Pain modeling and NSAID pharmacology in meat-type goats: Development, comparative efficacy, and pharmacokinetics

by

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B.S., Kansas State University, 2021

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

In recent years, there has been a growing trend among consumers of animal products to prioritize the ethical treatment of animals. Most animal products are derived from food-producing animals, including but not limited to cattle, pigs, poultry, sheep, and goats. Ensuring the ethical treatment of food animals includes addressing a range of animal welfare concerns, including pain recognition and pain alleviation. Pain responses in livestock animals are often challenging to interpret and assess objectively due to their complex nature and the inherent stoicism of prey animal species. This stoicism, while adaptive for survival, complicates the recognition and effective treatment of pain in food animal species, making pain management a significant welfare and veterinary challenge.

Goats have become increasingly popular in recent years, both as companion animals and for production purposes. However, compared to other livestock species, the timely and accurate recognition and treatment of pain in goats remains limited. Painful events in goats commonly arise from routine husbandry procedures such as disbudding and castration, as well as from health conditions like lameness or mastitis. While these events are challenging, effective pain management is essential for ensuring positive animal welfare and optimizing productivity. Efficient and timely pain management not only ensures the ethical treatment of livestock animals like goats but also helps address growing concerns among consumers of animal products regarding animal welfare.

This dissertation presents a comprehensive evaluation of three amphotericin B-induced lameness models to develop a reliable and repeatable transient synovitis model in meat-type goats. A species-specific grimace scoring system for goats is also introduced to support behavioral pain assessment. The analgesic efficacy of firocoxib and meloxicam is assessed

following surgical castration in meat-type goats. Additionally, the effectiveness of firocoxib, meloxicam, and transdermal flunixin, each administered at three different dosages, is evaluated after lameness induction with amphotericin B, using both behavioral assessments and pain-specific biomarkers. Finally, the pharmacokinetic profiles of firocoxib, meloxicam, and transdermal flunixin at varying dosages are reported using descriptive statistical analyses.

The results indicate that an amphotericin B lameness model, using a dose of 5 mg/0.25 mL, is a reliable and repeatable method for inducing transient synovitis in meat-type goats for research purposes. Pain assessment tools, including Visual Lameness Scoring (VLS), Visual Analogue Scoring (VAS), and Grimace Scoring, consistently indicated that lameness is a painful condition in goats. Additionally, the Grimace Scoring System developed specifically for evaluating facial expressions in goats during this study proved to be a successful tool for assessing pain-related grimacing behavior.

Our results suggest that pain experienced by male goats during surgical castration may be effectively alleviated through the administration of oral meloxicam. The current study provides evidence that surgical castration is a painful husbandry procedure for goats, as demonstrated by multiple pain assessment methods, including kinetic gait analysis, plasma cortisol levels, and Visual Analogue Scoring (VAS). Kinetic gait analysis revealed altered weight distribution, with reduced weight-bearing in the rear limbs compared to the front limbs following castration. Plasma cortisol concentrations peaked immediately after castration but decreased following analgesic intervention. Additionally, VAS scores were consistently higher at 24, 48, and 72 hours post-castration in goats treated with firocoxib compared to those treated with meloxicam or control goats, suggesting meloxicam may offer more effective pain relief.

In goats with experimentally induced lameness, analgesic interventions with transdermal flunixin at 3.3 mg/kg and 5.0 mg/kg were effective in reducing pain. Among treatments, the Mechanical Nociception Threshold (MNT) difference between lame and sound limbs was lowest in the high-dose transdermal flunixin group (5.0 mg/kg), indicating effective analgesia. Kinetic gait parameters, including stance time, stride length, peak force, and contact force, showed the smallest differences between lame and sound limb comparisons in the low-and high-dose transdermal flunixin groups (3.3mg/kg and 5.0mg/kg). Visual Lameness Scores (VLS) were reported to be lowest in the low-dose flunixin group on average (3.3 mg/kg), while Visual Analogue Scores (VAS) were lowest in the high-dose group on average (5.0 mg/kg). Descriptive pharmacokinetic data were collected for firocoxib, meloxicam, and transdermal flunixin at three dosage levels for each drug.

To conclude, these results indicate that pain-associated outcome measures previously used in livestock species such as cattle are also effective in assessing pain in goats. There is a clear animal welfare benefit for meat goats that receive analgesia prior to or during painful procedures, such as surgical castration or treatment for lameness. Further research is warranted to investigate species-specific pain indicators relevant to goats, and to optimize analgesic strategies for managing pain during procedures like castration or in cases of lameness. Such studies will help provide more effective pain control options for meat-type goats.

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Dedication

I would like to dedicate this dissertation to my parents Sheri and Jacob Weeder, and my siblings Brayden and Alexis Weeder. To my late grandparents Bob and Ethel Akin; this work is for you both, and I hope I have made you proud. To friends and colleagues who have offered me support, advice, or just a shoulder to cry on; thank you. I would also like to dedicate this dissertation to God and my Lord and Savior, Jesus Christ. Through Them, I can do all things.

I would, of course, like to dedicate this dissertation to the goats who participated in all my studies. They made the ultimate sacrifice for research, and without them I could not have completed the necessary work for my doctoral degree. I will always remember one little goat, Marbles. He is a big proponent behind my love for goats and drives my passion for goat research.

Chapter 1 - Literature Review

Introduction

In recent decades, interest in human-animal relationships has grown significantly (Hemsworth, 2007). A widely accepted societal view holds that animals can be used for production purposes, provided they are treated ethically and humanely by humans (Mellor, 2004). In many social contexts, both ethical principles and legal frameworks require humans to ensure the humane treatment of animals (Sneddon et al., 2014). Within the agriculture sector, the United States ranks as the world's largest beef producer and second-largest pork producer, with consistent growth in milk and egg production driven by global demands (USDA, 2025). As international trade increasingly prioritizes animal welfare, established guidelines support ethical animal treatment during global exchanges in animal products (OIE, 2008). Among these standards, appropriate pain management for farm animal species plays a crucial role in enhancing animal welfare (Steagall et al., 2021). This aligns with public expectations for ethically produced food worldwide and reinforces the importance of animal welfare standards in international trade.

In the U.S., the goat population has steadily grown from 2010-2023 (USDA, 2010; USDA, 2024), highlighting the potential for increased goat products like milk, meat, and fiber (Hart et al., 2019). This growth underscores an immediate need for improved pain recognition and management specific to goats. Despite this population growth, research on goats in regard to pain mitigation remains limited. Consequently, veterinarians often rely on pain management protocols designed for other livestock species, which may not effectively address the unique needs of goats. Without timely and adequate treatment, painful goats may experience compromised welfare and deteriorating health (Alvarez et al, 2009; Huxley, 2006; Hewson et al.,

2007). These welfare concerns not only threaten the economic viability of goat production but also heighten public scrutiny regarding the ethical treatment of food animals (Steagall et al., 2021).

Pain

Pain is an unpleasant physical and emotional experience associated with actual or potential tissue damage (Raja, 2020). Research shows that animals exhibit physiological and behavioral responses to pain that closely resemble those in humans (Allen et al., 2005; Sherwin, 2001). Recognizing that animals can feel pain creates an ethical responsibility for caretakers to prevent and manage it effectively (Weary et al., 2006). When left unmanaged, pain and inflammation can negatively impact an animal's overall welfare state (Gao and Hong, 2008). However, the inability to verbally communicate discomfort complicates both the evaluation of pain and the selection of appropriate analgesic therapies in animals (Livingston, 2010).

Pain is commonly categorized as either acute or chronic pain. A clear distinction between these types is essential for effectively managing pain in livestock animals. Acute pain is the result of actual tissue damage that resolves once fully healing has taken place (MacCarberg, 2023). If acute pain is left untreated, it can develop into chronic pain, which persists or recurs for more than three months. Chronic pain is often associated with maladaptive or long-term changes to the nervous system or psychological alterations (Treede et al., 2015; Herzberg et al., 2021; Merskey and Bogduk, 1994; May 2008). Differentiating between acute and chronic pain is critical to selecting the most appropriate analgesic strategies, which improve overall animal welfare and clinical outcomes (Loeser and Melzack, 1999; Chou and Huffman, 2007).

Misconceptions about Pain Throughout History

The recognition, diagnosis, and treatment of pain have long been subjects of controversy. During much of the 20th century, it was widely believed that human infants were incapable of feeling or adequately expressing pain. As a result, surgeries on newborns were sometimes carried out without effective pain relief (Chamberlain, 1999). Despite important advancements since then, neonatal pain management is still widely considered inadequate, with many infants often receiving insufficient or inconsistent pain relief during medical procedures (Carter and Brunkhorst, 2017).

The ability to experience pain is widely linked to the concept of sentience, defined as the capacity to have subjective experiences and feelings (Yeates, 2012). Many non-human animals, including mammals, birds, and even octopuses, possess neurological substrates needed to support conscious experiences, including pain (Low et al, 2012). Based on this understanding, most livestock animals are recognized as sentient beings capable of experiencing pain and other affective states. However, one of the greatest challenges in both animal science and veterinary medicine is accurately interpreting these emotional states, particularly pain (Weary et al., 2006). Animals cannot verbally communicate their discomfort, which makes assessing their pain inherently complex (Prunier et al., 2013). Despite this limitation, there is ample behavioral and physiological evidence that many animal species can experience a wide range of emotions, including pain. This highlights the urgent need for improved pain recognition and treatment across all animal species.

Animal Welfare

Animal welfare refers to the condition of an individual animal as influenced by its environment and can be assessed using measurable indicators (Broom, 1991). In recent years,

growing social and political interest in animal welfare has stemmed from increased public concern about how animals are treated in both personal and production contexts (Ohl, 2012). To effectively address these concerns, it is essential to understand animal welfare as a multidimensional concept encompassing three interrelated pillars: biological functions, affective states, and access to natural living conditions, which allows animals to express their innate and species-specific behaviors (Fraser et al., 1997). This section of literature review aims to explore these fundamental concepts to establish a comprehensive foundation for evaluating and improving welfare in farm animals, with particular attention to pain assessment in goats.

Animal welfare recognizes that an animal's welfare exists on a spectrum, ranging from negative, or poor, to positive, or good (Dawkins, 2008; Yeates and Main, 2008). However, accurately measuring affective states remains a challenge due to their subjective nature and animals' inability to verbally communicate these experiences (Duncan and Fraser, 1997; Mendl et al., 2009).

Animal welfare seeks to ensure that animals are treated humanely and that their well-being is safeguarded during human use, whereas animal rights philosophy advocates for the complete cessation of using animals for human purposes (Regan, 1983; Singer, 1975). These differing philosophical positions often create tension surrounding how animals should be treated in society. Navigating this tension requires a nuanced approach that considers both the ethical concerns of the public and the practical realities of animal use. To develop effective and sustainable welfare solutions, scientists must balance evidence-based research with the moral values and ethical concerns of society (Ohl, 2012).

History of Animal Welfare in Farm Animals

Understanding the historical development of agricultural animal welfare standards provides essential context for evaluating policies and identifying areas for reform. During World War II, small family farms gradually transitioned into larger-scale commercial operations to meet wartime food demands (McElveen, 1951). As a result, animals that were once raised in smaller numbers and spacious rural settings began to be produced in higher densities within confined spaces. With continued global population growth, governments have increasingly promoted intensified livestock production to meet rising food demands (FAO, 2006).

The publication of *Animal Machines* by Ruth Harrison in 1964 exposed the industrialization of animal agriculture to the public and brought attention to its negative consequences for animal welfare (Harrison, 1964). Harrison's work helped catalyze the modern animal welfare movement, stimulating widespread public debate and influencing policy reform in the UK and beyond (Fraser, 2008; Rushen, 2008). In response, the UK established the Brambell Committee in 1965, which led to the creation of the Five Freedoms; one of the first formal frameworks for evaluating and protecting animal welfare. This foundational model laid the groundwork for further animal welfare policy development.

In the United States, growing public concern over the ethical animal treatment led to the passage of the Animal Welfare Act (AWA) in 1966. Although originally focused on animals in research and exhibition, the AWA marked an important legislative milestone in animal welfare recognition. Although it has been improved in subsequent years, the AWA provides limited protection for farm animals, which has hindered improvements for livestock welfare under this legislation (Bickell, 2023).

Despite significant progress, challenges remain in addressing welfare concerns for farm animal species, including goats, across diverse production systems and regions. Examples of these challenges include limited access to veterinary care and resources in certain areas, difficulties monitoring welfare in large-scale intensive operations, economic pressures that hinder the adoption of improved welfare practices, cultural differences affecting public awareness and attitudes toward animal welfare, and wavering enforcement of welfare laws.

Animal Welfare Evaluation Frameworks

Animal welfare evaluation frameworks offer structured, theory-based approaches to assessing animal well-being, including the recognition and management of pain. In the context of goat welfare, these models assist researchers, veterinarians, and producers in systematically evaluating animal experiences by integrating biological, behavioral, and affective indicators. However, for these frameworks to be effective in practice, they must not only be scientifically robust but also feasible under field conditions. Understanding both the theoretical basis and practical application of these models is essential for improving pain assessment and overall welfare outcomes in goats.

One of the most widely recognized frameworks is the Five Freedoms, originating from the Brambell Report (1965) and later formalized by the UK Farm Animal Welfare Council (FAWC, 1979). The Five Freedoms assert that animals should be free from hunger, discomfort, pain, fear, and able to express natural behaviors. This model remains foundational in welfare audits, veterinary protocols, and farm assurance schemes (Webster, 2016). However, it has been criticized for focusing predominately on the absence of negative states and offering limited guidance on promoting positive welfare experiences (Browning, 2019; Ohl and van der Staay, 2012). For example, a goat housed in a clean, food-supplied pen may be free from pain or

discomfort. But, if said goat lacks opportunities to browse, interact socially, or move freely, its welfare still may be compromised.

Fraser's Three Circles Model (2003) advances this discussion by integrating biological functioning, affective states, and natural living. This model is particularly valuable in pain research, as it acknowledges emotional experiences such as fear and suffering alongside physical health. For instance, castration in goats might not significantly impact physiological parameters but can lead to considerable emotional distress. The Three Circles Model has been applied in both research design and on farm assessments, helping balance health, behavior, and emotional well-being. Its limitation lies in the difficulty of objectively measuring affective states objectively, particularly in species like goats, where behavioral expressions of pain are often subtle or misunderstood (Mellor et al., 2009; Dalla Costa et al., 2016).

The Five Domains Model (Beausoleil and Mellor, 2015; Mellor, 2016; Voogt et al., 2023) builds upon previous models by assessing nutrition, environment, health, and behavior (the four physical domains), all of which contribute to the fifth domain; the animal's mental state. This model explicitly incorporates positive experiences such as comfort, pleasure, and interest, making it one of the most comprehensive welfare assessment tools available. This model has been practically applied in welfare assessments for livestock and zoo animals (Mellor and Beausoleil, 2017). In goat welfare, the Five Domains Model has been proposed for use in extensive systems, employing both animal-based measures (e.g., posture, vocalizations, grimace scales) and resource-based indicators (e.g., shelter, flooring, access to browse). Despite its strengths, its application can be constrained by the need for extensive, multidimensional data; often impractical in time or resource limited field conditions.

The 3Rs Framework- Replacement, Reduction, and Refinement- originated in laboratory animal research (Russel and Burch, 1959) and is now increasingly integrated into livestock and field-based research ethics (Tannenbaum and Bennett, 2015). Although not a welfare assessment tool per se, the 3Rs promote ethical research design by minimizing animal pain and distress. In goat research, this includes using less invasive methods, ensuring effective analgesia, and avoiding terminal procedures when possible. The 3Rs thus reinforce the ethical imperative to prevent unnecessary suffering in studies intended to improve animal welfare.

Beyond these conceptual frameworks, effective welfare evaluation depends on integrating animal-based indicators (e.g., behavioral signs and physiological markers of pain), resource-based measures (e.g., housing quality and environmental enrichment), and management-based assessments (e.g., handling practices and health care protocols). This multidimensional approach allows for accurate monitoring and continuous improvement of goat welfare in both agricultural and research settings.

While no single model offers a comprehensive solution, each provides valuable insights into different aspects of animal welfare for evaluating and improving goat welfare. Combining these frameworks allows stakeholders to better understand, evaluate, and improve welfare conditions, particularly in the recognition and management of pain. Given that pain in goats remains under recognized and under treated, the thoughtful and practical application of these frameworks is essential for advancing both scientific knowledge and humane animal care.

Pain Associated Outcome Measurements

Pain is a highly individualized and subjective experience, which presents a significant challenge for objective measurement (DeVon et al., 2014). To address this challenge, researchers

have increasingly explored the use of biomarkers; measurable changes in cells, tissues, or body fluids that can indicate what is happening in the body, whether it is a normal process, a disease, or a response to treatment (Hulka et al., 1990). Biomarkers have the potential to enhance the development of effective pain management strategies, including pharmacological treatments and medical devices (Califf et al., 2018). The use of biomarkers to identify and quantify pain has been previously investigated, showing promise in both clinical and preclinical contexts (Marchi et al., 2009).

In animal models, pain biomarkers are particularly valuable in animal models, as self-reporting is impossible, and communication is strictly non-verbal (Livingston, 2010). While conducting research in animals, biomarkers offer a quantifiable and objective means of assessing pain, improving the ability of veterinarians and researchers to recognize pain responses and administer appropriate analgesia (Schwartzkopf-Genswein et al., 2012). These tools are especially important in livestock species, where subtle pain behaviors may go unnoticed, and effective pain mitigation is essential for both ethical and productivity-related reasons.

Infrared Thermography. Infrared thermography (IRT) is a non-contact imaging technique used to acquire and process thermal data based on the detection of infrared radiation (Maldague, 2001; Usamentiaga, 2014). As a non-invasive and objective method, IRT is particularly useful for detecting changes in surface temperature and tissue perfusion, which are often associated with inflammation and pain (Ramirez-GarciaLuna, 2022). IRT has been widely applied in livestock species, including cattle and goats, to assess inflammatory responses following painful procedures such as branding (Martin et al., 2022a), disbudding/dehorning (Martin et al., 2022b; Martin et al., 2022c; Hempstead et al., 2018a; Alvarez et al., 2019), castration (Kleinhenz et al., 2018; Curtis et al., 2024; Weeder et al., 2024), and lameness induction (Kleinhenz et al., 2019b;

Reppert et al., 2020; Weeder et al., 2023). Beyond pain related applications, IRT has also been utilized to assess udder health, heat stress, fever, hoof and limb conditions, transport stress, meat quality, and autonomic nervous system responses in cattle (Stokes et al., 2012; Zaninelli et al., 2018; Macmillam et al., 2019; Cuthbertson et al., 2020; Irdis et al., 2021), sheep (Castro-Costa et al., 2014; Perez de Diego et al., 2013; Joy et al., 2022; Byrne et al., 2019; Sutherland et al., 2020), and in goats (Korelidou et al., 2025; Reppert et al., 2020). Among these, lameness induction in livestock species has yielded the most consistent and significant increases in surface temperature, highlighting IRT's potential for detecting limb associated inflammation. This method offers several advantages, including that it is non-invasive, painless, rapid, and can be conducted with minimal disruption to the animal's natural state (Nahm, 2013). However, IRT is not without limits. Environmental factors such as sunlight, humidity, and wind can heavily influence the response of IRT, necessitating careful consideration or correction during data collection and analysis (Church et al., 2014). Moreover, while IRT can detect physiological changes linked to inflammation or stress, it does not directly measure pain. False positives may arise in situations where temperature changes are driven by non-painful factors, such as environmental heat exposure or acute stress responses.

Mechanical Nociception Threshold. Nociceptors are specialized sensory receptor neurons that detect potentially tissue damaging stimuli in the environment (Smith and Lewin, 2009). These receptors can be activated by mechanical, thermal, or chemical stimuli; initiating the process of nociception, which is the neural encoding of noxious events (Tracey, 2017). This technique involved the controlled application of quantifiable mechanical stimulus to a specific body region until a behavioral or physiological response is observed, at which point the stimulus is immediately withdrawn (Love, 2011). Pressure algometry has been utilized before to analyze

pain in cattle, sheep, pigs, and in goats, particularly during induced respiratory disease (BRD) (Martin et al., 2022d), lameness (Welsh and Nolan, 1995; Tapper et al., 2013; Kleinhenz et al., 2019b; Weeder et al., 2023), and disbudding (Adcock and Tucker, 2018; Kongara et al., 2023b). This technique is effective for differentiating between painful and non-painful tissues, making it a valuable tool in both research and clinical contexts (Haussler, 2020). However, a learning curve exists, and training is necessary to collect reliable data (Haussler et al., 2008; De Heus et al., 2010; Visser et al., 2019). Pressure algometry has been recognized to be a repeatable, semi-objective measure of pain that is low cost and portable (Haussler, 2020). Measurements should be limited to one consistent user to avoid variability, as inter-examiner reliability is poor in pain settings (Pelfort et al., 2015; Haussler et al., 2008; Visser et al., 2019). Despite its utility, mechanical nociceptive threshold (MNT) data can be influenced by several factors, including individual animal behavior, species-specific pain thresholds, and operator technique, which complicates cross-study comparisons. As such, continued refinement, validation, and standardization of pressure algometry protocols are needed to improve its reliability and broader application in animal welfare research.

Cortisol. Cortisol is a glucocorticoid steroid hormone secreted by the adrenal cortex in response to physiological or psychological stress, under the regulation of the hypothalamic pituitary adrenal (HPA) axis (Coetzee, 2011). Following exposure to a stressor, cortisol levels can remain elevated for several hours, reflecting the body's attempt to restore homeostasis (Kyrou et al., 2006). While adaptive in the short term, prolonged elevation of cortisol can have detrimental effects, including immunosuppression (Hopster et al., 1998; Tuchscherer et al., 2004), reduction in antibody levels (Roth, 1985) and impairment of reproductive function (Burnett et al., 2015). Cortisol has been widely studied as a biomarker of stress and pain in livestock. However, its

specificity as an indicator of pain is limited, as cortisol levels can increase due to non-painful stimuli such as hunger, circadian rhythm, environmental temperature, or handling (Kleinhenz et al., 2017). These confounding variables reduce its reliability as a standalone measure of pain. Therefore, while elevated cortisol can suggest that an animal is experiencing a stress response that may include pain, it should be interpreted cautiously and ideally used in combination with behavioral observations or other physiological markers to improve accuracy in welfare assessments.

Substance P. Substance P is a neuropeptide and neurotransmitter that plays a crucial role in the transmission of noxious stimuli and in modulating pain and inflammatory responses (DeVane, 2001; Suvas, 2017). Released from dorsal root ganglia in response to noxious stimuli, substance P facilitates communication between peripheral tissue and the central nervous system (Snijdelaar, 2000). This makes substance P a potential indicator of nociceptive activation rather than general stress. For example, plasma substance P concentrations remained high in cats handled and receiving periodontal treatment, even as cortisol concentrations significantly decreased (Zenter et al., 1996). Similarly, in calves undergoing cautery dehorning, concentrations of substance P decreased significantly after meloxicam administration. This demonstrates the biomarker's potential sensitivity to analgesic intervention (Allen et al., 2013). Because substance P is more closely associated with nociceptive signaling than with general stress responses, it may help researchers distinguish between painful stimuli and non-painful stressors in livestock species (Coetzee et al., 2007). However, despite its theoretical value, the use of substance P as a reliable biomarker of pain across food animal species has produced inconsistent results (Kleinhenz et al., 2019b; Martin et al., 2022d; Weeder et al., 2023; Weeder et al., 2024). This insinuates that further research is needed to validate this biomarker's use in

species such as cattle or goats. Additionally, the invasive nature of blood sampling required to measure substance P levels poses a methodological challenge, as the act of sampling may itself alter physiological responses and confound results.

Kinetic Gait Analysis. Lameness, broadly defined as the inability to express normal gait patterns in one or more limbs, is a widely accepted research model for studying pain in animals (Oehm et al., 2019). Traditional visual lameness scoring systems are often subjective and susceptible to poor inter-rater reliability, limiting their consistency and utility in research and clinical settings (Eriksson et al., 2020). To try and overcome this limitation, kinetic gait analysis has emerged as a more objective method for quantifying pain-related changes in locomotion. One such system is a commercially available pressure mat floor system produced by Tekscan (South Boston, USA), which allows for detailed assessment of gait dynamics (Coetzee et al., 2017). Animals are conditioned to walk across the pressure mat, and gait parameters such as contact pressure, impulse, stance time, and gait velocity are recorded and analyzed (Coetzee et al., 2014). This approach has been used across several livestock species, including cattle, sheep, goats, and horses, in studies investigating pain associated with castration, induced respiratory disease (BRD), lameness, dehorning, and postpartum recovery (Ishihara et al., 2009; Kleinhenz et al., 2018; Kleinhenz et al., 2019a; Kleinhenz et al., 2019b; Martin et al., 2022b; Martin et al., 2022d; Reppert et al., 2019; Viscardi et al., 2021; Weeder et al., 2023; Weeder et al., 2024). In cattle, kinetic parameters differences in weight distribution, total limb impulse, mean stance time, and gait velocity can indicate changes in locomotion associated with discomfort or impaired welfare (Chapinal et al., 2009; Kleinhenz et al., 2018; Kleinhenz et al., 2019a; Martin et al., 2022b; Martin et al., 2022d). In goats, differences in gait velocity, force, impulse, and contact pressure have been observed in response to painful procedures such as disbudding and

castration, demonstrating the sensitivity of this method to pain-related locomotor changes, independent of individual variation (Reppert et al., 2020; Weeder et al., 2023; Weeder et al., 2024). Notably, kinetic gait analysis was a pivotal outcome in the approval process of Banamine Transdermal Pour-On for cattle, underscoring its importance in evidence based analgesic evaluation (Merck Animal Health, 2024). The strengths of kinetic gait analysis include its semi-objective nature, ability to detect subtle asymmetries in limb use, and its validated application across multiple species. However, the method also has its limitations, such as the high cost of equipment, the complexity of data analysis software, and the requirement for animal training to ensure reliable and repeatable results.

Behavior Scoring. Physical injuries or illnesses can cause pain in animals (Weary et al., 2009). Since pain has a strong emotional component, behavioral evaluation plays a critical role in assessing pain responses (Bushnell et al., 2013). Observable signs such as reduced eating, drinking, or activity level can indicate illness or general malaise (Weary et al., 2009). Behavior scoring systems can support management decisions and welfare assessments, particularly when used for broad welfare monitoring and early detection of health concerns (Matheson et al., 2010) Observing behavior is a valuable assessment tool, as it requires minimal equipment and intervention (Johnston et al., 2022). This method offers a holistic view of animal welfare but may be confounded by non-pain related stressors, observer bias, or environmental influences. Additionally, limitations such as equipment costs, scorer training time, and the potential loss of nuanced behavioral information should be considered when implementing this approach.

Grimace Scoring. Facial expressions, such as changes in facial muscle tension and ear position, are increasingly recognized as sensitive and reliable indicators of pain and distress in livestock (Guesgen et al., 2016; Viscardi et al., 2017; McLennan et al., 2016).Grimace scoring, initially

developed for laboratory animals, has emerged as a promising method for detecting acute pain across species. It involves evaluating specific facial action units (FAUs), such as orbital tightening, cheek bulging, and changes in ear and nostril position, which typically occur involuntarily in response to pain (Langford et al., 2010). Grimace scales have been used before in several livestock species including cattle (Farghal et al., 2024), sheep (Viscardi et al., 2021), and pigs (Viscardi et al., 2017). However, research on their use of goats remains scarce. To date, only one study has applied grimace scoring to adult caprine patients (Weeder et al., 2023), highlighting a notable gap in the literature. One key advantage of grimace scoring is its practicality. It is less invasive and labor-intensive than many other pain assessment methods, requiring minimal equipment and allowing trained observers to use it effectively (Leach et al., 2012; Sotocinal et al., 2011). This makes it especially useful for identifying acute pain during or shortly after procedures such as castration, disbudding, or surgical interventions. Despite its benefits, this method also has limitations. Facial morphology varies across and within species, complicating the development of standardized scoring systems. Environmental factors, such as lighting, animal movement, or restraint, can hinder accurate observation and reduce reliability in field settings. Furthermore, grimace scoring is most effective for detecting acute pain; its sensitivity to chronic or low-grade discomfort remains uncertain (Langford et al., 2010; Viscardi et al., 2017). Given the importance of accessible and reliable pain assessment tools in animal welfare, further research is needed to validate and adapt grimace scales for goats.

Visual Lameness Scoring. When assessing lameness in large groups of livestock, it is essential to use a scoring system that is both quick and reliable (Thomsen, 2008). Most visual locomotion scoring systems use a five-point ordinal scale, where each score corresponds to a distinct level of lameness severity based on observable gait abnormalities (Sprecher et al., 1997; Flower and

Weary, 2006). These systems are particularly well-suited for herd-level surveillance for identifying animals with moderate to severe lameness that require intervention. Visual locomotion has been applied across several species, including cattle, horses, and goats (Kleinhenz et al., 2019b; Pfau et al., 2018; Reppert et al., 2020; Weeder et al., 2023). It offers a rapid method for detecting locomotor issues in the field. However, this approach has notable limitations. It is inherently subjective, often suffers from poor inter-observer reliability, and may fail to detect subtle or early-stage lameness. Additionally, multiple visual lameness scoring systems exist, many of which are slight modifications of earlier models (Thomsen, 2008).

Visual Analogue Scoring. The Visual Analogue Scoring (VAS) was originally developed for assessing pain in human patients (Haefeli and Elfering, 2006). It consists of a 100mm line, anchored by descriptors such as “no pain” at one end and “pain could not be any worse” at the other (Crichton, 2001). Human patients or trained observers mark a point along a line to represent perceived pain intensity. Compared to numerical rating scales, the VAS has been shown to detect more subtle changes in pain levels, making it particularly useful for evaluating lameness associated pain (Welsh et al., 1993; Hudson et al., 2004). VAS scoring has been used in a variety of livestock species. In cattle, it has been applied to assess pain associated with procedures and conditions such as castration, displayed abomasum, dehorning, lameness, mastitis, and uveitis (Tschoner et al., 2024; Martin et al., 2020; Martin et al., 2022c) sheep, (Welsh et al., 1993), and in goats following lameness induction and castration (Weeder et al., 2023; Weeder et al., 2024). The VAS has demonstrated high validity, retest reliability, and repeatability as an internal scale (Gupta et al., 2016; Rosier et al., 2002). VAS is best suited for research settings or clinical evaluations where subtle changes in pain intensity need to be captured, particularly in cases of lameness or post-surgical recovery. It offers a sensitive and

flexible method for pain assessment, buts its effectiveness depends heavily on the skill and consistency of trained observers. As such, it is not appropriate for large-scale herd level assessments or for use by untrained personnel. While originally developed for humans, VAS has become a valuable tool in animal pain research.

