

Pharmacokinetics of orally administered meloxicam in calves: Impact of breed and castration

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Abstract

No FDA-approved treatment exists to mitigate castration pain in cattle. However, meloxicam, a long-acting and cost-effective non-steroidal anti-inflammatory drug, may be administered extralabel to help attenuate pain and inflammation associated with this procedure. Although previous studies generally support the analgesic efficacy of meloxicam in cattle, reported outcomes have been variable. This variability may, in part, reflect differences in meloxicam pharmacokinetic disposition across studies, potentially influenced by covariates such as genetics (breed and sister species) and physiological status- factors that have largely been unaccounted for in existing research. This thesis aimed to use bioequivalence criteria in the absence of defined clinical significance to (1) evaluate the effect of breed on meloxicam pharmacokinetics and (2) evaluate the impact of surgical castration on the pharmacokinetics of meloxicam when administered at the time of the procedure. A tertiary aim of this thesis was to identify statistical outliers within each breed as potential candidates for genome-wide association studies to detect mutations associated with altered meloxicam metabolism and disposition. A total of 77 bull calves (3-5 months old, 75-130 kg) from three breeds (Angus n=25, Brahman n=25, and Holstein n=27) received a single oral dose of meloxicam (1 mg/kg), followed by plasma sampling at predetermined intervals over 168 h. After a washout period of at least 14 days, 41 of the calves were administered another single oral dose of meloxicam (1 mg/kg) at the time of surgical castration, with plasma sampling repeated over 168 h. Meloxicam concentrations were quantified using a validated ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. Noncompartmental pharmacokinetic and bioequivalence analyses revealed clear differences in meloxicam disposition across breeds and between physiological states. Brahman calves exhibited the greatest extent of absorption and the slowest

elimination, with markedly elevated area under the curve (AUC) and prolonged elimination half-lives ($t_{1/2}$) compared to Angus and Holstein calves. Holstein calves also showed increased AUC and extended $t_{1/2}$ relative to Angus. The only bioequivalent parameter among breeds was C_{Max} between Angus and Holstein calves; however, Holsteins reached C_{Max} earlier than both Angus and Brahman, and Brahman calves had a markedly increased C_{Max} compared to the other breeds. Following surgical castration, AUC and C_{Max} increased, while $t_{1/2}$ decreased compared to pre-castration values. Time to C_{Max} (T_{Max}) remained bioequivalent before and after castration. Additionally, attempts to identify statistical outliers using $t_{1/2}$ within each breed were successful, with a total of eighteen outliers identified (Angus $n=5$, Brahman $n=7$, Holstein $n=6$), evenly divided between rapid and slow metabolizers. These individuals may represent distinct metabolic phenotypes, warranting further genetic analysis to explore the metabolic and genetic factors that influence meloxicam disposition. Understanding these factors could ultimately affect its analgesic efficacy and withdrawal times. Lastly, this study reinforces the importance of evaluating drug pharmacokinetics in animals under the conditions for which they are intended, rather than in healthy animals alone, as physiological state may significantly alter drug disposition. Further research is needed to determine whether breed-based differences in meloxicam disposition translate into meaningful differences in pain mitigation, and to inform breed-specific dosing regimens and withdrawal times to optimize analgesic efficacy while ensuring food safety.

Table of Contents

List of Figures	vii
List of Tables	viii
Acknowledgements	ix
Chapter 1 - Literature Review	1
1.1 Pain Management in Cattle Following Castration	1
1.2 Meloxicam Use in Cattle: Therapeutic Use, Regulatory Considerations, and Pharmacokinetic Variability	2
1.2.1 Meloxicam for Pain Management	2
1.2.1.1 Regulatory Considerations for Use in Cattle	3
1.2.2 Pharmacokinetics of Meloxicam in Cattle	4
1.2.2.1 Known Variability in Pharmacokinetic Parameters and Influencing Factors	6
1.3 Breed-Dependent Variability in Drug Metabolism	7
1.3.1 Evidence of Genetic Differences in Disposition	7
1.3.1.1 Examples from Cattle and the Importance of Recognizing Breed Differences	8
1.3.2 Potential Role of Genetic Variability in CYP Enzymes	10
1.4 Influence of Stress, Pain, and Inflammation on Drug Disposition in Cattle	11
1.4.1 Surgical Castration-Induced Stress and Inflammation	11
1.4.1.1 Surgical and Inflammatory Modulation of Drug Disposition	11
1.4.2 Existing Evidence of Painful Procedures Altering NSAID Pharmacokinetics in Cattle	13
1.5 Identifying Outliers and Genetic Variability in Drug Metabolism	14
1.5.1 Interpreting Pharmacokinetic Differences: Statistical Significance, Clinical Significance, and Bioequivalence	14
1.5.2 Using Pharmacokinetic Data to Identify Polymorphisms	14
1.5.3 Genomic Sequencing to Identify Cytochrome-P450 Variants	15
1.6 Hypothesis, Objectives and Thesis Organization	17
Chapter 2 - Is Meloxicam Disposition Equivalent Between Cattle Breeds?	19
2.1 Introduction	19
2.2 Materials and Methods	21

2.2.1 Animal and Housing	21
2.2.2 Experimental Design and Sample Collection	21
2.2.3 Plasma Meloxicam Analysis.....	23
2.2.4 Pharmacokinetic and Statistical Analyses.....	25
2.3 Results.....	27
2.4 Discussion.....	35
Chapter 3 - Are Meloxicam Pharmacokinetics Bioequivalent When Administered to Calves	
Before vs During Surgical Castration?	42
3.1 Introduction.....	42
3.2 Materials and Methods.....	44
3.2.1 Animals and Housing.....	44
3.2.2 Experimental Design and Sample Collection	44
3.2.3 Plasma Meloxicam Analysis.....	45
3.2.4 Pharmacokinetic and Statistical Analyses.....	45
3.3 Results.....	46
3.4 Discussion.....	50
Chapter 4 - Discussion	54
References.....	56

List of Figures

Figure 2.1 (a) Linear and (b) semilogarithmic plots of mean meloxicam concentration-versus-time profiles after a single oral dose of meloxicam (1 mg/kg) to Angus (n=25), Brahman (n=25) and Holstein (n=27) bull calves (3-5 months of age). Mean concentrations were calculated using arithmetic means. Error bars represent the standard deviation.	29
Figure 2.2 Distribution of log-transformed elimination half-lives ($t_{1/2}$) of meloxicam determined in Angus (n=25), Brahman (n=25), and Holstein (n=27) bull calves administered a single oral dose (1 mg/kg). (a) histogram of all breeds; (b) histogram of Angus; (c) histogram of Brahman; (d) histogram of Holsteins; (e) probit plot of all breeds; (f) probit plot of Angus; (g) probit plot of Brahman; (h) probit plot of Holsteins. Anti-modes are indicated by gray lines in the probit plots.....	33
Figure 2.3 Letter value plot comparing log-transformed half-lives of meloxicam in Angus (n=25), Brahman (n=25), and Holstein (n=27) bull calves following single dose oral administration (1 mg/kg). Dashed lines correspond to the geometric means for each breed.	34
Figure 3.1 (a) Linear and (b) semilogarithmic plots of arithmetic mean meloxicam concentration-versus-time profiles after a single oral dose of meloxicam (1 mg/kg) to bull calves (n=41) before castration (REF) and at the time of castration (CAST) following a 14-day washout period. Error bars represent the standard deviation.	48

List of Tables

Table 1 Pharmacokinetic parameters for meloxicam determined from noncompartmental analysis in Angus (n=25), Brahman (n=25), and Holstein (n=27) bull calves administered a single oral dose (1 mg/kg).....	30
Table 2 Parallel bioequivalence calculations for log-transformed pivotal pharmacokinetic parameters for comparison of Angus (n=25), Brahman (n=25), and Holstein (n=27) bull calves administered a single oral dose of meloxicam (1 mg/kg).	31
Table 3 Pharmacokinetic parameters for meloxicam determined from noncompartmental analysis in Angus (n=15), Brahman (n=12), and Holstein (n=14) calves administered a single oral dose (1 mg/kg) during surgical castration.	49
Table 4 Average bioequivalence estimates for log-transformed pivotal pharmacokinetic parameters following oral administration of meloxicam (1 mg/kg) to bull calves (n= 41) in a crossover design. Comparisons were made between pre-castration (reference) and castration (test) conditions, with a minimum 14-day washout period treatments.	50

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Chapter 1 - Literature Review

1.1 Pain Management in Cattle Following Castration

An estimated 15 million cattle undergo castration annually to improve pen behavior and meat quality for human consumption.^{1,2} Despite being a commonly performed procedure, castration is known to cause pain attributed to acute inflammatory physiological response.²⁻⁷

There has been an increased focus on the use of analgesia in cattle in the past decade.⁸ The shift towards implementation of effective pain management in livestock has largely been influenced by consumers and producers.⁸ However, there are currently no FDA-approved analgesics to control castration pain in cattle. In contrast to other regions like Canada and Europe, the U.S. regulatory agency mandates rigorous evidence of efficacy before approving pain management drugs.⁹ As prey species, the instinctive masking of pain creates an additional barrier for objectively measuring pain in cattle.⁵ Since the passage of Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA), veterinarians have been permitted to prescribe drugs for extralabel use when an animal's health is threatened or when failure to treat the animal may result in suffering or death.¹⁰ The American Association of Bovine Practitioners has long-acknowledged that all methods of castration induce pain and recommends the combined use of local anesthetics and systemic pain relief (e.g. opioids, alpha-2 agonists, gabapentin, nonsteroidal anti-inflammatory drugs [NSAIDs]) as a proper pain management protocol.¹¹ Even so, a 2011 survey of over 660 veterinarians revealed that nearly 70% of calves undergoing castration at < 6 months of age do not receive analgesia, as castration was perceived to be one of the least painful procedures or conditions.¹² The gap between the professional recognition of the need for pain control and its real-world implementation highlights the need for effective pain management

strategies in cattle, as well as the availability of FDA-approved analgesics specifically indicated for castration-related pain.

1.2 Meloxicam Use in Cattle: Therapeutic Use, Regulatory Considerations, and Pharmacokinetic Variability

1.2.1 Meloxicam for Pain Management

In U.S. food animal production systems, the most commonly used analgesics are flunixin meglumine, aspirin, and meloxicam, all of which NSAIDs. NSAIDs are widely used for their antipyretic, analgesic, and anti-inflammatory properties achieved through the inhibition of cyclooxygenase (COX) enzymes (COX-1 and COX-2), which regulate inflammatory mediator production in the arachidonic acid cascade.^{8,13} Aspirin and flunixin meglumine are both carboxylic acid derivatives while meloxicam belongs to the oxicam class of NSAIDs, which are enolic acid derivatives. Flunixin and meloxicam have been shown to reduce pain and inflammation in cattle, particularly following procedures like castration and dehorning.¹⁴

Among these NSAIDs, meloxicam is often considered the best option for managing pain in cattle due to its affordability, ease of administration, and apparent long duration of action, providing analgesia for several days with a single dose.^{15,16} Unlike nonselective NSAIDs such as flunixin and aspirin, meloxicam acts by preferentially inhibiting COX-2, the COX isoform responsible for producing pro-inflammatory prostaglandins, as well as some that contribute to homeostatic functions. In contrast, COX-1 primarily produces prostaglandins essential for maintaining gastrointestinal and renal homeostasis. This COX-2-selectivity is thought to contribute to meloxicam's efficacy while reducing the risk of gastrointestinal side effects compared to nonselective NSAIDs.¹⁷ Meloxicam is FDA-approved for use in humans, dogs, and cats in oral, transmucosal, and injectable (IV, SQ) formulations, primarily for managing pain

associated with osteoarthritis, rheumatoid arthritis, musculoskeletal conditions, and control of postoperative pain and inflammation.^{13,18} It has also gained popularity in the U.S. for its efficacy in managing pain in food animals, including cattle, as discussed in the next section.

Meloxicam was approved for use in cattle in European countries in 1998, followed by approval in other countries including Canada and Australia.^{9,19} In the United States, meloxicam is available for extralabel use in cattle under AMDUCA, which allows meloxicam to be administered to cattle under veterinary supervision to address health risks.^{5,10}

Flunixin meglumine is FDA-approved for use in cattle to control pyrexia associated with bovine respiratory disease, endotoxemia, and acute bovine mastitis. It is also approved for the control of inflammation from endotoxemia and control of pain associated with foot rot.^{14,20} Despite meloxicam lacking FDA approval for use in cattle, it is often preferred over other NSAIDs due to its long elimination half-life ($t_{1/2}$). The $t_{1/2}$ of oral meloxicam in cattle (8.4 to 65.3 hours) results in prolonged drug exposure, whereas flunixin meglumine has a consistently shorter $t_{1/2}$ of 5–6 hours and may require multiple doses to maintain effective drug concentrations over the same time period making meloxicam a more convenient option for veterinarians and livestock producers.^{21–24}

1.2.1.1 Regulatory Considerations for Use in Cattle

Because meloxicam is not FDA-approved for the mitigation of procedural pain in cattle, its use in procedures like castration is considered extralabel use under AMDUCA. This designation places the responsibility on veterinarians to ensure appropriate use, including adherence to regulatory requirements such as establishing proper withdrawal intervals and preventing violative drug residues in edible tissues and animal products.

