% phosphoric acid were added to a 48-well non-tissue culture-treated plate. The plate was subsequently shaken for 20 min and centrifuged at $2,000 \times g$ for 30 min at 20°C. The supernatant was collected onto the Oasis HLB Prime µElution 96-well plate. Following a wash with 300 µL of 5% methanol, the target components were eluted with 50 µL of 90/10 acetonitrile/methanol and 50 µL of 0.1% formic acid was added to each well of the plate before the UPLC-MS/MS analysis. The calibration curve was linear from 0.05 to 100 ng/mL and accepted when the correlation coefficient exceeded 0.99. The LOD and LOQ were 0.025 and 0.05 ng/mL, respectively. The inter-day accuracy was 107.33%, 110.36%, 99.09%, and 99.88% at concentrations of 5, 10, 50, and 100 ng/mL. The inter-day precision was 2.21%, 1.37%, 0.77%, and 1.36% at concentrations of 5, 10, 50, and 100 ng/mL, respectively.

Statistical Analysis

Statistical analysis for complete blood count and serum chemistry was performed using computer software (JMP 17.2.0, SAS Inst. Inc., Cary, NC). Responses were analyzed using a mixed linear model with calf as the experimental unit. Calf nested in treatment group were designated as a random effect, with treatment, time, gender, treatment by time interaction and treatment by gender interaction designated as fixed effects. Histologic lesion scores were treated as categorical data and Fisher's exact test were used to compare scores. Statistical significance was set at $p \leq 0.05$ *a priori*.

Results

Serum Meloxicam

Mean ($\pm$ SD) serum meloxicam concentrations by treatment and day are presented in Table 3.1 and graphically in Figure 3.1. The highest concentrations were detected in the serum of animals 24 hours after the last oral dose (Day 4) irrespective of treatment group. The mean concentrations for the 5 mg/kg dose group were the highest at all time points compared to the 1 mg/kg dose group were always the lowest.

Table 3.1 Mean($\pm$ SD) serum meloxicam concentrations expressed as $\mu\text{g/mL}$ (n=8 for days 0-4, on days 5-7, n=5 for control and 1mg/kg groups, n=2 for 3 mg/kg group, and n=4 for 5 mg/kg group.

Tx Gp.	Day 0*	1*	2*	3	4	5	6	7
Control	0.00\$	0.00\$	0.00\$	0.00\$	0.00\$	0.00\$	0.00\$	0.00\$
1 mg/kg	0.00\$	3.35(0.98)	6.80(2.18)	8.95(1.58)	6.05(0.84)	4.30(0.63)	3.03(0.86)	1.83(0.58)
3 mg/kg	0.01\$	9.43(3.62)	15.39(2.87)	37.97(8.99)	15.08(1.90)	11.64(0.98)	8.84(0.88)	5.12(2.32)
5 mg/kg	0.00\$	12.77(3.93)	23.02(1.49)	63.36(16.26)	50.11(12.06)	18.04(13.78)	12.66(2.33)	9.69(2.45)

* Denotes drug administration days

\$ Means were quantifiable within the performance metrics of the assay and ranged 0.0006-0.0055 $\mu\text{g/mL}$.

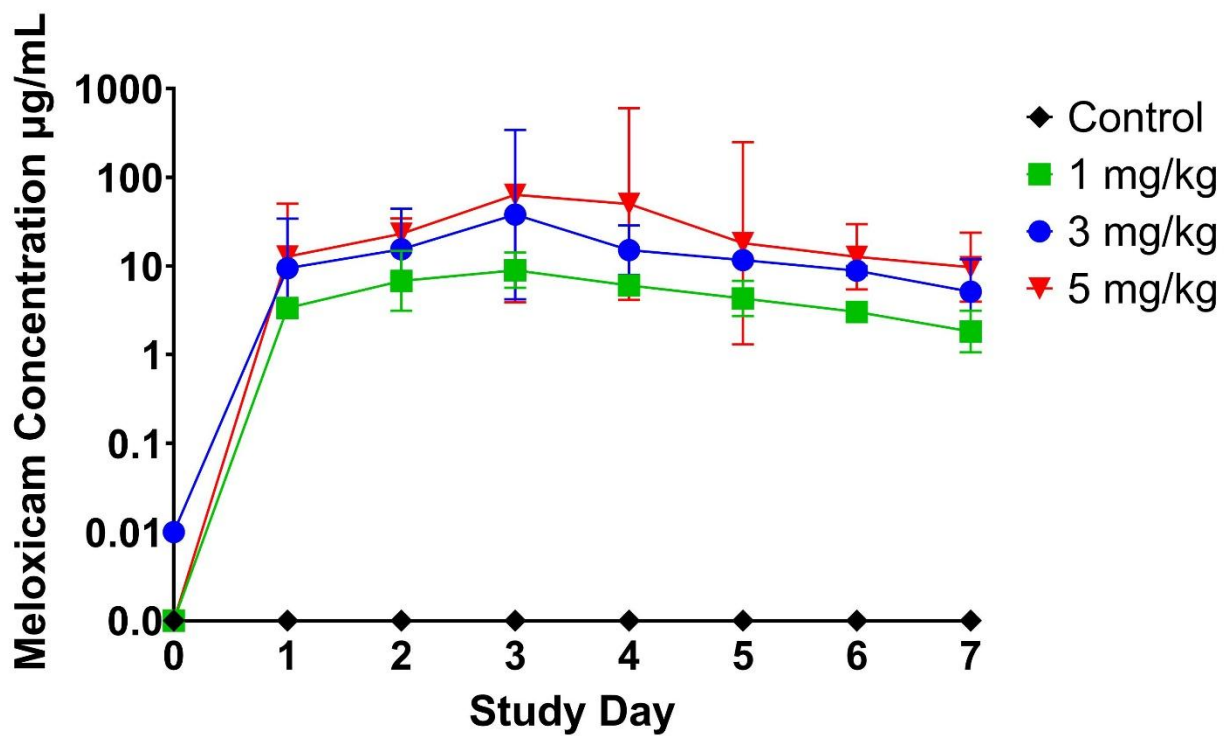


Figure 3.1 Mean($\pm$ SD) serum meloxicam concentrations (n=8 for days 0-4, on days 5-7, n=5 for control and 1mg/kg groups, n=2 for 3 mg/kg group, and n=4 for 5 mg/kg group).

Complete Blood Count

Summary complete blood count data is presented in Table 3.2. Mean hematocrit (HCT) and packed cell volume (PCV) concentrations were within the supplied reference intervals for all groups at all time points. While a statistical treatment by time effect was identified ($p=0.0485$) for PCV, all analyses were within the supplied reference ranges and were deemed biologically normal. Red blood cell counts (RBC) were consistently elevated above the reference for all groups and no treatment effect was identified. Similarly, all groups were microcytic and hypochromic at all time points, with no treatment effect identified. Additionally, white blood cell analyses were also within the supplied reference intervals at all time points. Platelets (PLT) were detected above the reference intervals at several time points, while protein was below the reference interval for all groups. However, no treatment effect was identified.

Table 3.2. Mean ($\pm$ SD) complete blood count results for Holstein calves administered 0, 1, 3, and 5mg/kg meloxicam per os for three consecutive days (n=8 per group for days -3 to 3, on day 7 n=5 for control and 1mg/kg groups, n=2 for 3 mg/kg group, and n=4 for 5 mg/kg group).

PARAMETER (UNIT) Reference Interval	Tx Gp.	-3	-1	3	7	<i>p</i> -value
HCT (%) 22-33	Control	30.50 (1.22)	31.63 (1.22)	29.75 (1.22)	29.77 (1.30)	0.0916
	1 mg/kg	32.00 (1.22)	31.88 (1.22)	30.63 (1.22)	29.61 (1.30)	
	3 mg/kg	29.13 (1.22)	30.25 (1.22)	28.75 (1.22)	27.38 (1.56)	
	5 mg/kg	31.50 (1.22)	30.38 (1.22)	29.75 (1.22)	31.51 (1.35)	
PCV (%) 22-33	Control	30.88 (1.32)	32.50 (1.32)	30.88 (1.32)	30.43 (1.40)	0.0485 ^s
	1 mg/kg	32.25 (1.32)	32.88 (1.32)	31.50 (1.32)	29.31 (1.40)	
	3 mg/kg	30.13 (1.32)	30.50 (1.32)	29.38 (1.32)	29.19 (1.68)	
	5 mg/kg	31.75 (1.32)	30.75 (1.32)	30.63 (1.32)	32.01 (1.45)	
RBC (M/μL) 5.10-7.60	Control	9.77* (0.34)	10.19* (0.34)	9.70* (0.34)	9.63* (0.36)	0.0847
	1 mg/kg	9.67* (0.34)	9.77* (0.34)	9.45* (0.34)	9.26* (0.36)	
	3 mg/kg	9.57* (0.34)	9.95* (0.34)	9.60* (0.34)	9.16* (0.43)	
	5 mg/kg	9.84* (0.34)	9.73* (0.34)	9.58* (0.34)	10.15* (0.37)	
HGB (g/dL) 8.5-12.2	Control	10.59 (0.45)	10.88 (0.45)	10.39 (0.45)	10.25 (0.47)	0.1256
	1 mg/kg	11.20 (0.45)	11.14 (0.45)	10.86 (0.45)	10.50 (0.47)	
	3 mg/kg	10.11 (0.45)	10.41 (0.45)	10.09 (0.45)	9.66 (0.54)	
	5 mg/kg	10.96 (0.45)	10.65 (0.45)	10.54 (0.45)	10.02 (0.48)	
MCV (fL) 38-50	Control	31.25* (0.66)	30.95* (0.66)	30.66* (0.66)	30.59* (0.67)	0.1379
	1 mg/kg	33.09* (0.66)	32.83* (0.66)	32.49* (0.66)	31.94* (0.67)	
	3 mg/kg	30.38* (0.66)	30.21* (0.66)	29.93* (0.66)	29.96* (0.69)	
	5 mg/kg	31.90* (0.66)	31.33* (0.66)	31.09* (0.66)	31.01* (0.67)	
MCH (pg) 14.0-18.0	Control	10.81* (0.26)	10.66* (0.26)	10.71* (0.26)	10.62* (0.26)	0.8264
	1 mg/kg	11.65* (0.26)	11.46* (0.26)	11.55* (0.26)	11.37* (0.26)	
	3 mg/kg	10.56* (0.26)	10.42* (0.26)	10.48* (0.26)	10.47* (0.27)	
	5 mg/kg	11.14* (0.26)	10.94* (0.26)	10.99* (0.26)	10.89* (0.26)	
MCHC (g/dL) 36.0-39.0	Control	34.60* (0.27)	34.48* (0.27)	34.90* (0.27)	34.77* (0.30)	0.9068
	1 mg/kg	35.21* (0.27)	34.90* (0.27)	35.51* (0.27)	35.60* (0.30)	
	3 mg/kg	34.74* (0.27)	34.48* (0.27)	34.99* (0.27)	34.98* (.040)	
	5 mg/kg	34.86* (0.27)	34.90* (0.27)	35.35* (0.27)	34.99* (0.32)	
WBC (K/μL) 4.9-12.0	Control	6.73 (0.59)	7.36 (0.26)	8.11 (0.26)	8.23 (0.73)	0.0999
	1 mg/kg	6.78 (0.26)	7.28 (0.26)	8.13 (0.26)	9.35 (0.73)	
	3 mg/kg	7.43 (0.26)	6.94 (0.26)	7.15 (0.26)	7.64 (1.12)	
	5 mg/kg	7.84 (0.26)	7.90 (0.26)	8.45 (0.26)	8.54 (0.81)	
LYMPH (K/μL) 1.6-5.6	Control	3.83 (0.44)	4.56 (0.44)	4.14 (0.44)	4.84 (0.50)	0.8457
	1 mg/kg	4.10 (0.44)	4.40 (0.44)	4.51 (0.44)	5.54 (0.50)	
	3 mg/kg	3.88 (0.44)	4.48 (0.44)	3.95 (0.44)	4.78 (0.68)	
	5 mg/kg	4.65 (0.44)	4.81 (0.44)	4.71 (0.44)	5.15 (0.53)	
MONO (K/μL) 0-0.8	Control	0.60 (0.10)	0.38 (0.10)	0.34 (0.10)	0.87* (0.13)	0.4452
	1 mg/kg	0.60 (0.10)	0.49 (0.10)	0.18 (0.10)	0.50 (0.14)	
	3 mg/kg	0.65 (0.10)	0.39 (0.10)	0.24 (0.10)	0.60 (0.21)	