Painful Events in Animal Production

Castration

Castration, the removal process or intentional ineffectiveness of the testicles in male livestock, is a common husbandry practice aimed at reducing aggressive behavior, enhancing meat quality, and preventing unintended breeding (Rault et al., 2011; Hood 1981; Stafford and Mellow, 2005). Despite its routine use, castration has been shown to be a painful procedure, particularly when performed without analgesia, as evidenced by observable physiological and behavioral stress responses in cattle (Coetzee et al., 2010; Coetzee, 2011). This husbandry procedure may be carried out through physical, chemical, or hormonal methods. All methods intended to result in tissue damage to the testicular tissue (Coetzee et al., 2010). Regardless of the method employed, all castration methods consistently cause inflammation, stress, suppression of immune function, and varying degrees of pain (Searle et al., 1999). Although inherently painful, castration in livestock species is frequently conducted without any form of pain management, raising significant animal welfare concerns.

Disbudding

Disbudding in livestock refers to the removal of horn epithelium, typically to prevent injuries to other animals and handlers (Sutherland et al., 2019). This routine husbandry procedure

has been widely acknowledged as a painful experience in goats and kids and is associated with reduced animal welfare (Alvarez et al, 2015; Hempstead et al., 2018a; Hempstead et al., 2018b; Alvarez et al., 2019). Thermal disbudding, commonly performed with a hot iron tool, has been linked to acute physiological pain, stress responses, pain-related behaviors, and elevated cortisol levels in goat kids (Molaei et al., 2015). When compared to calves, kids are more vulnerable to complications due to their thinner skulls and the younger age at which they are commonly disbudded. These factors can lead to complications and increased risk of thermal injury during thermal disbudding (Hempstead et al., 2020). Improper use of cautery disbudding can lead to severe consequences, including skull damage and a heightened risk of bacterial meningoencephalitis (Sanford, 1989; Thompson et al., 2005; Hempstead et al., 2020).

Lameness

Lameness is defined as a manifestation of pain or a mechanical defect, that results in a gait abnormality, characterized by limping (Ross and Dyson, 2011). Lame animals can cause economic loss to producers due to decreased productivity, cost of treatment, and culling of animals (Christodoulopoulos, 2009). Treatment of goats presenting with lameness is challenging due to an incomplete understanding of both physiological and behavioral pain responses (Weeder et al., 2023). Sole ulcers, subsolar abscesses, and interdigital dermatitis are all hoof lesions that are associated with causing lameness in goats (Hill et al., 1997; Christodoulopoulos, 2009; Crosby-Durrani et al., 2016). Common diseases such as Caprine Arthritis and Encephalitis (CAE), septic arthritis, laminitis, mycoplasmosis, or white muscle disease can cause chronic lameness in goats if not treated in a timely manner (MacKay, 2024). Lameness models have been used and developed in goats and cattle to mimic lameness conditions like foot rot or septic arthritis for research purposes (Berg et al., 1984; Reppert et al., 2020; Weeder et al., 2023;

Kotschwar et al., 2009). In these models, lameness was accomplished using an induction agent like ethylenediamine dihydriodide, amphotericin B, or kaolin-carrageenan into a joint or joint space.

AMDUCA

The Animal Medicinal Drug Use Clarification Act of 1994 (AMUDCA) legalizes extra label drug use in animals under the supervision of a veterinarian and in accordance with FDA regulations (Geyer, 1997). Because there is a limited number of approved drugs available for use, veterinarians often must utilize drugs in an extra-label manner in animals (Drew, 1998). Apart from pain associated with interdigital phlegmon in cattle, there are no drugs approved for pain control in livestock species. This includes pain associated with castration, dehorning, or other pain-producing conditions. AMDUCA states specifically "Extra-label drug use (ELDU) requires that some specific conditions be met which include: (1) ELDU is allowed only by or under the supervision of a veterinarian, (2) ELDU is allowed only for US Food and Drug Administration (FDA)–approved animal and human drugs; (3) ELDU is only permitted when the health of the animal is threatened and not for production purposes; (4) ELDU in feed is prohibited, and (5) ELDU is not permitted if it results in a violative drug residue in food intended for human consumption," (FDA, 1994; Coetzee, 2013). The AMDUCA policy allows veterinarians to practice medicine in an effective and safe manner without sacrificing the importance of overall public health (Geyer, 1997). Under AMDUCA, the prescribing veterinarian is responsible for establishing appropriate meat and milk withdrawal times to ensure the safety of consumers.

Analgesia

Analgesia is the absence of pain in a situation that would normally produce pain (Danneman, 1997). Despite advancements in veterinary medicine and increased awareness of animal sentience, pain in animals has historically been underrecognized and poorly managed. For instance, a study reviewing surgical cases in dogs at a veterinary teaching hospital between 1983 and 1989 found that only 40% of patients received any form of analgesia postoperatively (Hansen and Hardi, 1993). Although pain management in companion animals has since improved, the same progress has not been consistently reflected in livestock species, where economic, regulatory, and logistic barriers often constrain the routine use of analgesics.

In food producing animals such as cattle, pain management remains suboptimal due to several persistent challenges. These include a limited number of approved drugs, delayed onset or short duration of action, cost considerations, and the requirement for meat and milk withholding periods after drug administration (Kleinhenz et al., 2021). These issues create hesitation among producers and veterinarians, even when common husbandry procedures like disbudding, dehorning, and castration are known to be painful and to have long-term welfare implications.

In small ruminants, especially goats, the problem is more acute. Despite growing recognition that goats can experience pain, they remain underrepresented in pain management research. As of this writing, there are no FDA-approved analgesic drugs specifically labeled for pain relief in goats, including for common procedures such as castration, disbudding, or conditions like lameness. This lack of regulatory approval leads to reliance on extra-label drug use, which is permitted under the Animal Medicinal Drug Use Clarification Act (AMDUCA) but introduces concerns around dosing accuracy, safety, drug residues, and legal liability.

Moreover, while analgesia is a critical component of animal welfare, it is often inconsistently given, particularly in extensive or resource limited systems. As Roberston (2001) pointed out, many animals still go untreated or undertreated for pain despite the known benefits of analgesia on animal well-being, productivity, immune function, and recovery times. In goats, the underuse of analgesia may also be tied to limited validated tools for pain assessment, making it difficult for caregivers to recognize and respond to pain effectively.

The combination of regulatory limitations, practical constraints, and gaps in pain recognition creates a significant welfare issue in goat production systems. Addressing this problem requires developing approved and accessible analgesic options and better pain assessment methods that can reliably detect and quantify pain in goats under real-world conditions.

Mechanism of Action: Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Before 1971, the mechanism by which non-steroidal anti-inflammatory drugs (NSAIDs) relieved pain was not well understood. It was later discovered that arachidonic acid metabolism in the body leads to the production of prostaglandins and eicosanoids-molecules that play a key role in pain and inflammation pathways. NSAIDs work by inhibiting the cyclooxygenase (COX) pathway, which is essential for prostaglandin synthesis (Livingston et al., 2000). There are two recognized forms of the cyclooxygenase enzyme: COX-1 and COX-2 (Xie et al., 1991). COX-1 is involved in maintaining protective functions in the stomach and kidneys, whereas COX-2 is primarily induced in response to inflammatory stimuli and is responsible for producing prostaglandins that cause pain swelling, or inflammation. Different NSAIDs vary in their selectivity for COX-1 and COX-2. NSAIDs that selectively inhibit COX-2 are preferred for their

ability to reduce pain and inflammation while minimizing the gastrointestinal and renal side effects associated with COX-1 inhibition (Vane and Botting, 1998).

Common Systemic Analgesic Options in Livestock

Firocoxib

Firocoxib is a selective COX-2 inhibitor approved for use in horses (0.09-0.15 mg/kg) and dogs (2.27 mg/kg) (Boehringer Ingelheim, 2025a; Boehringer Ingelheim, 2025b) and is commonly prescribed for pain and inflammation associated with osteoarthritis (Rangel-Nava et al., 2019). This drug is not currently approved for use in humans, cattle, pigs, sheep, or goats. Firocoxib has been used in an extra-label manner in cattle, pigs, and sheep for pain in previous studies (Stock et al., 2014; Coetzee et al., 2019; Kongara et al., 2023a). This drug may be ideal for younger animals like calves, as it provides anti-inflammatory therapy while decreasing the risk for gastric ulcers (Marshall, 2009). The pharmacokinetics of firocoxib in goats have previously been researched. The mean bioavailability of oral firocoxib in adult goats has been reported to be 71% with a half-life of 21.51h (Stuart, 2019). Firocoxib at a dose of 1 mg/kg per os did not provide analgesia in goats following surgical castration or lameness induction (Weeder et al, 2024; Weeder et al., 2025.

Flunixin meglumine

Transdermal flunixin meglumine is FDA approved to treat pyrexia, pain associated with BRD and foot rot, and mastitis pain in beef and dairy cattle (Merck Animal Health, 2024). In cattle, the transdermal formulation is successful at treating pain and has been shown to have a longer half-life compared to the intravenous formulation (Kleinhenz et al., 2016, Kleinhenz et al., 2019b). The transdermal formulation of flunixin has been noted by producers to be easier to

administer when compared to the intravenous formulation (Meira et al., 2022). Flunixin meglumine transdermal is currently not approved for use in pigs, sheep, or goats. The transdermal formulation of this drug has been researched in an extra-label manner in horses, donkeys, pigs, alpacas, and goats (Knych et al., 2021; McLean et al., 2023; Lopez-Soriano et al., 2022; Reppert et al., 2019a; Reppert et al., 2019b). Intra-venous flunixin meglumine pharmacokinetics has been previously reported in adult meat goats with a half-life of 6.032h (range: 4.735-9.244h) and in adult dairy goats with a half-life of 3.6h (range: 2.0-5.0h) (Reppert et al., 2019a; Konigsson et al., 2003). The pharmacokinetics of flunixin meglumine transdermal was previously described in adult meat goats with a half-life of 43.12h and a bioavailability of 24.76% (Reppert et al., 2019a), young male goats with a half-life of 7.16+-2.06h (Graves et al., 2020), and in lactating dairy goats with a half-life of 21.63h (Meira et al., 2022).

Ketoprofen

Ketoprofen has been used to manage pain, pyrexia, inflammatory processes and musculoskeletal issues in both children and adults (Dowd et al., 2017; Gallelli et al., 2007). It has been reported to be used in cats and dogs for acute pain, osteoarthritis, and pyrexia (Maddison et al., 2008; Glew et al., 1996). The pharmacokinetic parameters of ketoprofen have been investigated in lactating goats with a reported half-life of 0.08+-0.05h (Musser et al., 1998). Separate KTP enantiomers of ketoprofen have been investigated in goats previously (Arifah et al., 2003). Ketoprofen has also been investigated in reducing pain in piglets after surgical castration (Viscardi and Turner, 2018) and on acute phase protein concentrations after castration in goats (Karademir et al., 2016).

Meloxicam

Meloxicam is used to treat conditions like soft tissue injuries, mastitis, and inflammatory conditions in small and large animals (Mathews, 2002). Meloxicam is also used in humans to treat osteoarthritis and pain (Cooper et al., 2019). This drug is not currently approved for use in goats. Meloxicam can be used in the U.S. in an extra-label manner in livestock species such as cattle, pigs, sheep, and goats (Coetzee et al., 2010; Viscardi et al., 2018; Crawford et al., 2023; Weeder et al., 2024). Inhibiting the COX-2 isoenzyme helps decrease the chance of gastric ulcers after NSAID administration in patients (Distel et al., 1995). Oral meloxicam pharmacokinetics have been reported in adult goats with a half-life of 11.8h and a bioavailability of 79% (Ingvast-Larsson et al., 2011). Meloxicam has been shown to have positive effect in goats undergoing surgical castration when given orally at 2 mg/kg initially and 1 mg/kg 24 and 48 hours later (Weeder et al., 2024).

Residue Profiles

Animal products are protein rich, which human beings often consume to fulfill their nutritional requirements for body function (Rokka et al., 2005). In the U.S., the FDA sets maximum permitted concentrations for veterinary drug residues as part of the drug approval process (Baynes et al., 2016). Drugs commonly given to animals to enhance production, treat diseases, and mitigate pain have associated risks to human health (Beyene, 2016). Depending on the drug's chemical and pharmacokinetic properties, residues may accumulate in animal tissue or milk (Rana et al., 2019). Food developed from animals must be monitored for drug residue violations (Reeves, 2010). Examples of food derived from animals that must comply with include meat products, tissue products, and milk products. Common drug groups that can cause residue concern in animal products include antibiotics or antimicrobials, anti-parasitic, sedative drugs, and nonsteroidal anti-inflammatory drugs (NSAIDS) (Beyene, 2016).

A residue or below tolerance level is considered safe for human consumption when food at that level is consumed daily for a human lifetime (Baynes et al., 2016). To achieve a below tolerance level, animals given a drug must go through a withdrawal period. The withdrawal period is described as the amount of time between the last administration of drug to the safe point for an animal to produce eggs, milk, or be slaughtered (Reeves, 2010). The FDA's Guidance for Industry #207: Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Marker Residue Depletion Studies to Establish Product Withdrawal Periods must be followed while completing research regarding drug withdrawal in animal tissue, animal meat, or animal milk (FDA, 2015). The required withdrawal interval is dependent on the drugs pharmacokinetics and depletion profile (Lees and Toutain, 2012).

FARAD

The Food Animal Residue Avoidance Databank (FARAD) was created in 1982 as an outreach program between cooperative state governments and the federal government (Payne et al., 1999). FARAD is a computerized databank needed to solve drug or residue contamination issues in food animals (Riviere et al., 1986). The FARAD program works to serve the public by involving food animal veterinarians to analyze and multiply information regarding residue withdrawal in food animal products (Sundlof et al., 1991). This comprehensive database has evolved into an expert led residue avoidance decision assistance program, with resources like telephone services, internet database assistance, and printed materials for food animal veterinarians to assess residue withdrawal times in animal products (Sischo et al., 1999). Veterinarians using drugs in an ELDU manner are encouraged to contact FARAD for assistance in applying meat and milk withdrawal intervals.

Conclusions

Effectively mitigating or managing pain in goats starts with proper identification of species-specific pain behaviors, identification of relevant pain biomarkers, and correct selection and usage of analgesia based on the level of pain displayed. The unique characteristics of the patients, such as age, weight, breed, sex, and source of pain should also be taken into consideration. Goats experiencing lameness or castration pain can benefit from analgesic intervention through AMDUCA. Withdrawal times for tissue and milk should be recognized and ensured for public safety with the help of FARAD and research knowledge around accurate residue profiles.

References

Adcock, S. J. J., & Tucker, C. B. (2018). The effect of disbudding age on healing and pain sensitivity in dairy calves. J Dairy Sci, 101(11), 10361-10373. https://doi.org/10.3168/jds.2018-14987

Allen, C., Hopewell, S., & Prentice, A. (2005). Non-steroidal anti-inflammatory drugs for pain in women with endometriosis. Cochrane Database Syst Rev (4), CD004753. https://doi.org/10.1002/14651858.CD004753.pub2

Allen, K. A., Coetzee, J. F., Edwards-Callaway, L. N., Glynn, H., Dockweiler, J., KuKanich, B.,Bergamasco, L. (2013). The effect of timing of oral meloxicam administration on physiological responses in calves after cautery dehorning with local anesthesia. J Dairy Sci, 96(8), 5194-5205. https://doi.org/10.3168/jds.2012-6251

Alvarez, L., Adcock, S. J. J., & Tucker, C. B. (2019). Sensitivity and wound healing after hot-iron disbudding in goat kids. J Dairy Sci, 102(11), 10152-10162. https://doi.org/10.3168/jds.2018-16062

Alvarez, L., De Luna, J. B., Gamboa, D., Reyes, M., Sánchez, A., Terrazas, A.,Galindo, F. (2015). Cortisol and pain-related behavior in disbudded goat kids with and without cornual nerve block. Physiol Behav, 138, 58-61. https://doi.org/10.1016/j.physbeh.2014.10.026

Alvarez, L., Nava, R. A., Ramírez, A., Ramírez, E., & Gutiérrez, J. (2009). Physiological and behavioural alterations in disbudded goat kids with and without local anaesthesia. Applied Animal Behaviour Science, 117(3), 190-196. https://doi.org/https://doi.org/10.1016/j.applanim.2009.01.001

Arifah, A. K., Landoni, M. F., & Lees, P. (2003). Pharmacodynamics, chiral pharmacokinetics, and PK-PD modelling of ketoprofen in the goat. J Vet Pharmacol Ther, 26(2), 139-150. https://doi.org/10.1046/j.1365-2885.2003.00455.x

Bain, M. (2024). Animal Welfare. In Welfare of Livestock/Production Animals. Merck Veterinary Manual.

Baynes, R. E., Dedonder, K., Kissell, L., Mzyk, D., Marmulak, T., Smith, G.,Riviere, J. E. (2016). Health concerns and management of select veterinary drug residues. Food Chem Toxicol, 88, 112-122. https://doi.org/10.1016/j.fct.2015.12.020

Beausoleil, N. J., & Mellor, D. J. (2015). Advantages and limitations of the Five Domains model for assessing welfare impacts associated with vertebrate pest control. N Z Vet J, 63(1), 37-43. https://doi.org/10.1080/00480169.2014.956832

Berg, J. N., Maas, J. P., Paterson, J. A., Krause, G. F., & Davis, L. E. (1984). Efficacy of ethylenediamine dihydriodide as an agent to prevent experimentally induced bovine foot rot. Am J Vet Res, 45(6), 1073-1078.

Beyene, T. (2016). Veterinary Drug Residues in Food-animal Products: Its Risk Factors and Potential Effects on Public Health. In (Vol. 7:1). Journal of Veterinary Science and Technology.

Bickell, E. G. (2023). The Animal Welfare Act: Background and Selected Issues. In.

Brambell, R. (1965). Report of the technical committee to enquire into the welfare of animals kept under intensive livestock husbandry systems. In. London: Her Majesty's Stationary Office.

Broom, D. M. (1991). Animal welfare: concepts and measurement. J Anim Sci, 69(10), 4167-4175. https://doi.org/10.2527/1991.69104167x

Browning, H. (2020). The Natural Behavior Debate: Two Conceptions of Animal Welfare. J Appl Anim Welf Sci, 23(3), 325-337. https://doi.org/10.1080/10888705.2019.1672552

Burnett, T. A., Madureira, A. M. L., Silper, B. F., Tahmasbi, A., Nadalin, A., Veira, D. M., & Cerri, R. L. A. (2015). Relationship of concentrations of cortisol in hair with health, biomarkers in blood, and reproductive status in dairy cows. Journal of Dairy Science, 98(7), 4414-4426. https://doi.org/https://doi.org/10.3168/jds.2014-8871

Bushnell, M. C., Ceko, M., & Low, L. A. (2013). Cognitive and emotional control of pain and its disruption in chronic pain. Nat Rev Neurosci, 14(7), 502-511. https://doi.org/10.1038/nrn3516

Byrne, D. T., Berry, D. P., Esmonde, H., McGovern, F., Creighton, P., & McHugh, N. (2019). Infrared thermography as a tool to detect hoof lesions in sheep. Transl Anim Sci, 3(1), 577-588. https://doi.org/10.1093/tas/txy132

Califf, R. M. (2018). Biomarker definitions and their applications. Exp Biol Med (Maywood), 243(3), 213-221. https://doi.org/10.1177/1535370217750088

Carr, D. B., & Goudas, L. C. (1999). Acute pain. Lancet, 353(9169), 2051-2058. https://doi.org/10.1016/S0140-6736(99)03313-9

Carter, B. S., & Brunkhorst, J. (2017). Neonatal pain management. Semin Perinatol, 41(2), 111-116. https://doi.org/10.1053/j.semperi.2016.11.001

Castro-Costa, A., Caja, G., Salama, A. A. K., Rovai, M., Flores, C., & Aguiló, J. (2014). Thermographic variation of the udder of dairy ewes in early lactation and following an Escherichia coli endotoxin intramammary challenge in late lactation. Journal of Dairy Science, 97(3), 1377-1387. https://doi.org/https://doi.org/10.3168/jds.2013-6978

Chamberlain, D. B. (1999). Babies Don't Feel Pain: A Century of Denial in Medicine. In (Vol. 1, pp. 145-168). Journal of Prenatal and Perinatal Psychology & Health 14.

Chapinal, N., de Passillé, A. M., & Rushen, J. (2009). Weight distribution and gait in dairy cattle are affected by milking and late pregnancy. J Dairy Sci, 92(2), 581-588. https://doi.org/10.3168/jds.2008-1533

Chou, R., & Huffman, L. H. (2007). Nonpharmacologic Therapies for Acute and Chronic Low Back Pain: A Review of the Evidence for an American Pain Society/American College of Physicians Clinical Practice Guideline. Annals of Internal Medicine, 147, 492-504.

Church, J. S., Hegadoren, P. R., Paetkau, M. J., Miller, C. C., Regev-Shoshani, G., Schaefer, A. L., & Schwartzkopf-Genswein, K. S. (2014). Influence of environmental factors on infrared eye temperature measurements in cattle. Res Vet Sci, 96(1), 220-226. https://doi.org/10.1016/j.rvsc.2013.11.006

Coetzee, J. F. (2011). A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the United States. Applied Animal Behaviour Science, 135, 192-213.

Coetzee, J. F. (2013). Assessment and management of pain associated with castration in cattle. Vet Clin North Am Food Anim Pract, 29(1), 75-101. https://doi.org/10.1016/j.cvfa.2012.11.002

Coetzee, J. F., Lubbers, B. V., Toerber, S. E., Gehring, R., Thomson, D. U., White, B. J., & Apley, M. D. (2007). Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. In (Vol. 69:6). American Veterinary Medical Association.

Coetzee, J. F., Mosher, R. A., Anderson, D. E., Robert, B., Kohake, L. E., Gehring, R.,Wang, C. (2014). Impact of oral meloxicam administered alone or in combination with gabapentin on experimentally induced lameness in beef calves. J Anim Sci, 92(2), 816-829. https://doi.org/10.2527/jas.2013-6999

Coetzee, J. F., Nutsch, A. L., Barbur, L. A., & Bradburn, R. M. (2010). A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. BMC Vet Res, 6, 12. https://doi.org/10.1186/1746-6148-6-12

Coetzee, J. F., Sidhu, P. K., Seagen, J., Schieber, T., Kleinhenz, K., Kleinhenz, M. D.,Lechtenberg, K. (2019). Transmammary delivery of firocoxib to piglets reduces stress and improves average daily gain after castration, tail docking, and teeth clipping1. J Anim Sci, 97(7), 2750-2768. https://doi.org/10.1093/jas/skz143

Cooper, C., Chapurlat, R., Al-Daghri, N., Herrero-Beaumont, G., Bruyère, O., Rannou, F.,Reginster, J. Y. (2019). Safety of Oral Non-Selective Non-Steroidal Anti-

Inflammatory Drugs in Osteoarthritis: What Does the Literature Say? Drugs Aging, 36(Suppl 1), 15-24. https://doi.org/10.1007/s40266-019-00660-1

Crawford, P. E., Hamer, K., Lovatt, F., Behnke, M. C., & Robinson, P. A. (2023). Improving analgesia provision for sheep: An analysis of farm medicine records and attitudes towards pain relief on sheep farms in Northern Ireland. Vet Rec Open, 10(2), e75. https://doi.org/10.1002/vro2.75

Cuthbertson, H., Tarr, G., Loudon, K., Lomax, S., White, P., McGreevy, P.,González, L. A. (2020). Using infrared thermography on farm of origin to predict meat quality and physiological response in cattle (Bos Taurus) exposed to transport and marketing. Meat Science, 169, 108173. https://doi.org/https://doi.org/10.1016/j.meatsci.2020.108173

D., F. (2003). Assessing Animal Welfare at the Farm and Group Level: The Interplay of Science and Values. In (Vol. 12 (4), pp. 433-443). Animal Welfare.

Danneman, P. J. (1997). Chapter 6 - Monitoring of Analgesia. In D. F. Kohn, S. K. Wixson, W. J. White, & G. John Benson (Eds.), Anesthesia and Analgesia in Laboratory Animals (pp. 83-103). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012417570-9/50009-X

Dawkins, M. S. (2008). The Science of Animal Suffering. Ethology, 114(10), 937-945. https://doi.org/https://doi.org/10.1111/j.1439-0310.2008.01557.x

De Heus, P., Van Oossanen, G., Van Dierendonck, M. C., & Back, W. (2010). A Pressure Algometer Is a Useful Tool to Objectively Monitor the Effect of Diagnostic Palpation by a Physiotherapist in Warmblood Horses. Journal of Equine Veterinary Science, 30(6), 310-321. https://doi.org/https://doi.org/10.1016/j.jevs.2010.04.010

DeVane, C. L. (2001). Substance P: A New Era, a New Role. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 21(9), 1061-1069. https://doi.org/https://doi.org/10.1592/phco.21.13.1061.34612

DeVon, H. A., Piano, M. R., Rosenfeld, A. G., & Hoppensteadt, D. A. (2014). The association of pain with protein inflammatory biomarkers: a review of the literature. Nurs Res, 63(1), 51-62. https://doi.org/10.1097/NNR.0000000000000013

Distel, M., Mueller, C., & Bluhmki, E. (1995). Side Effects of Anti-Inflammatory Drugs IV: Global Analysis of Gastrointestinal Safety of a New NSAID, Meloxicam. In (pp. 183-193).

Drew, M. L. (1998). Update on the Animal Medicinal Use Clarification Act of 1994 Regulations for Wildlife Veterinarians. In (pp. 163-167). 1998 Proceedings AAZV and AAWV Joint Conference.

E, D. C., F, D., D, L., P, S., S, B., E, C., M, M. (2023). Welfare assessment of horses: the AWIN approach. In: Cambridge University Press.

E, M. J., W, P. S., & B, C. D. (2008). Small Animal Clinical Pharmacology, Second Edition. Saunders Ltd. https://doi.org/https://doi.org/10.1016/B978-0-7020-2858-8.X5001-5

E.M., W., G., G., & A.M., N. (1993). Comparison of a visual analogue scale and a numerical rating scale for assessment of lameness, using sheep as a model. In.

Eriksson, H. K., Daros, R. R., von Keyserlingk, M. A. G., & Weary, D. M. (2020). Effects of case definition and assessment frequency on lameness incidence estimates. Journal of Dairy Science, 103(1), 638-648. https://doi.org/https://doi.org/10.3168/jds.2019-16426

Evelyn, M. (2024). Overview of Lameness in Goats. In. Merck Veterinary Manual.

FAO. (2006). World agriculture: towards 2030/2050. In. Global Perspective Studies Unit.

FAWC, F. A. W. C. (2009). Animal Welfare in Great Britain: Past, Present, and Future. In. FAWC. 17 Smith Square, London.

Farghal, M., Pajor, E., Luna, S. P. L., Pang, D., Windeyer, M. C., & Ceballos, M. C. (2024). Development of the calf grimace scale for pain and stress assessment in castrated Angus beef calves. Sci Rep, 14(1), 25620. https://doi.org/10.1038/s41598-024-77147-6

FDA. (1994). Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). In: In: FDA (ed.) No.21 CFR 530.

FDA. (2015). The FDA's Guidance for Industry #207: Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food Producing Animals: Marker Residue Depletion Studies to Establish Product Withdrawal Periods. In: FDA.

Flower, F. C., & Weary, D. M. (2006). Effect of hoof pathologies on subjective assessments of dairy cow gait. J Dairy Sci, 89(1), 139-146. https://doi.org/10.3168/jds.S0022-0302(06)72077-X

Fraser, D., Weary, D. M., Pajor, E. A., & Milligan, B. N. (1997). A Scientific Conception of Animal Welfare that Reflects Ethical Concerns. In. WBI Studies Repository.

Gallelli, L., Colosimo, M., Pirritano, D., Ferraro, M., De Fazio, S., Marigliano, N. M., & De Sarro, G. (2007). Retrospective evaluation of adverse drug reactions induced by nonsteroidal anti-inflammatory drugs. Clin Drug Investig, 27(2), 115-122. https://doi.org/10.2165/00044011-200727020-00004

Gao, H.-M., & Hong, J.-S. (2008). Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends in immunology, 29 8, 357-365.

Geyer, R. E. (1997). Extralabel Drug Use and Compounding in Veterinary Medicine. 52(3), 291-295.

Glew, A., Aviad, A. D., Keister, D. M., & Meo, N. J. (1996). Use of ketoprofen as an antipyretic in cats. Can Vet J, 37(4), 222-225.

Guesgen, M. J., Beausoleil, N. J., Leach, M., Minot, E. O., Stewart, M., & Stafford, K. J. (2016). Coding and quantification of a facial expression for pain in lambs. Behav Processes, 132, 49-56. https://doi.org/10.1016/j.beproc.2016.09.010

Haefeli, M., & Elfering, A. (2006). Pain assessment. Eur Spine J, 15 Suppl 1(Suppl 1), S17-24. https://doi.org/10.1007/s00586-005-1044-x

Hansen, B., & Hardie, E. (1993). Prescription and use of analgesics in dogs and cats in a veterinary teaching hospital: 258 cases (1983-1989). J Am Vet Med Assoc, 202(9), 1485-1494.

Harrison, R. (1964). Animal Machines. In: Vincent Stuart Ltd.

Hart, S., Merkel, R., & Gipson, T. (2019). Current Situation and Future Prospects of the US Goat Industry. In. AgEcon.

Haussler, K. K. (2020). Pressure Algometry for the Detection of Mechanical Nociceptive Thresholds in Horses. Animals (Basel), 10(12). https://doi.org/10.3390/ani10122195

Haussler, K. K., Behre, T. H., & Hill, A. E. (2008). Mechanical nociceptive thresholds within the pastern region of Tennessee Walking Horses. Equine Vet J, 40(5), 455-459. https://doi.org/10.2746/042516408X278166

Health, M. A. (2024). Banamine Transdermal (flunixin transdermal solution).

Hempstead, M. N., Shearer, J. K., Sutherland, M. A., Fowler, J. L., Smith, J. S., Smith, J. D.,Plummer, P. J. (2020). Cautery Disbudding Iron Application Time and Brain Injury in Goat Kids: A Pilot Study. Front Vet Sci, 7, 568750. https://doi.org/10.3389/fvets.2020.568750

Hempstead, M. N., Waas, J. R., Stewart, M., Cave, V. M., & Sutherland, M. A. (2018a). Evaluation of alternatives to cautery disbudding of dairy goat kids using physiological measures of immediate and longer-term pain. J Dairy Sci, 101(6), 5374-5387. https://doi.org/10.3168/jds.2017-13814

Hempstead, M. N., Waas, J. R., Stewart, M., Dowling, S. K., Cave, V. M., Lowe, G. L., & Sutherland, M. A. (2018b). Effect of isoflurane alone or in combination with meloxicam on the behavior and physiology of goat kids following cautery disbudding. J Dairy Sci, 101(4), 3193-3204. https://doi.org/10.3168/jds.2017-13507

Hemsworth, P. H. (2007). Ethical stockmanship. Aust Vet J, 85(5), 194-200. https://doi.org/10.1111/j.1751-0813.2007.00112.x

Herzberg, D. E., & Bustamante, H. A. (2021). Animals models of chronic pain. Are naturally occuring diseases a potential model for translational research? In. Austral journal of veterinary sciences.