The withdrawal period refers to the time between the final administration of a drug and when an animal can be slaughtered for food consumption or its products (e.g. milk) can enter the human food supply.²⁵ This interval is critical to ensure that drug residues do not exceed established safety thresholds that could pose risks to public health. In regions such as the European Union, meloxicam is approved for pain mitigation in cattle with a withdrawal period of 15 days for meat and 5 days for milk.²⁶ In contrast, meloxicam is not approved for use in cattle in the U.S., and its extralabel use requires veterinarians to assign an extended withdrawal interval, as the tolerance for residues is effectively zero.²⁷

1.2.2 Pharmacokinetics of Meloxicam in Cattle

Meloxicam is well absorbed following both parenteral and oral administration in cattle. Bioavailability is close to 100% after oral administration.²³ Meloxicam is reported to reach peak plasma concentrations (C_{Max}) of 1,450 to 3,610 ng/mL between 10.5 and 24 hours after oral dosing (1 mg/kg), indicating efficient absorption through the gastrointestinal tract.^{9,14,15} Because meloxicam is relatively lipophilic and weakly acidic, it favors passive diffusion in the largely neutral pH of the small intestine, the primary site of absorption in ruminants.¹⁸ Once absorbed, meloxicam is highly bound to plasma proteins, particularly albumin, with >95% of the drug typically bound.²⁸ This extensive binding limits the amount of free, pharmacologically active drug in circulation but may prolong systemic availability. Only the unbound fraction of meloxicam is able to cross biological membranes and reach target tissues. Despite the small free fraction, meloxicam remains an effective NSAID due to its potency.⁹ Reported volumes of distribution (V_d) for meloxicam in cattle range from 0.17 to 0.31 L/kg across various studies during intravenous administration, suggesting low tissue penetration.⁹

Across species, meloxicam undergoes extensive hepatic metabolism.⁹ Meloxicam is initially metabolized in the liver by cytochrome P450 (CYP) enzymes to 5'-hydroxy meloxicam (also known as 5'-hydroxymethyl meloxicam), which is further metabolized into 5'-carboxy meloxicam, through non-CYP routes.²⁹ At least two other inactive metabolites, possibly formed by peroxidase enzymes, are also reported in most species.^{30,31} Although the plasma metabolite profile has not yet been explicitly reported in cattle, European Medicines Agency (EMA) filings for meloxicam indicate that 5'-hydroxy meloxicam and 5'-carboxy meloxicam are found in the urine of cattle along with an unidentified polar metabolite (referred to as 'metabolite 1'), representing 5%, 2%, and 18% of the administered dose, respectively.³¹ The oxalyl metabolite (5-methylthiazol-2-yl carbonyl carboxylic acid) has also been reported in cattle urine. The EMA filing reports that the metabolite profile in cattle plasma and excreta is qualitatively similar to rats and minipigs.³¹ In minipigs, 5'-hydroxy meloxicam and 5'-carboxy meloxicam are reported to be the main metabolites while in rats, the primary metabolites are 5'-hydroxy meloxicam, 5'-carboxy meloxicam, and the oxalyl metabolite.³¹ Meloxicam metabolites are inactive. The specific CYP isoenzymes responsible for meloxicam metabolism to 5'-hydroxy meloxicam in cattle and other veterinary species have not been elucidated, but human CYP2C and CYP3A subfamilies are involved in the metabolism of meloxicam in humans.^{32,33}

In cattle, approximately 50% of meloxicam is excreted in the urine with only trace quantities of the parent drug reported in urine.³¹ Because it undergoes biotransformation into inactive metabolites prior to elimination, meloxicam clearance depends largely on the efficiency of metabolic pathways rather than on renal filtration of the parent drug. In cattle, reported clearance for oral meloxicam is consistently low at 0.10 mL/min/kg, consistent with the drug's long elimination half-life ($t_{1/2}$), reported to range from 8.4 to 65.3 hours.^{14,21} After oral

administration of meloxicam in cattle, the area under the concentration-time curve (AUC) is reported to be 36,000 to 216,000 h ng/mL.^{9,15,20}

1.2.2.1 Known Variability in Pharmacokinetic Parameters and Influencing Factors

Understanding variability in pharmacokinetic parameters is crucial for optimizing therapeutic regimens and ensuring consistent outcomes. In existing meloxicam pharmacokinetic studies in cattle, reported pharmacokinetic parameters show substantial variability.^{9,14,15,21} A recent review article found significant variability in pain assessment outcomes for cattle treated with meloxicam for procedural pain.⁹ Along with inconsistent methodologies across studies, extensive variability in pharmacokinetic parameters, with understudied variables; breed, age, sex, and physiological status, potentially contributing to this variability. Even when controlling for variables like breed, sex, or physiological status, pharmacokinetic variability can persist based on studies investigating meloxicam disposition in cattle, suggesting genetic factors may also influence drug disposition.²³ Studies report a wide range for the $t_{1/2}$ ranging from 8.4 to 65.3 hours, representing a nearly 8-fold difference, which would markedly influence dosing intervals and the duration of pain relief.^{21–23} The AUC, indicative of total drug exposure, is reported to be 36,000 to 216,000 h ng/mL), reflecting a 6-fold difference.^{9,15,20} Similarly, reported C_{Max} values range from 1,450 to 3,610 ng/mL (~2.5-fold difference) occurring at 10.5 to 24 hours (~2.3-fold difference) following oral administration.^{9,14,15} Variability in these parameters across individuals can affect both the duration of pain relief and the persistence of drug residues in tissues. Further research is needed to better understand intrinsic factors influence meloxicam pharmacokinetics to improve the effectiveness of pain management while ensuring food safety.

1.3 Breed-Dependent Variability in Drug Metabolism

1.3.1 Evidence of Genetic Differences in Disposition

CYP enzymes are polymorphic enzymes that play an essential role in drug metabolism. CYPs are primarily expressed in the liver and metabolize a wide variety of drugs, including meloxicam.^{29,34,35} Approximately 89% of commonly prescribed oral medications for humans are metabolized by CYP enzymes, with CYP3A4/5 accounting for the metabolism of the largest proportion of these drugs.³³ CYP enzyme expression and activity can be influenced by numerous factors, including genetic polymorphisms, sex, age, disease state, environmental exposures, co-administered drugs, and endogenous ligands.³⁴ As a result, variations in CYP expression and activity can directly affect pharmacokinetics, influencing drug exposure and ultimately, drug efficacy.³⁶ In addition to their role in drug metabolism, CYP enzymes also metabolize endogenous compounds essential for maintaining physiological homeostasis in the body.³⁵

Genetic factors account for up to 30% of the variability in drug metabolism observed in humans.³⁷ Variants and multiplications of alleles can significantly influence enzyme activity and, as a result, an individual's rate and extent of drug metabolism.³⁵ Extensive studies in humans over the last several decades have investigated the genetic causes underlying racial, ethnic, and population differences in drug disposition and response. For example, the main isoenzyme responsible for metabolizing the oxycam class of NSAIDs, CYP2C9, exhibits over 60 genetic variants.^{32,38,39} One of these variants, CYP2C*9 reduces enzyme activity in 25% of the Iranian population, 10% of Caucasians, and almost none of the African or Asian populations.³² Although genetic variability in drug metabolism is well documented in humans, it remains poorly studied in veterinary species, including cattle.

Most present-day cattle species are of the taurine (*Bos taurus*) and zebuine (*Bos indicus*) sister subspecies, which diverged from the now-extinct wild aurochs (*Bos primigenius*) between 300,000 and 2 million years ago.^{40–42} While both sister species share a common ancestor, they exhibit a wide range of physiological and developmental differences due to their distinct evolutionary paths, including heat tolerance, immune responses and tumor development, parasite resistance, metabolic hormone regulation, milk yield, teat development, and traits related to horn development and mastitis resistance.^{43–49} *B. indicus* breeds of cattle tend to be used primarily for beef production, although some will be used for dairy production, as well. On the other hand, *B. taurus* cattle breeds are commonly raised for both dairy and beef production. A critical distinction between the two sister species is the degree of genetic variation. *B. taurus* cattle generally exhibit lower genetic variation among breeds, whereas *B. indicus* cattle display higher genetic variation among breeds.⁵⁰ This increased variation among *B. indicus* breeds may reflect the evolution of more distinct traits, which could have implications for understanding pharmacokinetic differences between cattle breeds.

Given the physiological and genetic differences between these sister subspecies, it is reasonable to consider how genetics play a role in variable pharmacokinetics. Investigating these differences as an effect of breed is critical for optimizing pain management strategies and ensuring that each calf receives appropriate dosing to effectively mitigate procedural pain.

1.3.1.1 Examples from Cattle and the Importance of Recognizing Breed Differences

Breed-related differences in drug metabolism have been reported in cattle and may contribute to variability in drug efficacy and food safety. The earliest indications of such variation came from studies evaluating antipyrine, a probe drug commonly used to assess general CYP activity. Frisian calves were shown to eliminate antipyrine twice as fast as Blue White

Belgian calves.⁵¹ Similarly, when Black-and-White x Holstein-Frisian cross calves with varying admixtures were compared, those with 50% Holstein-Frisian genetic backgrounds eliminated antipyrine more rapidly than calves with 0-25% Holstein-Frisian backgrounds.⁵² Additionally, differences in the pharmacokinetics of ivermectin among cattle breeds (and sister species; *Bos indicus* and *Bos taurus*) have also been reported, further supporting the concept of breed-based variation in drug disposition.^{53,54} Following ivermectin administration, pharmacokinetic parameters including AUC, C_{Max}, and t_{1/2} were markedly elevated in *B. indicus* (Tabapuã) cattle compared to the *B. taurus* (Angus) cattle.⁵³ These differences suggest that *B. indicus* cattle may exhibit slower drug elimination and prolonged systemic exposure, which could influence both therapeutic efficacy and increase the risk of drug residues in edible tissues.

Given the central role of CYPs in drug metabolism, differences in their expression likely play a role in some of the observed breed- and sister-species-based variability. Multiple studies have demonstrated breed-dependent expression of CYP isoforms thought to be relevant to bovine drug metabolism.⁵⁵⁻⁵⁸ Hepatic microsomal CYP3A protein expression was found to be significantly lower in Piedmontese cattle compared to Limousin cattle.⁵⁵ A follow-up study evaluating Charolais, Piedmontese, and Blonde d'Aquitaine breeds found substantial differences in the expression of CYP1A, CYP2B, and CYP3A isoforms.⁵⁶ Similarly, Angus cows display significantly higher RNA expression of CYP2B and CYP2E, 3.4- and 2-fold greater, respectively, compared to Simmental cows.⁵⁷ Another investigation quantified hepatic mRNA expression of CYP3A28, CYP3A38, and CYP3A48 in Charolais, Piedmontese, Blonde d'Aquitaine, Marchigiana, and Valdostana breeds, revealing markedly lower expression in Piedmontese and Blonde d'Aquitaine cattle compared to Charolais, Marchigiana, and Valdostana breeds.⁵⁸ These findings are consistent across studies, indicating that some breeds (2.g.

Piedmontese) consistently show reduced CYP3A expression, while others (e.g. Charolais) exhibited elevated levels.^{55,56,58}

These breed-based differences in hepatic enzyme expression may have direct implications for pharmacokinetics, particularly in regard to drug clearance and elimination half-life. Failing to account for breed variability may confound study results. As such, recognizing and incorporating breed as a variable in pharmacology research may be critical for developing accurate, effective and safe drug dosing regimens.

1.3.2 Potential Role of Genetic Variability in CYP Enzymes

While breed-associated differences in CYP expression suggest a genetic basis, studies have only recently begun to characterize the functional implications of genetic variation within bovine CYP enzymes. Building on earlier findings (Section 1.3.1.1), one study investigated the functional impact of single nucleotide polymorphisms (SNPs) on CYP3A28, CYP3A38, and CYP3A48 genes in Piedmontese cattle.^{58,59} They identified 13 SNPs and evaluated their impact on enzyme activity using human CYP probe substrates, including testosterone and nifedipine. Some variants significantly reduced or enhanced enzyme activity, even those located outside the active site, demonstrating that subtle genetic changes can substantially alter drug metabolism.⁵⁹

Additional research has focused on CYP2D gene variability.^{60,61} A study in Japanese Black cattle identified two deletion variants of the CYP2D14 gene, which led to impaired metabolism of metoclopramide, resulting in higher plasma drug concentrations and prolonged half-lives.⁶⁰ A subsequent comparison with Holstein-Friesian cattle revealed that over half of Japanese Black cattle carried deletion variants, while the variants were rare in Holsteins.⁶¹ Together, these studies suggest that genetic differences may significantly impact drug disposition across cattle breeds.

1.4 Influence of Stress, Pain, and Inflammation on Drug Disposition in Cattle

1.4.1 Surgical Castration-Induced Stress and Inflammation

While breed-dependent differences are important, physiological factors such as stress, pain, and inflammation must also be considered when evaluating pharmacokinetic variability in cattle.