	5 mg/kg	0.54 (0.10)	0.20 (0.10)	0.31 (0.10)	0.74 (0.15)	
SEG (K/μL) 1.8-6.3	Control	2.19 (0.50)	2.18 (0.10)	3.30 (0.10)	2.55 (0.65)	0.8814
	1 mg/kg	1.79* (0.10)	2.14 (0.10)	3.14 (0.10)	2.94 (0.66)	
	3 mg/kg	2.78 (0.10)	1.91 (0.10)	2.81 (0.10)	2.07 (1.03)	
	5 mg/kg	2.36 (0.10)	2.59 (0.10)	3.01 (0.10)	2.11 (0.73)	
BASO (K/μL) 0-0.3	Control	0.06 (0.04)	0.13 (0.04)	0.13 (0.04)	0.12 (0.05)	0.8918
	1 mg/kg	0.10 (0.04)	0.11 (0.04)	0.13 (0.04)	0.18 (0.05)	
	3 mg/kg	0.06 (0.04)	0.08 (0.04)	0.10 (0.04)	0.10 (0.08)	
	5 mg/kg	0.09 (0.04)	0.14 (0.04)	0.21 (0.04)	0.13 (0.06)	
EOSIN (K/μL) 0-0.9	Control	0.04 (0.06)	0.14 (0.06)	0.23 (0.06)	0.06 (0.07)	0.7444
	1 mg/kg	0.19 (0.06)	0.14 (0.06)	0.16 (0.06)	0.08 (0.08)	
	3 mg/kg	0.09 (0.06)	0.11 (0.06)	0.08 (0.06)	0.04 (0.12)	
	5 mg/kg	0.16 (0.06)	0.20 (0.06)	0.28 (0.06)	0.11 (0.08)	
PLT (K/μL) 193-637	Control	682.38* (49.93)	663.85* (49.93)	538.38 (49.93)	474.71 (60.96)	0.5872
	1 mg/kg	607.50 (49.93)	615.38 (49.93)	550.50 (49.93)	530.38 (61.17)	
	3 mg/kg	707.00* (49.93)	664.25* (49.93)	577.75 (49.93)	603.36 (78.21)	
	5 mg/kg	640.89* (49.93)	591.89 (49.93)	571.63 (49.93)	558.23 (67.16)	
PROTEIN (g/L) 7.0-9.0	Control	6.14* (0.11)	6.18* (0.11)	6.11* (0.11)	6.21* (0.13)	0.6874
	1 mg/kg	6.30* (0.11)	6.26* (0.11)	6.08* (0.11)	6.25* (0.13)	
	3 mg/kg	6.13* (0.11)	6.20* (0.11)	5.93* (0.11)	6.04* (0.17)	
	5 mg/kg	6.23* (0.11)	6.25* (0.11)	6.01* (0.11)	6.36* (0.14)	
FIB (HEAT PPT) 300-700	Control	537.50 (47.82)	487.50 (47.82)	437.50 (47.82)	552.28 (59.71)	0.2971
	1 mg/kg	575.00 (47.82)	418.75 (47.82)	387.50 (47.82)	443.54 (60.38)	
	3 mg/kg	575.00 (47.82)	575.00 (47.82)	375.00 (47.82)	523.07 (92.89)	
	5 mg/kg	475.00 (47.82)	450.00 (47.82)	425.00 (47.82)	535.34 (66.81)	

* Denotes values outside of provided reference intervals, \$ indicates statistical significance.

Serum chemistry

Serum chemistry results are presented in Table 3.3. No abnormalities were identified in any treatment group at any time for electrolytes (Na, K, Cl), acid/base (bicarbonate), phosphorus (P), magnesium (Mg) or creatinine. Serum calcium was above the reference interval for all groups at the final time point irrespective of treatment, while mean serum protein was below the reference interval for all groups at all time points. In either case, no treatment effect was identified. Similarly, blood urea nitrogen (BUN) was below the reference interval for all groups at all time points, but did not display a treatment effect. Aspartate aminotransferase (AST), mean glucose (GLU), and gamma glutamyl transpeptidase (GGT) were all normal at all times for all groups with no treatment effect identified. Finally, creatinine kinase (CK) was below the

reference interval for several groups at multiple time points in contrast to both sorbitol dehydrogenase (SDH) and alkaline phosphatase (ALP), which were both elevated for many groups at most times. However, in all three cases, a treatment effect was not identified.

Table 3.3. Mean ($\pm$ SD) serum chemistry results for Holstein calves administered 0, 1, 3, and 5mg/kg meloxicam per os for three consecutive days (n=8 per group for days -3 to 3, on day 7 n=5 for control and 1mg/kg groups, n=2 for 3 mg/kg group, and n=4 for 5 mg/kg group).

Parameter (Unit) Reference Interval	Tx. Gp.	Day -3	Day -1	Day 3	Day 7	p-value
SODIUM (mmol/L) 138-155	Control	139.13 (0.50)	139.75 (0.50)	137.38 (0.50)	140.41 (0.63)	0.5911
	1 mg/kg	139.50 (0.50)	140.00 (0.50)	139.00 (0.50)	139.53 (0.64)	
	3 mg/kg	138.75 (0.50)	139.63 (0.50)	138.13 (0.50)	139.07 (0.98)	
	5 mg/kg	139.34 (0.50)	139.63 (0.50)	138.25 (0.50)	139.38 (0.71)	
POTASSIUM (mmol/L) 4.2-6.3	Control	5.15 (0.11)	4.85 (0.11)	4.75 (0.11)	4.72 (0.15)	0.7163
	1 mg/kg	5.25 (0.11)	4.64 (0.11)	4.80 (0.11)	4.46 (0.15)	
	3 mg/kg	5.05 (0.11)	4.79 (0.11)	4.90 (0.11)	4.78 (0.23)	
	5 mg/kg	5.38 (0.11)	4.99 (0.11)	4.86 (0.11)	4.79 (0.17)	
CHLORIDE (mmol/L) 92-117	Control	99.4 (0.49)	100.0 (0.49)	99.6 (0.49)	101.3 (0.60)	0.9745
	1 mg/kg	99.8 (0.49)	100.0 (0.49)	100.5 (0.49)	101.3 (0.61)	
	3 mg/kg	99.8 (0.49)	100.4 (0.49)	100.6 (0.49)	101.1 (0.92)	
	5 mg/kg	99.8 (0.49)	101.4 (0.49)	100.8 (0.49)	101.4 (0.67)	
BICARBONATE (mmol/L) 21-31	Control	28.13 (0.55)	29.00 (0.55)	25.00 (0.55)	26.86 (0.68)	0.7756
	1 mg/kg	27.25 (0.55)	28.38 (0.55)	25.25 (0.55)	26.64 (0.69)	
	3 mg/kg	27.63 (0.55)	28.38 (0.55)	24.13 (0.55)	26.05 (1.05)	
	5 mg/kg	27.38 (0.55)	28.38(0.55)	24.88 (0.55)	25.16 (0.76)	
CALCIUM TOTAL (mg/dL) 8.1-10.3	Control	10.01 (0.15)	10.15 (0.15)	10.21 (0.15)	10.41* (0.19)	0.9815
	1 mg/kg	9.93 (0.15)	10.13 (0.15)	10.35* (0.15)	10.39* (0.19)	
	3 mg/kg	9.89 (0.15)	10.14 (0.15)	10.18 (0.15)	10.54* (0.29)	
	5 mg/kg	10.10 (0.15)	10.14 (0.15)	10.23(0.15)	10.42* (0.21)	
PHOSPHORUS (mg/dL) 4.9-9.0	Control	7.65 (0.24)	7.74 (0.24)	7.40 (0.24)	7.50 (0.29)	0.0965
	1 mg/kg	7.64 (0.24)	7.85 (0.24)	8.28 (0.24)	7.80 (0.29)	
	3 mg/kg	7.38 (0.24)	7.53 (0.24)	8.08 (0.24)	6.53 (0.44)	
	5 mg/kg	7.76 (0.24)	7.54 (0.24)	7.83 (0.24)	7.37 (0.32)	
MG TOT (mg/dL) 1.9-3.0	Control	2.06 (0.05)	2.08 (0.05)	2.05 (0.05)	1.92 (0.06)	0.1796
	1 mg/kg	1.96 (0.05)	2.06 (0.05)	2.04 (0.05)	2.05 (0.06)	
	3 mg/kg	2.00 (0.05)	2.03 (0.05)	2.10 (0.05)	2.12 (0.09)	
	5 mg/kg	2.04 (0.05)	2.09 (0.05)	2.10 (0.05)	1.96 (0.07)	
PROTEIN (g/dL) 6.0-9.0	Control	5.49* (0.13)	5.51* (0.13)	5.45* (0.13)	5.41* (0.13)	0.9586
	1 mg/kg	5.49* (0.13)	5.55* (0.13)	5.36* (0.13)	5.39* (0.13)	
	3 mg/kg	5.34* (0.13)	5.39* (0.13)	5.23* (0.13)	5.29* (0.16)	
	5 mg/kg	5.48* (0.13)	5.54* (0.13)	5.40* (0.13)	5.51* (0.14)	
ALBUMIN (g/dL) 3.1-4.3	Control	3.13 (0.06)	3.08* (0.06)	3.10 (0.06)	3.08* (0.07)	0.7578
	1 mg/kg	3.20 (0.06)	3.14 (0.06)	3.10 (0.06)	3.12 (0.07)	
	3 mg/kg	3.19 (0.06)	3.09* (0.06)	3.10 (0.06)	3.12 (0.09)	
	5 mg/kg	3.19 (0.06)	3.15 (0.06)	3.06* (0.06)	3.04* (0.07)	
BUN (mg/dL) 9.0-24.0	Control	6.88* (0.43)	7.25* (0.43)	6.50* (0.43)	5.91* (0.53)	0.1636
	1 mg/kg	5.63* (0.43)	5.88* (0.43)	6.13* (0.43)	5.47* (0.54)	
	3 mg/kg	7.75* (0.43)	6.88* (0.43)	6.63* (0.43)	6.73* (0.82)	
	5 mg/kg	7.00* (0.43)	6.38* (0.43)	6.25* (0.43)	7.40* (0.59)	

CREATININE (mg/dL) 0.5-1.6	Control	0.875 (0.05)	0.800 (0.05)	0.813 (0.05)	0.690 (0.05)	0.7200
	1 mg/kg	0.825 (0.05)	0.738 (0.05)	0.763 (0.05)	0.634 (0.05)	
	3 mg/kg	0.900 (0.05)	0.825 (0.05)	0.838 (0.05)	0.754 (0.06)	
	5 mg/kg	0.863(0.05)	0.775 (0.05)	0.788 (0.05)	0.768 (0.05)	
AST (U/L) 53-156	Control	62.00 (5.31)	58.50 (5.31)	56.50 (5.31)	55.59 (6.22)	0.4797
	1 mg/kg	62.50 (5.31)	76.75 (5.31)	66.25 (5.31)	67.45 (6.25)	
	3 mg/kg	54.75 (5.31)	69.50 (5.31)	57.63 (5.31)	62.36 (9.00)	
	5 mg/kg	62.00 (5.31)	68.13 (5.31)	58.38 (5.31)	59.22 (6.78)	
CK (U/L) 171-357	Control	208.8 (27.4)	228.8 (27.4)	135.3* (27.4)	159.0* (34.4)	0.4075
	1 mg/kg	156.8* (27.4)	156.8* (27.4)	167.4* (27.4)	225.7 (34.9)	
	3 mg/kg	206.1 (27.4)	187.6 (27.4)	160.6* (27.4)	153.4* (54.0)	
	5 mg/kg	185.0 (27.4)	182.5 (27.4)	164.5* (27.4)	184.8 (38.6)	
SDH (U/L) 6.1-18.4	Control	32.7* (6.6)	31.0* (6.6)	25.3* (6.6)	23.4* (8.0)	0.2587
	1 mg/kg	22.9* (6.6)	42.0* (6.6)	29.0* (6.6)	31.7* (8.1)	
	3 mg/kg	16.1 (6.6)	43.0* (6.6)	17.9 (6.6)	40.5* (12.2)	
	5 mg/kg	23.9* (6.6)	31.8* (6.6)	16.6 (6.6)	19.5* (8.9)	
ALP (U/L) 21-81	Control	237.00* (32.49)	275.88* (32.49)	283.63* (32.49)	265.58* (37.40)	0.4655
	1 mg/kg	225.63* (32.49)	267.00* (32.49)	330.13* (32.49)	337.29* (37.49)	
	3 mg/kg	189.13* (32.49)	236.25* (32.49)	289.75* (32.49)	314.11* (52.59)	
	5 mg/kg	246.88* (32.49)	305.13* (32.49)	328.00* (32.49)	266.95* (40.39)	
GGT (U/L) 10-39	Control	27.6 (3.94)	24.1 (3.94)	22.4 (3.94)	20.0 (4.1)	0.2641
	1 mg/kg	31.3 (3.94)	28.1 (3.94)	25.5 (3.94)	20.5 (4.1)	
	3 mg/kg	25.8 (3.94)	24.4 (3.94)	20.0 (3.94)	24.6 (4.5)	
	5 mg/kg	23.4 (3.94)	21.6 (3.94)	19.1 (3.94)	18.0 (4.1)	
GLUCOSE (mg/dL) 29-73	Control	104.0* (5.4)	104.6* (5.4)	73.1* (5.4)	79.8* (6.6)	0.8571
	1 mg/kg	110.4* (5.4)	115.3* (5.4)	79.6* (5.4)	84.4* (6.7)	
	3 mg/kg	101.4* (5.4)	112.1* (5.4)	68.6 (5.4)	78.0* (10.0)	
	5 mg/kg	109.6* (5.4)	115.9* (5.4)	70.4 (5.4)	77.4* (7.3)	

* Denotes values outside of provided reference intervals.