Hewson, C. J., Dohoo, I. R., Lemke, K. A., & Barkema, H. W. (2007). Factors affecting Canadian veterinarians' use of analgesics when dehorning beef and dairy calves. Can Vet J, 48(11), 1129-1136.

Hood, D. E., & Tarrant, P. J. V. (1981). The Problem of Dark-Cutting in Beef. Current Topics in Veterinary Medicine and Animal Science

Hopster, H., van der Werf, J. T., & Blokhuis, H. J. (1998). Stress enhanced reduction in peripheral blood lymphocyte numbers in dairy cows during endotoxin-induced mastitis. Vet Immunol Immunopathol, 66(1), 83-97. https://doi.org/10.1016/s0165-2427(98)00189-5

Hudson, J. T., Slater, M. R., Taylor, L., Scott, H. M., & Kerwin, S. C. (2004). Assessing repeatability and validity of a visual analogue scale questionnaire for use in assessing pain and lameness in dogs. Am J Vet Res, 65(12), 1634-1643. https://doi.org/10.2460/ajvr.2004.65.1634

Hulka, B. S., Wilcosky, T. C., & Griffith, J. D. (1990). Biological markers in epidemiology. In.

Huxley, J. N. (2006). Current attitudes of cattle practitioners to pain and the use of analgesics in cattle. In. VetRecord.

Idris, M., Uddin, J., Sullivan, M., McNeill, D. M., & Phillips, C. J. C. (2021). Non-Invasive Physiological Indicators of Heat Stress in Cattle. Animals, 11(1). https://doi.org/10.3390/ani11010071

Ingelheim, B. (2025a). Equioxx (firocoxib). In.

Ingelheim, B. (2025b). PREVICOX (firocoxib) for Oesteoarthrits in Dogs.

Ingvast-Larsson, C., Högberg, M., Mengistu, U., Olsén, L., Bondesson, U., & Olsson, K. (2011). Pharmacokinetics of meloxicam in adult goats and its analgesic effect in disbudded kids. J Vet Pharmacol Ther, 34(1), 64-69. https://doi.org/10.1111/j.1365-2885.2010.01195.x

Ishihara, A., Reed, S. M., Rajala-Schultz, P. J., Robertson, J. T., & Bertone, A. L. (2009). Use of kinetic gait analysis for detection, quantification, and differentiation of hind limb lameness and spinal ataxia in horses. J Am Vet Med Assoc, 234(5), 644-651. https://doi.org/10.2460/javma.234.5.644

J, D. F., S, J. B., & J, M. A. (2017). Pharmacology and Therapeutics for Dentistry. Mosby.

Johnston, C. H., Whittaker, A. L., Franklin, S. H., & Hutchinson, M. R. (2022). The Neuroimmune Interface and Chronic Pain Through the Lens of Production Animals. Front Neurosci, 16, 887042. https://doi.org/10.3389/fnins.2022.887042

Joy, A., Taheri, S., Dunshea, F. R., Leury, B. J., DiGiacomo, K., Osei-Amponsah, R.,Chauhan, S. S. (2022). Non-invasive measure of heat stress in sheep using machine learning techniques and infrared thermography. Small Ruminant Research, 207, 106592. https://doi.org/https://doi.org/10.1016/j.smallrumres.2021.106592

Karademir, U., Akin, I., Erdogan, H., Ural, K., & Asici, G. S. (2016). Effect of Ketoprofen on acute phase protein concentrations in goats undergoing castration. BMC Vet Res, 12(1), 123. https://doi.org/10.1186/s12917-016-0748-y

Kleinhenz, M. D., Gorden, P. J., Burchard, M., Ydstie, J. A., & Coetzee, J. F. (2019). Rapid Communication: Use of pressure mat gait analysis in measuring pain following normal parturition in dairy cows. J Anim Sci, 97(2), 846-850. https://doi.org/10.1093/jas/sky450

Kleinhenz, M. D., Gorden, P. J., Smith, J. S., Schleining, J. A., Kleinhenz, K. E., Juarez, J. R.,Coetzee, J. F. (2019). Effects of transdermal flunixin meglumine on experimentally induced lameness in adult dairy cattle. J Dairy Sci, 102(7), 6418-6430. https://doi.org/10.3168/jds.2018-15091

Kleinhenz, M. D., Van Engen, N. K., Smith, J. S., Gorden, P. J., Ji, J., Wang, C.,Coetzee, J. F. (2018). The impact of transdermal flunixin meglumine on biomarkers of pain in calves when administered at the time of surgical castration without local anesthesia. Livestock Science, 212, 1-6. https://doi.org/https://doi.org/10.1016/j.livsci.2018.03.016

Kleinhenz, M. D., Viscardi, A. V., & Coetzee, J. F. (2021). Invited Review: On-farm pain management of food production animals. Applied Animal Science, 37(1), 77-87. https://doi.org/https://doi.org/10.15232/aas.2020-02106

Knych, H. K., Arthur, R. M., Gretler, S. R., McKemie, D. S., Goldin, S., & Kass, P. H. (2021). Pharmacokinetics of transdermal flunixin meglumine and effects on biomarkers of inflammation in horses. J Vet Pharmacol Ther, 44(5), 745-753. https://doi.org/10.1111/jvp.12993

Kongara, K., Purchas, G., Dukkipati, V., Venkatachalam, D., Ward, N., Hunt, H., & Speed, D. (2023). Pharmacokinetics and effect on renal function and average daily gain in lambs after castration and tail docking, of firocoxib and meloxicam. N Z Vet J, 71(6), 306-314. https://doi.org/10.1080/00480169.2023.2232337

Kongara, K., Singh, P., Venkatachalam, D., & Chambers, J. P. (2023). Pain Assessment in Goat Kids: Focus on Disbudding. Animals (Basel), 13(24). https://doi.org/10.3390/ani13243814

Korelidou, V., Grbovic, Z., Pavlovic, D., Simovic, I., Panic, M., Temenos, A., & Gelasakis, A. I. (2025). Clinical Assessment of Dairy Goats' Udder Health Using Infrared Thermography. Animals (Basel), 15(5). https://doi.org/10.3390/ani15050658

Kyrou, I., Chrousos, G. P., & Tsigos, C. (2006). Stress, visceral obesity, and metabolic complications. Ann N Y Acad Sci, 1083, 77-110. https://doi.org/10.1196/annals.1367.008

Königsson, K., Törneke, K., Engeland, I. V., Odensvik, K., & Kindahl, H. (2003). Pharmacokinetics and pharmacodynamic effects of flunixin after intravenous, intramuscular, and oral administration to dairy goats. Acta Vet Scand, 44(3-4), 153-159. https://doi.org/10.1186/1751-0147-44-153

Langford, D. J., Bailey, A. L., Chanda, M. L., Clarke, S. E., Drummond, T. E., Echols, S.,Mogil, J. S. (2010). Coding of facial expressions of pain in the laboratory mouse. Nat Methods, 7(6), 447-449. https://doi.org/10.1038/nmeth.1455

Leach, M. C., Klaus, K., Miller, A. L., Scotto di Perrotolo, M., Sotocinal, S. G., & Flecknell, P. A. (2012). The assessment of post-vasectomy pain in mice using behaviour and the Mouse Grimace Scale. PLoS One, 7(4), e35656. https://doi.org/10.1371/journal.pone.0035656

Lees, P., & Toutain, P. L. (2012). The role of pharmacokinetics in veterinary drug residues. Drug Test Anal, 4 Suppl 1, 34-39. https://doi.org/10.1002/dta.1374

Livingston, A. (2000). Mechanism of Action of Nonsteriodal Anti-Inflammatory Drugs. In (Vol. 30). The Veterinary Clinics of North America.

Livingston, A. (2010). Pain and Analgesia in Domestic Animals. In F. Cunningham, J. Elliott, & P. Lees (Eds.), Comparative and Veterinary Pharmacology (pp. 159-189). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-10324-7_7

Loeser, J. D., & Melzack, R. (1999). Pain: an overview. The Lancet, 353, 1607-1609.

Lopez-Soriano, M., Rocha Merenda, V., Esteves Trindade, P. H., Loureiro Luna, S. P., & Pairis-Garcia, M. D. (2022). Efficacy of transdermal flunixin in mitigating castration pain in piglets. Front Pain Res (Lausanne), 3, 1056492. https://doi.org/10.3389/fpain.2022.1056492

Love, E. J., Murrell, J., & Whay, H. R. (2011). Thermal and mechanical nociceptive threshold testing in horses: a review. Veterinary Anaesthesia and Analgesia, 38(1), 3-14. https://doi.org/10.1111/j.1467-2995.2010.00580.x

Low, P., Jaak, P., Diana, R., David, E., Van, S. B., & Christof, K. (2012). The Cambridge Declaration on Consciousness. In. Francis Crick Memorial Conference on Consciousness in Human and non-Human Animals: University of Cambridge.

Macmillan, K., Colazo, M. G., & Cook, N. J. (2019). Evaluation of infrared thermography compared to rectal temperature to identify illness in early postpartum dairy cows. Research in Veterinary Science, 125, 315-322. https://doi.org/https://doi.org/10.1016/j.rvsc.2019.07.017

Maldague, X. P. V. (2001). Theory and Practice of Infrared Technology for Nondestructive Testing.

Marchi, A., Vellucci, R., Mameli, S., Rita Piredda, A., & Finco, G. (2009). Pain biomarkers. Clin Drug Investig, 29 Suppl 1, 41-46. https://doi.org/10.2165/0044011-200929001-00006

Marshall, T. S. (2009). Abomasal ulceration and tympany of calves. Vet Clin North Am Food Anim Pract, 25(1), 209-220, viii. https://doi.org/10.1016/j.cvfa.2008.10.010

Martin, M. S., Kleinhenz, M. D., Edwards-Callaway, L. N., Engle, T. E., Guimaraes, O., Schafer, D. W.,Coetzee, J. F. (2022a). The effect of breed, sex, and oral meloxicam administration on pain biomarkers following hot-iron branding in Hereford and Angus calves. J Anim Sci, 100(3). https://doi.org/10.1093/jas/skac038

Martin, M. S., Kleinhenz, M. D., Viscardi, A. V., Curtis, A. K., Johnson, B. T., Montgomery, S. R.,Coetzee, J. F. (2022b). Effect of bupivacaine liposome suspension administered as a cornual nerve block on indicators of pain and distress during and after cautery dehorning in dairy calves. Journal of Dairy Science, 105(2), 1603-1617. https://doi.org/https://doi.org/10.3168/jds.2021-21004

Martin, M. S., Kleinhenz, M. D., Viscardi, A. V., Montgomery, S. R., Cull, C. A., Seagren, J. E.,Coetzee, J. F. (2022c). Comparison of lidocaine alone or in combination with a local nerve block of ethanol, bupivacaine liposome suspension, or oral meloxicam to extend analgesia after scoop dehorning in Holstein calves. JDS Commun, 3(3), 189-194. https://doi.org/10.3168/jdsc.2021-0178

Martin, M. S., Kleinhenz, M. D., White, B. J., Johnson, B. T., Montgomery, S. R., Curtis, A. K.,Coetzee, J. F. (2022d). Assessment of pain associated with bovine respiratory disease and its mitigation with flunixin meglumine in cattle with induced bacterial pneumonia. J Anim Sci, 100(2). https://doi.org/10.1093/jas/skab373

Mathews, K. A. (2002). Non-steriodal anti-inflammatory analgesics: a review of current practice. In. Journal of Veterinary Emergency & Critical Care.

May, A. (2008). Chronic pain may change the structure of the brain. Pain, 137(1), 7-15. https://doi.org/10.1016/j.pain.2008.02.034

McCarberg, B. (2023). Acute Pain in Perspective. J Fam Pract, 72(6 Suppl), S7-S12. https://doi.org/10.12788/jfp.0617

McElveen, J. V. (1951). Changes in Production During World War II by Size of Farm. In. AgEcon Search.

McLean, A. K., Falt, T., Abdelfattah, E. M., Middlebrooks, B., Gretler, S., Spier, S.,Knych, H. K. (2023). Transdermal Flunixin Meglumine as a Pain Relief in Donkeys: A Pharmacokinetics Pilot Study. Metabolites, 13(7). https://doi.org/10.3390/metabo13070776

McLennan, K. M., Rebelo, C. J. B., Corke, M. J., Holmes, M. A., Leach, M. C., & Constantino-Casas, F. (2016). Development of a facial expression scale using footrot and mastitis as models of pain in sheep. Applied Animal Behaviour Science, 176, 19-26. https://doi.org/https://doi.org/10.1016/j.applanim.2016.01.007

Meira, E. B. d. S., Wiloch, E. E., Nixon, E., Yeatts, J. L., Sheela, F. F., Smith, G. W., & Baynes, R. E. (2022). The pharmacokinetics of transdermal flunixin in lactating dairy goats. Journal of Dairy Science, 105(1), 549-559. https://doi.org/https://doi.org/10.3168/jds.2021-20460

Mellor, D. J. (2016). Updating Animal Welfare Thinking: Moving beyond the "Five Freedoms" towards "A Life Worth Living". Animals (Basel), 6(3). https://doi.org/10.3390/ani6030021

Mellor, D. J., & Beausoleil, N. J. (2017). Equine Welfare during Exercise: An Evaluation of Breathing, Breathlessness and Bridles. Animals (Basel), 7(6). https://doi.org/10.3390/ani7060041

Mellor, D. J., E., P.-K., & K.J., S. (2009). The Sciences of Animal Welfare. In: Oxford UK.

Mellor, D. J., & Littin, K. E. (2004). - Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. - 13, - S132.

Mendl, M., Burman, O. H. P., Parker, R. M. A., & Paul, E. S. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. Applied Animal Behaviour Science, 118(3), 161-181. https://doi.org/https://doi.org/10.1016/j.applanim.2009.02.023

Merskey, H. (1994). Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the Study of Pain, Subcommittee on Taxonomy. Pain. Supplement, 3, S1-226.

Molaei, M. M., Mostafavi, A., Kheirandish, R., Azari, O., & Shaddel, M. (2015). Study of disbudding goat kids following injection of clove oil essence in horn bud region. Vet Res Forum, 6(1), 17-22.

Musser J.M, B., K.L, A., & K.L., T. (1998). Pharmacokinetic parameters and milk concentrations of ketoprofen after administration as a single intravenous bolus dose to lactating goats. In (Vol. 21, pp. 358-363). J. vet. Pharmacol. Therap.

N., C. (2001). Information Point: Visual Analogue Scale (VAS). In (Vol. 10). Journal of Clinical Nursing: Blackwell Science Ltd.

N., G., S., G., A., A., S., A., & R., M. (2016). A comparison of visual analog scale and Wong Baker facial pain scale for pain measurement in post lower caesarean section case. In. International Journal of Reproduction, Contraception, Obstetrics, and Gynecology.

Nahm, F. S. (2013). Infrared thermography in pain medicine. Korean J Pain, 26(3), 219-222.

Oehm, A. W., Knubben-Schweizer, G., Rieger, A., Stoll, A., & Hartnack, S. (2019). A systematic review and meta-analyses of risk factors associated with lameness in dairy cows. BMC Vet Res, 15(1), 346. https://doi.org/10.1186/s12917-019-2095-2

Ohl, F., & van der Staay, F. J. (2012). Animal welfare: at the interface between science and society. Vet J, 192(1), 13-19. https://doi.org/10.1016/j.tvjl.2011.05.019

OIE. (2008). Terrestrial Animal Health Code 2008: Introduction to the recommendations for animal welfare. In (p. 1-2): World Organization for Animal Health (OIE).

Payne, M. A., Craigmill, A. L., Riviere, J. E., Baynes, R. E., Webb, A. I., & Sundlof, S. F. (1999). The Food Animal Residue Avoidance Databank (Farad): Past, Present and Future. Veterinary Clinics of North America: Food Animal Practice, 15(1), 75-88. https://doi.org/https://doi.org/10.1016/S0749-0720(15)30208-5

Pelfort, X., Torres-Claramunt, R., Sánchez-Soler, J. F., Hinarejos, P., Leal-Blanquet, J., Valverde, D., & Monllau, J. C. (2015). Pressure algometry is a useful tool to quantify pain in the medial part of the knee: an intra- and inter-reliability study in healthy subjects. Orthop Traumatol Surg Res, 101(5), 559-563. https://doi.org/10.1016/j.otsr.2015.03.016

Prunier, A., Mounier, L., Le Neindre, P., Leterrier, C., Mormède, P., Paulmier, V.,Guatteo, R. (2013). Identifying and monitoring pain in farm animals: a review. Animal, 7(6), 998-1010. https://doi.org/10.1017/S1751731112002406

Pérez de Diego, A. C., Sánchez-Cordón, P. J., Pedrera, M., Martínez-López, B., Gómez-Villamandos, J. C., & Sánchez-Vizcaíno, J. M. (2013). The use of infrared thermography as a non-invasive method for fever detection in sheep infected with bluetongue virus. The Veterinary Journal, 198(1), 182-186. https://doi.org/https://doi.org/10.1016/j.tvjl.2013.07.013

Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S.,Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: concepts,

challenges, and compromises. Pain, 161(9), 1976-1982. https://doi.org/10.1097/j.pain.0000000000001939

Ramirez-Garcialuna, J. L., Rangel-Berridi, K., Bartlett, R., Fraser, R. D., & Martínez-Jiménez, M. A. (2022). Use of Infrared Thermal Imaging for Assessing Acute Inflammatory Changes: A Case Series. Cureus, 14.

Rana, M. S., Lee, S. Y., Kang, H. J., & Hur, S. J. (2019). Reducing Veterinary Drug Residues in Animal Products: A Review. Food Sci Anim Resour, 39(5), 687-703. https://doi.org/10.5851/kosfa.2019.e65

Rangel-Nava, A., Ramírez-Uribe, J. M., Recillas-Morales, S., Ibancovichi-Camarillo, J. A., Venebra-Muñoz, A., & Sánchez-Aparicio, P. (2019). Pharmacological Regulation in the USA and Pharmacokinetics Parameters of Firocoxib, a Highly Selective Cox-2, by Pain Management in Horses. J Equine Vet Sci, 77, 36-42. https://doi.org/10.1016/j.jevs.2019.02.007

Rault, J.-L., Lay, D. C., & Marchant, J. N. (2011). Castration induced pain in pigs and other livestock. Applied Animal Behaviour Science, 135(3), 214-225. https://doi.org/https://doi.org/10.1016/j.applanim.2011.10.017

Rault, J. L., Hintze, S., Camerlink, I., & Yee, J. R. (2020). Positive Welfare and the Like: Distinct Views and a Proposed Framework. Front Vet Sci, 7, 370. https://doi.org/10.3389/fvets.2020.00370

Reeves, P. T. (2010). Drug residues. Handb Exp Pharmacol(199), 265-290. https://doi.org/10.1007/978-3-642-10324-7_11

Regan, T. (1983). The Case for Animal Rights. In: University of California Press.

Reppert, E. J., Kleinhenz, M. D., Montgomery, S. R., Bornheim, H. N., Magnin, G., Sidhu, P. K.,Coetzee, J. F. (2019a). Pharmacokinetics and pharmacodynamics of intravenous and transdermal flunixin meglumine in meat goats. J Vet Pharmacol Ther, 42(3), 309-317. https://doi.org/10.1111/jvp.12756

Reppert, E. J., Kleinhenz, M. D., Montgomery, S. R., Heiman, J., Sura, A., Bornheim, H. N.,Coetzee, J. F. (2019b). Pharmacokinetics and pharmacodynamics of intravenous and transdermal flunixin meglumine in alpacas. J Vet Pharmacol Ther, 42(5), 572-579. https://doi.org/10.1111/jvp.12800

Riviere, J. E., Craigmill, A. L., & Sundlof, S. F. (1986). Food Animal Residue Avoidance Databank (FARAD): An Automated Pharmacologic Databank for Drug and Chemical Residue Avoidance. J Food Prot, 49(10), 826-830. https://doi.org/10.4315/0362-028X-49.10.826

Robertson, S. A. (2001). Analgesia and Analgesic Techniques. Veterinary Clinics of North America: Exotic Animal Practice, 4(1), 1-18. https://doi.org/https://doi.org/10.1016/S1094-9194(17)30047-6

Rokka, M., Eerola, S., Perttilä, U., Rossow, L., Venäläinen, E., Valkonen, E.,Peltonen, K. (2005). The residue levels of narasin in eggs of laying hens fed with unmedicated and medicated feed. Mol Nutr Food Res, 49(1), 38-42. https://doi.org/10.1002/mnfr.200400042

Rosier, E. M., Iadarola, M. J., & Coghill, R. C. (2002). Reproducibility of pain measurement and pain perception. Pain, 98(1-2), 205-216. https://doi.org/10.1016/s0304-3959(02)00048-9

Ross, M., & Dyson, S. (2011). Diagnosis and Management of Lameness in the Horse. https://doi.org/10.1016/C2009-0-50774-X

Roth, J. A. (1985). Animal Stress: Cortisol as Mediator of Stress-Associated Immunosuppression in Cattle. In (pp. 225-243).

Rushen, J. (2008). Farm animal welfare since the Brambell report. Applied Animal Behaviour Science, 113(4), 277-278. https://doi.org/https://doi.org/10.1016/j.applanim.2008.01.019

S.M., M., J.A., R., K., M., Jack, M., Ison, S., Bunger, L., & Dwyer, C. M. (2010). Development and validation of on-farm behavioural scoring systems to assess birth assistance and lamb vigor. In. Cambridge Core.

Sanford, S. E. (1989). Ontario. Meningoencephalitis caused by thermal disbudding in goat kids. Can Vet J, 30(10), 832.

Schwartzkopf-Genswein, K. S., Fierheller, E. E., Caulkett, N. A., Janzen, E. D., Pajor, E. A., Gonzalez, L. A., & Moya, D. (2012). Achieving pain control for routine management procedures in North American beef cattle. In (Vol. 2, pp. 52-58). Animal Frontiers.

Searle, D., Dart, A. J., Dart, C. M., & Hodgson, D. R. (1999). Equine castration: review of anatomy, approaches, techniques, and complications in normal, cryptorchid and monorchid horses. Aust Vet J, 77(7), 428-434. https://doi.org/10.1111/j.1751-0813.1999.tb12083.x

Sherwin, C. M. (2001). Can invertebrates suffer? Or, how robust is argument-by-analogy? Animal Welfare, 10(Suppl), S103-S118. https://doi.org/10.1017/S0962728600023551

Singer, P. (1975). Animal Liberation: A New Ethics for Our Treatment of Animals. In: HarperCollins.

Sischo, W. M., Norman, H. S., & Kiernan, N. E. (1999). Use of the Food Animal Residue Avoidance Databank (FARAD). J Am Vet Med Assoc, 214(3), 344-350.

Smith, E. S., & Lewin, G. R. (2009). Nociceptors: a phylogenetic view. J Comp Physiol A Neuroethol Sens Neural Behav Physiol, 195(12), 1089-1106. https://doi.org/10.1007/s00359-009-0482-z

Sneddon, L. U., Elwood, R. W., Adamo, S. A., & Leach, M. C. (2014). Defining and assessing animal pain. Animal Behaviour, 97, 201-212. https://doi.org/10.1016/j.anbehav.2014.09.007

Snijdelaar, D. G., Dirksen, R., Slappendel, R., & Crul, B. J. P. (2000). Substance P. European Journal of Pain, 4.

Sotocinal, S. G., Sorge, R. E., Zaloum, A., Tuttle, A. H., Martin, L. J., Wieskopf, J. S.,Mogil, J. S. (2011). The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol Pain, 7, 55. https://doi.org/10.1186/1744-8069-7-55

Sprecher, D. J., Hostetler, D. E., & Kaneene, J. B. (1997). A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology, 47(6), 1179-1187. https://doi.org/https://doi.org/10.1016/S0093-691X(97)00098-8

Steagall, P. V., Bustamante, H., Johnson, C. B., & Turner, P. V. (2021). Pain Management in Farm Animals: Focus on Cattle, Sheep and Pigs. Animals (Basel), 11(6). https://doi.org/10.3390/ani11061483

Stevens, B. J., & Franck, L. (1995). Special needs of preterm infants in the management of pain and discomfort. J Obstet Gynecol Neonatal Nurs, 24(9), 856-862. https://doi.org/10.1111/j.1552-6909.1995.tb02571.x

Stock, M. L., Gehring, R., Barth, L. A., Wulf, L. W., & Coetzee, J. F. (2014). Pharmacokinetics of firocoxib in preweaned calves after oral and intravenous administration. J Vet Pharmacol Ther, 37(5), 457-463. https://doi.org/10.1111/jvp.12124

Stokes, J. E., Leach, K. A., Main, D. C. J., & Whay, H. R. (2012). An investigation into the use of infrared thermography (IRT) as a rapid diagnostic tool for foot lesions in dairy cattle. The Veterinary Journal, 193(3), 674-678. https://doi.org/https://doi.org/10.1016/j.tvjl.2012.06.052

Stuart, A. K., Kukanich, B., Caixeta, L. S., Coetzee, J. F., & Barrell, E. A. (2019). Pharmacokinetics and bioavailability of oral firocoxib in adult, mixed-breed goats. Journal of veterinary pharmacology and therapeutics.

Sundlof, S. F., Craigmill, A. L., & Riviere, J. E. (1991). Use of the food animal residue avoidance databank. J Am Vet Med Assoc, 198(5), 816-819.

Sutherland, M. A., Davis, B. L., Brooks, T. A., & Coetzee, J. F. (2012). The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia. J Anim Sci, 90(7), 2211-2221. https://doi.org/10.2527/jas.2011-4260

Sutherland, M. A., Huddart, F. J., & Stewart, M. (2019). Short communication: Evaluation of the efficacy of novel disbudding methods for dairy calves. J Dairy Sci, 102(1), 666-671. https://doi.org/10.3168/jds.2018-15230

Sutherland, M. A., Worth, G. M., Dowling, S. K., Lowe, G. L., Cave, V. M., & Stewart, M. (2020). Evaluation of infrared thermography as a non-invasive method of measuring the autonomic nervous response in sheep. PLoS One, 15(5), e0233558. https://doi.org/10.1371/journal.pone.0233558

Suvas, S. (2017). Role of Substance P Neuropeptide in Inflammation, Wound Healing, and Tissue Homeostasis. The Journal of Immunology, 199(5), 1543-1552. https://doi.org/10.4049/jimmunol.1601751

T., P., M.F., S. C., R., M., L., C., B., F., & M., R. (2018). Comparison of visual lameness scores to gait asymmetry in racing Thoroughbreds during trot in-hand. In: British Equine Veterinary Association.

Tannenbaum, J., & Bennett, B. T. (2015). Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. J Am Assoc Lab Anim Sci, 54(2), 120-132.

Tapper, K. R., Johnson, A. K., Karriker, L. A., Stalder, K. J., Parsons, R. L., Wang, C., & Millman, S. T. (2013). Pressure algometry and thermal sensitivity for assessing pain sensitivity and effects of flunixin meglumine and sodium salicylate in a transient lameness model in sows. Livestock Science, 157(1), 245-253. https://doi.org/https://doi.org/10.1016/j.livsci.2013.07.017

Thompson, K. G., Bateman, R. S., & Morris, P. J. (2005). Cerebral infarction and meningoencephalitis following hot-iron disbudding of goat kids. N Z Vet J, 53(5), 368-370. https://doi.org/10.1080/00480169.2005.36578

Thomsen, P. T., Munksgaard, L., & Tøgersen, F. A. (2008). Evaluation of a Lameness Scoring System for Dairy Cows. Journal of Dairy Science, 91(1), 119-126. https://doi.org/https://doi.org/10.3168/jds.2007-0496

Tracey, W. D. (2017). Nociception. Curr Biol, 27(4), R129-R133. https://doi.org/10.1016/j.cub.2017.01.037

Treede, R. D., Rief, W., Barke, A., Aziz, Q., Bennett, M. I., Benoliel, R.,Wang, S. J. (2015). A classification of chronic pain for ICD-11. Pain, 156(6), 1003-1007. https://doi.org/10.1097/j.pain.0000000000000160

Tschoner, T., Mueller, K. R., Zablotski, Y., & Feist, M. (2024). Pain Assessment in Cattle by Use of Numerical Rating and Visual Analogue Scales-A Systematic Review and Meta-Analysis. Animals (Basel), 14(2). https://doi.org/10.3390/ani14020351

Tuchscherer, M., Kanitz, E., Puppe, B., Tuchscherer, A., & Stabenow, B. (2004). Effects of postnatal social isolation on hormonal and immune responses of pigs to an acute endotoxin challenge. Physiol Behav, 82(2-3), 503-511. https://doi.org/10.1016/j.physbeh.2004.04.056

Usamentiaga, R., Venegas, P., Guerediaga, J., Vega, L., Molleda, J., & Bulnes, F. G. (2014). Infrared Thermography for Temperature Measurement and Non-Destructive Testing. Sensors (Basel, Switzerland), 14, 12305 - 12348.

USDA. (2010). Goat 2009, Part I: Reference of Goat Management Practices in the United States. In. Fort Collins, CO: 2009 USDA-APHIS-VS, CEAH.

USDA. (2024). Sheep and Goats (January 2024). In USDA, National Agricultural Statistics Service: National Agriculture Statistics Service (NASS), Agricultural Statistics Board.

USDA. (2025). Animal Production. In.

van der Staay, F. J., Goerlich, V. C., Meijboom, F. L., & Arndt, S. S. (2025). Animal welfare definitions, frameworks, and assessment tools: Advancing the measurement and laying the foundation for improved animal welfare through a three-step approach. Anim Welf, 34, e30. https://doi.org/10.1017/awf.2025.23

Vane, J. R., & Botting, R. M. (1998). Anti-inflammatory drugs and their mechanism of action. Inflamm Res, 47 Suppl 2, S78-87. https://doi.org/10.1007/s000110050284

Viscardi, A. V., & Turner, P. V. (2018). Use of Meloxicam or Ketoprofen for Piglet Pain Control Following Surgical Castration. Front Vet Sci, 5, 299. https://doi.org/10.3389/fvets.2018.00299

Visser, E. M., Menke, E. S., & van Loon, J. P. (2019). Pressure algometry for assessment of abdominal wall sensitivity in horses after ventral midline coeliotomy. Vet Anaesth Analg, 46(6), 820-828. https://doi.org/10.1016/j.vaa.2019.03.008

Voogt, A. M., Ursinus, D. T. H., Sijim, M., & Bongers, J. H. (2023). From the Five Freedoms to a more holistic perspective on animal welfare in the Dutch Animals Act. In. Frontiers in Animal Science.