Surgical castration is a routine yet acutely painful and stressful procedure in cattle that elicits a robust physiological response.^{2–7,62} The procedure activates the hypothalamic-pituitary-adrenal axis, resulting in elevated cortisol levels.^{63,64} Elevated cortisol promotes lipolysis, releasing free fatty acids from adipose tissue.⁶⁵ These free fatty acids are capable of displacing drugs from plasma protein binding sites, particularly albumin, increasing the unbound fraction of highly protein-bound drugs.⁶⁵

Castration also initiates a systemic inflammatory response. Increases in biomarkers of inflammation, such as substance P, haptoglobin, heat shock protein 90 (HSP90), and leukocyte mRNA expression of inflammatory interleukins (IL1A, IL1B, IL1RN, and IL6) have been noted following surgical castration.^{64,66} Surgical castration also increases total white blood cells, lymphocytes, and monocytes.⁶⁷ Notably, testosterone concentrations rapidly decrease following surgical castration, which can influence CYP activity, as sex hormones are known modulators of drug-metabolizing enzymes.⁶⁸ Additionally, stress-related hormonal cascades may disrupt gastrointestinal motility and blood flow may be altered, potentially altering oral drug absorption and disposition.⁶⁹

1.4.1.1 Surgical and Inflammatory Modulation of Drug Disposition

Physiological states involving stress, inflammation, and hormonal shifts have been shown to alter drug position across species. One of the primary mechanisms by which inflammation

affects pharmacokinetics is through the downregulation of hepatic CYP enzymes by pro-inflammatory cytokines.⁷⁰ IL-6 and IL1B, which are elevated during surgical procedures such as castration, have been shown to suppress the expression and activity of several CYP isoenzymes, including CYP3A4, CYP2C9, and CYP2C19 in humans.⁷⁰ This can lead to a temporary reduction in hepatic drug metabolism, increasing drug exposure and prolonging half-life.⁷⁰ Importantly, this inflammation-driven suppression of metabolic enzymes can override genetic influences, a phenomenon referred to as “phenoconversion,” in which an individual's rapid or intermediate metabolic phenotype is altered by environmental or physiological conditions.⁷⁰ Pro-inflammatory cytokine release has also been shown to modulate renal clearance. For example, one study demonstrated that surgical stress in humans led to altered pharmacokinetic profiles for multiple drugs due to inflammation-induced changes in metabolism and elimination.⁷¹

In humans, postoperative states reduced gastric emptying and altered blood volume and plasma protein levels, all of which can impact absorption and distribution.⁷¹ In pediatric surgical patients, methadone pharmacokinetics were affected by postoperative changes, including reduced clearance and altered volume of distribution, attributed, in part, to shifts in CYP activity and plasma protein binding.⁷² In veterinary species, ovariohysterectomy in dogs reduced the volume of distribution of meloxicam, and a trend of reduced meloxicam clearance and increased AUC was observed.⁷³ These findings suggest that the physiological changes following surgery, particularly those affecting the circulatory and metabolic systems, can significantly impact drug absorption, distribution, and clearance.

1.4.2 Existing Evidence of Painful Procedures Altering NSAID Pharmacokinetics in Cattle

Despite well-documented physiological changes following castration and other painful procedures in cattle, little is known about how these changes affect the pharmacokinetics of NSAIDs under field conditions. Studies evaluating NSAID efficacy and stress biomarkers following castration or dehorning often do not include pre-procedural or non-inflamed comparator groups, making it difficult to determine whether the pharmacokinetics of NSAIDs like meloxicam are altered. One study evaluated meloxicam pharmacokinetics in calves at the time of surgical castration, but did not include a pre-castration comparator group.^{15,62} While informative for castration kinetics, it leaves unresolved whether pain and inflammation altered meloxicam disposition, given that most pharmacokinetic studies in cattle are performed in non-inflamed, and otherwise healthy animals.

Only one study has directly demonstrated altered NSAID pharmacokinetics in the context of painful procedures. Transdermal flunixin meglumine (3.33 mg/kg) administered at the time of cautery dehorning in Holstein calves resulted in a significantly longer plasma half-life compared to calves that underwent sham dehorning without pain.⁷⁴ The study revealed that the $t_{1/2}$ of flunixin was notably extended in the dehorning group (10.09 hours) versus the sham group (7.16 hours).⁷⁴ Additionally, a treatment-by-time interaction indicated that pain altered the pharmacokinetic profile of flunixin over time.⁷⁴ These results suggest that pain associated with dehorning can influence the pharmacokinetics of transdermal flunixin, potentially modifying its therapeutic efficacy and duration of action.

1.5 Identifying Outliers and Genetic Variability in Drug Metabolism

1.5.1 Interpreting Pharmacokinetic Differences: Statistical Significance, Clinical Significance, and Bioequivalence

While variations in drug exposure are often reported as statistically significant (the likelihood that observed results are not due to random chance), it is essential to determine whether these differences are also clinically meaningful (impactful on patient outcome).^{53,54,71,72,74} This distinction is especially important in pharmacokinetic studies involving NSAIDs like meloxicam, where shifts in parameters may reflect changes in the rate and extent of drug exposure, but not necessarily alter clinical outcomes⁷⁵

In the absence of clinically meaningful differences in drug exposure, global federal regulatory bodies consider drug exposure endpoint differences to not be clinically (therapeutically) different from one another if the 90% confidence interval of the log-transformed ratio of test and reference treatment falls completely within the range of 80–125%.^{76,77} Although originally designed to compare different formulations of the same drug, bioequivalence framework can also be used to assess different populations such as male vs. female, healthy vs. impaired physiological states, or breed vs. breed. Applying bioequivalence principles in these contexts helps to determine whether observed variability may warrant change in dosing strategies in veterinary species.

1.5.2 Using Pharmacokinetic Data to Identify Polymorphisms

Pharmacokinetic datum is a powerful screening tool for detecting individuals or subpopulations with atypical drug metabolism. These individuals may represent fast or slow metabolizing phenotypes linked to genetic polymorphisms in drug-metabolizing enzymes, particularly CYPs.^{23,78} The ratio of parent drug to its metabolites, generally derived from the

AUCs of the parent drug and metabolite or single time-point concentration ratios between the parent drug and metabolite, reflects metabolic efficiency.⁷⁸⁻⁸⁰ A high parent-to-metabolite ratio may indicate impaired enzymatic activity or reduced expression of relevant CYP enzymes, while a low ratio could suggest accelerated metabolism.^{78,80} This approach is widely used to flag individuals for further investigation and provides functional insight into enzyme activity and drug clearance capacity. A study on celecoxib in beagle dogs identified extensive and poor metabolizers based on differences in elimination half-life and clearance, linking these phenotypes to CYP2D15 polymorphisms.^{78,80} This highlights the utility of pharmacokinetic screening to uncover genetic differences in drug metabolism.

Identifying fast and slow metabolizers in veterinary populations is critical for refining dosing strategies, preventing adverse drug reactions, and ensuring human food safety in food-producing animals.^{23,54,78} Studies in cattle and other species have reported outliers in pharmacokinetic parameters, including prolonged half-lives and altered drug exposure, as well as the identification of groups with varying metabolism, which may be indicative of underlying genetic polymorphisms.^{21,23,74,80,81} Genomic techniques described in the next section can help resolve this gap by confirming whether observed pharmacokinetic outliers reflect true genetic differences in drug metabolism.

1.5.3 Genomic Sequencing to Identify Cytochrome-P450 Variants

Pharmacogenomics aims to understand how genetic variation influences individual response to drugs. As with human medicine, this approach in veterinary medicine has the potential to guide precision dosing- ensuring that patients receive the right drug, at the right dose, at the right time. This is particularly relevant for drugs with narrow therapeutic indices or significant interindividual variability in metabolism. The most reliable way to detect genetic

polymorphisms, especially those affecting genes encoding drug-metabolizing enzymes like CYPs, is through DNA sequencing.³⁵ Several genomic technologies are available, each offering unique advantages depending on the research question.

Whole genome sequencing (WGS) provides an unbiased, comprehensive survey of the entire genome, including coding regions, regulatory elements, and intergenic areas. WGS can detect both common and rare variants and is especially useful when exploring complex, polygenic phenotypes. For example, WGS has been used to investigate breed-level genetic diversity in cattle and to better understand the genetic landscape within livestock populations.⁸² WGS can identify novel or breed-specific variants in drug metabolizing enzymes, transporters, and nuclear receptors that may impact pharmacokinetics. The cost of WGS continues to decline, making it an increasingly accessible tool for broader applications in veterinary pharmacogenomics.

Next-generation sequencing (NGS) is a broader term that includes WGS but also encompasses targeted sequencing approaches, such as whole-exome sequencing or gene panels.^{35,80} The targeted approach is more cost-effective than WGS for studies focused on a limited number of genes or when studying a large population.³⁵ For instance, NGS has been used to sequence the CYP2D15 gene in dogs, revealing breed-specific variants affecting probe drug metabolism.⁸⁰

Genome-wide association studies (GWAS) are a population-level approach used to associate specific genetic variants with phenotypic traits, including variation in CYP enzyme activity.³⁵ GWAS has traditionally relied on high-density SNP arrays to identify common variants across the genome. However, GWAS can also be conducted using WGS data, which increases resolution and enables the identification of rare or novel variants missed by array-based

methods.^{35,48} This integration of WGS and GWAS provides a more complete understanding of the genetic architecture underlying complex traits, such as breed-related differences in drug metabolism.^{80,83} In practice, GWAS often identifies genomic regions of interest, which can then be more deeply investigated with targeted sequencing approaches like NGS or WGS.⁴⁸ In cattle, GWAS has been utilized to identify SNPs associated with tick resistance.⁸³

Together, these genomic tools offer the potential to inform precision medicine in livestock by identifying genetic factors that influence drug disposition. This could ultimately guide more tailored and effective dosing strategies leading to enhanced efficacy and food safety in food animals.

1.6 Hypothesis, Objectives and Thesis Organization

Despite the widespread use of NSAIDs like meloxicam in cattle, there is limited understanding of how breed-specific genetic factors and physiological conditions affect drug metabolism. While population variability in drug metabolism is well-established in humans, breed-based variability has not been extensively studied in veterinary species, including cattle. Additionally, the impact of acute pain and inflammation on pharmacokinetics, particularly for drugs like meloxicam, remains underexplored. This knowledge gap poses a challenge to providing effective and tailored pain management strategies for cattle, which is essential for improving animal welfare and meeting regulatory requirements of drug use in food animals. By addressing these gaps, the research could lead to better-targeted therapeutic strategies and ultimately enhance the effectiveness of meloxicam in veterinary practice.

The research presented in this thesis investigates how breed and physiological status influence the pharmacokinetics of meloxicam in male calves. Specifically, it examines whether meloxicam disposition differs among Angus (*B. taurus*), Brahman (*B. indicus*), and Holstein (*B.*

taurus) breeds, and whether drug disposition is equivalent when administered prior to versus at the time of surgical castration.

The central hypothesis is that breed-related variation and physiological responses to surgical castration impact meloxicam disposition, potentially necessitating tailored dosing strategies to optimize analgesic efficacy.

The objectives of this work are to use bioequivalence criteria, in the absence of defined clinical significance, to:

1. Compare meloxicam pharmacokinetics in Angus, Holstein, and Brahman bull calves (Chapter 2).
2. Evaluate the impact of surgical castration on meloxicam disposition by comparing pharmacokinetics when administered before versus during the procedure (Chapter 3).

A tertiary objective is to identify statistical outliers within each breed as potential candidates for WGS followed by GWAS to detect genetic variants associated with altered meloxicam metabolism and disposition (Chapter 2).

By identifying breed- and physiology-related variability in meloxicam pharmacokinetics, these studies aim to inform tailored meloxicam use in cattle with the long-term goal of improving therapeutic outcomes, animal welfare, and human food safety.

Chapter 2 - Is Meloxicam Disposition Equivalent Between Cattle Breeds?

2.1 Introduction

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam class, frequently used in cattle to manage pain and inflammation associated with routine procedures such as castration and dehorning. Its long half-life, low cost, and anti-inflammatory properties have made it a favorable option for pain mitigation in food-producing animals.^{15,16} In the United States, there is currently no FDA-approved analgesic therapies specifically labeled for the mitigation of pain associated with castration so meloxicam is used in an extralabel manner.¹⁰ Despite its widespread use, variability in its ability to attenuate procedural pain has been noted, which may be due, in part, to variable pharmacokinetics in cattle.¹⁴ Limited data exist regarding sources of variability in meloxicam pharmacokinetics within cattle populations.

Pharmacokinetic variability in cattle can be influenced by several factors, including age, physiological status, route of administration, sex, and genetic background.¹⁴ Among these, breed-related genetic variation represents a potentially important but underexplored source of variability in drug disposition.^{53,84} Substantial evidence supports interbreed differences in the expression of cytochrome P450 (CYP) enzymes, which play a central role in the metabolism of many drugs, including meloxicam.⁵⁸⁻⁶¹ Studies evaluating other compounds, such as antipyrine and ivermectin, have demonstrated notable differences in pharmacokinetics among cattle breeds, suggesting that variability may exist for other CYP-metabolized drugs as well.⁵¹⁻⁵⁴

Meloxicam is predominantly cleared through hepatic CYP-mediated metabolism, initially forming 5'-hydroxy meloxicam as the primary metabolite, followed by conversion to 5'-carboxy meloxicam via a non-CYP pathway.²⁹ While the specific CYP isoenzymes responsible for

meloxicam metabolism in cattle have not been identified, data from human studies implicate enzymes from the CYP2C and CYP3A subfamilies.^{29,32,33} Consequently, genetic differences affecting the expression or activity of these enzymes could result in breed-related variability in meloxicam pharmacokinetics, with potential implications for analgesic efficacy, animal welfare, and withdrawal interval determinations.