Postmortem Lesions

Gross observation revealed multiple changes in the mucosa of the gastrointestinal tract in calves from each group during postmortem examination. Many calves were described as normal with no discernible macroscopic lesions. Lesions interpreted as minor included pinpoint to 2 mm irregular depressed areas of the pyloric mucosa, these areas were random and multifocal and interpreted as erosions with hyperemia. More severe lesions included depressed areas up to ~1cm in diameter in the mucosa, consistent with ulceration. The most severe gross lesions included larger up to ~3 cm in diameter irregular depressed areas of the pyloric mucosa with the

most severe lesions located on the torus pyloricus in all groups. The submucosa was exposed in calves with more severe ulcers and was hemorrhagic with the underlying submucosal tissue markedly thickened due to fibroplasia. Examples of macroscopic lesions observed as described are shown in Figure 3.2. The lesions above corresponded with a Type 1 ulcer in the grading system used; more severe lesions were not observed. Lesions consistent with bronchopneumonia were identified in some calves and considered unrelated to the treatments applied as the lesions were interpreted as chronic and were consistent with the respiratory signs noted during the acclimation phase of the project. No other lesions were identified on gross evaluation.

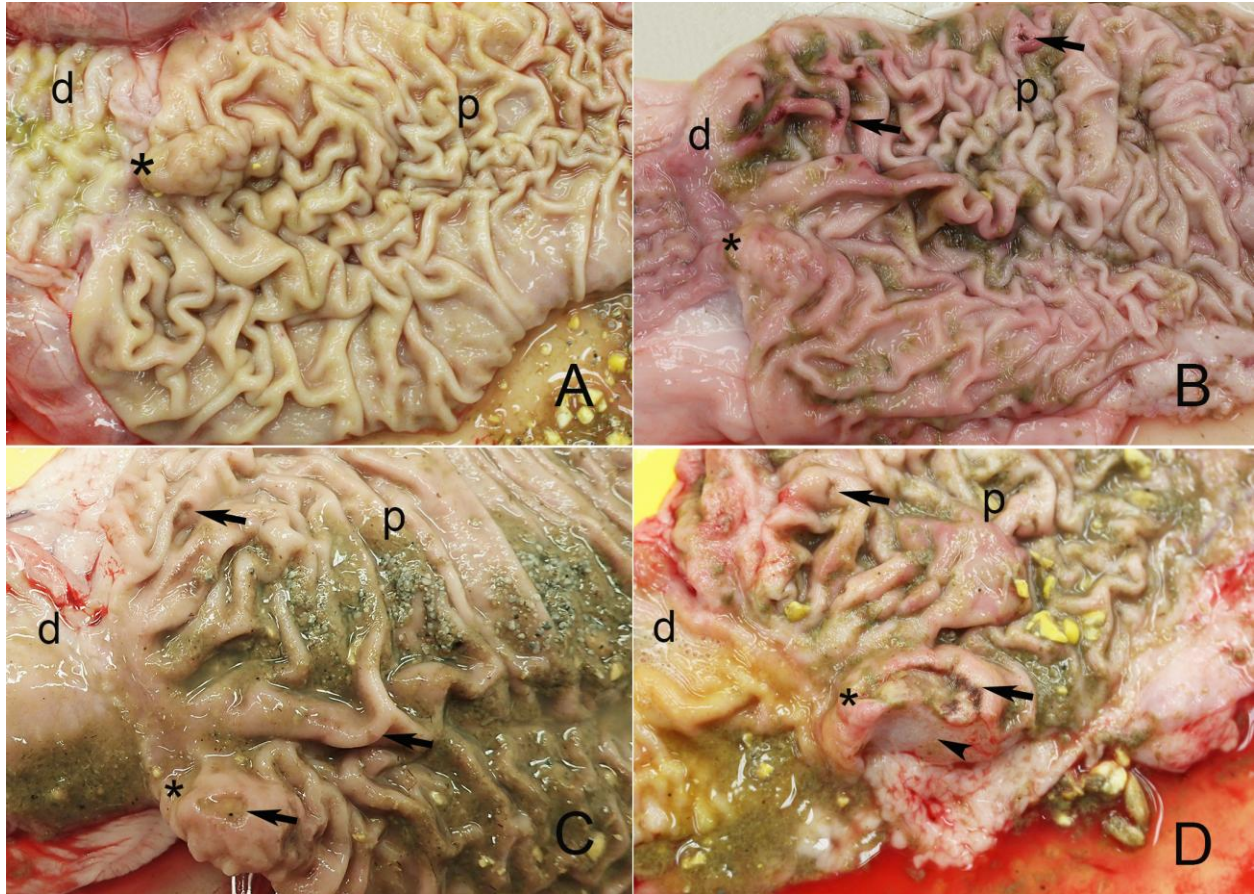


Figure 3.2 A. Abomasal pylorus (p) and duodenum (d). The torus pyloricus (*) and mucosa is normal. **B.** Abomasal pylorus (p) and duodenum (d). Multifocal and random pinpoint to 2mm diameter irregular depressed areas (arrows) are evident on the pyloric and duodenal mucosa, including the torus pyloricus (*). **C.** Abomasal pylorus (p) and duodenum (d). Multifocal and random pinpoint to 1cm diameter irregular depressed areas (arrows) are evident on the pyloric mucosa with the largest on the torus pyloricus (*). **D.** Abomasal pylorus (p) and duodenum (d). Multifocal and random pinpoint to 3cm diameter irregular depressed areas (arrows) are evident on the pyloric mucosa. The largest and most severe is on the torus pyloricus (*); this ulcer extends full thickness through the mucosal surface, the submucosa is exposed and hemorrhagic, and the underlying submucosa is markedly thickened due to dense white firm connective tissue (arrowhead).

Histopathology Findings

Organs evaluated microscopically included the liver, kidney, abomasum, torus, and duodenum. No lesions were observed in the liver or kidney in any animal and all appreciated lesions were isolated to the gastrointestinal tract. Examples of abomasal and torus lesions associated with the histologic scores are presented in Figure 3.3.

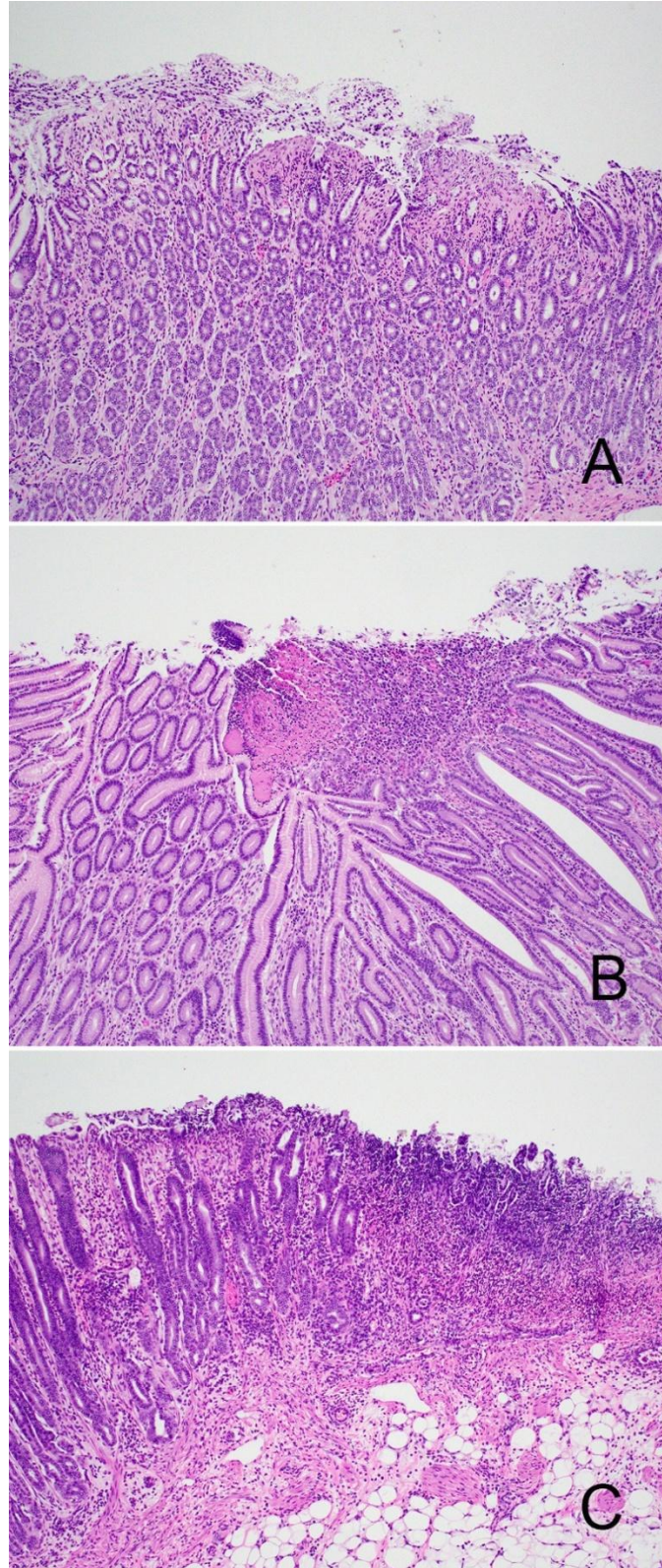


Figure 3.3. A. Abomasal pylorus (torus) grade 1A (10x): Abomasal pylorus (torus). There is superficial small intestinal mucosal injury extending from the surface epithelium to the

superficial mucosa (erosion), with loss of surface epithelium, superficial pyloric glands, and replacement by karyorrhectic and inflammatory debris. The mid to deeper pyloric glands are intact. Hematoxylin and eosin. B. Abomasal pylorus (torus) grade 1B (10x): Abomasal pylorus (torus). There is superficial small intestinal mucosal injury extending from the surface epithelium to the mid-mucosa (erosion), with loss of surface epithelium, superficial pyloric glands, and replacement by karyorrhectic debris and inflammatory cells. The deeper pyloric glands are intact. Hematoxylin and eosin. C. Abomasal pylorus grade 1C (10x): Abomasal pylorus (torus). There is full thickness small intestinal mucosal injury extending from the surface epithelium to the submucosa (ulceration), with loss of pyloric glands and replacement by immature fibrous and granulation tissue, karyorrhectic debris, fibrin, and inflammatory cells. Hematoxylin and eosin.

No perforations were appreciated on gross or microscopic examination thus, no animals received a 1D grade. At least one calf in all treatment groups including the placebo group developed ulceration at the abomasal torus pyloricus. One of eight control calves, four of eight calves in the 1 mg/kg group, three of eight calves in the 3 mg/kg dose group, and all eight calves in the 5 mg/kg dose group developed type 1c ulcers on the abomasal torus pyloricus by the end of the trial. One calf in both the 3 mg/kg and 5mg/kg group had ulcerations extend into the abomasal wall. No ulcerations were identified in the duodenum of any calf at any time point. The percentage of calves that developed each ulcer subtype on the abomasal torus is presented in Table 3.4. Generally, the most severe lesions were present on the torus pyloricus and there was a significant treatment effect where the high dose group developed more Type 1c lesions than the other groups ($p=0.0145$) shown in Figure 3.4.

Table 3.4 Contingency table of Type 1 abomasal torus pyloricus ulcer subtypes by treatment group and postmortem examination day. Percentages were calculated as number of calves with lesion type out of possible number of calves.

Tx Gp.	Necropsy Day	1a	1b	1c
Control	1	1 (33.3%)	1 (33.3%)	1 (33.3%)
Control	2	2 (40%)	3 (60%)	0
Total		3 (37.5%)	4 (50%)	1 (12.5%)
1 mg/kg	1	2 (66%)	0	1 (33.3%)
1mg.kg	2	0	2 (40%)	3 (60%)
Total		2 (25%)	2 (25%)	4 (50%)
3 mg/kg	1	1 (16.7%)	3 (50%)	2 (33.3%)
3mg/kg	2	0	1 (50%)	1 (50%)
Total		1 (12.5%)	4 (50%)	3 (37.5%)
5 mg/kg	1	0	0	4 (50%)
5 mg/kg	2	0	0	4 (50%)
Total		0	0	8 (100%)

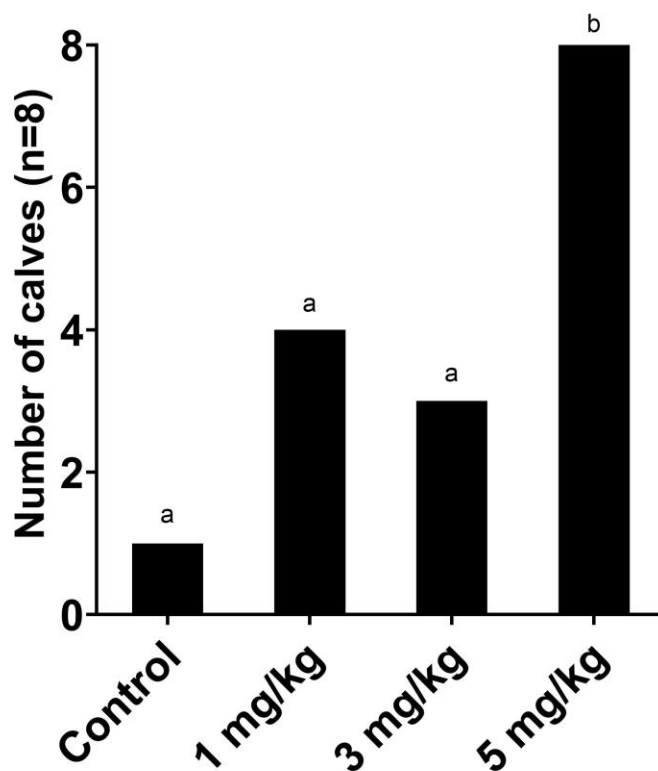


Figure 3.4 Number of calves with Type 1c ulcers on the abomasal torus within 8 days of meloxicam administration (n=8/treatment group).