Weary, D. M., Niel, L., Flower, F. C., & Fraser, D. (2006). Identifying and preventing pain in animals. Applied Animal Behaviour Science, 100(1), 64-76. https://doi.org/https://doi.org/10.1016/j.applanim.2006.04.013

Webster, J. (2016). Animal Welfare: Freedoms, Dominions and "A Life Worth Living". Animals (Basel), 6(6). https://doi.org/10.3390/ani6060035

Weeder, M. M., Kleinhenz, M. D., Reppert, E. J., Curtis, A. K., Viscardi, A. V., Leslie, A.,Coetzee, J. F. (2025). Comparison of multiple drug regimens for reduction of

synovitis-induced lameness pain in meat-type goats. Am J Vet Res, 1-10. https://doi.org/10.2460/ajvr.25.04.0134

Weeder, M. M., Kleinhenz, M. D., Reppert, E. J., Fritz, B. R., Viscardi, A. V., Montgomery, S. R.,Coetzee, J. F. (2023). Optimal lameness induction model development using amphotericin B in meat goats. *Transl Anim Sci, 7*(1), txad105. https://doi.org/10.1093/tas/txad105

Weeder, M. M., Kleinhenz, M. D., Reppert, E. J., Weaver, L. F., Johnson, B. T., Leslie, A. A.,Coetzee, J. F. (2024). Comparison of firocoxib and meloxicam for pain mitigation in goats undergoing surgical castration. *J Am Vet Med Assoc*, *262*(4), 498-505. https://doi.org/10.2460/javma.23.10.0575

Welsh, E. M., & Nolan, A. M. (1995). Effect of flunixin meglumine on the thresholds to mechanical stimulation in healthy and lame sheep. Research in Veterinary Science, 58(1), 61-66. https://doi.org/https://doi.org/10.1016/0034-5288(95)90090-X

WMS., R., & RL., B. (1959 (as reprinted 1992).). The principles of humane experimental technique. In. Wheathampstead (UK) Universities Federation of Animal Welfare.

Xie, W. L., Chipman, J. G., Robertson, D. L., Erikson, R. L., & Simmons, D. L. (1991). Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci U S A, 88(7), 2692-2696. https://doi.org/10.1073/pnas.88.7.2692

Yeates, J. W. (2012). Brain-pain: Do animals with higher cognitive capacities feel more pain? Insights for species selection in scientific experiments. In (p. 25-46): Graue Reihe.

Yeates, J. W., & Main, D. C. J. (2008). Assessment of positive welfare: A review. The Veterinary Journal, 175(3), 293-300. https://doi.org/https://doi.org/10.1016/j.tvjl.2007.05.009

Zaninelli, M., Redaelli, V., Luzi, F., Bronzo, V., Mitchell, M., Dell'Orto, V.,Savoini, G. (2018). First Evaluation of Infrared Thermography as a Tool for the Monitoring of Udder Health Status in Farms of Dairy Cows. Sensors (Basel), 18(3). https://doi.org/10.3390/s18030862

Zetner, K., W.D., R., J., W., P., K., & I., S. (1996). Pain relief after dental treatment in cats. In (Vol. 77, pp. 678-682): Praktische Tierarzt

Chapter 2 - Optimal lameness induction model development using amphotericin B in meat type goats

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Abstract

Lameness continues to be a critical health and welfare concern associated with goat production. Amphotericin B (amp B) is an antimicrobial successful in inducing transient lameness for research purposes previously in livestock animals. This study's objectives were to (1) identify which of three varying doses of amp B would be most effective in inducing lameness in meat type goats and (2) develop a facial grimace scale for goats. Lameness was produced by an intra-articular injection of amphotericin B into the left hind lateral claw distal interphalangeal joint with either a 5 mg/0.25 mL (high–low, 5 mg of amphotericin B in a volume of 0.25 mL), 5 mg/0.5 mL (high–high, 5 mg of amphotericin B in a volume of 0.5 mL), or a 2.5 mg/0.25 mL (low–low, 2.5 mg of amphotericin B in a volume of 0.25 mL). A saline treatment of 0.5 mL was used as control (0.9% sterile saline solution). Lameness response was analyzed by infrared thermography (IRT) at the induced joint, mechanical-nociception threshold (MNT), visual lameness scoring (VLS), a visual analogue scale (VAS), kinetic gait analysis (KGA), plasma cortisol (CORT), substance P (Sub P), and behavior scoring. The IRT and MNT values differed by timepoint ($P \leq 0.0001$). Results from VLS showed the HL treatment was the most effective at inducing lameness (6/6 goats became lame compared to HH 4/6 and LL 2/6). At 24, 48, and 72 h, VAS scores were significantly higher when comparing HL to all other treatment groups ($P = 0.0003$). Both behavior observers (1 and 2) reported a significant time effect ($P = 0.05$), with goats exhibiting more facial grimacing at 24 h post-lameness induction. From these data, an optimal dose for a repeatable lameness induction model in goats was acquired. An effective Goat Grimace Scale (GGS) was also developed to evaluate pain responses in goats.

Keywords: amp B, goat, grimace scale, lameness, pain

Introduction

Lameness is one of the most serious concerns amongst ruminant species (Christodoulopoulos, 2009). Sole ulcers, subsolar abscesses, and interdigital dermatitis are all hoof lesions associated with causing goat lameness (Hill et al., 1997; Christodoulopoulos, 2009; Crosby-Durrani et al., 2016). The economic impact of lame animals is reflected by decreased productivity, cost of treatment and premature culling of animals (Christodoulopoulos, 2009). Treatment of lame goats is challenging due to an incomplete understanding of physiologic and behavioral responses to lameness induced pain.

Amphotericin B (Amp B) has been shown to successfully induce acute transient synovitis and arthritis (McIlwraith et al., 1979; Coetzee et al., 2014; Reppert et al, 2020). Amphotericin B is a polyene antimicrobial that has been used in previous lameness models in horses and cattle (McIlwraith et al., 1979; Schulz et al., 2011). Lameness severity and clinical onset differs between species using Amp B. Duration of lameness in horses lasted from 3 d to 2 wk, while cattle peaked from 6-12 h; ending at or before 72 h (McIlwraith et al., 1979; Schulz et al., 2011). Little is known about lameness duration from associated amp B injection in small ruminants.

In a pilot study comparing Amp B and kaolin-carrageenan in goats, both treatment protocols produced lame goats, showing the effectiveness of Amp B as a lameness inducing agent (Reppert et al., 2020). Unfortunately, goats in both treatment groups became severely lame necessitating rescue analgesic intervention. These results insinuated the need for further dose refinement.

The meat goat industry is one of the fastest growing sectors of the U.S. livestock industry, indicating a need for a clearer understanding of lameness concerns in goat production (USDA, 2010). Mixed breed meat type goats were utilized during this study due to their

increasing prevalence in the United States (USDA, 2010). This study's objectives were to optimize an experimental lameness induction model using Amp B, along with developing a facial grimace scale for pain assessment in goats.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee at Kansas State University (Protocol #4387).

Animals and Study Design

Twenty-four intact male and female (21 male, 3 female) crossbred meat type goats were enrolled on this study. All goat were between 4 and 6 months of age and averaged a body weight of 19 kg (range 15 to 25 kg). Prior to study enrollment, all animals were found to be free of lameness, as determined by a trained individual using a visual lameness scoring (VLS) system. Goats were housed individually in raised pens with a grated floor and fed a complete mixed ration pellet, along with free access to water. This study was conducted over a 3-week period, with 1 week dedicated to acclimation and the following week focused on lameness induction, data collection, and the last week dedicated to post-study monitoring. Following the one-week acclimation period, a random number generator (random.org) was used to randomly assign each goat to one of four treatments:

- High dose-Low volume (HL; n = 6): 5 mg of amphotericin B in a volume of 0.25 mL
- High dose-High volume (HH; n = 6) 5 mg of amphotericin B in a volume of 0.5 mL
- Low dose-Low volume (LL; n = 6): 2.5 mg of amphotericin B in a volume of 0.25 mL
- Control (CNTL; n = 6): 0.5 mL of 0.9% sterile saline solution

Lameness Induction

Goats were separated into two groups to facilitate lameness induction. Groups 1 and 2 consisted of 12 individuals per group (2 females were in group 1, and 1 female was in group 2). An intravenous catheter (16 g × 7.5 cm, Mila International Inc., Florence, KY, USA) was aseptically placed in the right jugular vein of each goat. Catheters were used to facilitate the administration of Xylazine hydrochloride (0.1 mg/kg IV, Akron Inc., Lake Forest, IL, USA) to induce sedation as well as subsequent blood collections. Once sedated, hair on the lateral aspect of the distal limbs was clipped from hoof to fetlock joint. After clipping, the left lateral distal digit/claw was surgically scrubbed from the proximal interphalangeal joint to the coronary band. A 21 g × 2.54 cm needle was placed into the distal interphalangeal joint of the left lateral claw. All intra-articular injections were performed by a veterinarian (ER), blinded to treatment group, and skilled in arthrocentesis of small ruminant joints. Intra-articular injection was confirmed by lack of resistance to injection by the veterinarian. Resistance in this study was defined as the inability to distribute the full amount of treatment into the distal interphalangeal joint of the left lateral claw. If resistance was present during injection administration, the treatment was periarticular and recorded (n = 3). To ensure distribution of treatment throughout the joint after injection, the digit was flexed and extended five times. After injection completion, sedation was reversed using a single intramuscular injection of atipamezole hydrochloride (Zoetis INC, Kalamazoo MI, USA) at a dosage of 0.04 mg/kg. Each goat was monitored hourly for behavioral signs of distress or pain out to 10 h post-induction, and twice daily thereafter for 5 d.

Amphotericin B (X-Gen Pharmaceuticals Inc., Big Flats, NY) was prepared by adding 2.5 mL or 5 mL of sterile water for injection (Baxter Healthcare Corporation, Deerfield, IL,

USA) to achieve the needed concentrations for the high dose-low volume (HL) (20 mg/mL), high dose-high volume (HH) (10 mg/mL) or low dose-low volume (LL) (10 mg/mL) treatments.

Outcome Variables

Parameters for data collection included: behavioral scoring, facial grimace (GGS), infrared thermography (IRT), mechanical-nociception threshold (MNT), kinetic gait analysis (KGA), plasma cortisol (CORT) concentrations, plasma substance P concentrations (Sub P), visual lameness score (VLS), and visual analogue score (VAS) (Figure 2.1). All outcomes were collected at the following timepoints; baseline (−24 h prior to induction), 4, 6, 12, 24, 48, and 72 h post-induction of lameness. All observers were blinded to treatment during the study.

Behavior Scoring

High-definition video cameras (Sony Handycam HDR-CX405, Sony USA Inc., NY, USA) mounted on tripods were positioned in front of the goat pens, with goats in direct view. Every goat on study was video recorded for 30 min at each study time point (prior to induction (−24), 4, 6, 12, 24, 48, and 72 h). BORIS (Behavioral Observation Research Interactive Software v 7.7.3, Torino, Italy) was used to score goat behavior, along with a detailed ethogram adapted from Reppert et al. (2020) (Table 2.1). Raters had to obtain an ICC of 0.80 or higher in order to score behavior data. A total of 5,040 min (84 h) of continuous behavior scoring was included in the final analysis.

Facial Grimace Score

A high-definition video camera mounted on a tripod was placed at the end of the pressure mat walkway to video record goat faces when they walked across the pressure mat at every study timepoint (prior to induction (−24), 4, 6, 12, 24, 48, and 72 h). A maximum of three facial images were pulled from each video recording by an individual blinded to treatment and time

point. A total of 351 images (pre- and post-lameness induction) were assessed by an expert for facial grimace assessment. This person was not blinded to timepoint to ensure every goat had facial images to score for the two trained individuals. Four facial action units (FAUs) that changed in response to lameness (i.e., pain) in goats were identified: ear position, nostril shape and dilation, orbital tightening, and cheek tightening. Ear position was scored on a three-point scale (0 = symmetrical/forward ears, 1 = asymmetrical ears, and 2 = ears pulled back). Nostril shape and dilation were also scored on a three-point scale (0 = U-shaped nose and nostril dilation, 1 = intermediate nose shape, and 2 = V-shaped nose and nostril constriction). Orbital tightening and cheek tightening were scored on a two-point scale (0 = absent and 1 = present). Therefore, the maximum score on the Goat Grimace Scale (GGS) was 6. Facial images from goats enrolled in this study were used to create the GGS (Figure 2.2).

The Goat Grimace Scale with four Facial Action Units (FAUs), ear position, nose shape and dilation, orbital tightening, and cheek tightening.

Two individuals blinded to treatment and time point, with extensive experience in facial grimace assessment, scored all 351 images using the GGS. If an image could not be reliably scored, the individuals were instructed to exclude it from scoring. The GGS score for each image was calculated by summing the scores allotted to each FAU. The intraclass correlation coefficient (ICC) was assessed for the two scorers prior to analysis to determine rater reliability.

Infrared Thermography

Thermographic images of the dorso-lateral aspect of the lateral rear feet were taken at each timepoint; one of the left distal limb (affected) and one of the right distal limb (control) prior to induction (−24), 4, 6, 12, 24, 48, and 72 h. The infrared camera (FLUKE Ti580 IR Imager, Fluke Corp., WA, USA) was positioned at a 45° angle from the dorso-lateral aspect 1 m

from the coronary band of each limb. Images were analyzed to obtain the maximum temperatures for each distal limb with a research grade computer software (SmartView 4.3, Fluke Corp., Everett, WA, USA). The difference between the left distal limb (induced) and right distal limb (control) foot were determined as described by Kleinhenz et al. (2019). These differences were used for statistical analysis.

Mechanical-Nociception Threshold

All MNT measurements were taken with a handheld algometer (FPX 100, Wagner Instruments, Greenwich, CT) prior to induction (−24), 4, 6, 12, 24, 48, and 72 h. The MNT measured the lateral digit at the level of the coronary band at a point halfway between midline and the heel bulb, by applying slow, steady pressure until the goat responded by moving away from pressure or displaying a flinch response. The MNT measurements were taken on both the left (affected) distal limb and right (control) distal limb. The average of three readings for each distal limb at each time point was used for analysis as described by Kleinhenz et al. (2019). The difference between left (induced) and right (control) distal limbs MNT measurements (left distal limb minus right distal limb) was also determined for each time point. These differences were used for statistical analysis. The investigator measuring MNT was blinded to treatment and the algometer readings to prevent bias. A second investigator recorded algometer readings, also to prevent testing bias.

Kinetic Gait Analysis

A commercial pressure/force mat system (Strideway, Tekscan, Inc.) was used to record and analyze the gait of each goat at each timepoint (prior lameness induction (−24), 4, 6, 12, 24, 48, 72 h post-induction) using methods described by Reppert et al. (2020). Video analysis was used to ensure synchronization of each goat's gait while walking across the pressure mat system.

A research grade software (Strideway v 7.7, Tekscan, Inc., South Boston, MA, USA) was used to analyze different parameters of gait motion using methods described by Reppert et al. (stance time, stride length, contact force, impulse, contact pressure, and contact area).

Plasma Cortisol

Blood samples were collected from the intravenous catheter in each goat prior to induction (−24), and at 4, 6, 12, 24, 48, and 72 h post-lameness induction. Plasma cortisol concentrations from each sample were determined in duplicate using a radioimmunoassay (RIA) system using methods adapted from Kleinhenz et al., (2017). Plasma cortisol concentrations were determined using a commercially available RIA kit (MP Biomedicals, Irvine, CA) following manufacturer specifications with minor modifications as previously described (Martin et al., 2022); the standard curve was extended to include 1 and 3 ng/mL by diluting the 10 and 30 ng/mL manufacturer-supplied standards, 1:10, respectively. The standard curve ranged from 1 to 300 ng/mL. A low (25 ng/mL) and high (150 ng/mL) quality control (QC) was run at the beginning and end of each set to determine inter-assay variability. Plain 12 × 75 mm polypropylene tubes were used as blank tubes to calculate non-specific binding. Input for standards, QCs, and samples was adjusted to 50 µL. Samples were incubated at room temperature for 30 min before adding I-125. Manufacturer instructions were then followed. Tubes were counted on a gamma counter (Wizard2, PerkinElmer, Waltham, MA) for 1 min. The raw data file was then uploaded onto MyAssays Desktop software (version 7.0.211.1238, 21 Hampton Place, Brighton, UK) for concentration determination. Standard curves were plotted as a 4-parameter logistic curve. Samples with a coefficient of variation (CV) > 18% were re-analyzed. The intra- and inter-assay CV were determined to be 21.3% and 24.5%, respectively.

Plasma Substance P

Substance P (SP) concentrations were determined through RIA using methods described by Van Engen et al. (2014). The standard curve, ranging from 20 to 1,280 pg/mL, was created by diluting synthetic SP (Phoenix Pharmaceuticals, Burlingame, CA) with RIA Buffer (50 mM sodium phosphate dibasic heptahydrate, 13 mM disodium EDTA, 150 mM sodium chloride, 1 mM benzamidine hydrochloride, 0.1% gelatin, 0.02% sodium azide; pH 7.4). For analysis, 100 µL of sample, standard, or QC were aliquoted into plain 12 × 75 mm conical bottom tubes followed by 100 µL of Rabbit anti-SP primary antibody (1:20,000; Phoenix Pharmaceuticals). Iodine-125-SP tracer (custom iodination by PerkinElmer) was diluted with RIA buffer to 20,000 cpm, then 100 µL was added to the sample, standard, and QC tubes. Samples were then covered and stored at 4 °C for 48 h. At the end of the 48 h incubation, samples were placed on ice and 100 µL of normal rabbit plasma (1:80) and goat anti-rabbit secondary antibody (1:40; Jackson ImmunoResearch, West Grove, PA) were added to each tube. Samples were then incubated at room temperature for 10 min, placed back on ice, and 100 µL of blank bovine plasma was added to the standards and QCs. All tubes then had 1 mL of 12% polypropylene glycol in 0.85% sodium chloride added. Samples were centrifuged at 3,000 × g for 30 min at 4 °C and the supernatant aspirated. Tubes were counted on a gamma counter (Wizard2, PerkinElmer, Waltham, MA) for 1 min. The raw data file was then uploaded onto MyAssays Desktop software for concentration determination. Standard curves were plotted as a 4-parameter logistic curve. Samples with a CV > 18% were re-analyzed. The intra- and interassay CV were determined to be 18.8% and 30.02%, respectively.

Visual Lameness Scoring

A previously described VLS (Deeming et al., 2018) was used by an observer trained in lameness identification and severity. Each goat was scored at the following timepoints: prior to induction (−24), 4, 6, 12, 24, 48, and 72 h. Animals were walked through an alley system with non-slip flooring while traveling to the pressure mat system. Lameness was graded on a scale from 1 to 5 (1-normal gait, 2-uneven gait, 3-mildly lame, 4-moderately lame, and 5-severly lame).

Visual Analogue Scale

A VAS for pain was used to score each goat at each timepoint (prior to induction (−24), 4, 6, 12, 24, 48, and 72 h). An observer skilled in recognizing pain indicators scored each goat as they walked across the pressure mat walkway. Pain was graded on a scale from 1 to 5 (1-no pain, 2-slight pain, 3-mild pain, 4-moderate pain, and 5-severe pain). Pain was also graded with a 100-mm (10 cm) line anchored at each end by descriptors of "No Pain" or "Severe Pain". The evaluator marked the line between the 2 descriptors to indicate pain intensity. A millimeter scale was used to measure the score from the zero-anchor point to the evaluator's mark. Seven parameters were used to assess pain: depression, tail swishing or flicking, stance, head carriage, spinal alignment, movement, and ear carriage adapted from Martin et al. (2020). No pain was characterized by being alert and quick to show interest, no tail swishing, a normal stance, head carriage above spine level, a straight spine, moving freely around the pen and ears forward. Severe pain was characterized by being dull and showing no interest, more than three tail swishes per minute, legs abducted, head held below spine level, a curved spine, reluctant to move, and ears down.

Statistical Analysis

Plasma cortisol was log transformed for normality prior to statistical analysis. The outcome responses of IRT, KGA, and plasma cortisol were analyzed using linear mixed models with goat as the experimental unit. Goats nested in a treatment group were designated as a random effect, with treatment, time, treatment by time interaction, and replicate designated as fixed effects.

Visual lameness scores were treated as categorical data and Fisher's exact test was used to compare scores. All statistics, except for behavior data, were performed using statistical software (JMP Pro 14.0, SAS Institute, Cary, NC, USA). Post hoc tests were conducted on significant factors using the Tukey-Kramer adjustment. Statistical significance was set at $P \leq 0.05$.

Behavior results were analyzed using a generalized linear mixed model (GLIMMIX) with a beta distribution, including timepoint, treatment, group, and treatment by timepoint interaction in SAS (Statistical Analysis System 9.4, SAS Institute Inc., NC, USA). Time was a repeated measure, and group was designated as a random effect, with goat as the experimental unit. The same parameters were used to analyze results of the GGS, using a mixed model in SAS. Post hoc tests were conducted on significant factors using the Tukey-Kramer adjustment. Statistical significance was set at ($P \leq 0.05$).

Results

Behavior Scoring

Goats in the HH and LL groups spent significantly more time grooming across the observation period than goats in the HL group ($P = 0.02$) (Table 2.2). Similarly, goats in the HH and CNTL groups scratched their bodies using their horns or rear hooves more than HL goats ($P = 0.01$). Control goats were also observed licking the surfaces of their pen significantly more than HL goats ($P = 0.02$). All Amp B treatments were successful in inducing behavioral changes associated with lameness. Notably, the incidence of limping and postural alteration (standing on three legs) across the Amp B treatment goats did not differ significantly ($P > 0.05$).

Irrespective of treatment, goats spent significantly more time standing and drinking at baseline than at all other post-lameness induction time points ($P < 0.05$). Comparing only the post-lameness induction time points, goats spent significantly less time standing at 6 and 12 h than at 72 h ($P = 0.02$). Likewise, all goats spent significantly more time lying at 6 h post-lameness induction compared to 72 h ($P = 0.008$). The duration of standing and lying behavior did not return to baseline levels until 72 h post-lameness induction. Less maintenance behavior (grooming) was observed 4 h post-lameness induction compared to 24 and 72 h ($P < 0.0001$). Goats also ate significantly less at 24 h compared to 72h post-lameness induction ($P = 0.02$). None of the goats were seen exhibiting restless behavior, limping, or standing on three legs prior to lameness induction.

A few behavioral differences were found between the two groups of goats in this study. Compared to the goats in group 1, group 2 goats spent more time standing ($P = 0.001$), standing on three legs ($P = 0.02$), walking ($P = 0.03$), less time lying ($P = 0.008$), scratching ($P = 0.01$),

and rearing up on their home pen (P = 0.02). Group 1 goats spent significantly more time attentive to their affected hoof than group 2 goats (P = 0.02).

Infrared Thermography

Maximum IRT temperatures did not differ by treatment (P = 0.08) or treatment over time interaction (P = 0.14) but did differ by timepoint (P ≤ 0.0001) (Figure 2.3). Differences in temperature were consistently higher between LH (affected limb) and RH (control limb) when compared with baseline across all treatment groups at the 48 h timepoint only (HH: 5.03 °C [95% CI: (2.65 to 7.41) °C], (HL: 4.10 °C [95% CI: (1.72 to 6.48) °C], (LL: 6.08 °C [95% CI: (3.70 to 8.47) °C], and (CNTL: 2.48 °C [95% CI: (0.10 to 4.87) °C]. There were no treatment effects observed for the difference in the maximum temperature between the left distal limb and right distal limb (P = 0.08).

Mechanical-Nociception Threshold

MNT differed by treatment (P = 0.0023) and timepoint (P ≤ 0.0001), but a treatment over time interaction was not observed (P = 0.40; Figure 2.4). The HL, HH, and LL groups were significantly higher when compared with the CNTL group when evaluating difference in MNT response between LH (affected limb) and RH (control limb) (HH: −1.14 KgF [95% CI: −1.37 - (−0.91) kgF], (HL: −1.18 KgF [95% CI: −1.42 - (−0.94) KgF], (LL: −1.09 KgF [95% CI: −1.34 - (−0.86) KgF], and (CNTL: −0.54 KgF [95% CI: −0.78 - (−0.31) KgF]. All animals across all treatment groups had higher measurements across 24, 48, and 72 h timepoints when compared with baseline measurements (−24 h; P < 0.05).

Plasma Cortisol

Plasma cortisol did not differ by treatment (P = 0.87) but did differ by timepoint (P = 0.01) and treatment over time interaction (P = 0.04) (Figure 2.5). Cortisol levels were highest at

12 h [10.28 ng/mL (95% CI:7.60 to 12.95 ng/mL)] and at 24 h [7.77 ng/mL (95% CI:5.10 to 10.45 ng/mL)] when compared with baseline [6.09 ng/mL (95% CI: 3.41-8.76 ng/mL)].

The HH treatment group had the highest cortisol levels at 12 and 24 h [13.19 ng/mL (95% CI: 7.96 to 18.41 ng/mL)] and [10.55 ng/mL (95% CI: 5.33 to 15.78 ng/mL)] compared to the HL treatment group [7.01 ng/mL (95% CI: 1.78 to 12.24 ng/mL)] and [7.04 ng/mL (95% CI: 1.82 to 12.27 ng/mL)], the LL treatment group [12.71 ng/mL (95% CI: 7.48 to 17.93 ng/mL)], and [6.04 ng/mL (95% CI: 0.33 to 11.76 ng/mL)], and the CNTL group [8.20 ng/mL (95% CI: 2.50 to 13.91 ng/mL)] and [7.45 ng/mL (95% CI: 2.23 to 12.68 ng/mL)] at the same timepoints (12 and 24 h).

Plasma Substance P

Plasma substance P did not differ by treatment (P = 0.50), timepoint (P = 0.14), or treatment over time interaction (P = 0.76; Figure 2.6). The overall mean for plasma substance P by treatment was reported to be CNTL [390.33 pg/mL (95% CI: 233.39 to 547.27 pg/mL)], HH [429.49 pg/mL (95% CI: 272.55 to 586.43 pg/mL)], HL [390.73 pg/mL (95% CI: 233.78 to 547.67 pg/mL)], and LL [554.85 pg/mL (95% CI: 397.91 to 711.79 pg/mL)], respectively.

Kinetic Gait Analysis

Definitions of KGA parameters are in Table 2.3. KGA outcomes are shown in Table 2.4.

Stance Time (s)

Left distal limb stance time did not differ by treatment (P = 0.17) or treatment over time interaction (P = 0.08) but did differ by timepoint (P = 0.01). At 12 h, left distal limb stance time was reported as [25s (95%CI: 1 to 35 s)]. Stance time peaked at 72 h [32 s (95% CI: 15 to 88 s)] when compared to baseline measurements (−24) [18 s (95% CI: 13 to 24 s)]. Stance time increased among all treatment groups when compared to CNTL after lameness induction.

The right distal limb stance time did not differ by timepoint (P = 0.13), treatment (P = 0.08), or treatment over time interaction (P = 0.43).

Stride Length (cm)

Left distal limb stride length did not differ by treatment (P = 0.32) or treatment over time interaction (P = 0.29) but did differ by timepoint (P = 0.0065). Stride length was shortest at 48 h [64.43 cm (95% CI: 54-74 cm)] and longest at 6 h [86.12 cm (95% CI: 76 to 95 cm)] when compared with baseline measurements (−24) [84.50 cm (95% CI: 74 to 94 cm)].

Right distal limb stride length did not differ by treatment (P = 0.48) or treatment over time interaction (P = 0.26) but did differ by timepoint (P = 0.002). Stride length was shortest at 12 h [68.64 cm (95% CI: 60 to 77 cm)], and longest at 48 h [81.52 cm (95% CI: 73 to 90 cm)] when compared to baseline measurements (−24) at [94.68 cm (95% CI: 86 to 103 cm)].

Force (kg)

The left distal limb force did not differ by treatment over time interaction (P = 0.91) but did differ by treatment (P = 0.0034) and timepoint (P = 0.0002). HH treatment had the lowest force for left hind (treated limb) [1.91 kg (95% CI:1.47 to 2.35 kg)]. All treatments were lower in force when compared to CNTL [2.98 kg (95% CI: 2.54 to 3.41 kg)]. Force was lowest at 48 h [1.85 kg (95% CI: 1.27 to 2.42 kg)], but all timepoints were lower when compared to baseline measurements (−24 h) [3.55 kg (95% CI: 2.97 to 4.12 kg)].

The right distal limb force did not differ by treatment (P = 0.14) or timepoint (P = 0.43) but did differ by treatment over time interaction (P = 0.03). Right hind foot force for the HL group was significantly higher at 12 and 48 h [3.79 kg (95% CI: 2.68 to 4.91 kg)] and [3.48 kg (95% CI: 2.37 to 4.60 kg)], respectively) compared with baseline (−24), 4, 6, 24, and 72 h (≤3.06

kg; P = 0.03). Average force values for CNTL at 12 and 48 h were [1.83 kg (95% CI: 0.71 to 2.94 kg)] and [2.22 kg (95% CI: 2.36 to 4.60 kg), respectively] (P = 0.03).

Impulse (kg × s)

Left hind hoof impulse did not differ by treatment (P = 0.09), timepoint (P = 0.63), or treatment over time interaction (P = 0.33).

Right hind hoof impulse did not differ by treatment (P = 0.38) or timepoint (P = 0.56) but did differ by treatment over time interaction (P = 0.01). Right hind hoof impulse for the HL group was significantly higher at 12 [0.76 kg × s (95% CI: 0.54 to 0.97 kg × s)], 24 [0.56 kg × s (95% CI: 0.34 to 0.77 kg × s)]and 48 h [0.60 kg × s (0.38 to 0.81 kg × s) compared with −24, 4, 6, and 72 h (≤0.42 kg × s, respectively; P = 0.01). Average impulse values for CNTL at 12, 24, and 48 h [0.21 kg × s (95% CI: 0.004 to 0.42 kg × s)], [0.31 kg × s (95% CI: 0.10 to 0.52 kg × s), and [0.45 kg × s (95% CI:0.24 to 0.67 kg × s)], respectively; P = 0.01).

Contact Pressure (kg/cm^2)

Left hind hoof contact pressure did not differ by treatment (P = 0.07) or by treatment over time interaction. (P = 0.71) but did differ by timepoint (P = 0.03). Contact pressure was highest at 6 h [1.77 kg/cm^2 (95% CI: 1.52 to 2.02 kg/cm2)] and was lowest at 48 h [1.15 kg/cm^2 (95% CI: 0.90 to 1.4 kg/cm^2)].

Right hind hoof contact pressure did not differ by treatment (P = 0.054) or by timepoint (P = 0.87); however, treatment over time interactions were significant (P = 0.03). Right hind hoof contact pressure for the HL group was significantly higher at 12 [1.92 kg/cm^2 (95% CI:1.55 to 2.29 kg/cm^2)] and 48 h [1.66 kg/cm^2 (95% CI:1.10 to 1.85 kg/cm^2)] compared with −24 h, 4, 6, 24, and 72 h (≤1.60 kg/cm^2) (P = 0.03). Average force values for CNTL at 12 and 48 h [1.02

kg/cm^2 (95% CI: 0.64 to 1.39 kg/cm^2)] and [1.04 kg/cm^2 (95% CI:0.67 to 1.41 kg/cm^2)], respectively (P = 0.03).

Visual Lameness Scoring

Visual lameness scores differed only at 48 and 72 h post-lameness induction between all treatment groups (Figure 2.7). Differences included an increase or decrease in the number of individuals at a lameness score of level 2 or above. The HH treatment group had four individuals at a level 2 or higher at 48 h. At 72 h, there was only one individual at a grade 2 or higher in the HH treatment group. The HL treatment group had six individuals at a grade 2 or higher at 48 h. At 72 h, there were five individuals at a grade 2 or higher in the same treatment group. The LL treatment group had two individuals at a grade 2 or higher at 48 and 72 h. The CNTL group had no individuals that scored above a grade 1 at any timepoint during the duration of the study.