The primary objective of this study was to evaluate whether meloxicam pharmacokinetics differ among cattle breeds by comparing drug disposition in Angus (*B. taurus*), Holstein (*B. taurus*), and Brahman (*B. indicus*) bull calves following oral administration. In the absence of established clinically significant exposure thresholds for meloxicam in cattle, bioequivalence criteria were applied to assess differences in disposition between breeds.

A secondary aim of this work was to identify individual animals within each breed exhibiting outlier pharmacokinetic profiles suggestive of altered drug metabolism as potential candidates for future genome-wide association studies to identify genetic variants associated with altered meloxicam metabolism and disposition. Initially, this was to be accomplished by assessing parent-to-metabolite ratios as indirect markers of CYP activity; however, meloxicam metabolites were not detected at quantifiable concentrations in plasma. As a result, elimination half-life ($t_{1/2}$) was employed as a surrogate marker for metabolic variability.

By characterizing breed-related pharmacokinetic variability, this research aims to inform the development of more precise, breed-specific meloxicam dosing regimens, ultimately improving pain management, animal welfare, and human food safety in cattle production systems.

2.2 Materials and Methods

2.2.1 Animal and Housing

This study was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC #4661). The study was conducted at the Kansas State University Stocker Unit in Manhattan, KS, between July and September 2023. Holstein, Angus, and Brahman (n=27 per breed) weaned and vaccinated bull calves (weight range, 75-130 kg) at 3-5 months of age were purchased from Missouri, Kansas, and Texas, respectively, and were received between May and June 2023. Calves were acclimated to their housing for at least two weeks before the initiation of the study. They were housed in outdoor pens by breed, with dirt floors. Calves had *ad libitum* access to water and were fed once daily with a diet formulated to meet or exceed nutritional requirements set by the National Research Council at 2.2% of body weight per normal procedures at the study site.⁸⁵ Animals were evaluated by veterinarians at Kansas State University College of Veterinary Medicine to assure satisfactory health status prior to initiating the study and were identified with plastic numbered ear tags.

2.2.2 Experimental Design and Sample Collection

This was a parallel-group, single-dose bioequivalence study evaluating the pharmacokinetics of orally administered meloxicam in Angus, Holstein, and Brahman calves. To facilitate timely sample collection while accounting for potential variability in weight and age, animals were stratified by body weight at enrollment and assigned to one of five groups. The first four groups consisted of 18 calves (n=6 per breed), while the fifth group contained 9 calves (n=3 per breed). The heaviest animals were assigned to the first group to complete the study, with progressively lighter weight animals assigned to subsequent groups. This weight-based

stratification was implemented to minimize differences in body size and age at the time of the study while ensuring balanced breed representation within each group.

The FDA Center for Veterinary Medicine and International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VIHC) recommends enrolling a minimum of 12 subjects per ‘treatment’ group for blood level bioequivalence studies.⁸⁶ A more precise sample size calculation was determined based on prior research. The intersubject coefficient of variation for total drug exposure (area under the concentration time curve, AUC) in previous oral meloxicam pharmacokinetic studies in calves was approximately 10 to 30%.^{21–23,87} The sample size for a parallel bioequivalence design with an acceptance range of 0.81-1.25 and a μ_2/μ_1 value of 1.0 (indicating no difference) was estimated to be between 5 and 33 animals per group to achieve 80% power at an α value of 0.05.⁸⁸ Due to an ongoing concurrent study, a total of 27 animals per breed were available for enrollment in the study.

Sterile jugular catheters were placed approximately 16 hours prior to meloxicam administration to facilitate serial blood sampling for the first 24 h of the study. The target meloxicam dose was 1 mg/kg, with administered doses ranging from 150 to 405 mg. Meloxicam was administered by balling gun in gelatin boluses (size # 07, Torpac Inc., Fairfield, NJ) containing 15 mg meloxicam tablets (Mylan Pharmaceutical Inc., Morgantown, WV). Blood samples (6mL per time point), obtained from the jugular catheter or jugular or coccygeal venipuncture, were collected prior to meloxicam administration (0 hours), and at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120, and 168 hours following drug administration. Blood samples were immediately placed into 6mL lithium heparin blood tubes, gently inverted, then placed on

ice until centrifugation for 10 minutes at 2000 x g. plasma was then collected and stored at -80°C until analysis.

2.2.3 Plasma Meloxicam Analysis

Meloxicam and the internal standard, meloxicam-d₃, were purchased from Cayman Chemical (Ann Arbor, MI). Meloxicam metabolites, 5'-hydroxy meloxicam, and 5'-carboxy meloxicam were purchased from Toronto Research Chemicals (North York, ON). Analytical standards were dissolved in DMSO to create 1 mg/mL stocks. The meloxicam and metabolite stocks were further diluted in methanol to create working stocks and enrich untreated pooled bovine plasma to generate calibration curves at the following concentrations: 0.5, 1, 5, 10, 25, 50, 100, 500, 1000, and 2000 ng/mL for meloxicam and 5'-hydroxy meloxicam and 5, 10, 25, 50, 100, 500, 1000, and 2000 ng/mL for 5'-carboxy meloxicam. Quality control (QC) plasma samples were prepared by enriching untreated pooled bovine at the following concentrations: 1.5, 20, 40, 75, 750, and 1600 ng/mL for meloxicam and 5'-hydroxy meloxicam and 20, 40, 75, 750, and 1600 for 5'-carboxy meloxicam. An additional QC for meloxicam was included at 16 µg/mL for planned 10x dilution. A working solution of meloxicam-d₃ was created by dilution of the stock to 20 ng/mL with methanol. Plasma samples, calibration standards, and QCs were processed identically. Two hundred µL of -20°C internal standard solution was added to 25 µL of plasma to precipitate proteins followed by vortexing and centrifugation (15,000 x g at 6°C for 10 min). For negative controls, 25 µL of untreated pooled bovine plasma and zero-hour plasma samples were processed as described above except that methanol without internal standard was added instead for protein precipitation. Supernatant aliquots (150 µL) were transferred to 96-well plates containing 150 µL of ultra-pure water containing 0.1% formic acid and gently mixed by pipetting for analysis.

Meloxicam plasma concentrations were determined using ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UPLC-MS/MS). The analytical system consisted of an Acquity H-Class UPLC and Xevo TQD mass spectrometer (Waters Corp., Milford, MA). The autosampler was maintained at 6°C and the sample injection volume was 5 μ L. Separation was achieved with an ACQUITY UPLC BEH C18 2.1 x 50 mm, 1.7- μ m column (Waters Corp.) maintained at 50°C. A gradient mobile phase was used consisting of (A) 2mM ammonium acetate with 0.2% formic acid and (B) acetonitrile with 0.2% formic acid. The gradient was as follows: from 0 to 0.25 min, 95% A; from 0.25 to 1.0 min, decrease from 95% A to 10% A; from 1.0 to 1.25 min, maintain at 10% A; from 1.25 to 1.5 min, increase from 10% A to 95% A; and equilibration with 95% A from 1.5 min to 3 min. The total run time was 3.0 min with a flow rate of 0.4 mL/min. Under these conditions, the meloxicam, meloxicam-d₃, 5'-hydroxy meloxicam, and 5'-carboxy meloxicam retention times were 1.24, 1.24, 0.55, and 0.70 min, respectively. The mass spectrometer was operated in positive electrospray ionization mode, with multiple reaction monitoring of the following transitions: meloxicam, m/z 351.90 $\rightarrow$ 114.90; meloxicam-d₃, m/z 354.90 $\rightarrow$ 114.90; 5'-hydroxy meloxicam, m/z 368.00 $\rightarrow$ 130.90; and 5'-carboxy meloxicam, m/z 382.03 $\rightarrow$ 145.00. The UPLC-MS/MS system was controlled through MassLynx 4.2 software and Target Lynx 4.2 software was used to process the data (Waters Corp.) Quantification of meloxicam and metabolites was based on calibration curves constructed using the peak area ratio of meloxicam or metabolites to meloxicam-d₃, with 1/x weighted least-squares regression. Runs were accepted if at least six non-zero calibration levels were within 15% of nominal concentrations, except the lower limit of quantitation (LLOQ), which had to be within 20% of the nominal concentration, and 67% of QCs were within 15% of their nominal concentration. Plasma samples found to contain analyte concentrations exceeding

the upper limit of quantitation were diluted 10:1 in duplicate with blank plasma, processed as described above, and re-analyzed.

A fit-for-purpose validation was employed for the LC-MS/MS method.⁸⁹ The precision and accuracy for the bioanalytical method were within the acceptance criteria for the FDA Bioanalytical Method Validation Guidance for Industry.⁸⁹ Inter-assay accuracy (determined as the mean percent bias) and precision (determined as the mean percent relative standard deviation) for meloxicam and metabolites was based on QCs run in triplicate at each level on at least three separate days. Inter-assay accuracy for meloxicam, 5'-hydroxy meloxicam, and 5'-carboxy meloxicam ranged between -9.4 – 8.6%, -14 - 6.6%, and -12 - 9.0%, respectively. Inter-assay precisions for meloxicam, 5'-hydroxy meloxicam, and 5'-carboxy meloxicam ranged between 4.7 – 11%, 1.3 - 6.7%, and 4.7 - 8.6%, respectively.

2.2.4 Pharmacokinetic and Statistical Analyses

Pharmacokinetic, bioequivalence, and descriptive statistical analyses were performed using computer software (PKAnalix version 2024R1, Lixoft, Antony, France). Individual animal plasma concentration vs. time data were analyzed using noncompartmental analysis and were used to calculate the AUC from time 0 to the last measured concentration above the lower limit of quantitation (AUC_{0-last}), the AUC from time 0 extrapolated to infinity (AUC_{0-inf}), elimination half-life ($t_{1/2}$), maximum plasma concentration (C_{Max}), and time to C_{Max} (T_{Max}), as the pivotal parameters for bioequivalence.⁸⁶ Additional parameters determined included: the percent of AUC extrapolated from the last measured time point to infinity ($AUC_{\%Ex}$), the terminal elimination constant (λ_z), elimination half-life ($t_{1/2}$), and mean residence time (MRT). Plasma drug concentrations determined to be below the LLOQ were entered into the software as 'missing' as were missing time points. Pharmacokinetic parameters and plasma concentration vs

time data were summarized by descriptive statistics. Plasma concentration vs time profiles are presented as arithmetic mean $\pm$ standard deviation. Pharmacokinetic parameters, except for T_{Max} , are reported as the geometric mean, geometric mean coefficient of variation (%CV), and range. As a categorical variable, T_{Max} is presented as the median and range. The parallel bioequivalence model was used to calculate bioequivalence between breeds. Two breeds were considered bioequivalent if the 90% confidence intervals of the geometric mean ratio of log-transformed pivotal parameters of one breed to the second breed fell within the 0.8 to 1.25 (80-125%) limits, per the FDA's definition of bioequivalence.⁸⁶

RStudio version 2024 12.0+467 was used to identify outliers in each breed using log-transformed elimination half-lives and plots. R packages from the tidyverse collection were employed to carry out specific tasks in the analysis with their default settings, namely the ggplot2 package for data visualization and the dplyr package for data manipulation.⁹⁰ Shapiro-Wilk tests were used to evaluate normal distribution.⁹¹ The Freedman-Diaconis rule was utilized to determine bin width for frequency histograms for non-normal data.⁹² Three different methods were utilized to ensure identification of outliers from the log-transformed data on RStudio: quantile analysis, probit plotting (top 10% deviations), and median absolute deviation. Meloxicam plasma concentration-time curves, frequency histograms, and probit plots were created using Excel, version 2503 (Microsoft Corp). Probit plots were used to visually assess the distribution of log-transformed half-lives, with breaks in linearity interpreted as evidence of bimodality.⁹¹ A stacked letter value plot was created with the lvplot package on RStudio to compare $t_{1/2}$ among breeds to visualize distribution spread between the breeds.⁹³

2.3 Results

A total of 80 bull calves completed the study. During the post-dosing period, two meloxicam boluses were discovered on the alleyway floor beyond the squeeze chute. Upon subsequent plasma analysis, it was found that a total of four calves (two Angus and two Brahman) had no detectable meloxicam concentration at any time point. These animals were excluded from all pharmacokinetic and subsequent bioequivalence analyses. One Angus calf developed an ear tag infection, which was noted at the 96-hour sampling point. The site was cleaned and treated with 2% chlorhexidine antiseptic solution and wound-safe fly spray. One Brahman calf received two subcutaneous injections of enrofloxacin on days four and six of the study due to pyrexia and a suspected bovine respiratory disease. One Brahman calf was administered transdermal flunixin meglumine on day six due to lameness. Additionally, two calves died on the final day of the study (day seven): one Brahman calf, suspected to have pneumonia, died shortly after the 168-hour sample was collected, and one Angus calf was euthanized due to heat stress prior to the final sampling time point. This Angus calf was included in the analysis, with its last recorded sample at 120 hours. The final analysis population included 25 Angus, 25 Brahman, and 27 Holstein bull calves.