Discussion

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) that acts by inhibiting prostaglandin synthesis. Cyclooxygenase (COX) is an enzyme that is responsible for the first step in prostanoid production[17]. There are two main COX enzymes, COX-1 which is constitutively expressed in many tissues and COX-2 which is upregulated in the face of tissue damage and the ensuing inflammatory cascade[17-19]. COX-1 has been described as a housekeeping enzyme due to the prostaglandins produced from its activity being responsible for maintaining gastrointestinal mucosal integrity, platelet function, and renal blood flow[18,20]. Toxic exposures to NSAIDs manifest in adverse effects mainly involving the gastrointestinal and renal systems[21]. The COX-2 enzyme is inducible and produces prostaglandins that are responsible for pain and inflammation of damaged tissue[20]. Generally, it is accepted that the inhibition of COX-1 is responsible for the negative effects of NSAID use, while the inhibition of the COX-2 form is responsible for the therapeutic benefits. Therefore, it follows that selective inhibition of COX-2 could be a desirable characteristic of NSAIDs. Meloxicam has been shown to be a COX-2 preferential NSAID in some species, although not specifically in the bovine. It has demonstrated loss of selectivity with increasing doses[20,22,23]. The need for both COX-1 and COX-2 inhibition has been demonstrated to be necessary to develop gastric ulceration in rats[23].

Toxicity studies of meloxicam in cattle are lacking. Acute oral toxicity of meloxicam has been investigated in other species with wide ranges of lethal dose estimates from 83.5 mg/kg BW in rats to more than 1600 mg/kg in mini pigs[24]. In these studies, the primary organs damaged included the gastrointestinal tract and kidneys[24]. The no observed effect level (NOEL) for

meloxicam was determined in multiple species following prolonged oral administration in Wistar rats (0.2 mg/kg for 52 weeks) and minipigs (1 mg/kg for 52 weeks)[24]. Studies in calves demonstrated intravenous doses of 0.5 mg/kg and 1.5 mg/kg were well tolerated[24].

The plasma meloxicam concentrations detected in samples at time zero are similar for 2 groups (control and 5 mg/kg) as those detected throughout the trial for the control calves. These concentrations did not change for the duration of the study and the limit of quantitation was exceptionally low so these were interpreted as background noise but could also be due to carryover system contamination as the samples were analyzed randomly. The time zero means for the 1mg/kg and 3mg/kg were driven by a single animal in each group. Because the calves were acclimated for fourteen days and no meloxicam was administered to any animal before the first dose, this may reflect some sample contamination during analysis. Serum drug concentrations at 24 hours post administration of the first 1mg/kg oral dose are similar to previous work using the same dose [5,6,25-30]. The mean serum concentrations at 24 hours after the first administration were 2.81 and 3.81 times higher for the 3 mg/kg and 5 mg/kg dose groups, respectively, compared to the 1 mg/kg group. On day 3, the mean concentrations were 4.24 and 7.08 times higher than the 1 mg/kg dose group at that time for the 3mg/kg and 5mg/kg groups, respectively. These varying ratios suggest an altered absorption profile for the highest dose group. The highest mean concentration of the 1 mg/kg dose group was 8.95 ug/mL 24 hours following the last administered dose. This concentration is similar to the 3 mg/kg dose group four days following the last dose (8.84 ug/mL). The serum drug concentration for the 5 mg/kg dose group was still higher 5 days post exposure (9.69 ug/mL).

The unbalancing of treatment groups on Day 4 was due to an error in the randomization of calves to postmortem examination day and is a major weakness of this study. The ante-

mortem testing is completely balanced until that time. The single treatment effect identified by CBC was the packed cell volume. Reductions in packed cell volume can be caused by hemorrhage and would be consistent with a bleeding ulcer in the gastrointestinal tract. PCV was within normal reference intervals suggesting this difference was not biologically relevant. Previous work has suggested that type 1 abomasal ulcers can only be diagnosed at necropsy as they do not result in detectable clinical changes, which is consistent with our findings[16]. Importantly, the CBC reference intervals likely encompass cattle of all ages and breeds and there may be age or breed-specific differences that could potentially add some clarity to these results if developed.

No statistically significant treatment differences in any serum chemistry parameters were identified. The deviations from the provided reference interval for albumin, ALP, BUN, calcium, CK, glucose, total protein, and SDH are not due to the treatments applied. Like the CBC results, these deviations may reflect utilizing reference intervals that are not age-specific.

Lesions identified at postmortem examination were isolated to the gastrointestinal tract, and specifically located near the abomasal pylorus. Many control calves did not have grossly identifiable mucosal damage which is surprising given the reported prevalence of abomasal ulcers in European veal calves of over 70%; however, veal calves are fed a milk-only diet and the study population in this work was also fed starter grain[31]. There was a significant treatment effect on lesion development at the pylorus identified, with all calves in the highest dose group developing the most severe observed lesion grade. Interestingly, in most calves, the more severe damage occurred on the torus pyloricus. Previous work in veal calves has demonstrated the most common site of abomasal mucosal damage is the pyloric region[31]. Importantly, no gross changes were visible in any other organ of interest.

Histologic examination of grossly-identified lesions allowed a more detailed investigation of the mucosal injury. The number of tissue layers damaged was associated with treatment where the highest dose group had more severe deeper lesions than the other groups. No microscopic lesions were identified in the kidney or liver of the test population consistent with the lack of significant findings on the CBC or serum chemistry panels. Overall, routine diagnostic testing used to evaluate health in cattle failed to differentiate calves with or without gross lesions in the gastrointestinal tract. Further, many calves in the last euthanasia group demonstrated tissue healing within five days after stopping meloxicam administration. Every calf consumed their entire bottle at every feeding for the entire study duration, indicating no effect of treatment on appetite. Taken together, while the highest dose was associated with more damage to the gastrointestinal tract, that damage was minor and was being apparently healed in calves that lived longer once exposure ceased.

The work described herein may allow for expanded dosage selection in efficacy trials. Increasing dosages may allow for the appreciation of both analgesic efficacy and production benefits associated with meloxicam administration at the time of painful procedures in calves. This could lead to greater adoption by veterinarians and producers and increase alignment with consumer expectations.

References

1. Animal Welfare Institute: Survey of Consumer Attitudes about Pain Relief for Physical Alterations of Farm Animals. Available online: <https://awionline.org/sites/default/files/uploads/documents/Surveys-Consumer-Attitudes-Pain-Relief-Dairy-Practices.pdf> (accessed on 7/23/2025).
2. United States Food and Drug Administration. Guidance for Industry #123 Development of data supporting approval of NSAIDs for use in animals. Available online: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-123-development-data-supporting-approval-nsaids-use-animals> (accessed on 04/21).
3. United States Food and Drug Administration. Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). Available online: <https://www.fda.gov/animal-veterinary/guidance-regulations/animal-medicinal-drug-use-clarification-act-1994-amduca#animals> (accessed on 5/25/2025).
4. Heinrich, A.; Duffield, T.F.; Lissemore, K.D.; Millman, S.T. The effect of meloxicam on behavior and pain sensitivity of dairy calves following cautery dehorning with a local anesthetic. *Journal of Dairy Science* **2010**, *93*, 2450-2457, doi:<https://doi.org/10.3168/jds.2009-2813>.
5. Glynn, H.D.; Coetzee, J.F.; Edwards-Callaway, L.N.; Dockweiler, J.C.; Allen, K.A.; Lubbers, B.; Jones, M.; Fraccaro, E.; Bergamasco, L.L.; KuKanich, B. The pharmacokinetics and effects of meloxicam, gabapentin, and flunixin in postweaning dairy calves following dehorning with local anesthesia. *J Vet Pharmacol Ther* **2013**, *36*, 550-561, doi:10.1111/jvp.12042.

6. Allen, K.A.; Coetzee, J.F.; Edwards-Callaway, L.N.; Glynn, H.; Dockweiler, J.; KuKanich, B.; Lin, H.; Wang, C.; Fraccaro, E.; Jones, M.; et al. The effect of timing of oral meloxicam administration on physiological responses in calves after cautery dehorning with local anesthesia. *J Dairy Sci* **2013**, *96*, 5194-5205, doi:10.3168/jds.2012-6251.
7. Olson, M.E.; Ralston, B.; Burwash, L.; Matheson-Bird, H.; Allan, N.D. Efficacy of oral meloxicam suspension for prevention of pain and inflammation following band and surgical castration in calves. *BMC Vet Res* **2016**, *12*, 102, doi:10.1186/s12917-016-0735-3.
8. Meléndez, D.M.; Marti, S.; Pajor, E.A.; Sidhu, P.K.; Gellatly, D.; Moya, D.; Janzen, E.D.; Coetzee, J.F.; Schwartzkopf-Genswein, K.S. Effect of meloxicam and lidocaine administered alone or in combination on indicators of pain and distress during and after knife castration in weaned beef calves. *PLOS ONE* **2018**, *13*, e0207289, doi:10.1371/journal.pone.0207289.
9. Meléndez, D.M.; Marti, S.; Pajor, E.A.; Moya, D.; Gellatly, D.; Janzen, E.D.; Schwartzkopf-Genswein, K.S. Effect of subcutaneous meloxicam on indicators of acute pain and distress after castration and branding in 2-mo-old beef calves^{1,2}. *Journal of Animal Science* **2018**, *96*, 3606-3621, doi:10.1093/jas/sky245.
10. Kleinhenz, M.D.; Viscardi, A.V.; Coetzee, J.F. Invited Review: On-farm pain management of food production animals. *Applied Animal Science* **2021**, *37*, 77-87, doi:10.15232/aas.2020-02106.
11. United States Food and Drug Administration Center for Veterinary Medicine. Guidance for Industry 185-(VICH-GL43)-Target-Animal-Safety-for-Veterinary-Pharmaceutical-

- Products. Available online: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-185-vich-gl43-target-animal-safety-veterinary-pharmaceutical-products> (accessed on 7/23/2025).
12. Fritz, S.A.; Ensley, S.M.; Lawrence, J.R.; Van Engen, N.; Lin, Z.; Kleinhenz, M.D.; Wulf, L.W.; Rice, S.; Gorden, P.J.; Peterson, J.; et al. Pharmacokinetics, Milk Residues, and Toxicological Evaluation of a Single High Dose of Meloxicam Administered at 30 mg/kg per os to Lactating Dairy Cattle. *Veterinary Sciences* **2023**, *10*, 301.
 13. Clark, M.O.C.; Stahl, T.C.; Erickson, P.S. The effect of meloxicam on neonatal dairy calves: Immunoglobulin G uptake and preweaning performance. *Journal of dairy science*. **2020**, doi:10.3168/jds.2020-18501.
 14. Theurer, M.E.; White, B.J.; Coetzee, J.F.; Edwards, L.N.; Mosher, R.A.; Cull, C.A. Assessment of behavioral changes associated with oral meloxicam administration at time of dehorning in calves using a remote triangulation device and accelerometers. *BMC Vet Res* **2012**, *8*, 48, doi:10.1186/1746-6148-8-48.
 15. Braun, U.; Eicher, R.; Ehrensperger, F. Type 1 abomasal ulcers in dairy cattle. *Zentralbl Veterinarmed A* **1991**, *38*, 357-366, doi:10.1111/j.1439-0442.1991.tb01022.x.
 16. Anderson, N.V. *Veterinary gastroenterology*; 1981.
 17. Warner, T.D.; Mitchell, J.A. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. *The FASEB Journal* **2004**, *18*, 790-804, doi:<https://doi.org/10.1096/fj.03-0645rev>.
 18. Morita, I. Distinct functions of COX-1 and COX-2. *Prostaglandins & Other Lipid Mediators* **2002**, *68-69*, 165-175, doi:[https://doi.org/10.1016/S0090-6980\(02\)00029-1](https://doi.org/10.1016/S0090-6980(02)00029-1).