Peak lameness severity was noted at 48 h post-induction across all three treatment groups (HH, HL, and LL). Five of the six individuals in the HH treatment group were sound (no lameness observed) by 72h. One of six individuals was sound by the 72 h mark in the HL group. Four of six individuals in the LL group were sound by the 72 h mark.

Visual Analogue Scoring

The VAS outcomes differed by treatment (P = 0.04), timepoint (P < 0.0001), and treatment over time interaction (P = 0.0003) (Figure 2.8). Treatment groups did not differ at 4, 6, and 12 h post-induction. The HL treatment group differed from all groups at 24, 48, and 72 h post-induction. All other treatment groups differed from the CNTL group only at the 24 h post-induction. The second replicate group (group 2) was less lame when compared to the first replicate (group 1) (1.5 vs 0.4 cm).

Facial Grimace Score

The ICC between the two observers was found to be extremely poor (0.19); therefore, the GGS results were not pooled between observers and were analyzed and interpreted independently. Observer 1 was much more experienced in facial grimace development and assessment across multiple livestock species than Observer 2.

Observer 1: There was a significant time effect ($P = 0.05$), with goats exhibiting more facial grimacing at 24 h post-induction (2.47 ± 0.22) compared to baseline (1.36 ± 0.23; $P = 0.01$). A significant treatment effect was also found ($P = 0.02$), with goats in the HH treatment group grimacing more (2.36 ± 0.17) than goats in the LL treatment group (1.63 ± 0.17; $P = 0.02$). Group 1 goats grimaced significantly more (2.14 ± 0.12) than group 2 goats (1.74 ± 0.12; $P = 0.02$).

Observer 2: There was a significant time effect ($P = 0.006$), with goats exhibiting more facial grimacing at 24 h post-induction (2.79 ± 0.21) compared with baseline observations (1.65 ± 0.20; $P = 0.003$).

Discussion

This study demonstrated that the use of Amp B induces temporary, acute lameness in goats. Lameness among individuals was defined using parameters including GGS, VLS, VAS, IRT, MNT, KGA, CORT, and Sub P. Behavioral changes were also noted between the three Amp B treatment groups and the CNTL group.

Amphotericin B has been used to induce transient lameness in other species, including cattle and pigs (McIlwraith et al., 1979; Schulz et al., 2011; Coetzee et al., 2014; Muley et al., 2016). When compared with the CNTL group, Amp B produced consistent lameness in all three treatment groups when used in goats (18/24). No individuals on study received any type of rescue therapy (pain mitigation therapy (Meloxicam 1 mg/kg or pulled off study). Results from this study are consistent with severity and onset of lameness caused by Amp B when used in cattle with goats and cattle having shorter lameness durations compared to horses (Schulz et al., 2011; Coetzee et al., 2014). This provides justification for the need of species-specific lameness protocol treatments.

Notable behavioral changes are caused by lameness in goat and sheep species. Behaviors include, but are not limited to, longer lying bouts, a decrease in appetite, and abnormal social interactivity within a herd (Galindo and Broom, 2002; Bach et al., 2007; Ito et al., 2010; Blackie et al., 2011). In a previous lameness study in sheep, lame sheep and non-lame sheep displayed differences in walking, standing, and lying behaviors (Kaler et al., 2020). Goats displayed differences in walking, standing, and lying behaviors that were similar to behavior patterns shown in sheep in the aforementioned study (Kaler et al., 2020). Goats in this study also appeared restless in nature or vocalized more when compared to baseline (−24 h) behaviors.

From an animal welfare standpoint, behavioral indicators in response to pain are crucial for establishing effective treatment protocols for lame animals.

This is the first paper to describe a facial grimace scoring system in goats. The facial grimace scoring system is a viable and novel method to better understand pain responses in goats. This is especially important in individuals that do not look similar due to breed differences or crossbreeding. A grimace scale for sheep has been characterized before to assess pain induced facial expressions and was used as a reference in developing the Goat Grimace Scale in this study (Hager et al., 2017, Viscardi et al., 2021). The sheep grimace scale developed by Hager et al. (2017) was a reliable method to evaluate pain responses in sheep after surgery. Higher SGS were seen after one day post op compared to baselines in sheep on study. Higher facial grimacing scores compared to baselines were also seen after lameness induction in goats on this study, supporting that grimace scoring systems are useful in helping determine pain response behaviors. Higher grimace scores are indicative of an individual experiencing a higher grade of pain. Facial grimacing scores in this study were highest across all treatment groups at 24 h when compared to prior to baseline (−24 h). Results from this outcome measure supplied a novel way for researchers to quantify pain behavior in goats, leading to better identification and treatment of lame goats.

Objective measures of lameness detection used in this study included IRT and KGA. IRT was recently validated for detecting lameness associated with temperature differences in sheep with foot lesions (Byrne et al., 2019). Higher temperatures were noted in diseased hooves when compared to healthy hooves (Byrne et al., 2019). Similarly, higher temperatures have been reported in the hooves of dairy cattle with amphotericin induced lameness when compared to non-lame cattle (Kleinhenz et al., 2019; Warner et al., 2021). Our study identified similar higher

thermal changes between lame (Amp B) hooves compared to CNTL, suggesting that IRT was a reliable method for detecting induced lameness in goats. Higher thermal changes are indicative of a greater inflammatory response in body tissue, which is commonly seen in lame animals.

KGA was recently evaluated as a tool for characterizing gait in healthy goats (Rifkin et al., 2019). KGA demonstrated a difference between the left hind (treated limb) and right hind (untreated limb) across timepoints for parameters including stance time, stride length, and force. When comparing KGA to a previous pilot study (Reppert et al., 2020), stance time in this study was overall shorter in time between both left hind and right hind. Stride length was overall shorter in length on the left hind when compared to the previous pilot study (Reppert et al., 2020). Force, impulse, and contact pressure were all smaller in value on both limbs (left affected and right control) when compared with previous results by Reppert et al. (2020).

Plasma cortisol has been used as a measure of stress and inflammation associated with lameness induction in other livestock species (Coetzee et al., 2014). In a previous pilot study (Reppert et al., 2020), there was no evidence of differences in mean cortisol concentrations between treatment groups. Evidence from this study suggests that there is a treatment by timepoint effect seen between all three Amp B treatment groups and CNTL ($P = 0.04$). This suggests that cortisol values were positively or negatively affected based on the lameness induction treatment randomly assigned to each individual on trial.

Substance P is a recognized neuropeptide shown to be involved with the pain integration within areas of neuroaxis (Devane, 2001). There is no published data regarding substance P in either naturally occurring lameness or following induction in lameness models in goats. Substance P is a neuropeptide that has been shown to be associated with nociception in cattle (Coetzee et al., 2008). In a previous lameness study in cattle (Kleinhenz et al., 2019), there was

no evidence to suggest treatment differences concerning substance P concentrations. In horses, synovial concentrations of substance P were elevated in joints with osteoarthritis compared to non-diseased joints. Substance P levels have also been correlated to prostaglandin E2 concentrations in arthritic joints (Kirker-Head et al., 2000). Substance P results from this study highlight that there were no treatment differences between all three Amp B treatments when compared to CNTL.

When comparing the previous pilot study (Reppert et al., 2020), results from this trial showed several lameness inducing similarities in goats. In the pilot study (Reppert et al., 2020), a single dose of Amp B (10 mg/mL) was prepared for injection to individuals. In the current study, three varying dosages of amphotericin B (5 mg/0.25 mL, 0.25 mg/0.5 mL, 2.5 mg/0.25 mL) were prepared to assess their associated levels of lameness. The objective of varying dosages of Amp B was to determine an optimal dose for inducing lameness, while avoiding making individuals severely lame (VLS Score of 4+) or lame for an extended period (48 h + duration). The pilot study conducted prior to this trial (Reppert et al., 2020) produced severely lame goats (VLS score of 4+), which was half of the individuals (n = 3/6) on study. Many of these individuals required rescue analgesia given the level of severity and duration of lameness (VLS > 2, 48 h + duration, prolonged lying bouts, depression, and reduced feed intake). When comparing varying doses of Amp B in our trial, no goats exhibited lameness to the degree that required rescue intervention procedures when compared with Reppert et al. (2020).

Varying dosages and volumes of Amp B allowed us to evaluate and compare which treatment protocol would best induce lameness in goats for a brief period of 3 d. While all three treatments produced lame individuals, the treatment that produced lame goats most consistently

was the HL treatment group (5 mg/0.25 mL). This allowed researchers to induce lameness in all six goats with a smaller volume that needed to be administered into the intra-articular joint.

Conclusions

The first objective of this study sought to assess three different treatments of Amp B to determine which model was optimal for lameness induction in goats. Amphotericin B was able to induce acute lameness in meat goats across all three Amp B treatment groups, but only the HL treatment induced lameness in all six goats. The development of a goat grimace scale was also proven to be effective during this study to evaluate facial expressions in response to pain in goats. Lameness was characterized by using VLS, VAS, IRT, MNT, KGA, behavior, and facial grimace assessment. This study is one of only a few that has sought to investigate lameness concerns in growing goat populations. Limitations in this study include the number of individuals in each treatment group, and the overall number of individuals. A future study involving a larger population, and a longer observation period is needed to further prove the efficacy of Amp B in consistently inducing acute lameness.

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Conflict of Interest Statement

The author and co-authors do not have any conflicts of interest to disclose.

Figures

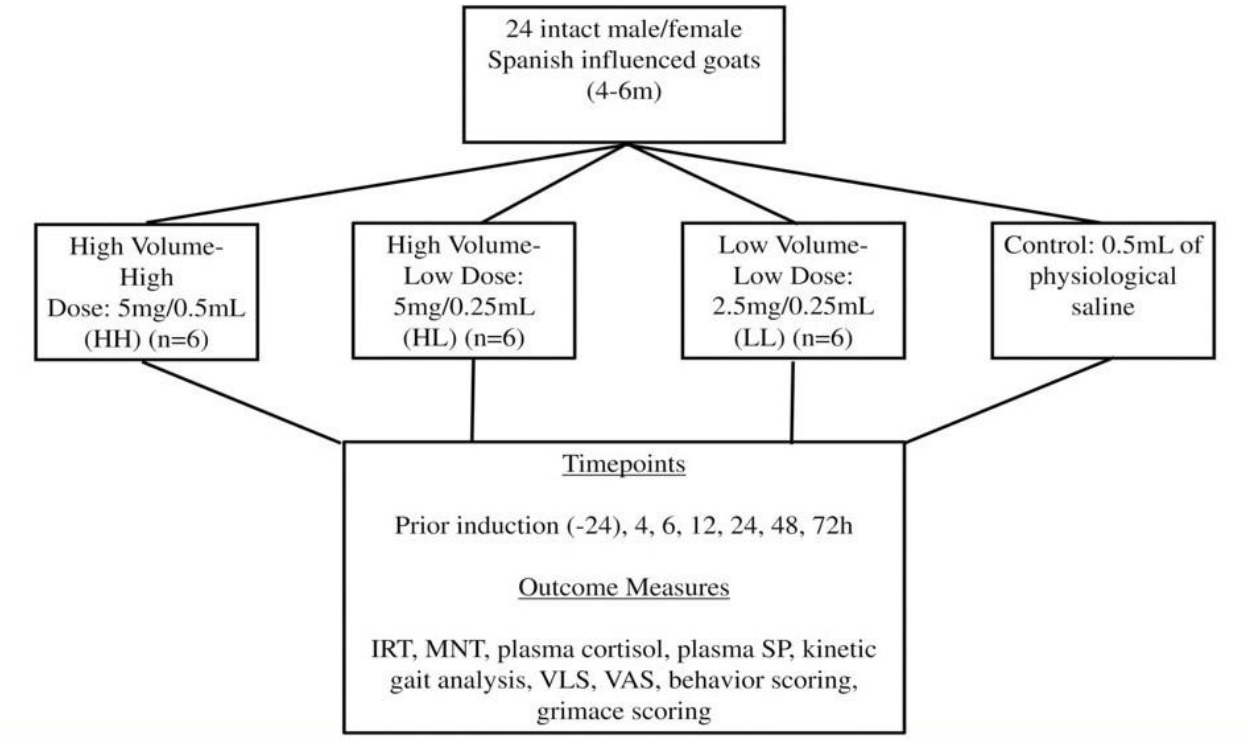

Figure 2.1. Flow chart of study events.

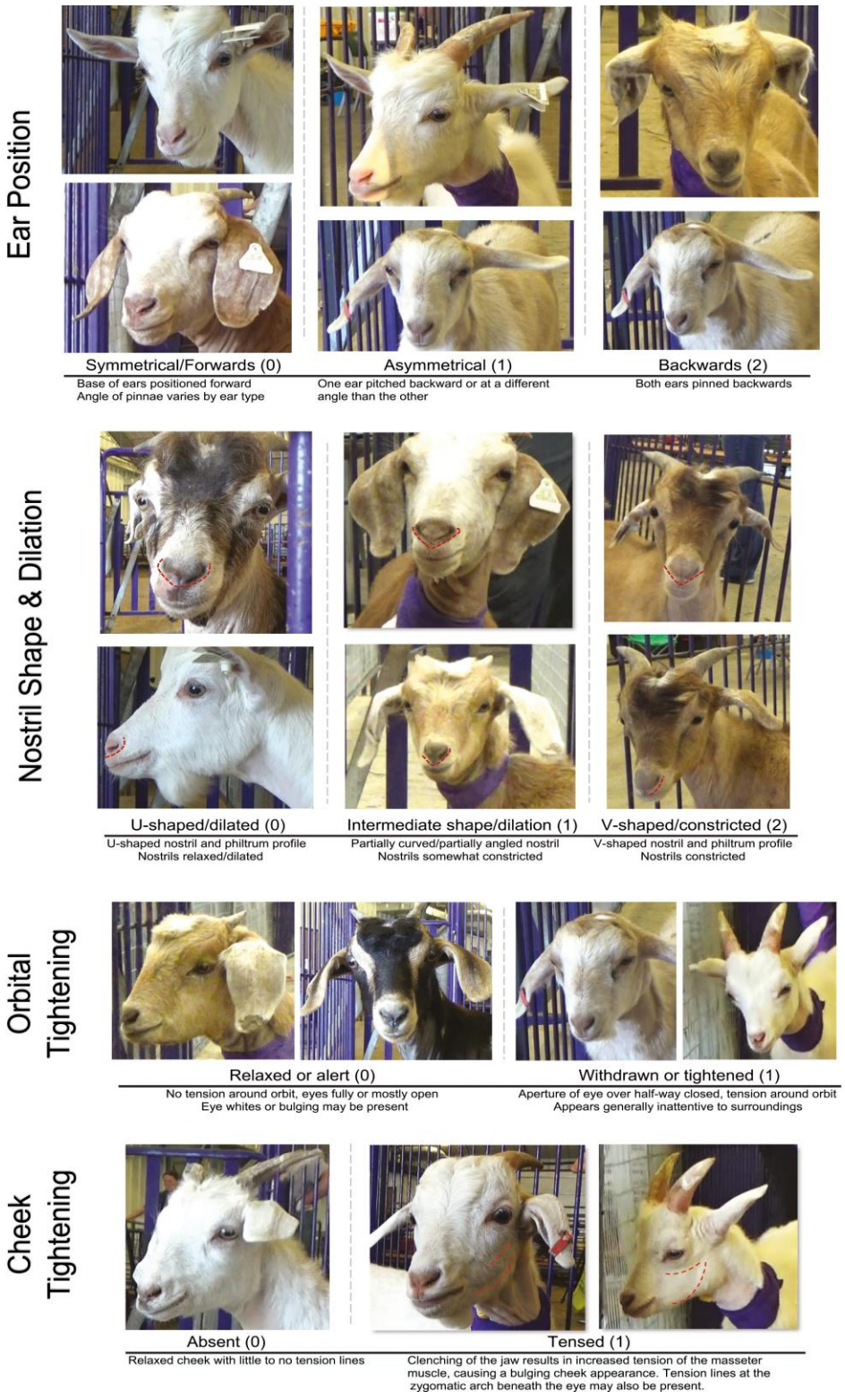

Figure 2.2 The Goat Grimace Scale with four Facial Action Units (FAUs), ear position, nose shape and dilation, orbital tightening, and cheek tightening.

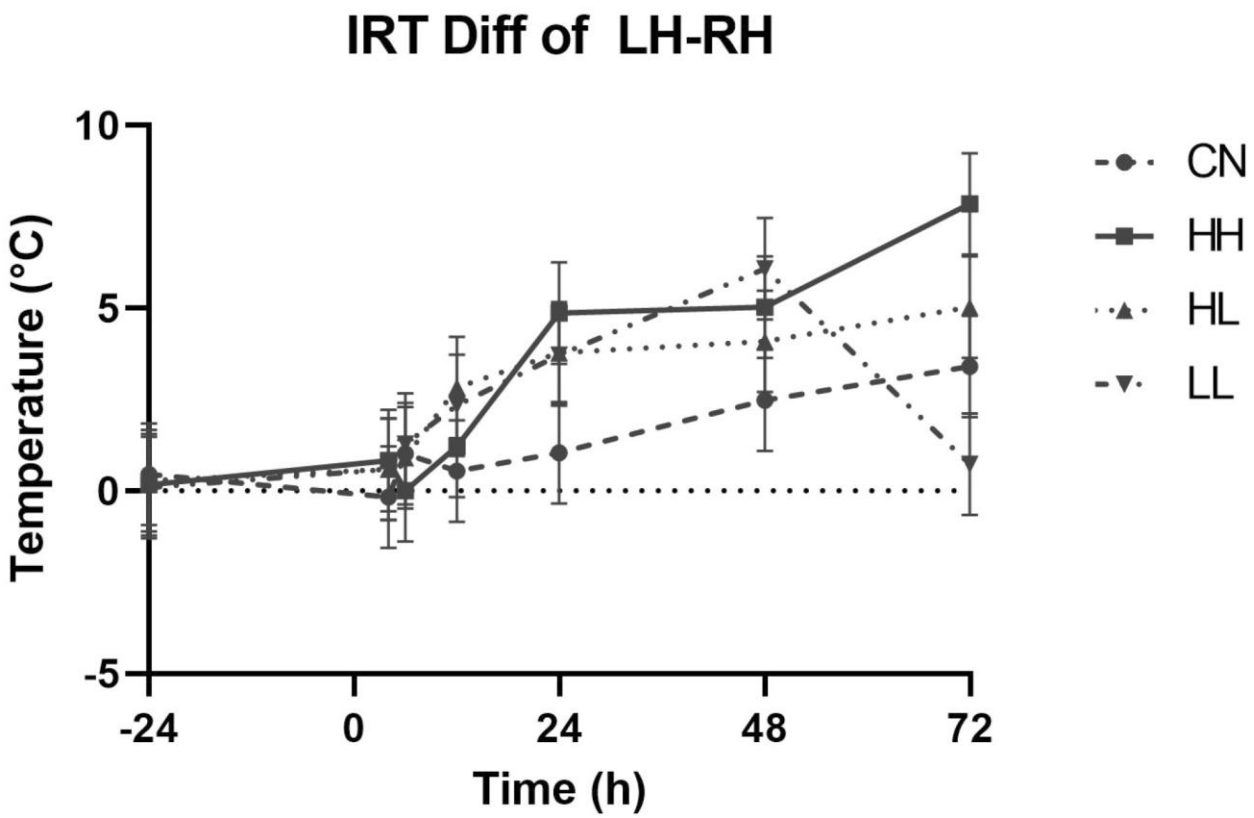

Figure 2.3 Mean (±SE) maximum temperatures (°C) from infrared thermography for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: n = 6), 5 mg/0.25 mL (HL: n = 6), 2.5 mg/0.25 mL (LL: n = 6) or a control induction using 0.5 mL of physiological saline (CNTL; n = 6).

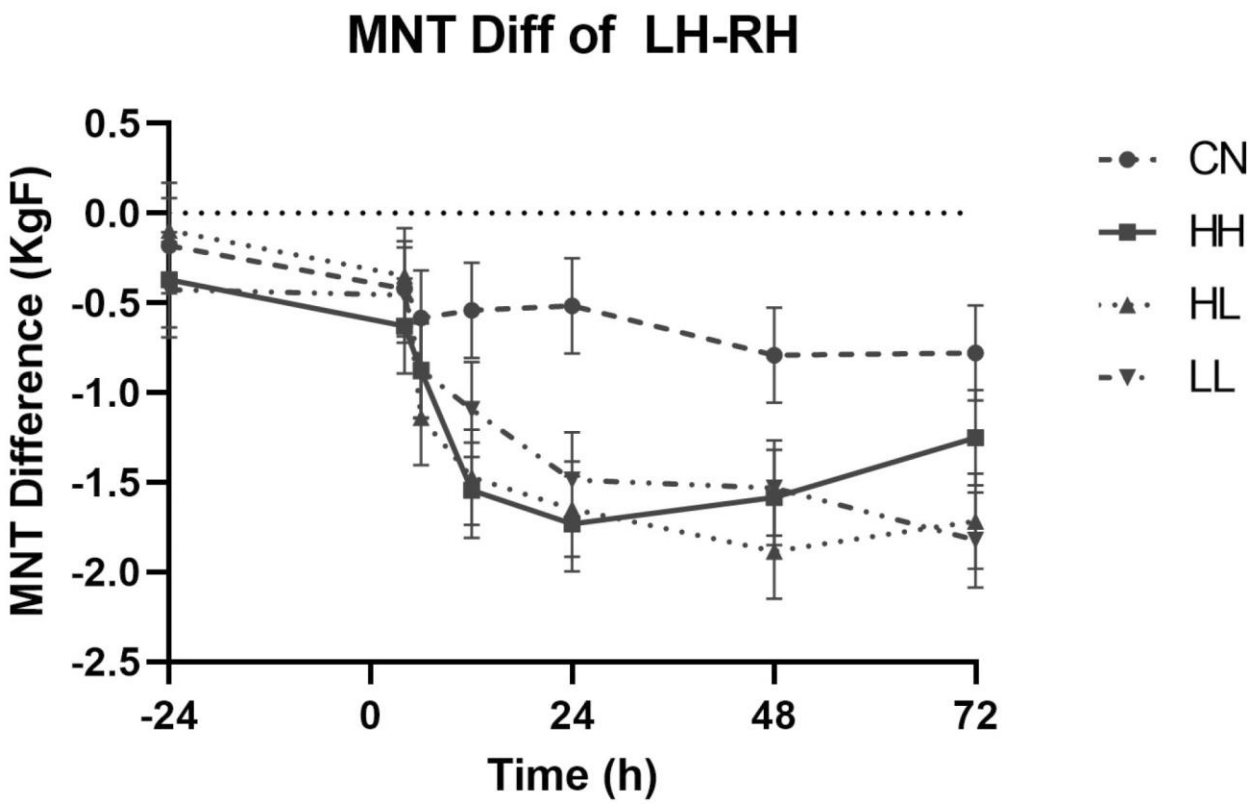

Figure 2.4 Mean (±SE) mechanical-nociception threshold differences between LH–RH for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: n = 6), 5 mg/0.25 mL (HL: n = 6), 2.5 mg/0.25 mL (LL: n = 6) or a control induction using 0.5 mL of physiological saline (CNTL; n = 6).

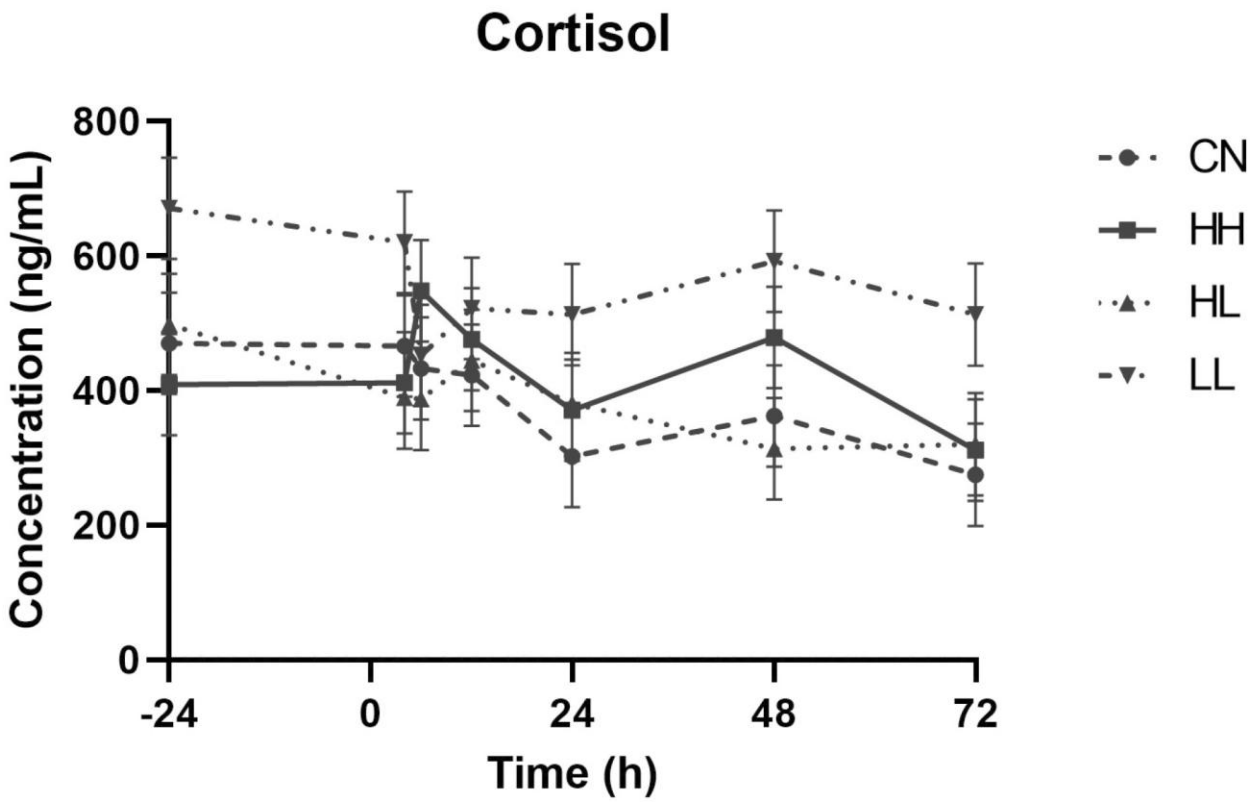

Figure 2.5 Mean (±SE) plasma cortisol concentrations (ng/mL) for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: n = 6), 5 mg/0.25 mL (HL: n = 6), 2.5 mg/0.25 mL (LL: n = 6) or a control induction using 0.5 mL of physiological saline (CNTL; n = 6).

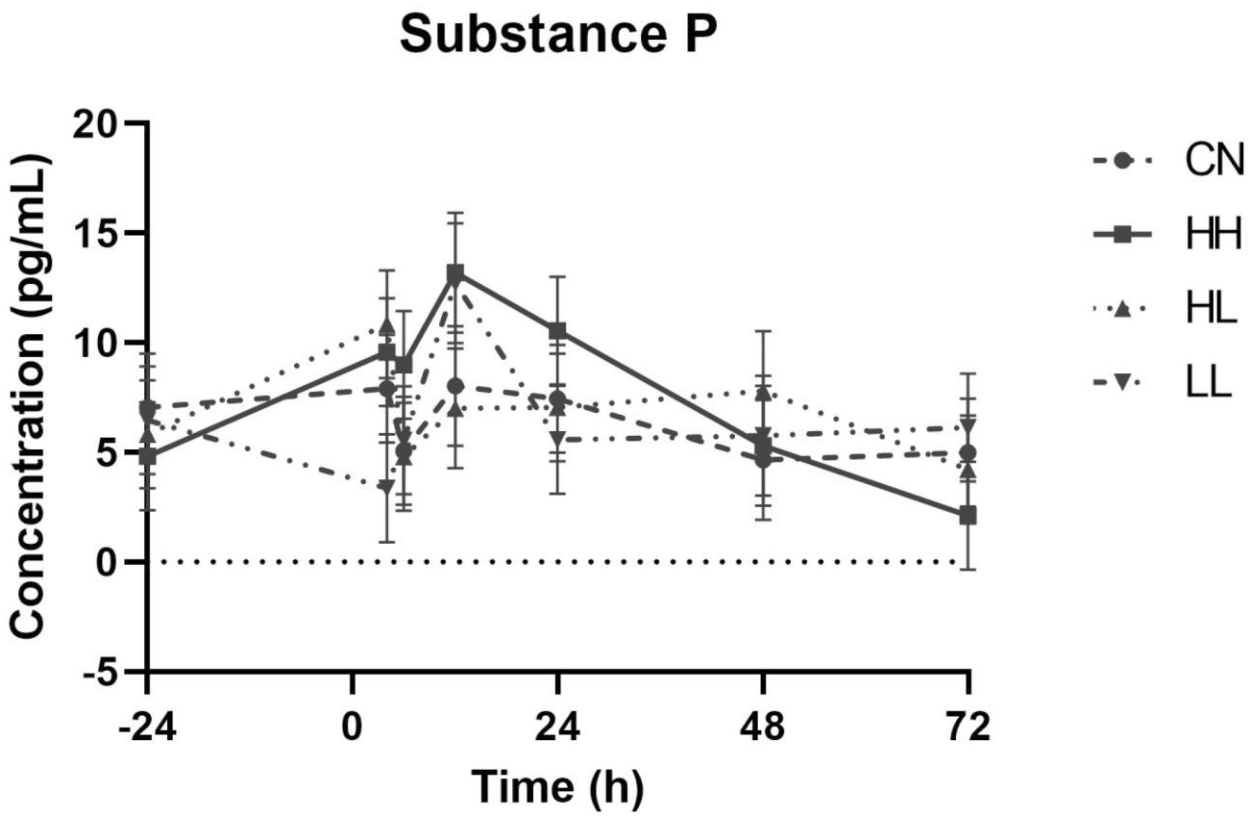

Figure 2.6 Mean (±SE) plasma substance P concentrations (ng/mL) for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: n = 6), 5 mg/0.25 mL (HL: n = 6), 2.5 mg/0.25 mL (LL: n = 6) or a control induction using 0.5 mL of physiological saline (CNTL; n = 6).