Upon UPLC-MS/MS analysis, neither 5'-hydroxy meloxicam nor 5'-carboxy meloxicam were detected in plasma samples. Consequently, the reported pharmacokinetic data centers exclusively on meloxicam concentrations. The mean plasma meloxicam concentration-time profiles for meloxicam are shown in Figure 2.1, and pharmacokinetic parameters are given in Table 1 for Angus, Brahman, and Holstein calves. The geometric means across all breeds were as follows: $t_{1/2}$ across breeds was 28.5 hours (range: 12.3 – 188.0), AUC_{0-inf} was 48,568.6 ng·h/mL (range: 24,402 – 330,012.7), and C_{Max} was 1,358 ng/mL (range: 827 – 4,708). The

median T_{Max} across all breeds was 8 hours (range: 4 - 16). Parallel bioequivalence results for breed comparisons are summarized in Table 2.

Figure 2.1 (a) Linear and (b) semilogarithmic plots of mean meloxicam concentration-versus-time profiles after a single oral dose of meloxicam (1 mg/kg) to Angus (n=25), Brahman (n=25) and Holstein (n=27) bull calves (3-5 months of age). Mean concentrations were calculated using arithmetic means. Error bars represent the standard deviation.

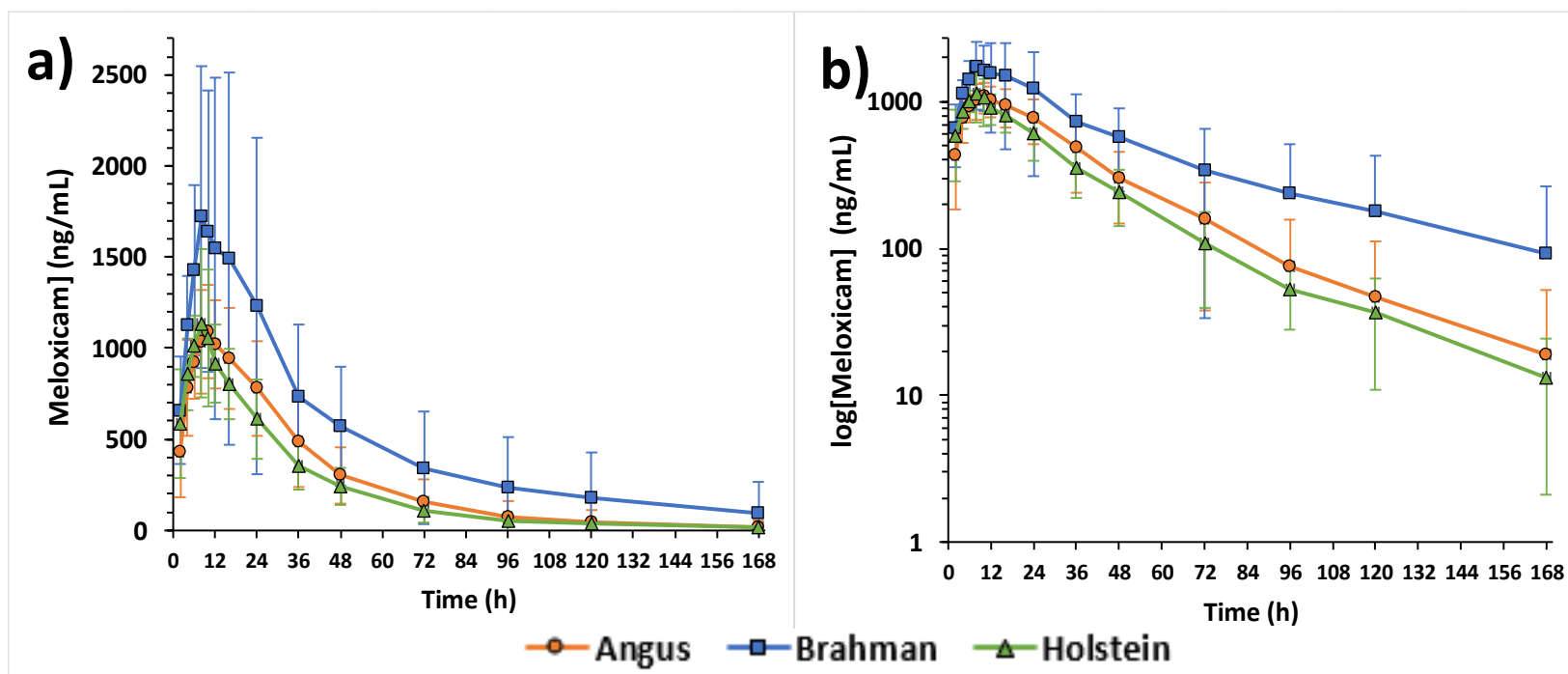


Table 1 Pharmacokinetic parameters for meloxicam determined from noncompartmental analysis in Angus (n=25), Brahman (n=25), and Holstein (n=27) bull calves administered a single oral dose (1 mg/kg).

Parameter	Angus			Brahman			Holstein		
	Mean	Range	%CV	Mean	Range	%CV	Mean	Range	%CV
AUC_{0-inf} (ng·h/mL)	42624.74	24401.95-99030.32	39.17	74606.34	26403.2-330012.73	65.76	36833.13	26693.99-61197.37	23.84
% extrapolated AUC	0.78	0.11- 25.89	306.04	1.8	0.11- 40.73	368.44	1.15	0.12- 20.12	307.9
AUC_{0-<i>t</i>last} (ng·h/mL)	41390.54	24339.19-94116.5	34.8	69891.17	26403.2-241523.75	55.64	35627.23	21323.3-59447.6	25.47
C_{Max} (ng/mL)	1186.65	845.63-1645.6	19.15	1805.14	1076.15-4708.35	41.84	1182.78	826.63-2839.9	24.18
Dose (mg)	342.7	225-405	12.2	233.2	195- 285	11.19	213.01	150- 240	9.48
T_{1/2} (h)	24.85	16.18- 73.5	41.68	31.7	12.32- 124.88	62.08	29.44	16.45- 188.01	61.77
k_{el} (1/h)	0.028	0.0094- 0.043	41.68	0.022	0.0056- 0.056	62.08	0.024	0.0037- 0.042	61.77
MRT_{0-inf} (h)	36.01	26.46- 89.8	32.77	46.27	22.5- 185.92	54.29	36.88	23.76- 113.95	30.74
T_{Max}* (h)	10	6- 16	23.51	10	5- 16	30.64	8	4- 16	30.94
AUC_{0-inf}/dose ((ng·h/mL)/mg)	124.38	108.45-244.52	43.89	319.92	135.40-1157.93	63.01	172.91	177.96-254.99	28.68
C_{Max}/dose ((ng/mL)/mg)	3.46	3.76- 4.06	23.62	7.74	5.52- 16.52	40.19	5.55	5.51- 11.83	30.85

*Categorical variable displays median instead of geometric mean.

Abbreviations and units of measurement: %CV = Geometric coefficient of variation; AUC_{0-inf}, area under the curve from 0 to ∞ (ng·h/mL); % extrapolated AUC, percentage of the area under the curve that is extrapolated; AUC_{0-*t*last}, observed area under the curve to the last measured time point (ng·h/mL); C_{Max}, maximum plasma concentration (ng/mL); Dose (mg), t_{1/2}, elimination half-life (h); k_{el}, elimination rate constant (1/h), MRT_{0-inf}, mean residence time extrapolated from 0 to ∞ (h); T_{Max}, time to maximum plasma concentration (h); AUC_{0-inf}/dose, the area under the curve from 0 to ∞ per unit dose ((ng·h/mL)/mg); C_{Max}/dose, the maximum plasma concentration per unit dose ((ng/mL)/mg).

Table 2 Parallel bioequivalence calculations for log-transformed pivotal pharmacokinetic parameters for comparison of Angus (n=25), Brahman (n=25), and Holstein (n=27) bull calves administered a single oral dose of meloxicam (1 mg/kg).

Brahman (test) vs Angus (reference)			
Parameter	Test: Reference Ratio	90% Confidence Interval Range	BE Criteria Met
AUC_{0-inf} (ng·h/mL)	175.03	(137.88, 222.19)	No
AUC_{0-tlast} (ng·h/mL)	168.86	(137.07, 208.01)	No
C_{Max} (ng/mL)	152.12	(130.9, 176.77)	No
T_{1/2} (h)	127.58	(100.92, 161.28)	No
T_{Max} (h)	91.51	(79.49, 103.52)	No
Holstein (test) vs Angus (reference)			
Parameter	Test: Reference Ratio	90% Confidence Interval Range	BE Criteria Met
AUC_{0-inf} (ng·h/mL)	86.41	(74.5, 100.23)	No
AUC_{0-tlast} (ng·h/mL)	86.08	(74.86, 98.97)	No
C_{Max} (ng/mL)	99.67	(90.2, 110.14)	Yes
T_{1/2} (h)	118.51	(94.39, 148.78)	No
T_{Max} (h)	79.37	(68.3, 90.43)	No
Holstein (test) vs Brahman (reference)			
Parameter	Test: Reference Ratio	90% Confidence Interval Range	BE Criteria Met
AUC_{0-inf} (ng·h/mL)	49.37	(39.73, 61.35)	No
AUC_{0-tlast} (ng·h/mL)	50.98	(42, 61.87)	No
C_{Max} (ng/mL)	65.52	(56.06, 76.58)	No
T_{1/2} (h)	92.89	(71.26, 121.08)	No
T_{Max} (h)	86.73	(73.67, 99.8)	No

Since metabolic ratios could not be calculated as indirect markers of CYP activity, $t_{1/2}$ was employed as a surrogate marker for metabolic variability. A frequency histogram and probit plot of log-transformed $t_{1/2}$ values for all calves and by breed are shown in Figure 2.2a–h. Normality was assessed using the Shapiro-Wilk test. The overall distribution was non-normal ($W = 0.89212$, $p = 8.198 \times 10^{-6}$), and this was also the case for Angus ($W = 0.84815$, $p\text{-value} = 0.001624$) and Holstein ($W = 0.84942$, $p\text{-value} = 0.00112$). Brahman data did not significantly deviate from normality ($W = 0.94822$, $p\text{-value} = 0.2285$). Visual inspection of the histograms and probit plots suggesting a mixed distribution, with evidence of breaks in linearity at the phenotypic extremes for all breeds and the overall dataset.⁹¹

A total of 18 outliers were identified across the three breeds (Angus $n=5$, Brahman $n=7$, Holstein $n=6$). Among these, the observed $t_{1/2}$ for outliers range from 16.18-17.15 hours to 66.84-73.5 hours in Angus calves, 12.32-17.38 hours to 60.04-124.88 hours in Brahman calves, and 16.45-17.62 hours to 58.57-188.01 hours in Holstein calves. A letter-value plot for each breed is shown in Figure 2.3 to visualize the log-transformed $t_{1/2}$ data and geometric means.

Figure 2.2 Distribution of log-transformed elimination half-lives ($t_{1/2}$) of meloxicam determined in Angus (n=25), Brahman (n=25), and Holstein (n=27) bull calves administered a single oral dose (1 mg/kg). (a) histogram of all breeds; (b) histogram of Angus; (c) histogram of Brahmans; (d) histogram of Holsteins; (e) probit plot of all breeds; (f) probit plot of Angus; (g) probit plot of Brahmans; (h) probit plot of Holsteins. Anti-modes are indicated by gray lines in the probit plots.