19. Lehmann, H.A.; Baumeister, M.; Lützen, L.; Wiegleb, J. Meloxicam: A toxicology overview. *InflammoPharmacology* **1996**, *4*, 105-123, doi:10.1007/BF02735465.
20. C. M. Modi, S.K.M., H.B. Patel, G.B. Dudhatra, Avinash Kumar, Madhavi Avale. *Toxicopathological overview of analgesic and anti-inflammatory drugs in animals*; Issue : 1: Volume Volume : 2, pp. 149-157.
21. Panchal, N.K.; Prince Sabina, E. Non-steroidal anti-inflammatory drugs (NSAIDs): A current insight into its molecular mechanism eliciting organ toxicities. *Food and Chemical Toxicology* **2023**, *172*, 113598, doi:<https://doi.org/10.1016/j.fct.2022.113598>.
22. Del Tacca, M.; Colucci, R.; Fornai, M.; Blandizzi, C. Efficacy and Tolerability of Meloxicam, a COX-2 Preferential Nonsteroidal Anti-Inflammatory Drug. *Clinical Drug Investigation* **2002**, *22*, 799-818, doi:10.2165/00044011-200222120-00001.
23. Wallace, J.L.; McKnight, W.; Reuter, B.K.; Vergnolle, N. NSAID-induced gastric damage in rats: Requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology* **2000**, *119*, 706-714, doi:<https://doi.org/10.1053/gast.2000.16510>.
24. EMEA. *Committee for Veterinary Medical Products: Meloxicam Summary Report (2)*; The European Agency for the Evaluation of Medicinal Products Website, 1999.
25. Coetzee, J.F.; KuKanich, B.; Mosher, R.; Allen, P.S. Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. *Vet Ther* **2009**, *10*, E1-8.
26. Malreddy, P.R.; Coetzee, J.F.; Kukanich, B.; Gehring, R. Pharmacokinetics and milk secretion of gabapentin and meloxicam co-administered orally in Holstein-Friesian cows. *J Vet Pharmacol Ther* **2013**, *36*, 14-20, doi:10.1111/j.1365-2885.2012.01384.x.
27. Gorden, P.J.; Burchard, M.; Ydstie, J.A.; Kleinhenz, M.D.; Wulf, L.W.; Rajewski, S.J.; Wang, C.; Gehring, R.; Mochel, J.P.; Coetzee, J.F. Comparison of milk and plasma

- pharmacokinetics of meloxicam in postpartum versus mid-lactation Holstein cows. *J Vet Pharmacol Ther* **2018**, *41*, 463-468, doi:10.1111/jvp.12488.
28. Shock, D.; Roche, S.; Olson, M. A Comparative Pharmacokinetic Analysis of Oral and Subcutaneous Meloxicam Administered to Postpartum Dairy Cows. *Vet Sci* **2019**, *6*, doi:10.3390/vetsci6030073.
 29. Meléndez, D.M.; Marti, S.; Pajor, E.A.; Sidhu, P.K.; Gellatly, D.; Janzen, E.D.; Schwinghamer, T.D.; Coetzee, J.F.; Schwartzkopf-Genswein, K.S. Pharmacokinetics of oral and subcutaneous meloxicam: Effect on indicators of pain and inflammation after knife castration in weaned beef calves. *PLoS One* **2019**, *14*, e0217518, doi:10.1371/journal.pone.0217518.
 30. Warner, R.; Ydstie, J.A.; Wulf, L.W.; Gehring, R.; Coetzee, J.F.; Mochel, J.P.; Gorden, P.J. Comparative Pharmacokinetics of Meloxicam Between Healthy Post-partum vs. Mid-lactation Dairy Cattle. *Front Vet Sci* **2020**, *7*, 548, doi:10.3389/fvets.2020.00548.
 31. Van Driessche, L.; Fecteau, G.; Arsenault, J.; Miana, L.; Chorfi, Y.; Villettaz-Robichaud, M.; Hélie, P.; Buczinski, S. Inter-Rater Reliability of Scoring Systems for Abomasal Lesions in Quebec Veal Calves. *Animals* **2023**, *13*, 1664.

Chapter 4 - Determining the Persistence of Xylazine and Ketamine in Cattle Tissue Following a Simulated Rendering Process

Original Citation: *Veterinary Sciences*. August 2025. doi:

<https://doi.org/10.3390/vetsci12080740>

© 2025 by the Authors

Scott A. Fritz¹, Michael D. Kleinhenz², Steve M. Ensley¹, Patrick J. Gorden³, Yuntao Zhang¹,
Johann F. Coetzee¹, and Michael D. Apley^{1,*}.

¹ Department of Anatomy and Physiology, Kansas State University College of Veterinary
Medicine, Manhattan, KS 66506, USA

² Veterinary Education, Research and Outreach (VERO), Texas A&M University, Canyon, TX
79015, USA

³ Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University,
Ames, IA 50011, USA

Abstract

Humane euthanasia is an endpoint for production animals succumbing to disease or trauma. Euthanasia performed with barbiturates or other anesthetic/sedative drugs observes zero withdrawal time and drug residues may remain in tissue. Carcasses may be submitted for rendering and rendered products can be used to manufacture pet foods. The purpose of this study was to determine the concentration of two drugs, xylazine and ketamine, which may be

used during the euthanasia process of food animals and to determine the fate of these drugs following a simulated rendering process using a commercial autoclave. Twelve cattle were administered xylazine or xylazine and ketamine prior to euthanasia via penetrating captive bolt and samples of muscle, fat, liver, and kidney were collected. Tissue samples were analyzed by LC-MS/MS both raw and following rendering. Parent compound xylazine and ketamine were detected in all tissues both before and after rendering. The highest concentrations were found in rendered kidney for both drugs and lowest in rendered and raw fat for xylazine and ketamine, respectively. Future confirmatory research using a large-scale commercial rendering facility is warranted.

Introduction

Humane euthanasia is an endpoint for some food animals experiencing disease or trauma. Multiple methods of euthanasia are approved by the American Veterinary Medical Association (AVMA) for use in bovids and small ruminants including the following: barbiturates and barbituric acid derivatives alone or physical methods like gunshot and penetrating or non-penetrating (calves) captive bolt together with adjunctive methods including exsanguination, pithing, or IV injection of potassium chloride[1]. Carcass management is an important consideration of euthanasia. Some carcasses can be submitted for a process known as rendering following euthanasia. According to the United States Department of Agriculture (USDA), “rendering is an off-site process that uses heat to convert animal carcasses into safe, pathogen-free feed protein and other valuable end products while reducing the negative effects of the carcasses on people and the environment[2].” The rendering industry in the United States is dominated by tube and disc continuous rendering systems[3]. In these systems, raw material at

ambient temperature is fed on a conveyor into the disc or tube cooker where it is cooked at 240-280 °F (~115-145 °C) for 30-40 minutes[3]. Moisture evaporates and liquid fat is separated from the solids following cooking. The solids go through a pressing process which removes more fat, and the fat and solid portions continue on in separate pathways.

Animals are often chemically restrained with sedative or anesthetic drugs for safety when using physical methods of euthanasia. Unfortunately, there are instances where sodium-pentobarbital-treated animal tissues have been consumed as raw or contaminated animal food products resulting in severe clinical outcomes in animals that consume these products[4-7]. Sodium pentobarbital has been shown to resist degradation during rendering[8]. Alternative drug options to chemically-restrain animals during euthanasia that do not pose a risk to animals consuming either the raw or rendered tissues of the euthanized animal are desirable. Xylazine and ketamine are drugs which may be used for chemical restraint during the euthanasia process.

Xylazine is an alpha-2 agonist with analgesic, muscle relaxation, and sedative effects. Ketamine is a dissociative anesthetic commonly used for chemical restraint, anesthesia, and pain management. Xylazine and ketamine are described as being highly lipophilic and consequently have large volumes of distribution, indicating they widely distribute throughout the body[9-11]. This rapid distribution into the tissues includes the central nervous system, contributing to the rapid clinical effects. These compounds are used to make animals calmer and easier to restrain in order to perform euthanasia, often via penetrating captive bolt or gunshot where animals need to remain still.

While some pharmacokinetic data exist for animals administered these drugs, there are no data on the tissue concentrations of xylazine or ketamine in cattle tissue immediately after administration[9,10]. Anesthetic drugs are typically considered low risk compounds for residues

in food products due to their typical short term use, short half-lives, often intravenous administration, low doses needed for the desired effect, and more veterinary oversight compared to some antibiotic compounds[12]. The Food Animal Residue Avoidance Database (FARAD) suggests meat withdrawal intervals of 3 and 5 days for ketamine and xylazine respectively[13,14]. These withdrawal times apply to animals harvested for human consumption. Both ketamine and xylazine have been detected in tissues collected from horses euthanized after utilizing these drugs for chemical restraint[15]. The concentration of xylazine and ketamine in cattle tissue immediately following administration, as in the case of sedation for euthanasia, is unknown. Cattle are more sensitive to xylazine than horses and smaller doses are used to achieve the same anesthetic effect[12]. Currently, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) tests for ketamine and xylazine as part of its multi-drug screening and confirmation assay[16]. The listed applicability levels for both ketamine and xylazine in kidney and muscle are 20 ng/g and 1 ng/g, respectively for the screening assay[16]. The confirmation assay lists applicability levels for ketamine at 20 ng/g in muscle and kidney while xylazine is listed as “N.A.”.

While xylazine and ketamine are typically administered intravenously for the purpose of restraint, the exposure in animals consuming the rendered products from treated animals is primarily oral. Oral bioavailability data for these drugs has not been fully described in all species. Cats administered 2.5 mg/kg xylazine, and 10 mg/kg ketamine *per os* concurrently exhibited sedative effects similar to intramuscular administration of the same combination suggesting active drug is absorbed systemically following ingestion[17]. In humans, extensive first-pass metabolism of ketamine occurs in the liver following oral administration resulting in a low oral bioavailability calculated at 17%[18].

The stability of some veterinary drugs in cooked beef or after exposure to various levels of environmental heat has been reported[19-22]. Changes in concentrations of some veterinary drugs in milk products has been shown to significantly increase or decrease depending on the process used to produce the products[23]. However, the persistence of xylazine and ketamine following rendering is unknown which creates a significant data gap in recommending their use in euthanasia protocols for food animals. If these drugs are destroyed by the rendering process, they could be used to chemically restrain food animals during the euthanasia procedure with negligible risk of inadvertent exposures through rendered products.

Objectives

To address potential food safety issues of the use of xylazine and ketamine for sedation in association with food animal euthanasia, this study had two objectives. 1) Determine the tissue concentrations of xylazine and ketamine in tissues of cattle immediately following administration of these drugs as part of the euthanasia procedure. 2) Determine the persistence of these drugs in cattle tissue following a simulated rendering process.

Materials and Methods

Tissues for this project were procured from animals from previously completed research projects (KSU IACUC #-4937, ISU IACUC #-22-217). Group one consisted of six 10-14-week-old beef/dairy crossbred calves that weighed 59-68 kg and were intravenously administered 0.2 mg/kg xylazine prior to penetrating captive bolt euthanasia. Group two consisted of six adult female lactating Holstein cows that weighed 660-868 kg and received intravenous ketamine (2 mg/kg) and xylazine (0.5 mg/kg) mixed together in a syringe prior to penetrating captive bolt

once the cows reached an unconscious state. For both treatment groups, samples collected included liver, kidney, muscle, and fat from each individual animal. Muscle was collected from the semimembranosus and semitendinosus muscles of the hind limbs. Perirenal fat was collected from group 2 while the fat samples from group one were collected from multiple locations, including the perirenal fat, to obtain enough sample weight. Animals were euthanized and tissue samples were collected and frozen at -80° C until analysis. Samples were thawed on a benchtop at room temperature and ten subsamples for each animal were created including the following:

1. RAW – individual tissues (fat, liver, muscle, kidney) not subject to heating prior to drug analysis
2. RENDER - individual tissues (fat, liver, muscle, kidney) subject to heating prior to drug analysis
3. Raw – COMP - all tissues combined prior to drug analysis
4. RENDER – COMP – all tissues combined prior to heating and drug analysis

For the rendered samples, 20g of each tissue were weighed into glass beakers and sealed with aluminum foil. For the combined sample, 5g of each of the four tissues were weighed into a beaker for a total of 20g of tissue. All rendered samples were rendered as one piece of tissue to mimic the rendering process with the exception of the combined samples which were composed of four pieces weighing five grams each. All rendered samples were processed on a single autoclave run. For the raw samples, 2g of each tissue were weighed into 50 mL conical tubes. The combined raw sample included 2g of each sample type. Raw samples were homogenized and analyzed without further treatment.

Rendering

To mimic the continuous rendering process most often used in the US, a commercial autoclave (Model: Lancer LSS 275, Getinge, Wayne, NJ, USA) was utilized. A custom cycle was created to achieve temperatures of 240 °F (115.56 °C) for 30 minutes at atmospheric pressure. The autoclave run lasted for 33:09 minutes at 240° F with a minimum temperature of 240.5 °F (115.83 °C) and maximum temperature of 242.1 °F(116.72 °C). Temperature was monitored by the autoclave system at each minute of the run. Following the autoclave procedure, samples were homogenized and submitted for analysis via UPLC-MS/MS.

Drug Analysis

Drug analysis was adapted from previously described work analyzing the same compounds in similar matrices[24,25].

Chemicals and Reagents

Pharmaceutical standards ketamine, ketamine-d4, xylazine, and xylazine-d6 were purchased from Sigma-Aldrich (St. Louis, MO, USA). LC/MS grade methanol, formic acid and acetonitrile were purchased from Fisher Scientific (Waltham, MA, USA).

Stock Solutions

The ketamine and xylazine stock solutions were prepared in methanol at 1 mg/mL and stored in a -80 °C freezer. The ketamine-d4 and xylazine-d6 were in methanol at 100 µg/mL and stored in a -80 °C freezer.

Standard Working Solutions

The standard stock solutions were moved out of the -80 °C freezer and thawed to room temperature on a benchtop before use (20 min). For daily analysis, the ketamine and xylazine stock solutions were diluted with methanol to get a mixture of ketamine and xylazine at 10 µg/mL. Subsequently, the mixture of ketamine and xylazine was further diluted to get a set of concentrations at 2.5, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, and 5000 ng/mL in methanol.