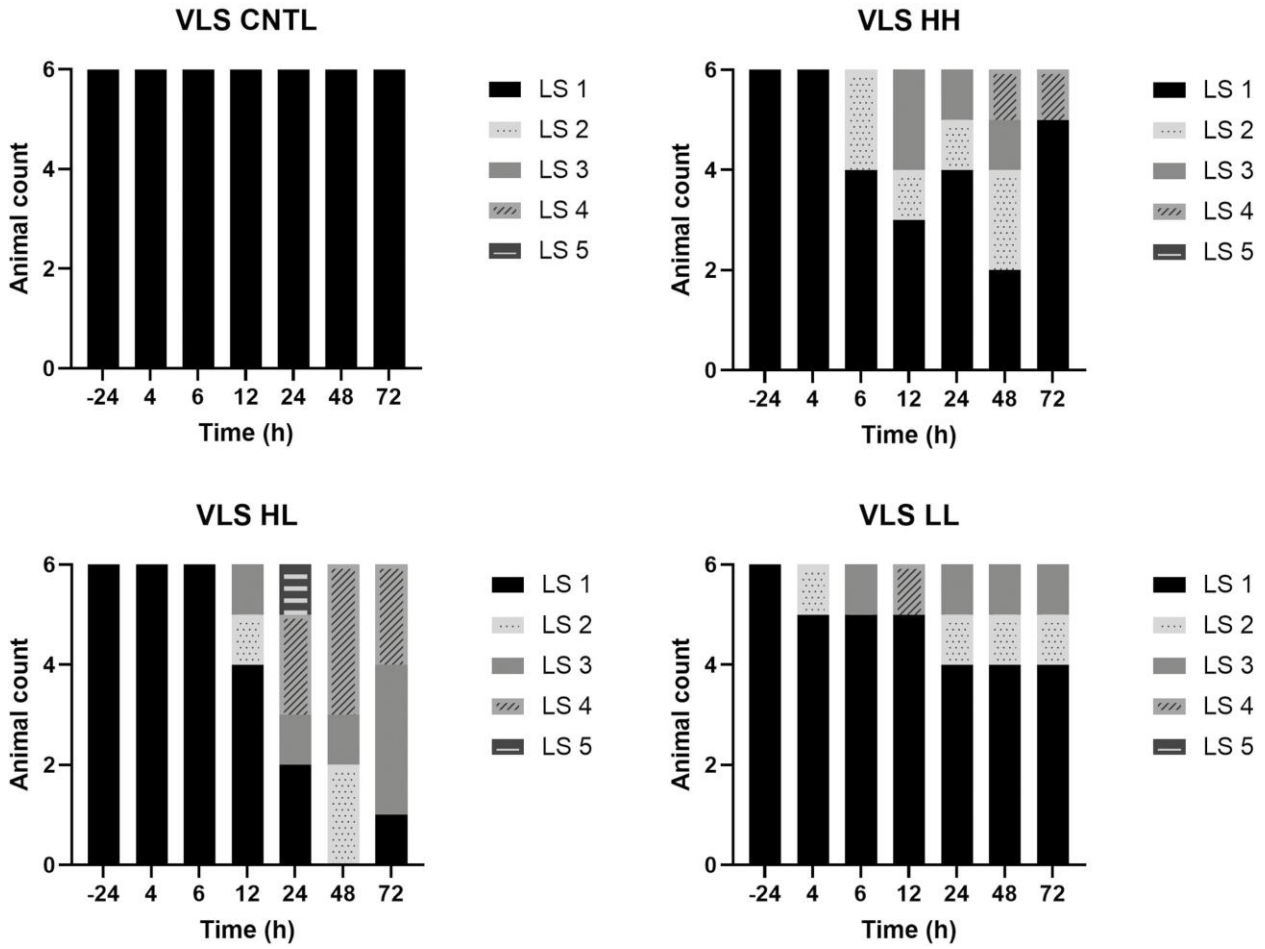

Figure 2.7 Visual lameness scores for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: n = 6), 5 mg/0.25 mL (HL: n = 6), 2.5 mg/0.25 mL (LL: n = 6) or a control induction using 0.5mL of physiological saline (CNTL; n = 6).

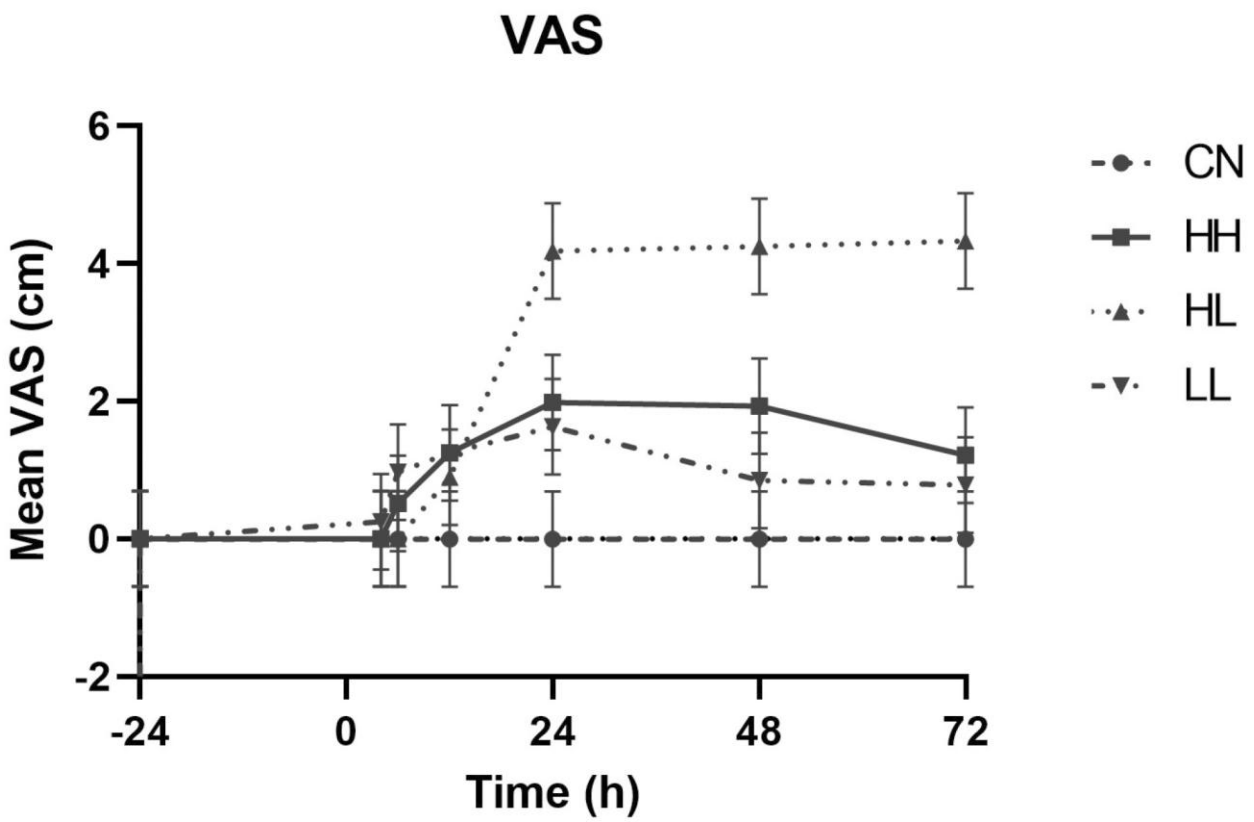

Figure 2.8 Visual analog scores (VAS) for goats with induced lameness using Amp B at varying dosages of 5 mg/0.5 mL (HH: n = 6), 5 mg/0.25 mL (HL: n = 6), 2.5 mg/0.25 mL (LL: n = 6), or a control induction using 0.5 mL of physiological saline (CNTL; n = 6).

Tables

Table 2.1. Ethogram used to score goat behavior. Behaviors were grouped into the following: maintenance, locomotion, oral behavior, social interaction, and pain behavior.

Behaviors, Description

Maintenance,

Eating, Ingesting food provided at feed bunk

Drinking, Consuming water from nozzle

Defecating, Passing fecal matter in standing or lying position

Urinating, Passing urine in standing or lying position

Sleeping, Lying down, eyes closed

Scratching, Using horns or rear hoof to scratch the body

Ruminating, Regurgitating, chewing, and swallowing food

Pawing, Moving front limb in a digging motion against the ground or feed bunk

Grooming, Licking or rubbing body or head against pen

Locomotion,

Walking, Moving forward at a normal pace

Limping, Walking with one or more legs not supporting body weight

Standing, Body weight supported by four legs, no forward movement

Standing on 3 legs, Body weight supported by three legs; non-weight bearing on one limb. No forward movement

Sitting, Body weight supported by hindquarters and front legs

Lying, Recumbent, body on ground

Rearing, Body weight supported by back legs. Front legs on fence or feed bunk

Kneeling, Body weight supported by front carpal joints and hind legs

Oral behavior,

Licking, Moving tongue over surfaces or pen mates

Chewing, Nibbling at substrates or conspecific in nearby pen

Sniffing, Inhaling air close to object or conspecific in nearby pen

Social interaction,

Playing, Running, trotting, galloping, or springing

Butting, Head-to-head or head-to-body contact with conspecific in nearby pen

Allo-grooming, Licking or rubbing body against conspecific in nearby pen

Agonistic, Biting or fighting other littermates (e.g., head-knock)

Pain behavior,

Restlessness, Repeated sitting, standing, or walking for short durations, unsettled

Attention to hoof, Guarding or constant attention to injured foot or limb

Tail wagging, Tail movement from side to side (or up and down)

Foot flicking, Moving back limb in a flicking/kicking motion

Table 2.2 Pre-treatment and post-treatment proportional mean durations (presented in seconds ± SE) of behavioral analysis outcomes across all time points for goats with experimentally induced lameness using Amp B in the following dosages of 5mg/0.5ml (HH: n=6), 5mg/0.25ml (HL: n=6), 2.5mg/0.25ml (LL: n=6) or a control induction using 0.5mL of physiological saline (CNTL; n=6).

	Pre-Lameness Induction		Post-Lameness Induction				
Behavior[c]	Trt P-value	Pre-Treatment	Trt P-value	Time P-value	HH (n=6)	HL(n=6)	LL(n=6)	CNTL(n=6)
Grooming	0.47	108.8 ± 22.2	0.02	0.0004	120.4 ± 13.8[a]	60.9 ± 14.7[b]	113.0 ± 13.6[a]	111.7 ± 13.6[ab]
Scratching	0.51	49.7 ± 16.1	0.01	0.43	55.9 ± 7.2[a]	22.8 ± 7.8[b]	47.5 ± 7.3[ab]	52.2 ± 7.0[a]
Lying	0.60	734.6 ± 436.5	0.15	0.005	1132.0 ± 115.7	1137.0 ± 107.0	914.2 ± 106.2	858.6 ± 110.8
Standing	0.70	1634.6 ± 90.8	0.20	<.0001	797.5 ± 120.4	680.7 ± 129.2	868.5 ± 109.8	1035 ± 108.1
Licking	0.88	33.3 ± 8.7	0.02	0.03	16.6 ± 11.1	-	15.9 ± 12.7	38.0 ± 7.7
Eating	0.69	132.1 ± 53.1	0.12	0.02	342.7 ± 48.4	234.3 ± 49.2	321.0 ± 45.7	210.5 ± 43.3
Drinking	-[d]	135.5 ± 22.6	0.09	0.001	21.9 ± 7.0	-	11.3 ± 4.3	23.0 ± 4.2

[a,b]Means with different superscripts in the same row differ significantly ($P < 0.05$)

[c]Only behavior variables that were significant post-treatment are presented

[d]Dash indicates behavior was not observed

Table 2.3 Definitions of Kinetic Gait Analysis biomechanical markers.

Outcome	Definition (unit)
Stance Time	The time that passes in a gait cycle of one extremity (s).
Stride Length	The distance between two successive placements of the same extremity (cm).
Force	The maximum force measured for a single step from a single extremity (kg).
Impulse	The maximum force applied per unit of time measured (kg x s).
Contact area	The peak pressure measured from a singular footfall (kg/cm^2).

Table 2.4 Overall mean (95% Confidence Interval) outcome measures from kinetic gait analysis in goats with induced lameness using Amp B at varying dosages of 5mg/0.5ml (HH: n=6), 5mg/0.25ml (HL: n=6), 2.5mg/0.25ml (LL: n=6) or a control induction using 0.5mL of physiological saline (CNTL; n=6).

Parameter	LS[1] means				*P*-values		
	HH(n=6)	HL(n=6)	LL(n=6)	CNTL(n=6)	Treatment	Time	Treatment × time
Left hind foot							
Stance time, s	0.27 (0.23-0.31)	0.21 (0.17-0.25)	0.25 (0.21-0.29)	0.25 (0.21-0.29)	0.17	0.01	0.08
Stride length, cm	74.29 (66.87-81.92)	81.86 (74.44-89.29)	73.54 (66.12-80.96)	79.53 (72.11-86.95)	0.33	0.0065	0.29
Force, kg	1.91 (1.48-2.35)	2.87 (2.43-3.31)	2.70 (2.27-3.14)	2.98 (2.55-3.42)	0.003	0.0002	0.91
Impulse, kg × s	0.32 (0.24-0.41)	0.44 (0.35-0.53)	0.46 (0.37-0.55)	0.46 (0.38-0.55)	0.086	0.64	0.33
Contact pressure, kg/cm^2	1.26 (1.07-1.45)	1.47 (1.29-1.66)	1.61 (1.43-1.80)	1.47 (1.28-1.66)	0.07	0.036	0.71
Right hind foot							

Stance time, s	0.28 (0.22-0.33)	0.23 (0.18-0.28)	0.31 (0.26-0.37)	0.23 (0.18-0.28)	0.08	0.13	0.43
Stride length, cm	78.87 (72.44-85.29)	82.66 (76.24-89.08)	79.38 (72.96-85.81)	75.47 (69.05-81.90)	0.48	0.0021	0.26
Force, kg	2.10 (1.68-2.52)	2.77 (2.35-3.19)	2.24 (1.82-2.66)	2.45 (2.02-2.87)	0.14	0.43	0.032
Impulse, kg × s	0.39 (0.31-0.47)	0.46 (0.38-0.54)	0.44 (0.36-0.52)	0.38 (0.30-0.46)	0.38	0.56	0.01
Contact pressure, kg/cm^2	1.36 (1.22-1.51)	1.43 (1.29-1.57)	1.44 (1.30-1.58)	1.19 (1.05-1.33)	0.05	0.87	0.03

[1] Least Squares

References

Bach, A., Dinarés, M., Devant, M., & Carré, X. (2007). Associations between lameness and production, feeding and milking attendance of Holstein cows milked with an automatic milking system. *J Dairy Res*, *74*(1), 40-46. https://doi.org/10.1017/S0022029906002184

Blackie, N., Amory, J., Bleach, E., & Scaife, J. (2011). The effect of lameness on lying behaviour of zero grazed Holstein dairy cattle. *Applied Animal Behaviour Science*, *134*(3), 85-91. https://doi.org/https://doi.org/10.1016/j.applanim.2011.08.004

Byrne, D. T., Berry, D. P., Esmonde, H., McGovern, F., Creighton, P., & McHugh, N. (2019). Infrared thermography as a tool to detect hoof lesions in sheep. *Transl Anim Sci*, *3*(1), 577-588. https://doi.org/10.1093/tas/txy132

Christodoulopoulos, G. (2009). Foot lameness in dairy goats. *Res Vet Sci*, *86*(2), 281-284. https://doi.org/10.1016/j.rvsc.2008.07.013

Coetzee, J. F., Lubbers, B. V., Toerber, S. E., Gehring, R., Thomson, D. U., White, B. J., & Apley, M. D. (2008). Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. *Am J Vet Res*, *69*(6), 751-762. https://doi.org/10.2460/ajvr.69.6.751

Coetzee, J. F., Mosher, R. A., Anderson, D. E., Robert, B., Kohake, L. E., Gehring, R.,…Wang, C. (2014). Impact of oral meloxicam administered alone or in combination with gabapentin on experimentally induced lameness in beef calves. *J Anim Sci*, *92*(2), 816-829. https://doi.org/10.2527/jas.2013-6999

Crosby-Durrani, H. E., Clegg, S. R., Singer, E., Angell, J. W., Evans, N. J., Carter, S. D., Duncan, J. S. (2016). Severe Foot Lesions in Dairy Goats Associated with Digital Dermatitis Treponemes. *J Comp Pathol*, *154*(4), 283-296. https://doi.org/10.1016/j.jcpa.2016.04.001

Deeming, L. E., Beausoleil, N. J., Stafford, K. J., Webster, J. R., & Zobel, G. (2018). Technical note: The development of a reliable 5-point gait scoring system for use in dairy goats. *J Dairy Sci*, *101*(5), 4491-4497. https://doi.org/10.3168/jds.2017-13950

DeVane, C. L. (2001). Substance P: a new era, a new role. *Pharmacotherapy*, *21*(9), 1061-1069. https://doi.org/10.1592/phco.21.13.1061.34612

Galindo, F., & Broom, D. M. (2002). Effects of lameness of dairy cows. *J Appl Anim Welf Sci*, *5*(3), 193-201. https://doi.org/10.1207/S15327604JAWS0503_03

Hill, N. P., Murphy, P. E., Nelson, A. J., Mouttotou, N., Green, L. E., & Morgan, K. L. (1997). Lameness and foot lesions in adult British dairy goats. *Vet Rec*, *141*(16), 412-416. https://doi.org/10.1136/vr.141.16.412

Häger, C., Biernot, S., Buettner, M., Glage, S., Keubler, L. M., Held, N.,Bleich, A. (2017). The Sheep Grimace Scale as an indicator of post-operative distress and pain in laboratory sheep. *PLoS One*, *12*(4), e0175839. https://doi.org/10.1371/journal.pone.0175839

Ito, K., von Keyserlingk, M. A., Leblanc, S. J., & Weary, D. M. (2010). Lying behavior as an indicator of lameness in dairy cows. *J Dairy Sci*, *93*(8), 3553-3560. https://doi.org/10.3168/jds.2009-2951

Kaler, J., Mitsch, J., Vázquez-Diosdado, J. A., Bollard, N., Dottorini, T., & Ellis, K. A. (2020). Automated detection of lameness in sheep using machine learning approaches: novel insights into behavioural differences among lame and non-lame sheep. *R Soc Open Sci*, *7*(1), 190824. https://doi.org/10.1098/rsos.190824

Kirker-Head, C. A., Chandna, V. K., Agarwal, R. K., Morris, E. A., Tidwell, A., O'Callaghan, M. W.,Kumar, M. S. (2000). Concentrations of substance P and prostaglandin E2 in synovial fluid of normal and abnormal joints of horses. *Am J Vet Res*, *61*(6), 714-718. https://doi.org/10.2460/ajvr.2000.61.714

Kleinhenz, M. D., Gorden, P. J., Smith, J. S., Schleining, J. A., Kleinhenz, K. E., Juarez, J. R.,Coetzee, J. F. (2019). Effects of transdermal flunixin meglumine on experimentally induced lameness in adult dairy cattle. *J Dairy Sci*, *102*(7), 6418-6430. https://doi.org/10.3168/jds.2018-15091

Kleinhenz, M. D., Van Engen, N. K., Gorden, P. J., Ji, J., Walsh, P., & Coetzee, J. F. (2017). Effects of transdermal flunixin meglumine on pain biomarkers at dehorning in calves. *J Anim Sci*, *95*(5), 1993-2000. https://doi.org/10.2527/jas.2016.1138

Martin, M., Kleinhenz, M., Kleinhenz, K., Reppert, E., Blasi, D., Parks, T.,Coetzee, J. (2020). Comparison of the effect of tildipirosin administered alone or in combination with transdermal flunixin on the performance, health, activity, and well-being of transported feedlot calves on arrival at the feedlot. *Transl Anim Sci*, *4*(1), 452-459. https://doi.org/10.1093/tas/txaa005

Martin, M. S., Kleinhenz, M. D., Viscardi, A. V., Curtis, A. K., Johnson, B. T., Montgomery, S. R.,Coetzee, J. F. (2022). Effect of bupivacaine liposome suspension administered as a local anesthetic block on indicators of pain and distress during and after surgical castration in dairy calves. *J Anim Sci*, *100*(1). https://doi.org/10.1093/jas/skab378

McIlwraith, C. W., Fessler, J. F., Blevins, W. E., Page, E. H., Rebar, A. H., Van Sickle, D. C., & Coppoc, G. L. (1979). Experimentally induced arthritis of the equine carpus: clinical determinations. *Am J Vet Res*, *40*(1), 11-20.

Muley, M. M., Krustev, E., & McDougall, J. J. (2016). Preclinical Assessment of Inflammatory Pain. *CNS Neurosci Ther, 22*(2), 88-101. https://doi.org/10.1111/cns.12486

Reppert, E. J., Kleinhenz, M. D., Viscardi, A., Montgomery, S. R., Crane, A. R., & Coetzee, J. F. (2020). Development and evaluation of two different lameness models in meat goats, a pilot study. *Transl Anim Sci, 4*(4), txaa193. https://doi.org/10.1093/tas/txaa193

Rifkin, R. E., Grzeskowiak, R. M., Mulon, P. Y., Adair, H. S., Biris, A. S., Dhar, M., & Anderson, D. E. (2019). Use of a pressure-sensing walkway system for biometric assessment of gait characteristics in goats. *PLoS One, 14*(10), e0223771. https://doi.org/10.1371/journal.pone.0223771

Schulz, K. L., Anderson, D. E., Coetzee, J. F., White, B. J., & Miesner, M. D. (2011). Effect of flunixin meglumine on the amelioration of lameness in dairy steers with amphotericin B-induced transient synovitis-arthritis. *Am J Vet Res, 72*(11), 1431-1438. https://doi.org/10.2460/ajvr.72.11.1431

USDA. (2010). Goat 2009, Part I: Reference of Goat Management Practices in the United States. In. Fort Collins, CO: 2009 USDA-APHIS-VS, CEAH.

Van Engen, N. K., Stock, M. L., Engelken, T., Vann, R. C., Wulf, L. W., Karriker, L. A.,Coetzee, J. F. (2014). Impact of oral meloxicam on circulating physiological biomarkers of stress and inflammation in beef steers after long-distance transportation. *J Anim Sci, 92*(2), 498-510. https://doi.org/10.2527/jas.2013-6857

Viscardi, A. V., Reppert, E. J., Kleinhenz, M. D., Wise, P., Lin, Z., Montgomery, S.,Coetzee, J. F. (2021). Analgesic Comparison of Flunixin Meglumine or Meloxicam for Soft-Tissue Surgery in Sheep: A Pilot Study. *Animals (Basel), 11*(2). https://doi.org/10.3390/ani11020423

Warner, R., Kleinhenz, M. D., Ydstie, J. A., Schleining, J. A., Wulf, L. W., Coetzee, J. F., & Gorden, P. J. (2021). Randomized controlled trial comparison of analgesic drugs for control of pain associated with induced lameness in lactating dairy cattle. *J Dairy Sci, 104*(2), 2040-2055. https://doi.org/10.3168/jds.2020-18563

Chapter 3 - Comparison of firocoxib and meloxicam for pain mitigation in goats undergoing surgical castration

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Abstract

Objective

This study sought to determine whether firocoxib (FIRO) or meloxicam (MEL) was effective at providing analgesia after surgical castration in goats.

Animals

18 intact male crossbred goats (6 to 8 months old) were enrolled with a mean weight of 32.6 (± 2.9) kg.

Methods

Surgical castration was done under injectable anesthesia by a licensed veterinarian. Twelve bucks were surgically castrated and given either FIRO (n = 6) or MEL (n = 6). Six bucks served as controls (CNTLs) and were not castrated. Outcome measurements included visual analogue scale, infrared thermography, plasma cortisol, plasma substance P, and kinetic gait analysis. All outcome measurements were obtained at –24, 4, 8, 24, 48, and 72 hours.

Results

All 3 treatments were significantly different from each other at the 24- and 48-hour time points, with MEL animals having lower visual analogue scale scores when compared to FIRO animals; CNTL animals exhibited the lowest plasma cortisol levels (3.19 ng/mL; 95% CI, –1.21 to 7.59 ng/mL) followed by FIRO (7.45 ng/mL; 95% CI, 3.10 to 11.80 ng/mL) and MEL (10.24 ng/mL; 95% CI, 5.87 to 14.60 ng/mL). FIRO had an average mean decrease in gait velocity change (–54.17 cm/s; 95% CI, –92.99 to –15.35 cm/s), while MEL had an increase in gait velocity when compared to baseline values (14.54 cm/s; 95% CI, –24.27 to 53.36 cm/s). Control animals had an average mean of –3.06 cm/s (95% CI, –41.88 to 35.75 cm/s).

Clinical Relevance

Results from this study showed that there were some analgesic effects from administering MEL when compared to bucks that received a placebo treatment (CNTL).

Introduction

Surgical castration is a frequent practice in livestock management. Castration is commonly performed on intact male food animals to reduce aggression, prevent indiscriminate breeding, and improve carcass quality (Needham et al., 2017). Castration has been cited as a source of pain and stress in cattle (Kleinhenz et al., 2018), but a specific pain scale to evaluate and demonstrate postoperative pain in goats had not been developed until recent years. Analgesia is used to provide pain relief and reduce stress in livestock animals undergoing castration. There are currently no approved analgesic drugs for surgical castration in goats in the US. Veterinarians must extrapolate analgesic data from other livestock species to treat pain in goats. Sheep and cattle are commonly used as references, but specific pain protocols are needed to accurately assess and treat pain responses in goats.

For male goats intended to be kept as pets, castration is recommended closer to sexual maturity (6 to 8 months old) to allow for increased urethral diameter to reduce incidence of blockage in the male urethra (Makhdoomi and Gazi, 2013). Blockages in the male urinary tract, often referred to as obstructive urolithiasis, is a condition that is complex and multifactorial (Makhdoomi and Gazi, 2013). Obstructive urolithiasis of male ruminants is difficult to manage, causing significant economic losses and compromising animal welfare (Cook, 2023; Fortier et al., 2004). Surgery is the most common treatment for obstructive urolithiasis (Reppert et al., 2016), which insinuates welfare concerns for the animal and economic burdens for owners. Although urolithiasis is a multifactorial condition that is mainly due to diet components, maximizing urethral diameter in small ruminants intended as pets is encouraged to help prevent this condition. Therefore, it is important to prevent blockages from occurring in pet goats by castrating at an older age when compared to production animals.

Firocoxib (FIRO) is an NSAID that is a fast-acting cyclooxygenase-2 selective inhibitor (Kvaternick et al., 2007). Cyclooxygenase plays a significant role in the production of prostaglandins, which are produced by mammalian tissues and facilitate intercellular communication of various processes (Fitzpatrick, 2004; Wilson et al., 2004). FIRO has been shown to be useful in inhibiting pain responses in horses and in dogs. The pharmacokinetics of FIRO in goats has been previously described in older adult female goats using a paste formulation with a bioavailability of 71% (Stuart et al., 2019). Orally administered FIRO at 0.5 mg/kg is rapidly absorbed, with a Tmax of 0.77 hours and an oral bioavailability of 77%. FIRO also has a long terminal half-life of 27 hours (Stuart et al., 2019).

Meloxicam (MEL) is an oxicam-class NSAID that is a preferential inhibitor of cyclooxygenase-2 (Coetzee et al., 2009). It is used commonly in livestock production due to its longer half-life (10.7 hours) and high oral bioavailability (96%) (Glynn et al., 2013; Karademir et al., 2016). MEL has been shown previously to help mitigate pain responses in livestock animals, such as cattle and sheep, along with small animal species, including dogs and cats, following oral dosing.

The objective of this study was to investigate the analgesic effects of FIRO and MEL administered orally before surgical castration in goats using associated pain biomarkers previously described in cattle (Kleinhenz et al., 2018). We hypothesized that either FIRO or MEL, when administered before surgical castration, would provide some analgesia benefits by lowering visual analogue scale (VAS) scores, decreasing infrared ocular temperatures, lowering cortisol values and substance P (Sub P), and increasing stride length and gait velocity.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee at Kansas State University (protocol No. 4627).

Animals and study design

Eighteen intact male crossbred goats (6 to 8 months old) were enrolled with a mean weight (± SD) of 32.6 ± 2.9 kg. All individuals on trial had two (2) descended testicles, as determined by a licensed veterinarian. Goats were kept in group housing (pens) and were fed exclusively a diet of brome hay ad libitum. Goats had free access to water and minerals for supplementation. The study was conducted over a 2-week period, with 1 week dedicated to acclimation and 1 week focused on castration, data collection, and poststudy monitoring. Each individual enrolled in the study (goat) was both the experimental and observational unit. Following the 1-week acclimation period, a random number generator (random.org) was used to assign each of the goats to 1 of the 3 treatments:

FIRO-castration (n = 6)—Surgical castration was performed. FIRO (Equioxx Tablets; Boehringer Ingelheim Animal Health) was administered at 1 mg/kg through oral tablet administration (gelatin bolus administered with balling gun [Torpac]) at induction (0 hours) and then after at 0.5 mg/kg orally at 24 and 48 hours after castration.

MEL-castration (n = 6)—Surgical castration was performed. MEL (Zydus Pharmaceuticals Inc.) was administered at 2 mg/kg through oral administration (gelatin bolus administered with balling gun) at induction (0 hours) and then after at 1 mg/kg orally at 24 and 48 hours after castration.

Control (n = 6)—Surgical castration was not performed. A placebo treatment was administered to individuals orally (whey protein powder in gelatin bolus administered with

balling gun) at induction (0 hours) and then after at 1 mg/kg orally at 24 and 48 hours after sedation.

Castration procedure

Twelve goats were castrated for the study. All goats, including control (CNTL) goats, were fasted for 24 hours prior to the procedure. All goats were sedated with xylazine hydrocholoride (0.04 mg/kg, IV; Akron Inc) and ketamine (2.0 mg/kg, IV; Putney Inc). Goats that were castrated were taken to a surgical suite. The castration site was clipped and sterilely prepped for surgery. Both spermatic cords were blocked with lidocaine injection (1 mL/cord; 2 mL total; 2%; MWI Animal Health) prior to surgical castration when animals were under general anesthesia. Two licensed veterinarians performed and assisted during all castration procedures. Animals were monitored for respiratory rate and heart rate under anesthesia and after surgery until they regained full consciousness. Following the castration procedure, or after 10 minutes of time for the CNTL goats, each goat was administered an anesthetic reversal agent, atipamezole, at 0.05 mg/kg IM (Zoetis Inc).

Outcome variables

All outcomes were measured for each goat at –24, 4, 8, 24, 48, and 72 hours. Outcomes of interest included VAS, infrared thermography (IRT), blood plasma cortisol and Sub P, and kinetic gait analysis (KGA; Figure 3.1).

Visual analogue scale

A VAS score was adapted from Martin et al., 2022, to quantify pain in goats based on visual parameters correlated to pain. VAS consisted of a 100-mm line with “NO PAIN” anchoring the left side and “SEVERE PAIN” to the right. The following parameters were used to assess pain: depression, tail swishing or flicking, full body stance, head carriage, spinal

alignment, and movement. No pain was characterized by being alert and quick to show interest, no tail swishing, a normal stance, head carriage above spine level, a straight spine, moving freely around the pen, and ears forward. Severe pain was characterized by being dull and showing no interest, more than 3 tail swishes/min, legs abducted, head held below spine level, a curved spine, and reluctant to move. The study utilized a trained observer (masked to treatment group) to score the level of pain, from 0 to 100 mm, based on visual parameters. Scores were taken prior to (–24), and 4, 8, 24, 48, and 72 hours after castration. A millimeter scale was used to measure the score from the zero-anchor point to the evaluator's mark.