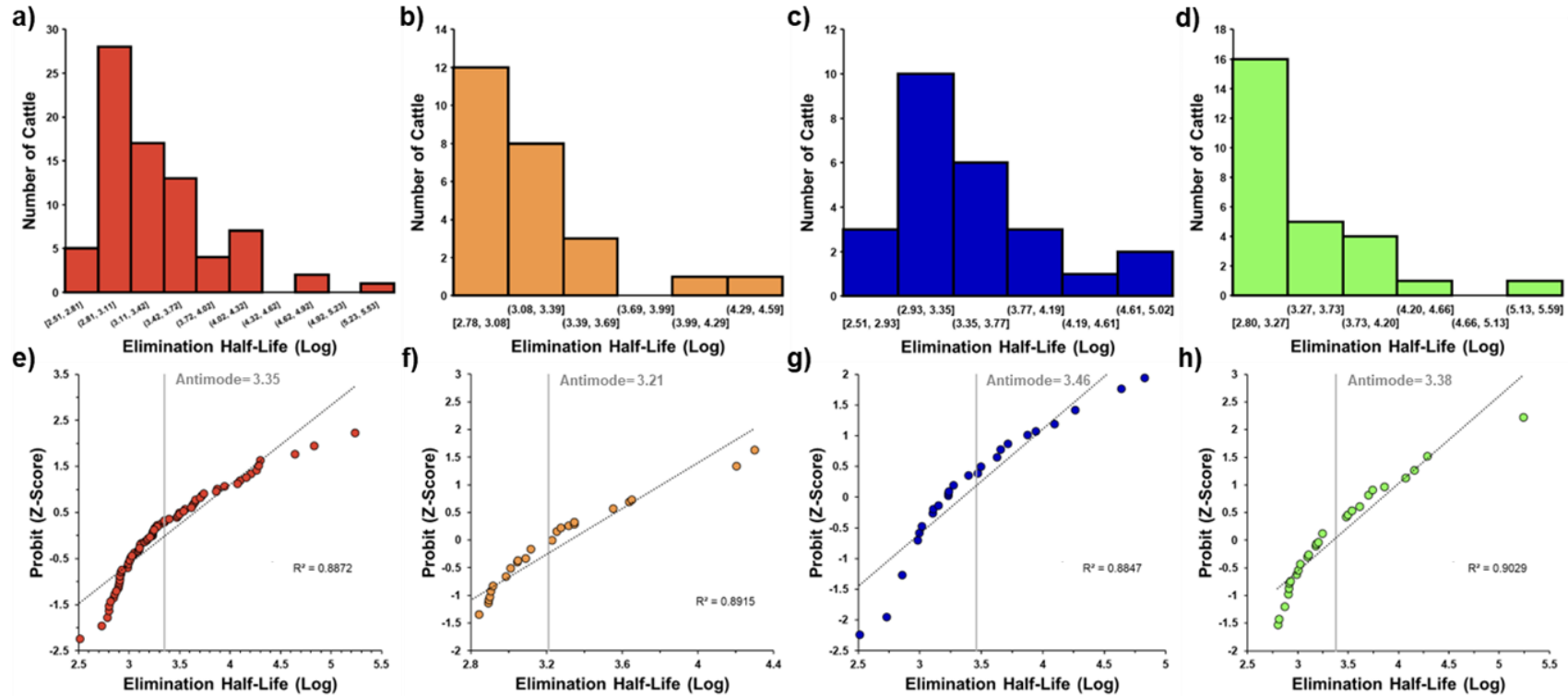
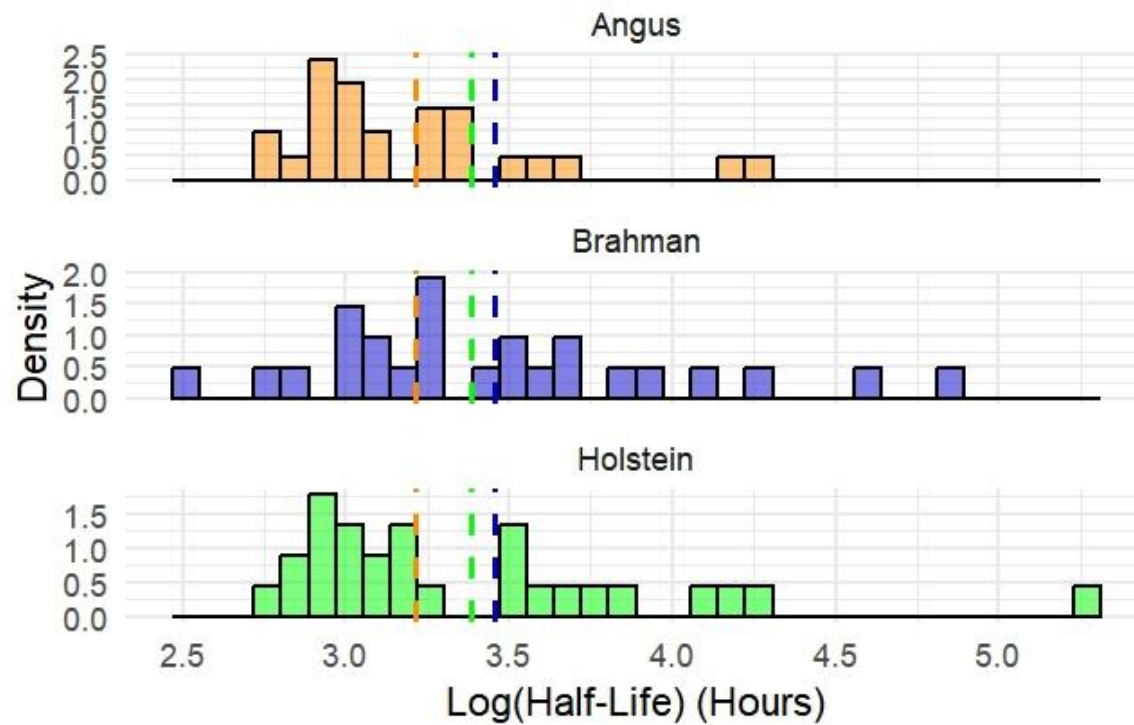


Figure 2.3 Letter value plot comparing log-transformed half-lives of meloxicam in Angus (n=25), Brahman (n=25), and Holstein (n=27) bull calves following single dose oral administration (1 mg/kg). Dashed lines correspond to the geometric means for each breed.



2.4 Discussion

This study evaluated the effect of breed on the pharmacokinetics of orally administered meloxicam (1 mg/kg) in Angus, Brahman, and Holstein bull calves. In the U.S., no FDA-approved analgesic therapies are currently labeled for the mitigation of pain associated with castration in cattle, highlighting a critical gap in food animal pain management. While breed-related differences in drug disposition have been documented in cattle for a small number of drugs, including ivermectin, this is the first study to assess breed effects on meloxicam pharmacokinetics.^{53,94} Importantly, in the absence of clinically meaningful differences in drug exposure for cattle, we employed bioequivalence analysis rather than traditional difference testing to compare pharmacokinetic parameters across breeds in an attempt to detect clinically potentially meaningful differences between breeds. The results of this study demonstrate that meloxicam disposition is not bioequivalent among Angus, Holstein, and Brahman breeds, with the exception of CMax values between Angus and Holstein calves (both *B. taurus*).

Previous studies examining meloxicam pharmacokinetics in cattle have predominantly focused on the Holstein breed. In these Holstein studies, the $t_{1/2}$ ranged from approximately 8.4 to 65.3 hours, representing a 7.8-fold variability.^{21–23} In the current study, the $t_{1/2}$ in Holstein calves ranged from 16.5 to 188 hours, representing an 11.4-fold variability. This study examined 27 Holstein calves, a larger sample size compared to previous meloxicam pharmacokinetic studies in cattle, which included 6 to 12 animals.⁹ The increased sample size may provide a more accurate representation of the Holstein bull calf population, although all calves in this study were sourced from a single supplier in Missouri, and their genetic relatedness was unknown. Future studies examining intra-breed variability should endeavor to include animals from multiple geographic and genetic backgrounds.

When comparing pharmacokinetic parameters, Holstein calves in this study exhibited an average dose-normalized AUC_{0-inf} of 172.92 (ng·h/mL)/mg and a dose-normalized C_{Max} of 5.55 (ng/mL)/mg, both notably lower than those derived from previous studies. For example, one study administering oral meloxicam (1 mg/kg) to six 3-month-old Holstein calves (~165 kg) reported an estimated dose-normalized AUC of 1044.19 (ng·h/mL)/mg and a C_{Max} of 19.7 (ng/mL)/mg, with a median T_{Max} of 12 hours (range 10-12 hours) and $t_{1/2}$ of 27.5 hours.²¹ Similarly, another administered 0.5 mg/kg orally to six Holstein calves (4-7 months old, 95-168 kg) reported a dose-normalized AUC_{0-inf} of 1342 (h·ng/mL)/mg and a C_{Max} of 30.2 (ng/mL)/mg, with a median T_{Max} of 18 hours (range 10-24 hours) and $t_{1/2}$ of 29.9 hours..²³ In both studies, meloxicam tablets were crushed and administered in water via a stomach tube, while in the current study, intact meloxicam tablets were administered in a gelatin bolus. Interestingly, despite differences in administration method and animal body weight, the median T_{Max} in the current study was 8 hours (range 4-16 hours) and the average $t_{1/2}$ was 29.4 hours, which was similar to the first comparator studies.^{21,23} The comparable T_{Max} values across studies suggests that the rate of absorption was similar, indicating that the gelatin bolus delivered the meloxicam for systemic absorption. Had the bolus failed to fully dissolve or resulted in delayed absorption, a lower C_{Max} , prolonged T_{Max} , and similar AUC_{0-inf} would have been expected. The lower AUC_{0-inf} observed in the present study, despite similar T_{Max} and $t_{1/2}$ values, suggests that factors other than absorption rate, such as inter-individual variability in bioavailability, may have contributed to the differences in drug exposure between studies. Because the majority of published studies on meloxicam pharmacokinetics have been conducted in Holstein cattle, comparisons across these studies are more robust for this breed than for Angus or Brahman cattle, making further investigation into breed-specific variability in these other cattle breeds particularly important.

Breed-specific variability in drug disposition was evident in this study. When comparing $t_{1/2}$, AUC_{0-inf} , and C_{Max} values, Brahman calves consistently exhibited higher values than Holstein and Angus calves. The intra-breed variability for $t_{1/2}$ was markedly greater in Brahman and Holstein calves, with a 10.1-fold range in Brahmans and 11.4-fold range in Holsteins, compared to a narrower 4.5-fold range in Angus calves. Greater intra-breed variability for Brahman compared to Holstein and Angus calves for AUC_{0-inf} and C_{Max} values were also observed. For AUC_{0-inf} , variability was 12.5-fold in Brahmans, 4.1-fold for Angus, and 2.3-fold for Holsteins. For C_{Max} , the variability was 4.4-fold for Brahman, 4.3-fold for Holstein, and 1.9-fold for Angus calves. These findings may suggest that variability in drug elimination and systemic exposure may be more pronounced in certain breeds.

Comparable breed- and sister species-related pharmacokinetic differences have been reported in ivermectin, where Tabapuã (*B. indicus*) cattle showed higher AUC, C_{Max} , and $t_{1/2}$ values than Angus cattle.⁵³ The authors of the study speculated that differences in fat composition and intestinal p-glycoprotein might explain these findings.⁵³ Although meloxicam and ivermectin differ in their physiochemical properties, both drugs undergo CYP-mediated metabolism, albeit to different extents.^{53,95} About 50% of ivermectin residues in cattle are metabolites formed by CYP enzymes, breed differences in metabolism likely contribute as well.^{95,96} Supporting this, a follow-up tissue residue study that did not measure metabolites found higher hepatic parent ivermectin concentration necessitating longer withdrawal times in Nelore (*B. indicus*) cattle compared to Angus x Nelore crosses. The elevated parent compound concentrations in the liver, which contains the highest concentration of ivermectin metabolites of all tissues and accounts for 23% of radioactive ivermectin residues in cattle, may reflect breed-related differences in hepatic metabolism.^{54,96} Taken together, the observed pharmacokinetic

differences in this study likely reflect a combination of physiological factors and breed-related genetic differences in drug metabolism, warranting further investigations to clarify their respective contribution and potential clinical implications in additional drugs used in cattle.

Although the LC-MS/MS method used in this study was developed and validated to quantify the meloxicam metabolites 5'-hydroxy meloxicam and 5'-carboxy meloxicam with 80- and 8-fold greater sensitivity, respectively, than a previously published HPLC method validated for rat plasma, neither metabolite was quantifiable in the plasma samples collected.⁹⁷ This was unexpected given prior evidence from EMA filings indicating that cattle produce both metabolites, and that their plasma and excreta metabolite profiles are qualitatively similar to those observed in rats.³¹ In rats administered meloxicam orally at 2 mg/kg, 5'-hydroxy meloxicam and 5'-carboxy meloxicam were detected in plasma at concentrations ranging from approximately 7-33 ng/mL and 2.5-8.5 ng/mL, respectively.⁹⁷ In contrast, consistent with our findings, a metabolic profiling study in cats reported no detectable metabolites in plasma; only the parent drug was observed, with metabolites only measurable in urine and feces.⁷⁹ The EMA filing for cattle also noted a polar metabolite, referred to as "metabolite 1", comprising 18% of the administered dose in urine, though no structural details were provided.³¹ Interestingly, Boehringer Ingelheim, the drug sponsor for the EMA submission, conducted metabolic profiling across species and identified a polar metabolite in humans, also referred to as "metabolite 1," 2-[hydroxyacetyl]amino]-5-thiazolecarboxylic acid, thought to be produced from 5'-carboxy meloxicam.^{31,98-100} The absence of detectable metabolites in plasma, despite the sensitivity of our analytical method, suggests that meloxicam metabolites may be rapidly eliminated. In vitro studies using bovine liver microsomes and hepatocytes are needed to better characterize meloxicam metabolism in cattle. Given that approximately 50% of an administered dose is

eliminated in urine, future studies should prioritize urine-based analyses of parent-to-metabolite ratios to assess metabolic phenotypes, consistent with established practices in human medicine, such as the use of dextromethorphan/dextrorphan urinary ratios to evaluate CYP2D6 activity.⁸⁰ Given the absence of quantifiable plasma metabolites, the study pivoted to using $t_{1/2}$ as a surrogate marker for metabolic variability rather than the planned parent-metabolite ratios.