Internal Standard Working Solutions

The internal standard stock solutions were moved out of the -80 °C freezer and thawed to room temperature on a benchtop before use (20 min). For daily analysis, the ketamine-d4 and xylazine-d6 stock solutions were diluted with methanol to get a mixture of ketamine-d4 and xylazine-d6 at 1 µg/mL.

Sample Preparation

2 g of each homogenized tissue sample was put into a 50 mL centrifuge tube, and 2 g of anhydrous sodium sulfate was added. A volume of 100 µL of internal standard working solution (1 µg/mL) was added. The samples were extracted with 10 mL of ammonia modified acetonitrile (5%, v/v). After vortexing with a multiple tube Vortexer (FischerBrand™, Fischer Scientific, Waltham, MA, USA) at 2000 rpm for 30 min, the samples were centrifuged at 3500 rpm for 10 min at 4° C. A volume of 3 mL of supernatant was transferred into a 15 mL centrifuge tube. A volume of 1 mL of hexane was added into the supernatant to remove the remaining fat matrix. After vortexing with a Multiple Tube Vortexer at 2000 rpm for 30 min, the samples were centrifuged at 3500 rpm for 10 min at 4° C. A volume of 2 mL of lower layer fraction was

transferred into a glass tube and concentrated by drying at 45 °C until dry. The dried sample was reconstituted in 0.2 mL of 0.1% of formic acid/acetonitrile (30/70) and vortexed for 30 seconds. The reconstituted solution was passed through a 0.22 µm filter by centrifuging at 13,000 RPM for 10 min at 4° C and analyzed by UPLC-MS/MS.

Instrumentation

The analysis was carried out by UPLC–MS/MS utilizing a Waters Acquity Ultra Performance LC with a Waters column manager and heater/cooler, binary system manager, sample manager coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer equipped with electrospray ionization (Waters Acquity Ultra Performance LC, Xevo TQ-S MS/MS, Waters Co., Milford, MA, USA). A Waters Acquity UPLC BEH C18 Column (130Å, 1.7 µm, 2.1 mm ×100 mm) was utilized combined with eluents composed of mobile phase A (0.1% formic acid in Milli-Q water) and mobile phase B (acetonitrile). The flow rate was 0.4 mL/min. The following gradient program was used: from 0-1 min phase A/phase B at 82%/18% respectively, at 3 min 65%/35%, at 3.01 min 82%/18% for 1.99 min. Total run time was 5 min. The MS/MS ionization mode used was positive electrospray ionization. The operating parameters for the mass spectrometer were as follows: capillary voltage was 3.70 kV, source and desolvation temperatures were 150° C and 600° C, respectively. The cone energy was set at 16 V. Nitrogen was used as the desolvation gas and cone gas. Helium was used as the collision gas. The collision gas flow was 0.15 mL/min. Desolvation and cone gas flows were 900 and 150 L/h, respectively. Data analysis and quantification were performed using the Waters MassLynx and TargetLynx software 4.1, respectively.

Method Validation

The developed method was validated in accordance with the selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, and precision.

Selection and tuning of the parent and daughter ions as well as analyte-dependent parameters, such as collision energy and Dwell, were performed by combined infusion of individual pharmaceutical solutions at a concentration of 1000 ng/mL in 0.1% of formic acid/acetonitrile (30/70). The mass spectra for the ketamine, ketamine-d4, xylazine, and xylazine-d6 were obtained individually. The selection of the parent ion for each analyte was accomplished. These parameters were later used to constitute the Multiple Reaction Monitoring (MRM) method as follows. The quantification was conducted using the MRM mode with transitions at m/z 238.0938 $\rightarrow$ 179.1188 (qualifier) and m/z 238.0938 $\rightarrow$ 125.1085 (quantifier) for ketamine, m/z 242.1776 $\rightarrow$ 129.0691 (quantifier) and m/z 242.1776 $\rightarrow$ 183.1397 (qualifier) for ketamine-d4 (internal standard). For xylazine, the quantification was conducted using the MRM mode with transitions at m/z 221.149 $\rightarrow$ 164.0811 (qualifier) and m/z 221.149 $\rightarrow$ 90.0736 (quantifier) for xylazine, and m/z 227.149 $\rightarrow$ 90.0765 (quantifier) and m/z 227.149 $\rightarrow$ 170.1366 (qualifier) for xylazine-d6 (internal standard).

To prepare calibration standard curves, a volume of 100 μ L of each standard working solution and 50 μ L of internal standard working solution were added into 850 μ L of 0.1% of formic acid/acetonitrile solution (30/70), to get a set of standard concentrations at 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 250, 500 ng/mL. The internal standard concentration was 50 ng/mL. To prepare QC samples, 2 g of blank bovine muscle, fat, liver, and kidney were analyzed with no alteration. Positive control samples were spiked with 200 ng ketamine and xylazine standards to get the concentrations of ketamine and xylazine at 100 ng/g, and then 100 ng of ketamine-d4 and

xylazine-d6 were spiked to get the concentrations of ketamine-d4 and xylazine-d6 at 50 ng/g so that the internal standards in the calibration standard solutions, the QC samples, and the test samples were at the same concentration of 50 ng/g. For ketamine, the calibration curve was linear from 0.25 to 500 ng/mL and accepted with the correlation coefficient (R^2) at least 0.99. For xylazine, the calibration curve was linear from 0.25 to 500 ng/mL and accepted with the correlation coefficient R^2 at least 0.99. For both compounds, the LOD and LOQ were 0.1 and 0.25ng/mL, respectively.

The inter-day accuracy and precision were assessed by analyzing two replicated QC samples at 100 ng/g on three separate days. The recovery ration (%) and relative standard deviation (RSD %) were used to evaluate the accuracy and precision. For xylazine spiked in bovine muscle, fat, liver and kidney, the accuracy was 99.98%, 99.62%, 100.52%, and 100.87% at the concentration of 100 ng/g, respectively. The inter-day precision was 4.29%, 1.28%, 0.17%, and 1.75% at the concentration of 100 ng/g, respectively. For ketamine spiked in bovine muscle, fat, liver and kidney, the accuracy was 103.88%, 109.60%, 106.72%, and 105.63% at the concentration of 100 ng/g, respectively. The inter-day precision was 1.39%, 3.44%, 1.50%, and 1.04% at the concentration of 100 ng/g, respectively.

Results

Tissue Concentrations

Table 4.1 summarizes the individual and mean tissue concentrations of xylazine and ketamine. Kidney contained the highest concentration of xylazine in all individuals, regardless of treatment group. Liver and muscle contained similar xylazine concentrations followed by fat with relatively low concentrations. Group 1 received 0.2 mg/kg xylazine IV and the highest

detected kidney concentration was 1562 ng/g in raw, and 2033.5 ng/g in rendered tissue. Group 2 received 0.5 mg/kg xylazine IV and the highest detected kidney concentration was 4093.6 ng/g in raw and 6866.8 ng/g in rendered tissue. Ketamine was found in the highest concentrations in the kidney, followed by muscle, liver, and fat. Group 2 received 2 mg/kg ketamine IV, and the highest kidney concentration was 3321.3ng/g in raw and 4528.2 ng/g in rendered tissue. Rendered tissue contained higher drug concentrations than the raw counterpart with the exception of xylazine in fat.

Table 4.1. Individual and Mean($\pm$ SD) xylazine and ketamine concentrations (ng/g) from individual and composite raw and rendered bovine tissue samples (n=6/treatment group).

Treatment Group	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6	Mean (SD)
Group 1 Xyl. Raw							
Muscle	222.4	129.6	142.4	99.4	109.8	138.5	140.35(43.51)
Fat	152.0	47.8	52.4	56.5	72.7	42.4	70.63(41.17)
Liver	288.0	457.8	251.7	345.6	167.0	213.6	287.28(103.58)
Kidney	1562.0	506.7	1308.5	749.3	1180.5	898.9	1034.32(388.06)
Composite	558.6	291.1	433.0	384.9	398.0	364.0	404.93(88.86)
Group 1 Xyl. Rendered							
Muscle	303.0	172.0	181.9	123.0	141.4	162.8	180.68(63.61)
Fat	121.6	52.1	57.3	58.5	76.6	49.6	69.28(27.32)
Liver	378.2	828.4	318.1	451.5	212.5	268.3	409.50(221.50)
Kidney	2033.5	836.1	1747.3	1401.6	1823.9	1514.7	1559.52(419.55)
Composite	649.8	414.7	535.0	354.0	422.6	373.6	458.28(112.95)
Group 2 Xyl. Raw							
Muscle	409.5	66.5	29.7	489.3	261.1	568.3	304.07(223.07)
Fat	68.9	20.3	6.2	95.4	40.8	192.2	70.63(67.78)
Liver	754.4	31.8	16.2	543.5	538.7	641.3	420.98(317.45)
Kidney	3629.0	657.0	190.7	2858	2979.7	4093.6	2401.33(1602.72)
Composite	1320.7	169.0	71.2	1885.3	1289.2	1847.3	1097.12(798.10)
Group 2 Xyl. Rendered							
Muscle	509.3	82.3	41.0	597.6	339.0	629.8	366.50(257.14)
Fat	63.3	20.3	6.3	77.3	34.9	218.5	70.10(77.34)
Liver	1087.7	35.8	20.4	192.1	715.3	834.0	480.88(456.38)
Kidney	4633.9	914.5	251.0	6866.8	3729.5	5525.8	3653.58(2602.96)
Composite	1573.2	214.9	73.8	2271.8	1096.2	1746.4	1162.72(874.96)
Group 2 Ket. Raw							
Muscle	1474.2	156.5	328.9	1653	1028.2	1827.3	1078.02(701.69)
Fat	215.5	52.6	44.8	461.2	160.8	1214.4	358.22(446.01)
Liver	2261.1	66.2	65.4	819.3	971.4	1401.7	930.85(836.90)
Kidney	2591.6	232.0	165.7	1304.9	1442.2	3321.3	1509.62(1259.98)
Composite	1591.9	140.5	166.3	1808.9	850.4	1969.8	1087.97(819.14)
Group 2 Ket. Rendered							
Muscle	1995.2	186.0	499.6	2340.8	1031.0	2247.6	1383.37(935.61)
Fat	292.4	74.0	56.6	477.1	161.1	1624.3	477.58(597.37)
Liver	2790.2	93.4	102.1	2661.4	1345.4	2087.8	1513.38(1209.50)
Kidney	3528.2	316.2	235.1	3884.2	1840.3	4528.2	2388.70(1862.60)
Composite	1930.8	162.6	210.3	2174.3	977.6	2421.6	1312.87(1000.44)

Raw vs. Rendered Tissue

Group one individual tissue concentrations of xylazine in raw vs. rendered tissue are compared in Figure 4.1. Rendered tissue concentrations were higher than raw tissue except in fat.

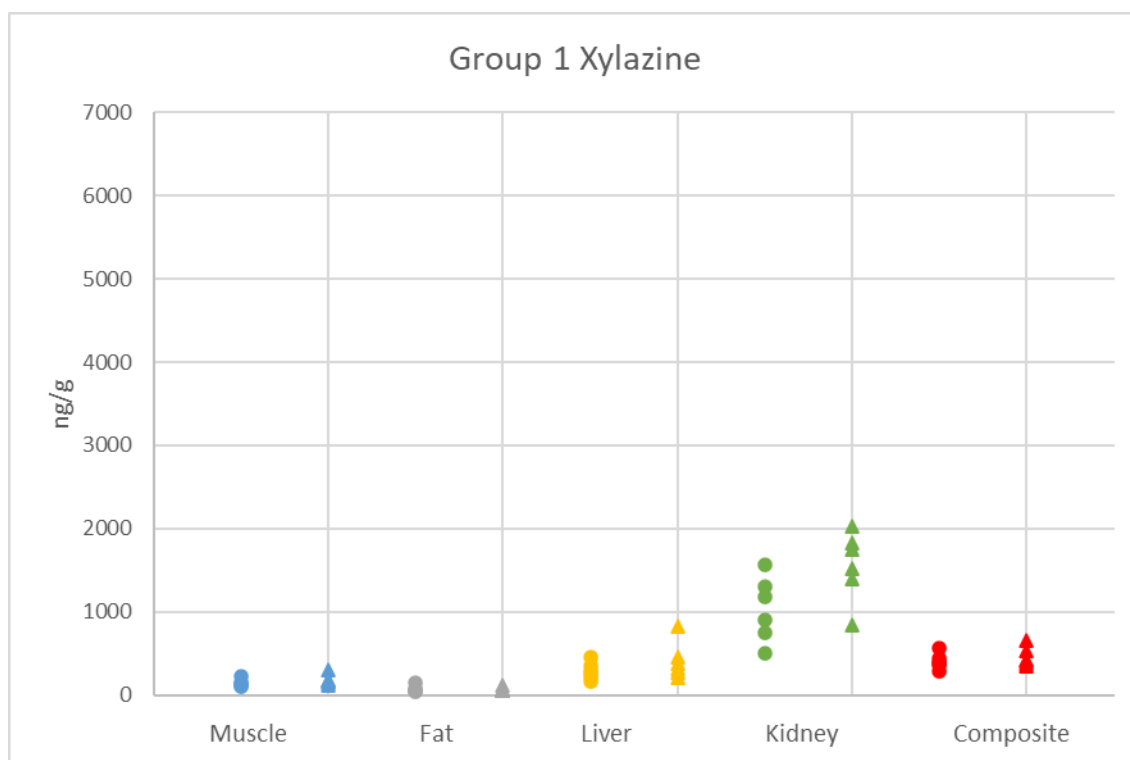


Figure 4.1. Individual Group 1 xylazine concentrations in raw and rendered bovine tissue (n=6). • = Raw Tissue Δ = Rendered Tissue

Group two individual tissue concentrations of xylazine in raw vs. rendered tissue are compared in Figure 4.2. Rendered tissue concentrations were higher in rendered than raw tissue except for fat.