IRT imaging

Infrared thermography of the left medial canthus of each individual on study was obtained prior to castration (–24) as well as at 4, 8, 24, 48, and 72 hours afterwards. The image was taken noninvasively and occurred prior to blood sampling for each time point with a commercially available IRT camera (TiX850 Thermal Camera; Fluke Corp). Images were stored on flash memory card after image capture. The maximum temperature of the medial canthus was recorded with SmartView, version 4.3 (Fluke Corp).

Plasma cortisol

Blood samples were collected via jugular venipuncture from each goat (6 mL of whole blood) at –24, 4, 8, 24, 48, and 72 hours. Blood was collected in a 3-mL heparinized vacutainer (Greiner Bio-One North America Inc) and placed on ice until plasma cortisol levels could be processed. Plasma cortisol concentrations from each sample were analyzed in duplicate with a radioimmunoassay (RIA) system using methods adapted from Kleinhenz et al., (2017). Plasma cortisol concentrations were determined with a commercially available RIA kit (MP Biomedicals) following manufacturer specifications with minor modifications as previously

described (Martin et al., 2022). The standard curve was created and extended to include 1 and 3 ng/mL by diluting the 10- and 30-ng/mL manufacturer-supplied standards, 1:10 respectively. The standard curve ranged from 1 to 300 ng/mL. Two unique CNTLs (25 and 150 ng/mL) were performed at the beginning and end of each sample set to determine interassay variability. Plain 12 X 75-mm polypropylene tubes served as blank tubes to calculate nonspecific binding. From each sample, 50 µL, including standards and quality control (QC), was utilized for analysis. Samples were incubated at room temperature for 30 minutes before addition of radioactive iodine (I-125) per manufacturer instructions. Tubes were counted on a γ counter (Wizard2; PerkinElmer). Raw data values were uploaded onto MyAssays Desktop, version 7.0.211.1238 (MyAssays Ltd), for concentration determination. Standard curves were plotted as a 4-parameter logistic curve. Samples with a coefficient of variation (CV) > 18% were reanalyzed. The intra-assay and interassay CV were determined to be 26.30% and 10.42%, respectively.

Plasma Sub P

Blood was collected at the same time for both cortisol and Sub P (6 mL total separated into 2 different 3-mL tubes). Blood was collected in a 3-mL EDTA vacutainer (Greiner Bio-One North America Inc) and placed on ice until plasma Sub P levels could be processed. Time from collection to processing was 2 hours or shorter for all samples. Sub P concentrations were determined through RIA via methods described by Kleinhenz et al., (2017). A standard curve (range, 20 to 1,280 pg/mL) was created by diluting synthetic secreted protein (SP; Phoenix Pharmaceuticals) with RIA buffer (50mM sodium phosphate dibasic heptahydrate, 13mM disodium EDTA, 150mM sodium chloride, 1mM benzamidine hydrochloride, 0.1% gelatin, 0.02% sodium azide; pH 7.4). For analysis, 100 µL of sample, standard, or QC was aliquoted into plain 12 X 75-mm conical bottom tubes followed by 100 µL of rabbit anti-SP primary

antibody (1:20,000; Phoenix Pharmaceuticals). Iodine-125-SP tracer (custom iodination by PerkinElmer) was diluted with RIA buffer to 20,000 counts/min. After, 100 µL was added to the sample, standard, and QC tubes. Samples were covered and stored at 4 °C for 48 hours. At the end of the 48-hour incubation, samples were placed on ice, and 100 µL of normal rabbit plasma (1:80) and goat anti-rabbit secondary antibody (1:40; Jackson ImmunoResearch) were added to each tube. Samples were then incubated at room temperature for 10 minutes and placed back on ice, and 100 µL of blank bovine plasma was added to the standards and QCs. All tubes then had 1 mL of 12% polypropylene glycol in 0.85% sodium chloride added. Samples were centrifuged at 3,000 X g for 30 minutes at 4 °C, and the supernatant was aspirated. Tubes were counted on a γ counter (Wizard2; PerkinElmer) for 1 minute. The raw data file was then uploaded onto MyAssays Desktop software for concentration determination. Standard curves were plotted as a 4-parameter logistic curve. Samples with a CV > 18% were reanalyzed. The intra-assay and interassay CV were determined to be 6.6% and 7.96%, respectively.

Kinetic gait analysis

Commercially available floor mat–based pressure/force measurement system (Walkway; Tekscan Inc) was utilized to record and analyze the gait of each goat. Measurements for KGA were collected at –24, 4, 8, 24, 48, and 72 hours, respectively. Goats were freely walked across the mat in a chute-like fashion at each time point. Video synchronization was used to ensure consistent gait among individuals for each time point. Data from pressure force measurements were analyzed with Walkway, version 7.7 (Tekscan Inc). Data collected, including contact pressure, contact area, impulse, and stance phase duration of each foot, were assessed via the methods described in Kleinhenz et al., (2019). Study definitions for outcomes are presented in Table 3.1.

Statistical analysis

Plasma cortisol and Sub P data were log transformed to normalized data prior to statistical analysis. The outcome responses of IRT, KGA, and plasma cortisol were analyzed via linear mixed models with individual goat as the experimental unit in JMP, version 15.1 (SAS Institute Inc). Goats nested in a treatment group were designated as a random effect, with treatment, time, treatment-by-time interaction, and replicate designated as fixed effects. F tests were utilized for testing the significance of main effects and interactions. Pairwise comparisons with significant overall differences were performed using the Tukey test for honestly significant difference. Statistical significance was determined a priori at $P \leq 0.05$. Data were presented as least squares means.

Results

VAS scoring

The VAS outcomes did not differ by treatment ($P = 1.00$) but did differ by time point ($P < 0.0001$) or treatment-by-time-point interaction ($P < 0.0001$; Figure 3.2). VAS scores for animals in the CNTL group stayed consistent throughout the study around the 0-mm mark. VAS scores for animals in the FIRO and MEL groups were similar at the 4-hour time point. All 3 treatments were significantly different from each other at the 24- and 48-hour time points, with MEL animals having lower VAS scores when compared to FIRO animals.

Infrared thermography

The IRT maximum temperatures did not differ by treatment ($P = 0.08$) or treatment-over-time interaction ($P = 0.41$) but did differ by time point ($P \leq 0.0001$). These data are summarized in Table 3.2. Highest temperatures were seen at the 48-hour time point for FIRO (39.45 °C; 95% CI, 38.99 to 39.90 °C; least squares mean followed by 95% CI), the 72-hour time point for MEL (38.92 °C; 95% CI, 38.48 to 39.37 °C), and the 72-hour time point for CNTL (39.35 °C; 95% CI, 38.90 to 39.80 °C). The lowest temperatures were seen for FIRO at the –24-hour time point (37.95 °C; 95% CI, 37.49 to 38.40 °C), MEL at the –24-hour time point (37.99 °C; 95% CI, 37.55 to 38.43 °C), and CNTL at the 24-hour time point (38.37 °C; 95% CI, 37.91 to 38.82 °C).

Plasma cortisol

Plasma cortisol differed by treatment ($P = 0.0128$), by time point ($P < 0.001$), and by a treatment-over-time interaction ($P = 0.003$; Table 3.2; Figure 3.2). CNTL animals exhibited the lowest plasma cortisol levels (3.19 ng/mL; 95% CI, –1.21 to 7.59 ng/mL), followed by FIRO (7.45 ng/mL; 95% CI, 3.10 to 11.80 ng/mL) and MEL (10.24 ng/mL; 95% CI, 5.87 to 14.60 ng/mL). FIRO plasma cortisol levels were lowest at 72 hours (4.13 ng/mL; 95% CI, –1.27 to

9.54 ng/mL) and highest at 4 hours (16.31 ng/mL; 95% CI, 10.90 to 21.72 ng/mL). MEL plasma cortisol levels were lowest at 8 hours (4.63 ng/mL; 95% CI, –1.07 to 10.32 ng/mL) and highest at 4 hours (18.56 ng/mL; 95% CI, 13.15 to 23.97 ng/mL). CNTL plasma cortisol levels were lowest at 4 hours (1.32 ng/mL; 95% CI, –4.39 to 7.04 ng/mL) and highest at –24 hours (10.42 ng/mL; 95% CI, 5.00 to 15.82 ng/mL).

Plasma Sub P

Plasma Sub P did not differ by treatment (P = 0.62), by time point (P = 0.054), or by a treatment-by-time-point interaction (P = 0.25; Table 3.2). The overall mean plasma Sub P by treatment was reported to be FIRO (503.45 pg/mL; 95% CI, 372.84 to 634.05 pg/mL), MEL (586.05 pg/mL; 95% CI, 455.45 to 716.66 pg/mL), and CNTL (572.77 pg/mL; 95% CI, 442.17 to 703.37 pg/mL), respectively.

Kinetic gait analysis

Kinetic gait analysis outcomes are shown in Table 3.1 with select outcomes shown in Figure 3.3.

Stance time (s)—Front limb stance time did not differ by treatment (P = 0.28), by treatment-by-time-point interaction (P = 0.99), or by time point (P = 0.47).

The rear limb stance time did not differ by treatment (P = 0.06), by time point (P = 0.95), or by treatment-by-time-point interaction (P = 0.28).

Stride length (cm)—Front limb stride length differed by treatment (P = 0.01) but did not differ by a treatment-by-time-point interaction (P = 0.59) or by time point (P = 0.12). FIRO had a shorter overall stride length when compared to baseline measurements (–19.76 cm; 95% CI, –30.90 to –8.61 cm). MEL had the longest overall stride length when compared to baseline

measurements (5.62 cm; 95% CI, –5.52 to 16.77 cm). CNTL stride length was slightly shorter when compared to baseline measurements (–1.81 cm; 95% CI, –12.96 to 9.32 cm).

Rear limb stride length differed by treatment (P = 0.03) but did not differ by treatment-by-time-point interaction (P = 0.14) or by time point (P = 0.62). FIRO had a shorter overall stride length when compared to baseline measurements (–18.24 cm; 95% CI, –30.31 to –6.17 cm). MEL had the fixed overall stride length when compared to baseline measurements (3.76 cm; 95% CI, –8.30 to 15.83 cm). CNTL stride length was slightly longer when compared to baseline measurements (0.16 cm; 95% CI, –11.90 to 12.23 cm).

Force (kg)—The front limb force did differ by treatment (P = 0.02) but did not differ by time point (P = 0.80) or by a treatment-by-time-point interaction (P = 0.91). FIRO had a lower overall force when compared to baseline measurements (–1.98 kg; 95% CI, –3.56 to –0.40 kg). MEL and CNTL had a slightly stronger force when compared to baseline measurements (0.89 kg [95% CI, –0.68 to 2.47 kg] and 0.62 kg [95% CI, –0.95 to 2.20 kg], respectively).

The rear limb force differed by treatment (P = 0.02) but did not differ by time point (P = 0.23) or by a treatment-by-time-point interaction (P = 0.46). FIRO had a lower overall force when compared to baseline measurements (–1.13 kg; 95% CI, –2.04 to –0.23 kg). MEL and CNTL had a slightly stronger force when compared to baseline measurements (0.26 kg [95% CI, –0.64 to 1.16 kg] and 0.66 kg [95% CI, –0.23 to 1.57 kg], respectively).

Impulse (kg x s)—Front foot impulse did not differ by treatment (P = 0.11), by time point (P = 0.97), or by treatment-by-time-point interaction (P = 0.37).

Rear foot impulse differed by treatment (P = 0.01) but did not differ by time point (P = 0.92) or by a treatment-by-time-point interaction (P = 0.18). FIRO had a slightly lower impulse measurement when compared to baseline measurements (–0.06 kg x s; 95% CI, –0.39 to 0.27 kg

x s). MEL had the lowest overall impulse measurement when compared to baseline measurements (–0.54 kg x s; 95% CI, –0.88 to –0.20 kg x s). CNTL stride length was a slightly higher impulse measurement when compared to baseline measurements (0.21 kg x s; 95% CI, –0.12 to 0.55 kg x s).

Contact pressure (kg/cm^2)—Front foot contact pressure did not differ by treatment (P = 0.06), by time point (P = 0.72), or by a treatment-by-time-point interaction (P = 0.42).

Rear foot contact pressure did not differ by treatment (P = 0.10), by time point (P = 0.35), or by a treatment-by-time-point interaction (P = 0.58).

Gait distance (cm)—Gait distance change from baseline values (–24h) did not differ by treatment (P = 0.43), by time point (P = 0.78), or by a treatment-by-time-point interaction (P = 0.81).

Gait velocity change from baseline (cm/s)—Gait velocity change from baseline values (–24 hours) differed by treatment (P = 0.045) but did not differ by time point (P = 0.82) or by a treatment-by-time effect (P = 0.56). FIRO had an average mean decrease in gait velocity change (–54.17 cm/s; 95% CI, –92.99 to –15.35 cm/s), while MEL had an increase in gait velocity when compared to baseline values (14.54 cm/s; 95% CI, –24.27 to 53.36 cm/s). CNTL animals had an average mean of –3.06 cm/s (95% CI, –41.88 to 35.75 cm/s).

Discussion

The primary objective of this study was to determine whether FIRO or MEL were effective at providing analgesia in goats after surgical castration. The MEL dosing regimen was the current treatment plan utilized by clinicians at Kansas State's Veterinary Health Center for goats of this age. Data from this study suggests that both FIRO and MEL can provide some analgesic effect when administered to goats orally prior to surgical castration.

The differences in IRT over time are likely due to environmental influences (Church et al., 2013). The animals were group housed with access to outdoor runs. A weather event (thunderstorm) occurred on the first day, keeping the ambient temperature cooler than subsequent study days. When this paper was written, no record of Sub P levels in goats was reported in the literature. FIRO had lower Sub P values at all time points included in this study after castration when compared to MEL and CNTL values. These data indicate that Sub P may not be a reliable biomarker for castration pain in goats.

MEL-treated individuals displayed lower VAS scores when compared to FIRO-treated individuals, indicating they appeared to the observer to be in less pain. MEL had similar or positive gait changes when compared to CNTL animals. The average for stride length increased slightly from baselines in both front stride length and rear stride length in the MEL group. MEL and CNTL individuals had a slightly stronger force when compared to baseline measurements in both front limb force and hind limb force. MEL had the lowest overall impulse measurement when compared to baseline measurements. MEL-treated goats also had an increase in gait velocity when compared to baseline values. CNTL animals had a slight decrease in gait velocity, suggesting that individuals in the MEL group were experiencing less pain compared to FIRO, as their pressure mat outcomes more closely resembled those of the CNTL group.

This is the first report to compare MEL and FIRO in goats following surgical castration. A study by Brusin et al. (2022), compared MEL to flunixin in goats for post castration analgesia. That study found flunixin to be superior to MEL for pain sensitivity based on von Frey filament tests. Following ring castration and tail-docking in sheep, MEL-treated sheep had lower average daily gains compared to FIRO sheep, with the first 2 weeks following the procedures being the most pronounced (Kongara et al., 2023). A paper comparing FIRO and MEL following castration in horses found no differences between the 2 drugs on stiffness and preputial swelling (Gobbi et al., 2020).

Strengths of the study reported here include utilizing the novel pain measurement outcomes KGA and plasma Sub P. These two outcomes have not been utilized in goat pain management research previously. Additionally, KGA was used as the pivotal outcome for the pain label approval of transdermal flunixin (U.S. FDA, 2024). MEL was effective at reducing visually assessed pain behaviors and positively affecting gait measurements. Visual pain behaviors were scored by VAS. Pain behaviors result in an increase in VAS score (higher score = more pain behaviors shown). Animals given MEL seemed more comfortable after surgical castration based on behavior parameters of VAS scoring when compared to FIRO. The average scores of MEL were more comparable to CNTL averages when looking at VAS scores. When pressure mat values were looked at, MEL had values that most closely resembled individuals of the CNTL group, suggesting that MEL provided sufficient analgesia after surgical castration.

FIRO and MEL used for pain control following castration are considered extra label drug uses in the US. Prescribing veterinarians in the US are encouraged to contact the Food Animal Residue Avoidance Database for assistance in determining a meat withdrawal interval. Veterinarians outside the US are encouraged to contact regulatory officials for assistance in

determining when FIRO or MEL concentrations are below maximum residue limits for their country.

Limitations of this study included the small sample size, not using a castrated CNTL group for comparison purposes (a group that was castrated and did not receive postoperative analgesia), and the unknown pharmacokinetics of FIRO and MEL dosing regimens used in this group of goats. Sample size for the current study was determined from data regarding cortisol in goats and cattle following surgical castration, and a sample size of 6 animals/treatment group was determined. An increase in sample size would give more statistical power in the statistical analysis. A castrated CNTL group was not used, as castration is a common husbandry practice proven to be painful to livestock animals of varying species, including goats. With animal welfare considerations in mind, a castrated CNTL group was not implemented. Additional work is needed to provide prescribing veterinarians meat withdrawal intervals for goats treated with MEL or FIRO.

Based on data collected from this study, there is evidence to suggest that MEL can provide some analgesia after surgical castration in goats. This is indicated by changes in VAS and KGA. These data show that administration of MEL perioperatively in goats provided varying degrees of pain relief. FIRO lowered plasma cortisol at later time points, but treated goats had higher VAS scores and persistent changes in gait following castration. Further research is needed to better understand the most effective analgesic drug for goats following painful husbandry procedures such as castration.

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Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

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Figures

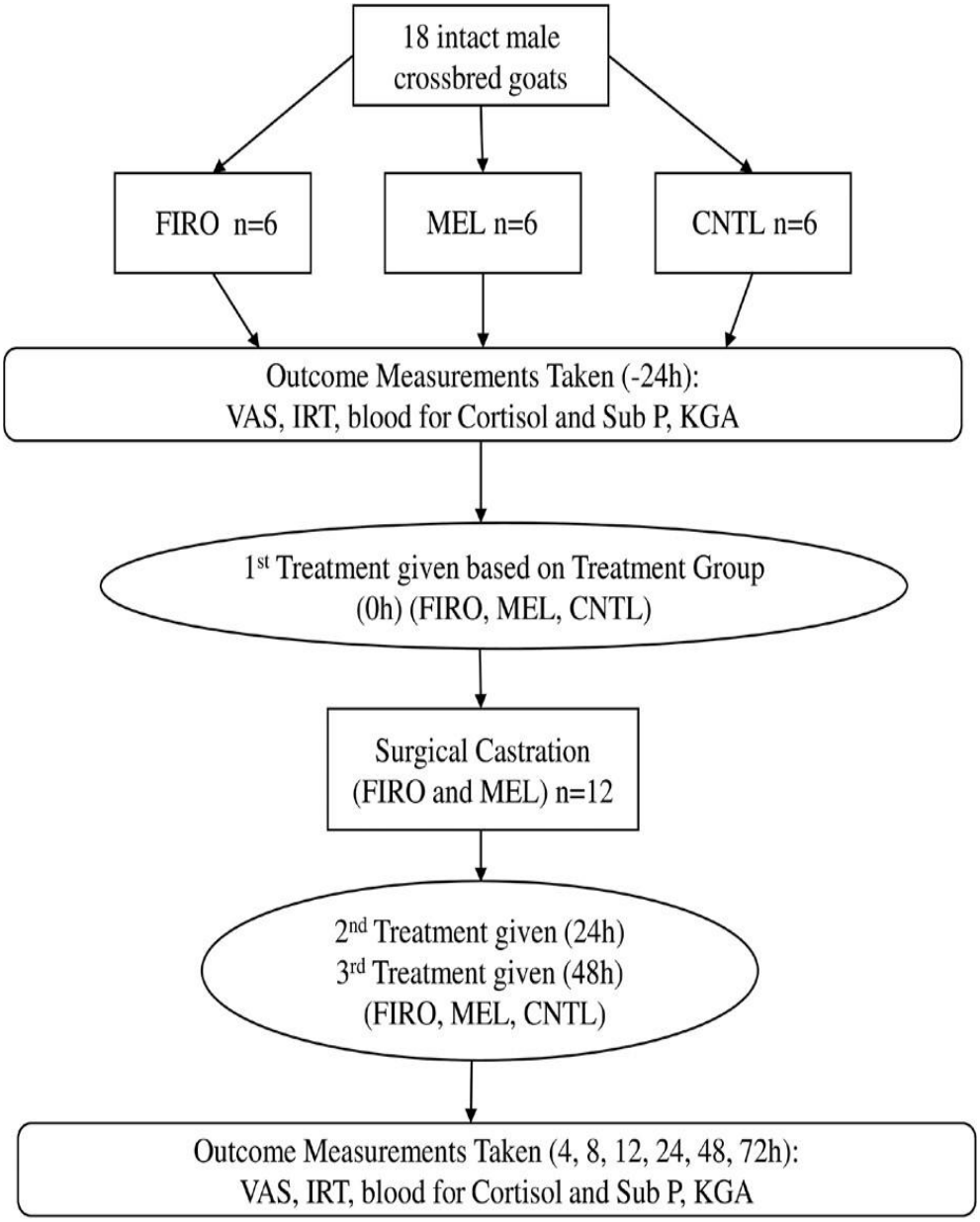


Figure 3.1. Flowchart outlining study events. CNTL = Control. FIRO = Firocoxib. IRT = Infrared thermography. KGA = Kinetic gait analysis. MEL = Meloxicam. Sub P = Substance P. VAS = Visual analogue scale.

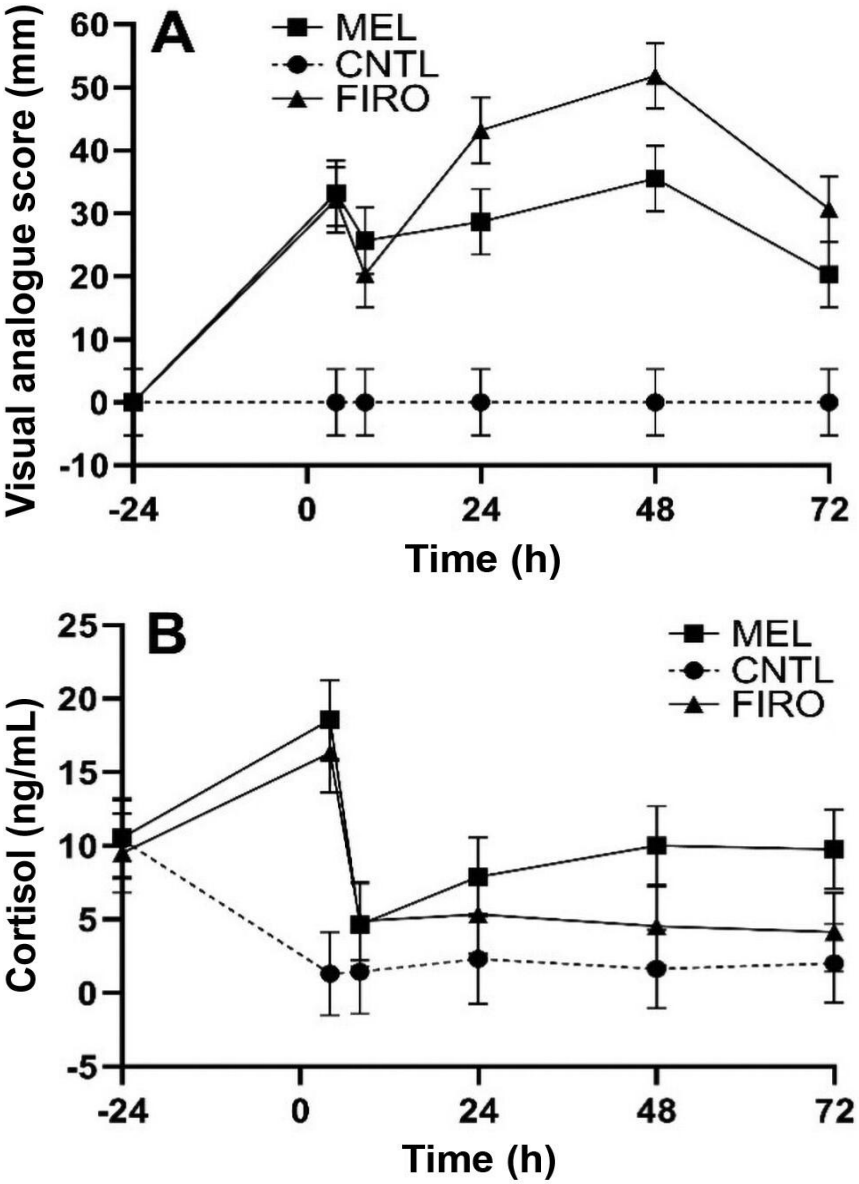
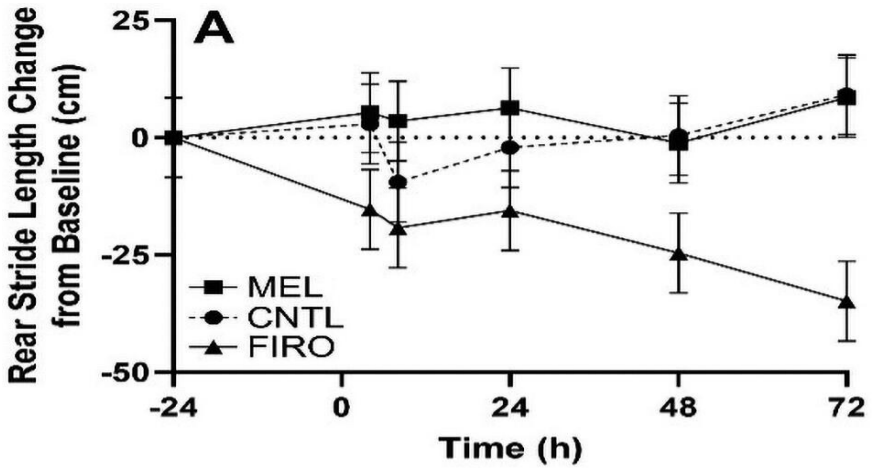
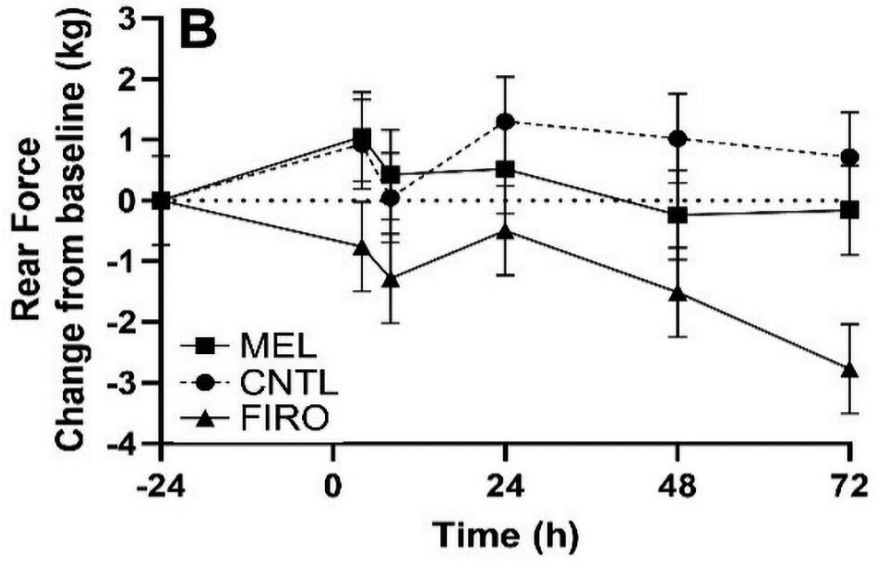


Figure 3.2. Visual analogue scores (A) and cortisol concentrations (B) over time for goats that underwent surgical castration and were administered FIRO (triangles; n = 6) or MEL (squares; n = 6) and goats kept intact (not castrated) serving as controls and administered a placebo treatment (i.e., CNTL goats; circles; n = 6).


A
Rear Stride Length Change from Baseline (cm)
25
0
-25
-50
MEL
CNTL
FIRO
-24
0
24
48
72
Time (h)


B
Rear Force Change from baseline (kg)
3
2
1
0
-1
-2
-3
-4
MEL
CNTL
FIRO
-24
0
24
48
72
Time (h)

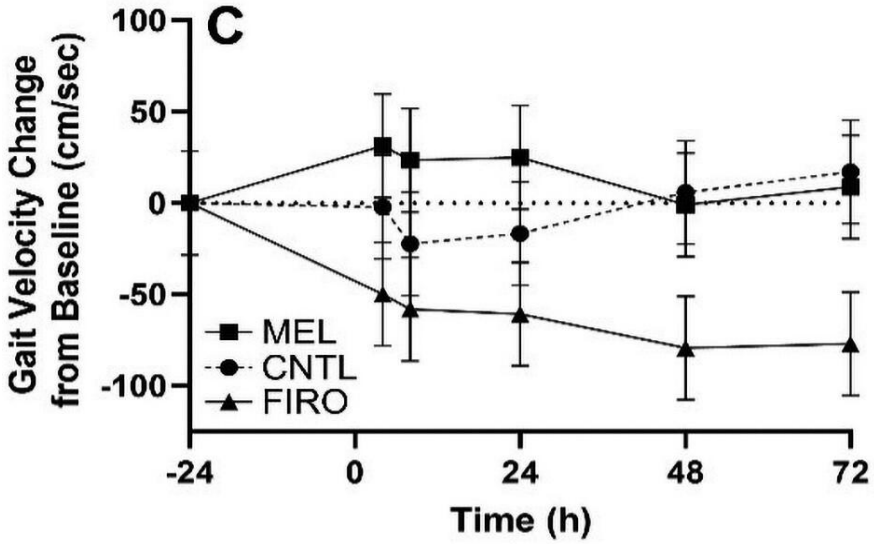

C
Gait Velocity Change from Baseline (cm/sec)
100
50
0
-50
-100
MEL
CNTL
FIRO
-24
0
24
48
72
Time (h)

Figure 3.3. Kinetic gait analysis outcome expressed as change from baseline for rear stride length (A), rear force (B), and gait velocity (C) for goats that underwent surgical castration and were administered FIRO (triangles; n = 6) or MEL (squares; n = 6) and goats kept intact (not castrated) serving as controls and administered a placebo treatment (i.e., CNTL goats; circles; n = 6).

Tables

Table 3.1. Definitions of Kinetic Gait Analysis biomechanical markers.

Outcome	Definition (unit)
Stance Time	The time that passes in a gait cycle of one extremity (s).
Stride Length	The distance between two successive placements of the same extremity (cm).
Force	The maximum force measured for a single step from a single extremity (kg).
Impulse	The maximum force applied per unit of time measured (kg x s).
Contact area	The peak pressure measured from a singular footfall (kg/cm^2).
Gait Distance	The distance along the line of progression, from posterior of the first left or right front stance to posterior of the last left of right front stance (cm).
Gait Velocity	The gait distance is divided by gate time (cm/sec).

Table 3.2. Overall mean (95% CI) infrared thermography temperature (°C) of the left medial canthus (eye), cortisol (ng/mL), and substance P (pg/mL) in goats that underwent surgical castration and were administered firocoxib (FIRO; n = 6) or meloxicam (MEL; n = 6) and goats kept intact (not castrated) serving as controls and administered a placebo treatment (CNTL; n = 6).