Outlier analysis based on $t_{1/2}$ identified a greater number of outliers in Brahman (n=7) and Holstein (n=6) calves compared to Angus (n=5). Additionally, as mentioned above, the intra-breed variability in $t_{1/2}$ was markedly greater in Brahman and Holstein calves than Angus calves, with Angus displaying a narrower range of $t_{1/2}$ and fewer outliers, suggesting more consistent drug disposition in this breed. In contrast, the higher frequency of both long and short $t_{1/2}$ in Holstein and Brahman calves may reflect underlying genetic polymorphisms affecting drug metabolism. While trends in the probit plot suggested a mixed distribution pattern, larger sample sizes for each breed would be robustly characterized phenotypes, as human phenotyping studies typically enroll a few hundred participants to reliably define population distributions.¹⁰¹

Genetic polymorphisms influencing drug metabolism are not uniformly distributed across breeds or populations, potentially contributing to interindividual and inter-breed variability in drug disposition and therapeutic responses.¹⁰² Some breeds may have a larger proportions of individuals with exceptionally rapid or slow meloxicam metabolism as has been noted in human studies.¹⁰² Notably, the potential for distinct metabolizer phenotypes based on $t_{1/2}$ has been previously described in Holsteins. This study is the first to document outliers in both Brahman and Angus calves. One prior investigation of subcutaneous meloxicam pharmacokinetics in postpartum dairy cows observed three distinct elimination phased in concentration-time profiles: rapid, intermediate, and slow, which may correspond to metabolizer phenotypes.⁸¹ Other studies

have reported individual outliers with markedly prolonged (43.29 hours) or shortened (9.7 hours) $t_{1/2}$ values compared to study means, attributing such variability to potential polymorphisms in CYP genes.^{21,23} Further research is warranted to determine whether underlying genetic mechanisms account for these outliers, and if so, whether such polymorphisms occur at meaningful frequencies and have clinically significant effects on drug disposition and response.

This study is not without limitations. The cattle representing each breed were sourced from limited geographic locations and may not fully reflect the genetic diversity of their respective breeds. As such, extrapolation of these findings across broader breed populations should be made with caution. Additionally, the study did not evaluate clinical outcomes, precluding conclusions about the therapeutic relevance of the observed pharmacokinetic differences. While bioequivalence analysis was employed under the assumption that lack of bioequivalence may indicate a potential for clinical difference, this cannot be confirmed without efficacy data. The original study design aimed to evaluate parent to metabolite ratios as markers of metabolism, but these metabolites were undetectable in plasma. In response, $t_{1/2}$ was used as a surrogate for metabolic clearance. However, because $t_{1/2}$ is influenced by both clearance and volume of distribution, and without intravenous data to estimate clearance independent of bioavailability, the observed intra-breed variability in $t_{1/2}$ must be interpreted cautiously. Finally, this study focused exclusively on bull calves, and the effects of sex, age, or physiological status on meloxicam pharmacokinetics among breeds remains unknown. As such, it is unclear whether the breed-related differences identified here would extend to females, adult animals, or cattle under diseased or stressed conditions.

Despite these limitations, this study provides novel insights into breed-related pharmacokinetic differences in cattle and underscores the importance of considering intrinsic

factors when evaluating drug disposition in food animals. The absence of FDA-approved analgesic options for the mitigation of castration-associated pain in U.S. cattle further amplifies the clinical and regulatory relevance of this work. Our results suggest a one-size-fits-all approach to meloxicam dosing in cattle may be suboptimal and that breed-specific or individualized dosing strategies could improve therapeutic outcomes and reduce violative drug residues. Future investigations should prioritize the evaluation of drug disposition in additional breeds, age groups, and physiological states, including diseased animals. The potential need for breed-specific withdrawal times also warranted exploration. Moreover, integrating pharmacodynamic endpoints will be critical for establishing the clinical significance of pharmacokinetic variability. Finally, efforts to elucidate the genetic basis for interindividual variability in meloxicam metabolism may inform breed-specific dosing strategies and support regulatory efforts aimed at improving pain management practices in food animals and human food safety.

Chapter 3 - Are Meloxicam Pharmacokinetics Bioequivalent When Administered to Calves Before vs During Surgical Castration?

3.1 Introduction

Castration is a common and necessary management practice in the cattle industry performed to reduce aggressive behavior, improve meat quality, and facilitate animal handling; however, it is a highly stressful and painful procedure for the animals, eliciting nociceptive, inflammatory, and stress responses.²⁻⁷ In recent year, consumer concern for animal welfare and evolving industry standards have driven growing interest in identifying effective pain management strategies for routine husbandry procedures.⁸ Among available pharmacological options, non-steroidal anti-inflammatory drugs (NSAIDs) are frequently administered to mitigate pain and inflammation in calves undergoing castration. Although there is general consensus that NSAIDs improve welfare outcomes for painful husbandry procedures in cattle, there are currently no FDA-approved NSAIDs for this specific indication in the United States, and optimal analgesia protocols remain under investigation.⁸

In both human and veterinary medicine, characterization of drug pharmacokinetics is typically conducted in healthy research subjects, with the implicit assumption that the resulting dose-exposure relationships will adequately reflect those occurring in the intended patient population.¹⁰³ However, there is ample evidence that this assumption may not hold true.¹⁰³ Pathophysiological states such as pain, inflammation, and stress can influence drug absorption, distribution, metabolism, and elimination, potentially altering pharmacokinetics in a clinically relevant manner.^{64,65} Despite this, few veterinary studies have examined how these factors influence drug disposition in animals undergoing clinical procedures or experiencing these conditions. This knowledge gap may have important implications for pain management strategies

in cattle, where procedures like castration trigger marked physiological changes that could influence drug pharmacokinetics.

Meloxicam, a preferential cyclooxygenase-2 (COX-2) inhibitor NSAID with anti-inflammatory and analgesic properties commonly used in veterinary medicine to manage pain associated with surgical and inflammatory conditions.^{8,13} Its pharmacokinetic profile is characterized by high oral bioavailability, a long half-life, and extensive protein binding.^{15,16} These features contribute to prolonged analgesic effects, making it a popular choice for managing pain associated with castration.^{15,16} However, physiological alterations induced by castration, including acute inflammation, tissue trauma, endocrine stress responses, altered blood flow, altered gastrointestinal motility, and augmentation in plasma proteins, could possibly affect the absorption and distribution of meloxicam.^{6,65,66,74} Additionally, inflammatory cytokines and mediators, released during acute inflammation, such as prostaglandins, are known to modulate cytochrome P450 (CYP) enzymes, which are responsible for the metabolism of many drugs, including meloxicam.^{63,64} While meloxicam pharmacokinetics have been described in healthy calves, the potential impact of surgical castration on meloxicam disposition has not been investigated.

The objective of this study was to compare the pharmacokinetics of oral meloxicam administered to bull calves prior to and again at the time of surgical castration. In the absence of established clinically significant exposure thresholds for meloxicam in cattle, bioequivalence criteria were applied to assess differences in meloxicam disposition between these two physiologic states. The findings from this study will contribute to the evidence base for developing effective, evidence-based pain management protocols for castration in cattle.

3.2 Materials and Methods

3.2.1 Animals and Housing

This study was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC #4661). Following a minimum 14-day washout period from prior drug administration, 41 bull calves (Angus, n = 15; Brahman, n = 12; and Holstein, n = 14) from the study described in Chapter 2 were enrolled in this study. Enrollment numbers reflect the simultaneous conduct of a complementary study evaluating the effect of breed on the ability of meloxicam to attenuate castration pain, which informed the available population.

3.2.2 Experimental Design and Sample Collection

This study used a 2-period, open-treatment, single-sequence design. FDA Center for Veterinary Medicine and the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products recommends that a minimum of 6 animals be enrolled in a blood level crossover bioequivalence study.⁸⁶ In the absence of previously published crossover data for meloxicam pharmacokinetics in cattle, an intra-subject coefficient of variation of 30% (representing the intrasubject coefficient of variation cutoff for a highly variable drug used by the FDA) was used. The sample size for a crossover bioequivalence design, with an acceptance range of 0.8-1.25 and a μ_2/μ_1 value of 1.0 (indicating no difference), was estimated to be 32 animals to achieve 80% power at an α value of 0.05. Since additional animals were enrolled in the complementary study conducted concurrently with this study, additional animals were included in the study (n=41).

As outlined in section 2.2.2, the process for group stratification, catheter placement, drug administration, and blood collection and processing were consistent between studies. Cohort assignments from the study described in Chapter 2 were maintained. Each calf received a single

oral dose of meloxicam (1 mg/kg), ranging from 150 to 480 mg, administered via balling gun, immediately prior to surgical castration ($t = 0$ hours). Blood samples were collected beginning at the time of dosing and continued for up to 168 hours post-administration.

Two experienced large animal clinicians from the Kansas State University Livestock Services section performed all surgical castrations using the same protocol. All castrations were performed with the calves standing in a hydraulic chute for restraint without systemic sedation or general anesthesia. Lidocaine (2%) was injected subcutaneously in three points around the scrotum (5-10 mL total). A scalpel was used to incise the distal 1/3 of the scrotum. Through blunt dissection of the testes, the spermatic cord was extracted, stripped, and twisted to complete the castration procedure. Instruments were dunked in a chlorhexidine disinfectant solution in between animals. Iodine was applied to the incision, which was left open to heal by second intention. The area was sprayed with a wound-safe fly repellent.

3.2.3 Plasma Meloxicam Analysis

Meloxicam working stocks, plasma standard solutions, and QCs were prepared as described in section 2.2.3. The same LC-MS/MS system was used to determine meloxicam plasma concentrations, and the acceptance criteria were identical to that previously described.

3.2.4 Pharmacokinetic and Statistical Analyses

3.2.4 Pharmacokinetic and Statistical Analyses

Pharmacokinetic, bioequivalence, and descriptive statistical analyses were performed using the same software and approaches described in Section 2.2.4. Individual plasma concentration-time data were analyzed using noncompartmental analysis (PKAnalix version 2024R1, Lixoft, Antony, France) to determine the same pharmacokinetic parameters as in Chapter 2, including AUC_{0-last} , $AUC_{0-\infty}$, C_{Max} and T_{Max} , $AUC\%Ex$, λ_z , $t_{1/2}$ and MRT. As in

Chapter 2, plasma concentrations below the LLOQ or from missed samples were treated as missing. Plasma concentration-time profiles are presented as arithmetic mean $\pm$ standard deviation. Pharmacokinetic parameters, except for T_{Max} , are reported as the geometric mean, geometric mean coefficient of variation (%CV), and range. As a categorical variable, T_{Max} is presented as the median and range.

In the current study, a crossover bioequivalence model was used to assess within-animal differences in meloxicam pharmacokinetics before and at the time of surgical castration. Pre-castration data (Chapter 2) served as the reference condition, and data collected at the time of castration served as the test condition. Bioequivalence analysis focused on the pivotal parameters (AUC_{0-last} , $AUC_{0-\infty}$, C_{Max} and T_{Max} , and $t_{1/2}$) and were considered bioequivalent if the 90% confidence intervals of the geometric mean ratio (castration:pre-castration) for the log-transformed values fell within the FDA-defined bioequivalence range of 0.8 to 1.25.⁸⁶

3.3 Results

Following the washout period, 41 individual cattle were orally administered 1 mg/kg meloxicam again to investigate differences in drug disposition as a result of castration. One Angus calf died on day 6 of the study, and necropsy findings were consistent with bovine respiratory disease. One Holstein calf was removed from the study on day 4. Data from both calves were included in the study.

The mean plasma meloxicam concentration-time profiles for meloxicam are shown in Figure 3.1. The pre-and post-castration pharmacokinetic parameters are reported in Table 3. AUC_{0-inf} , $AUC_{0-tlast}$, and C_{Max} were greater in the post-castration samples, but $t_{1/2}$ was shorter following castration. Crossover bioequivalence results are summarized in Table 4 as a ratio and 90% confidence interval range for AUC_{0-inf} , $AUC_{0-tlast}$, C_{Max} , $t_{1/2}$, and T_{Max} , in which each

parameter investigated lacked bioequivalence in calves before and after surgical castration, except for T_{Max} .

Figure 3.1 (a) Linear and (b) semilogarithmic plots of arithmetic mean meloxicam concentration-versus-time profiles after a single oral dose of meloxicam (1 mg/kg) to bull calves (n=41) before castration (REF) and at the time of castration (CAST) following a 14-day washout period. Error bars represent the standard deviation.