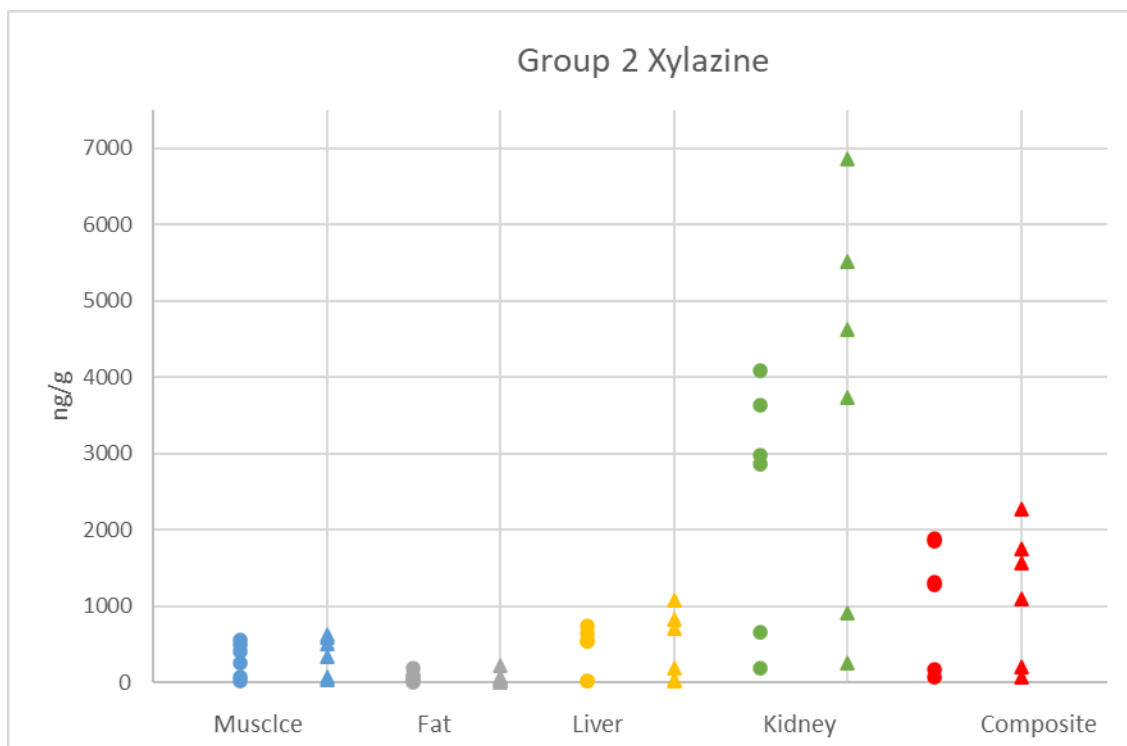


Figure 4.2 Individual Group 2 xylazine concentrations in raw and rendered bovine tissue (n=6). ● = Raw Tissue Δ = Rendered Tissue

Group two individual tissue concentrations of ketamine in raw vs. rendered tissue are compared in Figure 4.3. Rendered tissue concentrations were higher than raw tissue.

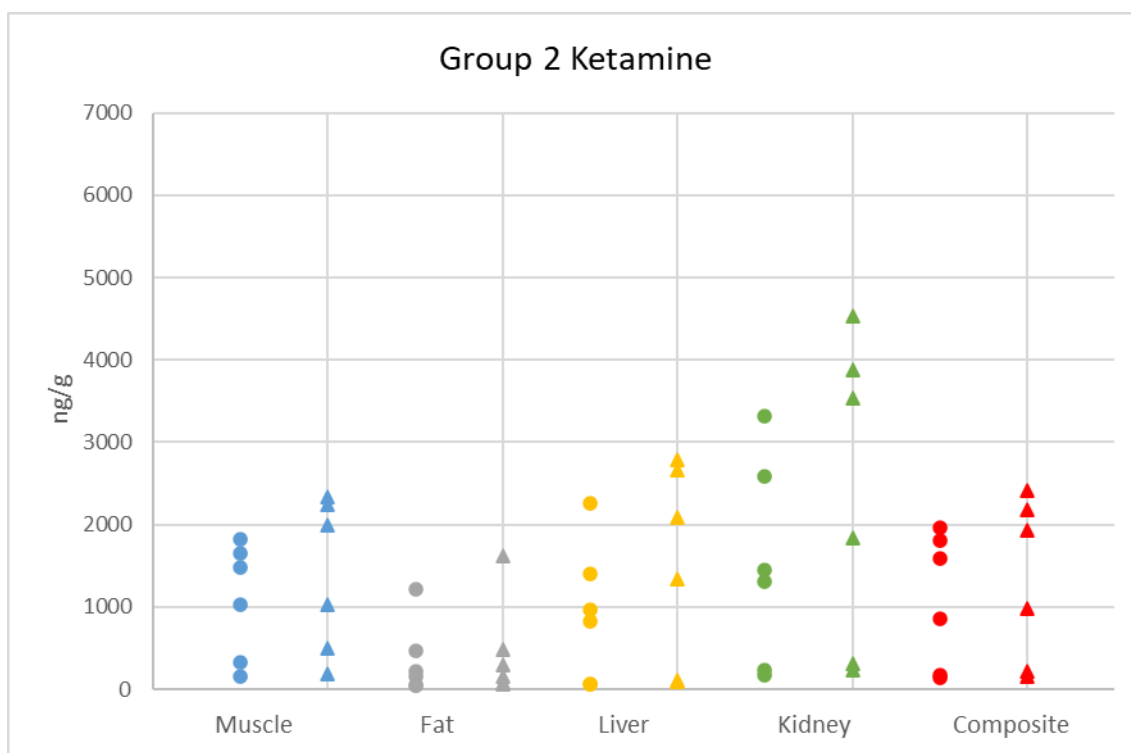


Figure 4.3. Individual Group 2 ketamine concentrations in raw and rendered bovine tissue (n=6). ● = Raw Tissue Δ = Rendered Tissue

Percent change in drug concentrations for each treatment group are presented in Table 4.2. Nearly all analyzed samples exceed the USDA-FSIS applicability concentrations of xylazine (20 ng/g) and ketamine (1 ng/g) in animals euthanized immediately following administration.

Tissue Concentration Changes

Table 4.2. Mean(±SD) percent change in individual xylazine and ketamine concentrations between raw and rendered cattle tissue samples (n=6/treatment group).

Treatment Group	Muscle	Fat	Liver	Kidney	Composite
Group 1 Xylazine	24.83(9.00)	-4.38(11.92)	13.48(39.62)	49.82(44.59)	8.33(16.58)
Group 2 Xylazine	27.79(6.60)	4.04(12.65)	37.02(21.64)	56.47(21.79)	13.86(17.79)
Group 2 Ketamine	28.50(18.37)	23.35(17.33)	72.15(74.61)	62.65(66.30)	20.26(4.37)

* Percent change was calculated using the formula: $[(\text{Rendered} - \text{Raw})/\text{Raw}] * 100$.

Moisture Loss

Sample weight reductions following rendering are presented in Table 4.3. All sample types reduced by similar degrees with the exception of the composite samples.

Table 4.3. Mean (SD) percent change in sample weight attributed to moisture loss during the simulated rendering process (n=12).

Tissue	Muscle	Fat	Liver	Kidney	Composite
% Change	-11.52% (1.82)	-12.65% (3.96)	-12.43% (2.20)	-11.90% (1.20)	-16.85% (2.19)

* Percent change was calculated using the formula: [(After Render – Before Render)/Before Render)] * 100.

Discussion

Tissue concentrations of xylazine were highest in kidney from both treatment groups consistent with the reported primary urinary excretion and short half-life[9]. Group 2 had higher overall xylazine concentrations compared to group 1 consistent with the larger dose administered. Ketamine was also highest in kidney, but liver and muscle contained similar concentrations while fat contained less. Mean raw muscle xylazine concentrations were 140.35 ng/g and 304.07 ng/g for treatment groups one and two, respectively. The concentrations were similar to previous studies that detected 103.1-112.4 ng/g xylazine in muscle of horses following euthanasia[15]. The mean muscle ketamine concentration in the present study was 1078.02 ng/g which is similar to the 1176.5-1756.6 ng/g reported in a previous study in horses[15]. These drugs are known to have large volume of distribution which is a desirable characteristic in a fast-acting sedative. These drugs are also known to have relatively short half-lives and are rapidly excreted following administration in horses, cattle, sheep, and dogs[9,10]. Drug metabolism in exposed animals may produce some drug metabolites that were not evaluated in the present study due to the brief time between administration and euthanasia.

Both drugs persisted through a simulated rendering process and the concentrations were higher in the rendered tissue compared to their raw counterparts. The exception was xylazine in fat which decreased slightly after rendering in the group receiving xylazine alone. Part of this increase can likely be attributed to moisture loss as all tissue weights reduced by about 12% in weight after rendering. Importantly, in modern commercial rendering systems, moisture and fat

are removed from solid cooked tissue during and immediately after cooking, respectively. The autoclave rendering model deployed in the present work would not allow moisture to fully escape and the tissues were cooked in glass beakers which did not allow liquid fat to be fully separated. Other studies have shown variable changes in oxytetracycline and tylosin concentrations in other matrices that were dependent upon the process used[26]. The composite samples weight reduced by about 17% which may be due to smaller 5g pieces of each tissue being included, potentially making the moisture extraction more efficient than the 20g samples due to increased surface area related to sample volume. The decrease in drug concentration in fat despite a 12.65% mean moisture loss may suggest that xylazine in fat is reduced by the rendering process. Importantly, the concentration of xylazine in fat after rendering is not zero and would exceed the 1ng/g applicability threshold for USDA-FSIS screening.

The concentrations in rendered tissue may be considered in the context of the doses needed for sedation in species that may be fed rendered product. Oral doses of 2.5 mg/kg xylazine, and 10 mg/kg ketamine were used concurrently in cats to evaluate their sedative effects in a previous study[17]. These doses were used as target doses along with the highest detected rendered tissue concentrations detected(6866.80 ng/g xylazine in kidney, and 4528.20 ng/g ketamine in kidney) in the present study to crudely represent the highest possible exposure. A 10-pound (4.54kg) cat would need to ingest 11.36 mg of xylazine to reach the 2.5 mg/kg dose which translates to 3.64 pounds (1.66kg) of rendered bovine kidney containing 6866.8 ng/g xylazine in one sitting. The 10 mg/kg ketamine dose results in a calculated exposure of 22.2 pounds (10kg) of rendered bovine kidney containing 4528.2 ng/g ketamine. This represents 36.4% and 222% of bodyweight respectively, both scenarios are not physiologically likely for an animal that consumes 2-4% body weight per day. The dosage calculations were performed based on concurrent dosing; individual dosing of each drug alone may result in differing levels of sedation. While toxicity is unlikely, inadvertent inclusion of sedative drugs in rendered products should be avoided.

The parent compounds xylazine and ketamine were found in tissues of cattle following administration during euthanasia followed by a simulated rendering process. Confirmation of these findings should be carried-out in large scale commercial rendering facilities. Differences between rendering procedures and individual facility components may affect the detection of these drugs. Drug metabolites were not evaluated in the present work, future work determining

the rate at which these drugs are metabolized in a euthanasia scenario and the potential metabolites produced is needed. Future research detailing the oral bioavailability of these drugs in animals consuming food that utilizes rendered product is warranted.

References

1. AVMA. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Available online: <https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf> (accessed on 5/7/2025).
2. USDA. Carcass Management Course: Rendering Module. Available online: <https://www.aphis.usda.gov/sites/default/files/7-rendering.pdf> (accessed on 05/27/2025).
3. Meeker, D.L. *Essential Rendering: All About the Animal By-Products Industry*; Kirby Lithographic Company, Inc.: Alexandria, VA, 2006; p. 295.
4. Buchweitz, J.P.; Johnson, M.; Jones, J.L.; Lehner, A.F. Development of a Quantitative Gas Chromatography-Tandem Mass Spectrometry Method for the Determination of Pentobarbital in Dog Food. *Journal of Agricultural and Food Chemistry* **2018**, *66*, 11166-11169, doi:10.1021/acs.jafc.8b04178.
5. Kaiser, A.M.; McFarland, W.; Siemion, R.S.; Raisbeck, M.F. Secondary pentobarbital poisoning in two dogs: a cautionary tale. *Journal of Veterinary Diagnostic Investigation* **2010**, *22*, 632-634, doi:10.1177/104063871002200423.
6. Jurczynski, K.; Zittlau, E. Pentobarbital poisoning in Sumatran tigers (*Panthera tigris sumatrae*). *Journal of Zoo and Wildlife Medicine* **2007**, *38*, 583-584, doi:10.1638/2006-0051r.1.
7. Bischoff, K.; Jaeger, R.; Ebel, J.G. An unusual case of relay pentobarbital toxicosis in a dog. *Journal of Medical Toxicology* **2011**, *7*, 236-239, doi:10.1007/s13181-011-0160-8.
8. O'Connor, J.J., Stowe, Clarence M., Robinson, Robert R. Fate of Sodium Pentobarbital in Rendered Products. *American Journal of Veterinary Research* **1985**, *46*, 1721-1724, doi:<https://doi.org/10.2460/ajvr.1985.46.08.1721>.

9. Garcia-Villar, R.; Toutain, P.L.; Alvinerie, M.; Ruckebusch, Y. The pharmacokinetics of xylazine hydrochloride: an interspecific study. *Journal of Veterinary Pharmacology and Therapeutics* **1981**, 4, 87-92, doi:10.1111/j.1365-2885.1981.tb00715.x.
10. Sellers, G.; Lin, H.C.; Riddell, M.G.; Ravis, W.R.; Lin, Y.J.; Duran, S.H.; Givens, M.D. Pharmacokinetics of ketamine in plasma and milk of mature Holstein cows. *Journal of Veterinary Pharmacology and Therapeutics* **2010**, 33, 480-484, doi:10.1111/j.1365-2885.2010.01159.x.
11. Jenkins, W.L. Disposition of Anesthetic and Anesthetic-Related Agents in Ruminants. *Veterinary Clinics of North America: Food Animal Practice* **1986**, 2, 527-552, doi:[https://doi.org/10.1016/S0749-0720\(15\)31205-6](https://doi.org/10.1016/S0749-0720(15)31205-6).
12. Papich, M.G. Drug Residue Considerations for Anesthetics and Adjunctive Drugs in Food-Producing Animals. *Veterinary Clinics of North America: Food Animal Practice* **1996**, 12, 693-706, doi:[https://doi.org/10.1016/S0749-0720\(15\)30393-5](https://doi.org/10.1016/S0749-0720(15)30393-5).
13. Craigmill, A.L.; Rangel-Lugo, M.; Damian, P.; Riviere, J.E. Extralabel use of tranquilizers and general anesthetics. *Journal of the American Veterinary Medical Association* **1997**, 211, 302-304.
14. Smith, G. Extralabel Use of Anesthetic and Analgesic Compounds in Cattle. *Veterinary Clinics: Food Animal Practice* **2013**, 29, 29-45, doi:10.1016/j.cvfa.2012.11.003.
15. Aleman, M.; Davis, E.; Knych, H.; Guedes, A.; Smith, F.; Madigan, J.E. Drug Residues after Intravenous Anesthesia and Intrathecal Lidocaine Hydrochloride Euthanasia in Horses. *Journal of Veterinary Internal Medicine* **2016**, 30, 1322-1326, doi:10.1111/jvim.14372.

16. United States Department of Agriculture Food Safety and Inspection Service: Screening and Confirmation of Animal Drug Residues by UHPLC-MS-MS. Available online: https://www.fsis.usda.gov/sites/default/files/media_file/documents/CLG-MRM3.04.pdf (accessed on 7/3/2025).
17. Grove, D.M.; Ramsay, E.C. Sedative and physiologic effects of orally administered alpha 2-adrenoceptor agonists and ketamine in cats. *Journal of the American Veterinary Medical Association* **2000**, *216*, 1929-1932, doi:10.2460/javma.2000.216.1929.
18. Clements, J.A.; Nimmo, W.S.; Grant, I.S. Bioavailability, pharmacokinetics, and analgesic activity of ketamine in humans. *Journal of Pharmaceutical Sciences* **1982**, *71*, 539-542, doi:10.1002/jps.2600710516.
19. Cooper, K.M.; Whelan, M.; Danaher, M.; Kennedy, D.G. Stability during cooking of anthelmintic veterinary drug residues in beef. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment* **2011**, *28*, 155-165, doi:10.1080/19440049.2010.542775.
20. Foertsch, M.J.; T., M.J.; J., H.N.; Dario, R.J.; Ann, S.; Mueller, P.E.W.; and Droege, C.A. Ketamine Stability over Six Months of Exposure to Moderate and High Temperature Environments. *Prehospital Emergency Care* **2022**, *26*, 422-427, doi:10.1080/10903127.2021.1934203.
21. Frida Mgonja, R.M., Faith Mabiki, and Kennedy Choongo. Effect of heat treatment on oxytetracycline residues in beef. *American Journal of Research Communication* **2017**, *5*, 1-13.
22. Planche, C.; Chevolleau, S.; Noguier-Meireles, M.H.; Jouanin, I.; Mompelat, S.; Ratel, J.; Verdon, E.; Engel, E.; Debrauwer, L. Fate of Sulfonamides and Tetracyclines in Meat

- during Pan Cooking: Focus on the Thermodegradation of Sulfamethoxazole. *Molecules* **2022**, *27*, doi:10.3390/molecules27196233.
23. Adegbeye, M.J.; Adetuyi, B.O.; Igirigi, A.I.; Adisa, A.; Palangi, V.; Aiyedun, S.; Alvarado-Ramírez, E.R.; Elghandour, M.M.M.Y.; Molina, O.M.; Oladipo, A.A.; et al. Comprehensive insights into antibiotic residues in livestock products: Distribution, factors, challenges, opportunities, and implications for food safety and public health. *Food Control* **2024**, *163*, 110545, doi:<https://doi.org/10.1016/j.foodcont.2024.110545>.
 24. Zheng, X.; Mi, X.; Li, S.; Chen, G. Determination of xylazine and 2,6-xylidine in animal tissues by liquid chromatography-tandem mass spectrometry. *Journal of Food Science* **2013**, *78*, T955-959, doi:10.1111/1750-3841.12144.
 25. Mella, M.; Schweitzer, B.; Botch-Jones, S.; Mallet, C. Analysis of ketamine and xylazine in complex matrices using two-dimensional liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* **2018**, *32*, 295-305, doi:10.1002/rcm.8041.
 26. Hassan, H.F.; Saidy, L.; Haddad, R.; Hosri, C.; Asmar, S.; Jammoul, A.; Jammoul, R.; Hassan, H.; Serhan, M. Investigation of the effects of some processing conditions on the fate of oxytetracycline and tylosin antibiotics in the making of commonly consumed cheeses from the East Mediterranean. *Veterinary World* **2021**, *14*, 1644-1649, doi:10.14202/vetworld.2021.1644-1649.

Chapter 5 - Overall Conclusions and Future Research

In Chapters Two and Three of this dissertation, the safety of meloxicam doses exceeding those currently used in cattle was evaluated in multiple age classes. A single large oral dose that was thirty times greater than oral doses on labelled products in Canada and the oral doses most often evaluated and recommended for ELDU in the United States was administered to adult lactating dairy cows. Commonly-used clinical antemortem health monitoring analyses failed to identify any deviations from normal reference ranges in the test subjects related to treatment. Post-mortem evaluations of cows receiving this dose were unremarkable suggesting that a single large dose of meloxicam is well-tolerated by adult cows. Pharmacokinetic investigation of drug concentrations in both serum and milk reflected the administration of a large dose. Maximum concentrations in serum were approximately thirty times higher than earlier work using a dose of thirty times less and were similar to adult sows receiving the same dose. Maximum concentrations in serum were achieved longer from administration than previous work with small doses reflecting longer times are needed to absorb more drug. Elimination half-lives were similar to earlier work suggesting that excretion remains first-order. Similar to the serum drug concentrations, milk concentrations were significantly higher and took longer to achieve than earlier work with smaller doses. The 30 mg/kg oral dose resulted in serum drug concentrations higher than 1 mg/kg doses until five days post-administration. Taken together, this work may suggest that cows given oral doses of 30 mg/kg meloxicam could experience longer duration analgesia than cows receiving 1 mg/kg orally with low risk of adverse effects.

Chapter Three detailed a target animal safety study of oral meloxicam administration in young Holstein calves. The study was designed using FDA Guidance for Industry documents describing target animal safety studies to evaluate non-steroidal anti-inflammatory drugs in food

animals. The dosage selections were based on these documents and used 1 mg/kg as the recommended oral dose based on earlier research and approved product labelling in Canada. Serum drug concentrations were used to show the different doses were absorbed by the study population. Similar to Chapter Two, increasing the dose increased the maximum concentration detected in serum and the time above 1 mg/kg C_{max} was lengthened for both 3 mg/kg and 5 mg/kg doses. Chapters Two and Three both suggest that maximum concentrations are roughly equivalent to multiples of a 1 mg/kg dose at 24 hours. Chapter Three also used thorough antemortem health evaluations similar to Chapter Two. Again, while some statistically significant treatment effects were shown, the clinical significance of those differences is questionable. Age-specific reference data may limit the utility of common clinical analyses in young calves. Chapter Two is the first work describing lesions associated with excessive meloxicam administration in cattle. There was a significant treatment effect identified with all calves in the highest dose group developing gastric ulcers on the torus pyloricus. Failure to detect gastric ulcers with biomedical screening and the lack of effects on appetite suggest that these lesions are physiologically minor. No perforating ulcers were found. There were also no other appreciable lesions either grossly or microscopically that were associated with treatment. This work suggests that while higher and longer administration of meloxicam in young calves was associated with gastric ulceration, the overall adverse effects were relatively minor.

Future research in this area could focus on more targeted dosage selection with a couple caveats. First, objective and repeatable measures of pain need to be developed, validated, and approved by regulatory authorities and this work is continually being improved. Until regulatory bodies accept pain assessment techniques, determination of efficacy of analgesic protocols will continue to be a challenge to the industry. Standardizing study design to assess pain relief

interventions could help make demonstration of analgesic efficacy more streamlined. The body of research to date is inconsistent regarding study design, outcome measures, and findings. The importance of these two steps cannot be overstated if securing FDA-approval for NSAID use to control pain in food animals is desired.

Assuming those two objectives above are met, there exists unlimited potential in the development of targeted analgesic drug regimens depending on the class of animal in question. For the sake of simplicity for this dissertation, four distinct classes of cattle are used to demonstrate the potential for research in this area. The four classes include: Adult dairy cattle that are handled daily by caretakers in current production systems, young dairy cattle that may experience castration and/or dehorning and are also handled relatively frequently, adult beef cows that are handled infrequently, and beef calves that may be branded, castrated, and/or dehorned and handled infrequently.

For meloxicam alone, different combinations of dose and timing of administration could be deployed to achieve maximum duration analgesia with minimal risk of adverse effects. For the lactating dairy cow, administration of smaller doses with more days between administration could reduce the amount of drug needed to achieve longer duration of analgesia. This could potentially reduce the withdrawal interval for milk. For adult beef cows, a single dose between 1-30 mg/kg may be able to provide analgesia for multiple days without having to handle the animal again. Potentially, a single large dose of meloxicam at the time of parturition could increase cow comfort and also has the potential for trans-mammary delivery to offspring which could be castrated at that time further reducing drug use while maximizing animal welfare. Dairy and beef calves may tolerate a single dose of 5 mg/kg and experience multiple days of pain relief following administration which may translate into production benefits like increased

weaning weights. Production benefits are essential to document in order for increased adoption in management systems as multiple surveys have shown that regardless of clinical efficacy or welfare improvements, return on investment remains a significant concern for producers and veterinarians. A significant consideration of having multiple analgesic regimens is then creating multiple withdrawal intervals to ensure adulterated products do not enter the food chain. Providing analgesia to cattle in general is a multi-faceted consideration and there likely exists a balance between efficacy of the drug for the condition, safety of the regimen in the class of animal presented, return on investment for producers, and food safety concerns.

Chapter Four evaluated the concentrations of xylazine and ketamine in edible tissues of cattle immediately following administration as part of the euthanasia process and detailed the fate of these drugs following a simulated rendering process. Kidney had the highest concentrations of both drugs while fat contained the least. Both xylazine and ketamine survived the rendering process and concentrations actually increased in most tissues. These tissues all reduced in weight by about 12% following the simulated rendering process and the increase in drug concentrations likely partially represents water leaving the tissue but drug staying behind. The concentrations detected were relatively low and when evaluated in the context of doses needed to cause sedation in cats, the likelihood of animals consuming enough drug through rendered products is minimal.

While xylazine and ketamine appear to survive the rendering process, the concentration in edible tissues is relatively low. Demonstration of the oral dose of both xylazine and ketamine necessary to result in changes in mentation in domestic species that may ingest rendered products would be a useful data set. This would allow for the determination of tolerable levels of drug in rendered products. FSIS already has applicability limits for these drugs in human food;

determining these levels for pet foods could allow these drugs to be used for euthanasia of food animals and allow for those animals to enter the rendering process. This would protect the safety of consumers while simultaneously securing the safety of people charged with humane euthanasia of food animals and a valuable revenue stream for producers who are currently challenged with thin profit margins in many production systems. Reaching an agreement between these multiple sectors would be mutually beneficial for all.