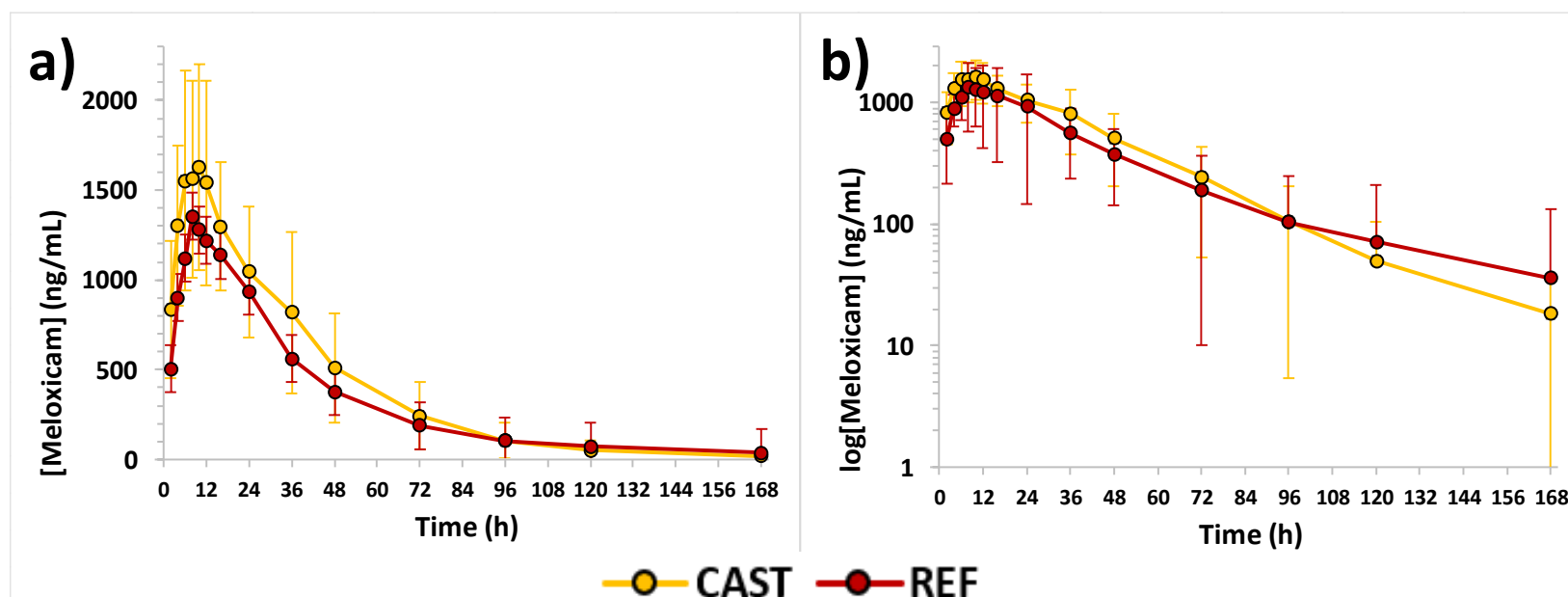


Table 3 Pharmacokinetic parameters for meloxicam determined from noncompartmental analysis in Angus (n=15), Brahman (n=12), and Holstein (n=14) calves administered a single oral dose (1 mg/kg) during surgical castration.

Parameter	Pre-Castration			Post-Castration		
	Mean	Range	% CV	Mean	Range	% CV
AUC_{0-inf} (ng·h/mL)	49090.1	26586.7- 330013	51.69	62398.6	27651.4- 137223	37.84
% extrapolated AUC	0.99	0.13- 26.81	287.13	0.49	0.063- 6.63	213
AUC_{0-tlast} (ng·h/mL)	47464.9	21302.2- 241524	48.11	61701.3	26112.1- 133640	38.35
C_{max} (ng/mL)	1379.88	845.63- 4708.35	39.6	1893.85	1000.2- 3351.45	28.73
Dose (mg)	261.14	150- 405	24.78	293.87	210- 480	23.89
T_{1/2} (h)	27.76	16.41- 185	57.02	19.69	9.29- 54.13	37.35
k_{el} (h ⁻¹)	0.025	0.0037- 0.042	57.02	0.035	0.013- 0.075	37.35
MRT_{0-inf} (h)	38.55	23.76- 126.78	38.77	33.1	19.46- 53.27	25.27
T_{max} (h) *	8	6- 16	27.18	10	4- 36	46
AUC_{0-inf}/dose ((ng·h/mL)/mg)	187.98	177.2- 814.8	2.09	212.33	131.7- 285.9	1.58
C_{max}/dose ((ng/mL)/mg)	5.28	5.6- 11.6	1.6	6.44	4.8- 6.98	1.2

*Categorical variable displays median instead of geometric mean.

Abbreviations and units of measurement: %CV = Geometric coefficient of variation; AUC_{0-inf}, area under the curve from 0 to ∞ (ng·h/mL); % extrapolated AUC, percentage of the area under the curve that is extrapolated; AUC_{0-tlast}, observed area under the curve to the last measured time point (ng·h/mL); C_{Max}, maximum plasma concentration (ng/mL); Dose (mg), t_{1/2}, elimination half-life (h); k_{el}, elimination rate constant (1/h), MRT_{0-inf}, mean residence time extrapolated from 0 to ∞ (h); T_{Max}, time to maximum plasma concentration (h); AUC_{0-inf}/dose, the area under the curve from 0 to ∞ per unit dose ((ng·h/mL)/mg); C_{Max}/dose, the maximum plasma concentration per unit dose ((ng/mL)/mg).

Table 4 Average bioequivalence estimates for log-transformed pivotal pharmacokinetic parameters following oral administration of meloxicam (1 mg/kg) to bull calves (n= 41) in a crossover design. Comparisons were made between pre-castration (reference) and castration (test) conditions, with a minimum 14-day washout period treatments.

Parameter	Castrated vs Reference		
	Ratio	90% CI Range	BE Criteria Met
AUC_{0-inf} (ng·h/mL)	127.11	(110.79, 145.83)	No
AUC_{0-tlast} (ng·h/mL)	129.99	(113.91, 148.35)	No
C_{Max} (ng/mL)	137.25	(122.47, 153.81)	No
T_{1/2} (h)	70.91	(60.13, 83.63)	No
T_{Max} (h)	108.59	(95.03, 122.14)	Yes

3.4 Discussion

This study evaluated the effect of surgical castration on the pharmacokinetics and bioequivalence of orally administered meloxicam (1 mg/kg) in male calves, using a crossover design to compare drug disposition before and at the time of the procedure. Effective pain management in cattle remains complicated by the absence of FDA-approved analgesics labeled specifically for husbandry procedures such as castration.⁹ An often overlooked issue in veterinary medicine is the assumption that pharmacokinetic data obtained from healthy animals can be directly applied to clinical populations experiencing altered physiology.¹⁰³ This study addresses that critical gap by assessing whether meloxicam pharmacokinetics remain bioequivalent when administered prior to versus at the time of surgical castration. The findings demonstrate that meloxicam pharmacokinetics are not bioequivalent when administered during castration, underscoring the importance of disposition in the target population to inform evidence-based pain management strategies.

When comparing oral meloxicam pharmacokinetics administered at the time of surgical castration in calves to a previous study, our results align with and expand upon those of Meléndez et al., which examined meloxicam pharmacokinetics in 12, 7-8-month-old Angus cross

calves (1 mg/kg PO) undergoing surgical castration.¹⁵ In the current study, the post-castration $AUC_{0-inf}/dose$ was 212.33 (ng·h/mL)/mg, and $C_{Max}/dose$ was 6.44 (ng/mL)/mg with Meléndez et al. reporting an $AUC_{0-inf}/dose$ was 291 (ng·h/mL)/mg, and $C_{Max}/dose$ was 7.1 (ng/mL)/mg.¹⁵ The average $t_{1/2}$ in the Meléndez study was 15.6 hours and while the $t_{1/2}$ of this study was slightly longer at 19.7 hours, it was within the range of reported values.¹⁵ The median T_{Max} in the current study was 10 hours which was considerably earlier than the 24 hours reported previously.¹⁵ Notably, the population in the current study was larger and more diverse, incorporating three breeds and two sister species, providing a broader representation of the male calf population.

In this study, AUC and C_{Max} were elevated when meloxicam was administered at the time of castration, while the $t_{1/2}$ was shorter compared to the pre-castration state. T_{Max} remained bioequivalent. Although veterinary literature evaluating the effect of surgery, pain, and inflammation on NSAID disposition is limited, findings from this study align with existing data while also diverging in meaningful ways. For example, A pilot study in eight dogs undergoing ovariohysterectomy assessed intravenous meloxicam pharmacokinetics administered during the procedure and 10 days prior. While no statistically significant differences were found, trends included increased AUC_{0-inf} and decreased $t_{1/2}$ during surgery, attributed to an approximate 25% decrease in clearance and a 43% decrease in volume of distribution.⁷³ Notably, since meloxicam was administered orally in the present study, the clearance and volume of distribution could not be directly assessed. Furthermore, comparing apparent clearance (CL/F and apparent volume of distribution (Vd/F) would be inappropriate given the differences in AUC, implying bioavailability (F) may have varied between pre- and at the time of castration. The ovariohysterectomy study also speculated that altered meloxicam protein binding, possibly due to drug-drug or drug-hormone interactions during surgery, may have contributed to observed

changes, as they ruled out changes in serum albumin between states.⁷³ Drug-drug interactions were unlikely in the present study where only local lidocaine was administered. No explanation was given by the authors of the ovariohysterectomy study as to why AUC was increased in dogs undergoing surgery.⁷³ Like the present study, the ovariohysterectomy study shared the same limitation of a single-sequence design, potentially introducing a period effect, but was thought unlikely by the authors as they utilized a 10-day washout.⁷³ The current study utilized a minimum of 14 days washout.

In contrast, a study examining pharmacokinetics of transdermal flunixin meglumine, another NSAID, in 6-8-week old male Holstein calves undergoing cautery dehorning using a two sequence, two period crossover design found a significantly longer $t_{1/2}$ and lower C_{Max} during dehorning, while AUC and T_{Max} did not differ.⁷⁴ However, the 14-day washout used may have been insufficient given that lesions from cautery dehorning can take several weeks to heal.¹⁰⁴ Thus, animals that were dehorned first and then exposed to a sham dehorning treatment would have still been healing from the procedure, which may have obscured results. Additionally, differences in drug administration route (transdermal vs. oral) and physicochemical properties between the two NSAIDs likely contributed to the contrasting pharmacokinetic responses.

The physiological changes induced by surgical castration, including stress, pain, inflammation, and possible alterations in gastrointestinal motility or protein binding, may, partly explain the observed changes in meloxicam disposition.^{28,65,70} While this study provides valuable insight into the impact of these effects, several limitations of this study should be acknowledged. Direct measures of stress, pain, or inflammation were not performed, precluding mechanistic evaluation of their pharmacokinetic impact. Additionally, serum albumin concentrations were not measured during each study period, and unbound (free) meloxicam concentrations were not

assessed, limiting conclusions about changes in protein binding or abundance. Lastly, because meloxicam was administered orally to better reflect field use, volume of distribution and clearance could not be directly evaluated to give more insight into which pharmacokinetic process was contributing to alteration of $t_{1/2}$.

This study demonstrated that surgical castration alters the pharmacokinetics of meloxicam in male calves, with increased AUC and C_{Max} and a decreased $t_{1/2}$ following administration at the time of castration. These findings suggest that stress, pain, and inflammation induced by castration may meaningfully influence drug disposition, potentially requiring adjustments to dosing regimens to optimize pain management. Future studies should explore the complex interactions between pathophysiological changes, protein binding, and drug metabolism to better understand how altered physiological states impact pharmacokinetics. Further research is also needed to determine whether similar effects occur in female cattle undergoing painful procedures or in animals with other inflammatory conditions. Collectively, these findings reinforce the importance of studying pharmacokinetics in the actual target population rather than extrapolating from healthy animals, as disposition changes under clinical conditions could impact therapeutic efficacy. Continued investigation into NSAID pharmacokinetics in stressed, inflamed, or clinically ill cattle is essential to advance evidence-based, effective pain management protocols in livestock.

Chapter 4 - Discussion

This thesis provides important new insights into the pharmacokinetics of orally administered meloxicam in calves, demonstrating that both breed and inflammatory state are significant determinants of drug disposition. In Chapter 2, pharmacokinetic analyses revealed non-bioequivalent meloxicam profiles among Angus, Holstein, and Brahman bull calves exhibited non-bioequivalent meloxicam disposition. Notably, Brahman and Holstein calves exhibited longer elimination half-lives compared to Angus calves, suggesting potential breed-specific differences in metabolic capacity or drug clearance mechanisms. These findings expand upon prior reports by evaluating breed effect under controlled conditions and identifying pharmacokinetic differences with potential implications for both analgesic efficacy and drug residue avoidance.

In Chapter 3, surgical castration was shown to significantly alter meloxicam pharmacokinetics, resulting in a reduced $t_{1/2}$, but enhanced extent of absorption relative to pre-castration administration. This is the first controlled study to evaluate the impact of an acute inflammatory state on meloxicam disposition in calves. The findings underscore the importance of assessing pharmacokinetics in the target population, as physiological changes associated with inflammation and tissue injury can substantially modify drug absorption, distribution, metabolism, and excretion.

Collectively, these data support the conclusion that intrinsic factors like breed and inflammatory state independently influence meloxicam pharmacokinetics in calves.^{9,15,20,21,23} Importantly, while bioequivalence limits were exceeded in most pivotal parameter comparisons, further research is warranted to determine whether lack of bioequivalence translates to clinically

meaningful variations in analgesic efficacy or withdrawal intervals of different breeds or physiological states.

Additionally, while this study focused on bull calves, expanding research to include additional breeds, females, younger calves, and adult cattle would further refine our understanding of how breed, sex, age, and physiologic state influence meloxicam pharmacokinetics. Continued study of how altered physiological states, such as disease or stress, affect drug disposition will also be important for informing evidence-based therapeutic protocols.

Further investigations should incorporate urinary metabolite quantification and genomic sequencing to identify functional polymorphisms in cytochrome P450 genes associated with NSAID metabolism. Such research will be essential for developing breed-specific dosing strategies and withdrawal time guidelines, thereby improving pain management practices while ensuring food safety in cattle production systems.

This thesis lays the groundwork for a more precision- and evidence-based approach to analgesic drug use in food animals and highlights the importance of accounting for genetic and physiologic variability when designing therapeutic protocols in livestock.

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