	LS[1] means (95% Confidence Interval)			*P*-values		
Parameter	FIRO	MEL	CNTL	Treatment	Time	Treatment × time
Infrared thermography °C	37.95 (37.49-38.40)	37.99 (37.55-38.43)	38.60 (38.15-39.05)	0.08	< 0.0001	0.41
Cortisol (ng/mL)	7.45 (3.10-11.80)	10.24 (5.87-14.60)	3.19 (-1.21-7.59)	0.0128	< 0.0001	0.003
Substance P (pg/mL)	503.45 (372.84-634.05)	586.05 (455.45-716.6)	572.77 (442.17-703.37)	0.05	0.25	0.62

[1] Least Squares

Table 3.3.Overall mean (95% Confidence Interval) outcome measures from kinetic gait analysis in goats who underwent surgical castration and were administered firocoxib (FIRO) (n=6) or meloxicam (MEL) (n=6), or goats kept intact (not castrated) serving as controls and administered a placebo treatment (CNTL) (n=6).

	LS[1] means (95% Confidence Interval)			*P*-values		
Parameter	FIRO	MEL	CNTL	Treatment	Time	Treatment × time
Front foot						
Stance time, s	0.001 (-0.07-0.07)	-0.05 (-0.12-0.02)	0.03 (-0.04-0.10)	0.28	0.99	0.47
Stride length, cm	-19.76 (-30.90- (-8.61))	5.61 (-5.52-16.77)	-1.81 (-12.96-9.32)	0.01	0.59	0.12
Force, kg	-1.98 (-3.56-(-0.40))	0.89 (-0.68-2.47)	0.62 (-0.95-2.20)	0.02	0.80	0.11
Impulse, kg × s	0.13 (-0.37-0.64)	-0.47 (-0.98-0.03)	0.21 (-0.29-0.72)	0.11	0.97	0.37
Contact pressure, kg/cm^2	-20.52 (-39.86-(-1.45))	-0.98 (-20.32-18.35)	12.40 (-6.93-31.74)	0.06	0.72	0.42
Rear foot						
Stance time, s	0.01 (-0.04-0.08)	-0.07 (-0.13-0.004)	0.03 (-0.03-0.09)	0.06	0.95	0.28
Stride length, cm	-18.24 (-30.31- (-6.17)	3.76 (-8.30-15.83)	0.16 (-11.90-12.23)	0.03	0.62	0.14
Force, kg	-1.13 (-2.04-(-0.23)	0.26 (-0.64-1.16)	0.66 (-0.23-1.57)	0.02	0.23	0.46
Impulse, kg × s	-0.06 (-0.39-0.27)	-0.54 (-0.88-(-0.20))	0.21 (-0.12-0.55)	0.01	0.92	0.18
Contact pressure, kg/cm^2	-17.51 (-35.61-0.58)	1.16 (-16.93-19.26)	9.72 (-8.38-27.82)	0.10	0.35	0.58
Gait Distance Change (cm)	2.91 (-11.65-17.48)	-7.40 (-21.96-7.16)	-8.88 (-23.45-5.68)	0.43	0.78	0.81
Gait Velocity Change (cm/sec)	-54.17 (-92.99-(-15.35))	14.54 (-24.27-53.36)	-3.06 (-41.88-35.75)	0.045	0.82	0.56

[1] Least Squares

References

Brusin, V., Ceballos, M. C., Trindade, P. H. E., Rocha Góis, K. C., Conde, G., Tessarine Barbosa, V.,Paranhos da Costa, M. J. R. (2022). Flunixin Meglumine Is Superior to Meloxicam for Providing Analgesia after Surgical Castration in 2-Month-Old Goats. Animals (Basel), 12(23). https://doi.org/10.3390/ani12233437

Całkosiński, I., Dobrzyński, M., Rosińczuk, J., Dudek, K., Chrószcz, A., Fita, K., & Dymarek, R. (2015). The use of infrared thermography as a rapid, quantitative, and noninvasive method for evaluation of inflammation response in different anatomical regions of rats. Biomed Res Int, 2015, 972535. https://doi.org/10.1155/2015/972535

Coetzee, J. F., KuKanich, B., Mosher, R., & Allen, P. S. (2009). Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. Vet Ther, 10(4), E1-8.

Coetzee, J. F., Shearer, J. K., Stock, M. L., Kleinhenz, M. D., & van Amstel, S. R. (2017). An Update on the Assessment and Management of Pain Associated with Lameness in Cattle. Vet Clin North Am Food Anim Pract, 33(2), 389-411. https://doi.org/10.1016/j.cvfa.2017.02.009

Cook, M. (2023). Urinary Calculi of Small Ruminats. In. Veterinary Clinics of North America: Food Animal Practice.

DM, M. (2013). Obstructive urolithiasis in ruminants-A review. In G. MA (Ed.), (pp. 233-238). Vet World.

Fitzpatrick, F. A. (2004). Cyclooxygenase enzymes: regulation and function. Curr Pharm Des, 10(6), 577-588. https://doi.org/10.2174/1381612043453144

Fortier, L. A., Gregg, A. J., Erb, H. N., & Fubini, S. L. (2004). Caprine obstructive urolithiasis: requirement for 2nd surgical intervention and mortality after percutaneous tube cystostomy, surgical tube cystostomy, or urinary bladder marsupialization. Vet Surg, 33(6), 661-667. https://doi.org/10.1111/j.1532-950X.2004.04089.x

Fraccaro, E., Coetzee, J. F., Odore, R., Edwards-Callaway, L. N., Kukanich, B., Badino, P.,Bergamasco, L. (2013). A study to compare circulating flunixin, meloxicam and gabapentin concentrations with prostaglandin E_2 levels in calves undergoing dehorning. Res Vet Sci, 95(1), 204-211. https://doi.org/10.1016/j.rvsc.2013.01.018

Glynn, H. D., Coetzee, J. F., Edwards-Callaway, L. N., Dockweiler, J. C., Allen, K. A., Lubbers, B.,KuKanich, B. (2013). The pharmacokinetics and effects of meloxicam, gabapentin, and flunixin in postweaning dairy calves following dehorning with local anesthesia. J Vet Pharmacol Ther, 36(6), 550-561. https://doi.org/10.1111/jvp.12042

Gobbi, F. P., Di Filippo, P. A., Mello, L. M., Lemos, G. B., Martins, C. B., Albernaz, A. P., & Quirino, C. R. (2020). Effects of Flunixin Meglumine, Firocoxib, and Meloxicam in

Equines After Castration. J Equine Vet Sci, 94, 103229. https://doi.org/10.1016/j.jevs.2020.103229

Gofur, M. R. Effect of testosterone on penile and urethral development in Black Bengal goat. In (Vol. 17, Issue 2, pp. 133-139). Bangl. J. Vet Med.

Huber, J., Arnholdt, T., Möstl, E., Gelfert, C. C., & Drillich, M. (2013). Pain management with flunixin meglumine at dehorning of calves. J Dairy Sci, 96(1), 132-140. https://doi.org/10.3168/jds.2012-5483

Karademir, U., Erdogan, H., Boyacioglu, M., Kum, C., Sekkin, S., & Bilgen, M. (2016). Pharmacokinetics of meloxicam in adult goats: a comparative study of subcutaneous, oral, and intravenous administration. N Z Vet J, 64(3), 165-168. https://doi.org/10.1080/00480169.2015.1124811

Kleinhenz, M. D., Gorden, P. J., Smith, J. S., Schleining, J. A., Kleinhenz, K. E., Juarez, J. R.,Coetzee, J. F. (2019). Effects of transdermal flunixin meglumine on experimentally induced lameness in adult dairy cattle. J Dairy Sci, 102(7), 6418-6430. https://doi.org/10.3168/jds.2018-15091

Kleinhenz, M. D., Van Engen, N. K., Gorden, P. J., Ji, J., Walsh, P., & Coetzee, J. F. (2017). Effects of transdermal flunixin meglumine on pain biomarkers at dehorning in calves. J Anim Sci, 95(5), 1993-2000. https://doi.org/10.2527/jas.2016.1138

Kleinhenz, M. D., Van Engen, N. K., Gorden, P. J., Kleinhenz, K. E., Kukanich, B., Rajewski, S. M.,Coetzee, J. F. (2018). The impact of pain on the pharmacokinetics of transdermal flunixin meglumine administered at the time of cautery dehorning in Holstein calves. Vet Anaesth Analg, 45(6), 849-857. https://doi.org/10.1016/j.vaa.2018.06.008

Kleinhenz, M. D., Van Engen, N. K., Gorden, P. J., KuKanich, B., Rajewski, S. M., Walsh, P., & Coetzee, J. F. (2016). The pharmacokinetics of transdermal flunixin meglumine in Holstein calves. J Vet Pharmacol Ther, 39(6), 612-615. https://doi.org/10.1111/jvp.12314

Kongara, K., Purchas, G., Dukkipati, V., Venkatachalam, D., Ward, N., Hunt, H., & Speed, D. (2023). Pharmacokinetics and effect on renal function and average daily gain in lambs after castration and tail docking, of firocoxib and meloxicam. N Z Vet J, 71(6), 306-314. https://doi.org/10.1080/00480169.2023.2232337

Kvaternick, V., Pollmeier, M., Fischer, J., & Hanson, P. D. (2007). Pharmacokinetics and metabolism of orally administered firocoxib, a novel second generation coxib, in horses. J Vet Pharmacol Ther, 30(3), 208-217. https://doi.org/10.1111/j.1365-2885.2007.00840.x

Lizarraga, I., & Chambers, J. P. (2012). Use of analgesic drugs for pain management in sheep. N Z Vet J, 60(2), 87-94. https://doi.org/10.1080/00480169.2011.642772

M.D., K. (2018). The impact of transdermal flunixin meglumine on biomarkers of pain in calves when administered at the time of surgical castration without local anesthesia. In V. E. N. K. S. J. S. G. P. J. J. J. W. C. P. S.C.B (Ed.), (Vol. 212, pp. 1-6). Livestock Science.

Martin, M. S., Kleinhenz, M. D., Edwards-Callaway, L. N., Engle, T. E., Guimaraes, O., Schafer, D. W.,Coetzee, J. F. (2022). The effect of breed, sex, and oral meloxicam administration on pain biomarkers following hot-iron branding in Hereford and Angus calves. J Anim Sci, 100(3). https://doi.org/10.1093/jas/skac038

Reppert, E. J., Streeter, R. N., Simpson, K. M., & Taylor, J. D. (2016). Retrograde catheterization of the urinary bladder in healthy male goats by use of angiographic catheters. Am J Vet Res, 77(11), 1295-1299. https://doi.org/10.2460/ajvr.77.11.1295

Schulz, K. L., Anderson, D. E., Coetzee, J. F., White, B. J., & Miesner, M. D. (2011). Effect of flunixin meglumine on the amelioration of lameness in dairy steers with amphotericin B-induced transient synovitis-arthritis. Am J Vet Res, 72(11), 1431-1438. https://doi.org/10.2460/ajvr.72.11.1431

Stewart, M., Stookey, J. M., Stafford, K. J., Tucker, C. B., Rogers, A. R., Dowling, S. K.,Webster, J. R. (2009). Effects of local anesthetic and a nonsteroidal antiinflammatory drug on pain responses of dairy calves to hot-iron dehorning. J Dairy Sci, 92(4), 1512-1519. https://doi.org/10.3168/jds.2008-1578

Stock, M. L., Coetzee, J. F., KuKanich, B., & Smith, B. I. (2013). Pharmacokinetics of intravenously and orally administered meloxicam in sheep. Am J Vet Res, 74(5), 779-783. https://doi.org/10.2460/ajvr.74.5.779

Stuart, A. K., KuKanich, B., Caixeta, L. S., Coetzee, J. F., & Barrell, E. A. (2019). Pharmacokinetics and bioavailability of oral firocoxib in adult, mixed-breed goats. J Vet Pharmacol Ther, 42(6), 640-646. https://doi.org/10.1111/jvp.12795

T, N. (2017). Castration of male livestock and the potential of immunocastration to improve animal welfare and production traits: Invited Review. In L. H (Ed.), (Vol. 47 (6), pp. 731-742). South African Journal of Animal Science.

Tekeli, I. O., Turk, E., Durna Corum, D., Corum, O., & Uney, K. (2020). Effect of castration procedure on the pharmacokinetics of meloxicam in goat kids. J Vet Pharmacol Ther, 43(5), 429-434. https://doi.org/10.1111/jvp.12872

US, FDA. (2017). Freedom of information summary original new animal drug application Banamine Transdermal. In. US FDA CVM.

Viscardi, A. V., Reppert, E. J., Kleinhenz, M. D., Wise, P., Lin, Z., Montgomery, S.,Coetzee, J. F. (2021). Analgesic Comparison of Flunixin Meglumine or Meloxicam for Soft-Tissue Surgery in Sheep: A Pilot Study. Animals (Basel), 11(2). https://doi.org/10.3390/ani11020423

Webster, H. B., Morin, D., Jarrell, V., Shipley, C., Brown, L., Green, A.,Constable, P. D. (2013). Effects of local anesthesia and flunixin meglumine on the acute cortisol response, behavior, and performance of young dairy calves undergoing surgical castration. J Dairy Sci, 96(10), 6285-6300. https://doi.org/10.3168/jds.2012-6238

Wilson, J. E., Chandrasekharan, N. V., Westover, K. D., Eager, K. B., & Simmons, D. L. (2004). Determination of expression of cyclooxygenase-1 and -2 isozymes in canine tissues and their differential sensitivity to nonsteroidal anti-inflammatory drugs. Am J Vet Res, 65(6), 810-818. https://doi.org/10.2460/ajvr.2004.65.810

Chapter 4 - Comparison of multiple drug regimens for reduction of synovitis induced lameness pain in meat type goats

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Abstract

Objective: This study evaluated the analgesic effects of firocoxib, meloxicam, and transdermal flunixin in meat type goats with experimentally induced lameness.

Methods: Forty-four crossbred castrated meat type goats with an average weight of 35kg (range: 25-43kg), were enrolled from June to December 2022. Lameness was induced with amphotericin B injected in the distal interphalangeal joint of the left hind lateral claw. After a washout period, lameness was reproduced in the right hind limb. Eleven treatment groups were implemented: firocoxib (0.5, 1.0, 2.0 mg/kg), meloxicam (1.0, 2.0, 3.0 mg/kg, transdermal flunixin (3.3, 4.2, 5.0 mg/kg) and positive and negative control Outcome measurements included infrared-thermography (IRT), mechanical nociception threshold (MNT), plasma cortisol, plasma substance p, kinetic gait analysis (KGA), visual lameness scoring (VLS), and visual analog scoring (VAS)and were collected at –24, 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72h relative to lameness induction.

Results: Transdermal flunixin at 3.3 mg/kg and 5.0 mg/kg demonstrated the greatest analgesic effects from outcome measurements collected.

Conclusion: Transdermal flunixin meglumine provides the most effective analgesia for goats with induced lameness, suggesting it may be the preferred option for pain relief in goats with lameness symptoms.

Clinical Relevance: Transdermal flunixin meglumine at 3.3 mg/kg or 5.0 mg/kg provided the best results for pain mitigation in goats with induced lameness.

Keywords: firocoxib, flunixin, goat, lameness, meloxicam

Introduction

Lameness remains a persistent welfare and management concern in livestock production. Unsound animals can cause economic loss to livestock producers and insinuate animal welfare concerns (Coetzee et al., 2017). In goats, common causes of lameness can include foot issues including sole ulcers, subsolar abscesses and interdigital dermatitis (Hill et al., 1997; Christodoulopoulos, 2009; Crosby-Durrani et al., 2016). Due to the wide range of causes and subsequent varying severity, lameness can be a difficult condition to treat and manage in livestock species. Timely and appropriate intervention of lame goats can improve animal welfare and increase economic productivity for producers.

Amphotericin B (amp B) is a polyene antibiotic previously used to induce acute lameness in cattle and horses (Kotschwar et al., 2009; Coetzee et al., 2014). Amp B produces synovitis by destruction of lysosomes and can vary in severity between species when administered into a joint space (McIlwraith et al., 1979; Reppert et al., 2020). A recent study established that Amphotericin B can produce a repeatable and reliable lameness model in meat type goats for research purposes (Weeder et al., 2023).

The American Medicinal Drug Use Act of 1994 (AMDUCA) legalizes extra-label drug use in animals by veterinarians (Geyer, 1997). Due to limited drug approvals in goats, veterinarians often must utilize Non-Steroidal Anti-Inflammatory (NSAIDs) in an extra-label manner to prevent unnecessary suffering (Drew, 1998). The AMDUCA policy allows veterinarians to practice medicine in an effective and safe manner without sacrificing the importance of overall public health (Geyer, 1997).

The pharmacokinetics of firocoxib, flunixin, and meloxicam have been described before (Rangel et al., 2019; Stuart et al., 2019; Coetzee, 2013; A MK, 2002; Ingvast-Larsson et al.,

2011; Lees et al., 1991; Kleinhenz et al., 2016; Kleinhenz et al., 2019; Reppert et al., 2019; Graves et al., 2020; Meira et al., 2022). Currently, data regarding the optimal dose for each drug when administered to caprine patients is unknown. These NSAIDS are available in formulations that are easy and practical for owners to administer on-farm. The objectives of the study were to evaluate the efficacy of firocoxib, meloxicam, and transdermal flunixin to mitigate pain in meat type goats after lameness induction with amphotericin B, and to determine an optimal dose of each drug by evaluating three different doses. The hypothesis of this study is that firocoxib, meloxicam, and flunixin transdermal will provide analgesia to crossbred meat type goats with amphotericin B induced lameness.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee at Kansas State University (Protocol #4387).

A flow chart of chronological study events is represented in Figure 4.1.

Animals and Study Design

Goats were housed individually in raised pens with a grated floor and provided water, brome hay, and pelleted grain ad lib. Animals on study were housed individually due to the study's multiple collection timepoints and then returned to pasture after study completion. This study was conducted over a 12-wk period, with a single replicate (twenty-two goats in one replicate) completed in a three-wk period (four replicates total). One wk was dedicated to acclimation, the following wk included lameness induction and data collection, and the last wk was dedicated to post-study monitoring. Following the one-wk acclimation period, a random number generator (Haahr and Svend, 1999-2025) was used to randomly assign each goat to one of the following eleven treatments. Each goat was administered the same treatment for both left and right limb lameness induction.

FIRO-LOW (n = 8): Induced lameness with Amphotericin B. Firocoxib tablet at 0.5mg/kg in oral bolus with topical placebo down back.

FIRO-MID (n = 8): Induced lameness with Amphotericin B. Firocoxib tablet at 1.0mg/kg in oral bolus with topical placebo down back.

FIRO-HIGH (n = 8): Induced lameness with Amphotericin B. Firocoxib tablet at 2.0mg/kg in oral bolus with topical placebo down back.

MEL-LOW (n = 8): Induced lameness with Amphotericin B. Meloxicam tablet at 1.0mg/kg in oral bolus with topical placebo down back.

MEL-MID (n = 8): Induced lameness with Amphotericin B. Meloxicam tablet at 2.0mg/kg in oral bolus with topical placebo down back.

MEL-HIGH (n = 8): Induced lameness with Amphotericin B. Meloxicam tablet at 3.0mg/kg in oral bolus with topical placebo down back.

FLU-LOW (n = 8): Induced lameness with Amphotericin B. Whey protein in oral bolus with 113 3.3mg/kg topical flunixin down back.

FLU-MID (n = 8): Induced lameness with Amphotericin B. Whey protein in oral bolus (oral 115 placebo) with 4.2mg/kg topical flunixin down back.

FLU-HIGH (n = 8): Induced lameness with Amphotericin B. Whey protein in oral bolus (oral placebo) with 5.0mg/kg topical flunixin down back.

POS-CNTL (n = 8): Induced lameness with Amphotericin B. Whey protein in oral bolus (oral 119 placebo) with topical placebo down back.

NEG-CNTL (n = 8): No induced lameness (saline injection into joint). Whey protein in oral bolus (oral placebo) with topical placebo down back.

Lameness Induction

Lameness was induced twice per goat-once for the left hind limb, and once for the right hind limb. This was done for comparison purposes, as each goat served as its own control. The dose of amphotericin B used for lameness induction was based on previous work (Weeder et al., 2024). Negative control animals received 0.5 mL of sterile saline during the lameness induction process that served as a control induction (Nova-Tech Inc. Grand Island NE, US). A minimum one-wk washout period (range: 2-4 wk) between left and right hind limb lameness induction was implemented for every individual on study. All goats were monitored for resolution of lameness after the study period. No goats were lame prior to being enrolled or re-enrolled on study.

An intravenous catheter (16 G x 7.5 cm, Mila International Inc., Florence, KY, US) was aseptically placed in the right jugular vein of each goat. Catheters were used to facilitate the administration of xylazine hydrochloride (0.1mg/kg IV, Akron Inc., Lake Forest, IL, USA) to induce sedation and facilitate blood collection. For each lameness induction, the hair on the abaxial side of the lateral digit from the fetlock to the coronary band was clipped. The area was aseptically prepared. A 21 G x 1in needle was placed into the distal interphalangeal joint of the left or right lateral claw. All intra-articular injections were performed by a licensed veterinarian (ER), blinded to treatment groups, and skilled in arthrocentesis of small ruminant joints. Intra-articular injection was confirmed by lack of resistance to injection by the veterinarian. Resistance in this study was defined as the inability to distribute the full amount of treatment into the distal interphalangeal joint of the left lateral claw. If resistance was present during injection administration, the treatment was periarticular and recorded (n=3). To ensure distribution of treatment throughout the joint after injection, the induced digit was flexed and extended five times. After injection completion, sedation was reversed using a single intramuscular injection of atipamezole hydrochloride (Zoetis INC, Kalamazoo MI, USA) at a dosage of 0.04mg/kg. Each goat was monitored hourly for behavioral signs of distress or pain out to 10h post-induction, and twice daily thereafter for 5d, and then daily for 7d. Goats with visual lameness scores of 4 or greater for longer than 48h were eligible for rescue analgesia, which consisted of flunixin meglumine at 2.2 mg/kg IV. Rescue analgesia was not needed for any goat on study but was considered during study planning.

Amphotericin B (X-Gen Pharmaceuticals Inc., Big Flats, NY) was prepared (Baxter Healthcare Corporation, Deerfield, IL, USA) to achieve the needed concentrations for the high dose-low volume (HL) (5mg/0.25mL) previously used (Weeder et al., 2023).

Treatment Administration

Eleven different treatments were administered. Treatment was only given once during left and right hind limb lameness inductions. Firocoxib (Equioxx Tablets; Boehringer Ingelheim Animal Health), meloxicam (Zydus Pharmaceuticals Inc.) flunixin transdermal (Banamine Transdermal, Merck Animal Health, Madison, NJ) were each given at three different dosages. Each drug and dosage combination were considered a treatment, totaling nine analgesic treatments. A positive (lameness induction with oral and topical placebos) and negative control (sham lameness induction with oral and topical placebos) were also assigned for comparison purposes.

Outcome Variables

Parameters for data collection included: infrared thermography (IRT), mechanical nociception threshold (MNT), plasma cortisol concentrations (CORT), plasma substance P concentrations (Sub P), kinetic gait analysis (KGA), visual lameness score (VLS), and visual analogue score (VAS) (Fig. 4.1). All outcomes were collected at the following timepoints; baseline (-24h prior to induction), 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36,48, 60, and 72h post-induction of lameness. All observers were blinded to treatment during the study's completion.

Infrared Thermography

Thermographic images of the dorsolateral aspect of the lateral rear digits were taken at each timepoint; one of the left distal limb and one of the right distal limb digits prior to induction (-24), 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72h. Left and right limbs were induced at different points in the study. The infrared camera (FLUKE Ti580 IR Imager, Fluke Corp., WA, USA) was positioned at a 45-degree angle from the dorsolateral aspect 1m from the coronary band of each limb. Images were analyzed to obtain the maximum, average, and minimum

temperatures for each distal limb with a research grade computer software (SmartView 4.3, Fluke Corp., Everett, WA, USA). The difference between the lame distal limb and sound distal limb foot were determined as previously described (Kleinhenz et al., 2019). These differences were used for statistical analysis.

Mechanical Nociception Threshold

All MNT measurements were taken with a handheld algometer (FPX 100, Wagner Instruments, Greenwich, CT) prior to induction (-24), 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72h. The MNT measured the lateral digit at the level of the coronary band at a point halfway between midline and the heel bulb, by applying slow, steady pressure until the goat responded by moving away from pressure or displaying a flinch response. The MNT measurements were taken on both the left distal limb and right distal limb. The animal was gently restrained with a hand under the chin to prevent forward or backward motion. The average of three readings for each distal limb at each time point was used for analysis as previously described (Kleinhenz et al., 2019). The difference between lame and sound distal limbs MNT measurements (lame distal limb minus sound distal limb) were also determined for each time point. These differences were used for statistical analysis. The investigator measuring MNT was blinded to treatment and the algometer readings to prevent bias. A second investigator recorded algometer readings.

Plasma Cortisol

Blood samples were collected from the intravenous catheter in each goat prior to induction (-24), 0, 1, 2, 3 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72h post lameness induction. Plasma cortisol concentrations from each sample were determined in duplicate using a radioimmunoassay (RIA) system using adapted methods (Kleinhenz et al., 2017). Plasma cortisol concentrations were determined using a commercially available RIA kit (MP Biomedicals,

Irvine, CA) following manufacturer specifications with minor modifications as previously described (Martin et al., 2022); the standard curve was extended to include 1 and 3 ng/mL by diluting the 10 and 30 ng/mL manufacturer-supplied standards, 1:10 respectively. The standard curve ranged from 1 to 300 ng/mL. A low (25 ng/mL) and high (150 ng/mL) quality control (QC) were run at the beginning and end of each set to determine inter-assay variability. Plain 12 x 75 mm polypropylene tubes were used as blank tubes to calculate non-specific binding. Input for standards, QCs, and samples was adjusted to 50µL. Samples were incubated at room temperature for 30 min before adding I-125. Manufacturer instructions were then followed. Tubes were counted on a gamma counter (Wizard2, PerkinElmer, Waltham, MA) for 1 min. The raw data file was then uploaded onto MyAssays Desktop software (version 7.0.211.1238, 21 Hampton Place, Brighton, UK) for concentration determination. Standard curves were plotted as a 4-parameter logistic curve. Samples with a coefficient of variation (CV) ≥18% were re-analyzed. The intra-assay and inter-assay CV were determined to be 21.3% and 24.5%, respectively.

Plasma Substance P

Substance P (SP) concentrations were determined through RIA using methods previously described (Martin et al., 2020). The standard curve, ranging from 20 to 1,280 pg/mL, was created by diluting synthetic SP (Phoenix Pharmaceuticals, Burlingame, CA) with RIA Buffer (50mM sodium phosphate dibasic heptahydrate, 13mM disodium ethylenediaminetetraacetic acid (EDTA), 150mM sodium chloride, 1mM benzamidine hydrochloride, 0.1% gelatin, 0.02% sodium azide; pH 7.4). For analysis, 100 µL of sample, standard, or QC were aliquoted into plain 12 x 75mm conical bottom tubes followed by 100 µL of Rabbit anti-SP primary antibody (1:20,000; Phoenix Pharmaceuticals). Iodine-125-SP tracer (custom iodination by Revvity) was

diluted with RIA buffer to 20,000 cpm, then 100 µL was added to the sample, standard, and QC tubes. Samples were then covered and stored at 4°C for 48h. At the end of the 48h incubation, samples were placed on ice and 100 µL of normal rabbit plasma (1:80) and goat anti-rabbit secondary antibody (1:40; Jackson ImmunoResearch, West Grove, PA) were added to each tube. Samples were then incubated at room temperature for 10 min, placed back on ice, and 100 µL of blank bovine plasma was added to the standards and QCs. All tubes then had 1 mL of 12% polypropylene glycol in 0.85% sodium chloride added. Samples were centrifuged at 3,000 x g for 30 minutes at 4°C and the supernatant aspirated. Tubes were counted on a gamma counter (Wizard2, PerkinElmer, Waltham, MA) for 1 min. The raw data file was then uploaded onto MyAssays Desktop software for concentration determination. Standard curves were plotted as a 4-parameter logistic curve. Samples with a CV ≥18% were re-analyzed. The intra-assay and interassay CV were determined to be 18.8% and 30.02%, respectively.

Kinetic Gait Analysis

A commercial pressure/force mat system (Strideway, Tekscan, Inc.) was used to record and analyze the gait of each goat at each timepoint (prior lameness induction (-24), and 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72h post induction) using methods previously described (Reppert et al., 2020; Weeder et al., 2024). Video analysis was used to ensure synchronization of each goat's gait while walking across the pressure mat system. A research grade software (Strideway v 7.7, Tekscan, Inc., South Boston, MA, USA), was used to analyze different parameters of gait motion using methods previously described (Reppert et al., 2019) (stance time, stride length, contact force, impulse, contact pressure, and contact area). Gait velocity and gait distance were also recorded on each individual run. Post induction timepoints were compared to –24h for statistical analysis purposes.

Visual Lameness Scoring

Using previously described methods (Deeming et al., 2018), VLS was performed by an observer trained in lameness identification and severity. Each goat was scored at the following timepoints: prior to induction (-24), 0, 1, 2, 3, 4, 6, 8, 10,12, 24, 36, 48, 60, and 72h. Animals were walked through an alley system with non-slip flooring while traveling to the pressure mat system. Lameness was graded on a scale from 1 to 5 (1-normal gait, 2-uneven gait, 3-mildly lame, 4-moderately lame, 5- severely lame). Counts for animals scoring a 2 or above were used for VLS comparisons.

Visual Analogue Scale

A visual analogue scale for pain was used to score each goat at each time point (prior to induction (-24), 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72h). An observer skilled in recognizing pain indicators scored each goat as they walked across the pressure mat walkway. Pain was graded with a 100 mm (10 cm) line anchored at each end by descriptors of "No Pain" or "Severe Pain". The evaluator marked the line between the two descriptors to indicate pain intensity. A millimeter scale was used to measure the score from the zero-anchor point to the evaluator's mark. Seven parameters were used to assess pain: depression, tail swishing or flicking, stance, head carriage, spinal alignment, movement, and ear carriage adapted from a previous study (Martin et al., 2020). No pain was characterized by being alert and quick to show interest, no tail swishing, a normal stance, head carriage above spine level, a straight spine, moving freely across the pressure mat and ears forward. Severe pain was characterized by dullness and showing no interest, more than three tail swishes per minute, legs abducted, head held below spine level, a curved spine, reluctance to move, and ears down.

Statistical Analysis

A sample size of sixty-eight (68) animals was needed to reach a statistical power of 0.80 for critical outcome measurements in this study, including but not limited to contact force from KGA analysis (sigma=0.57, true difference of 10.25kg). Forty-four animals were used twice on study, totaling eighty-eight (88) animal total. Six (6) animals were retained as substitutions.

Plasma cortisol and Sub P data were log transformed to normalized data prior to statistical analysis. Animal nested within treatment was the random effect. Treatment, time, treatment by time interaction, and replicate were fixed effects. IRT, MNT, and VAS outcome responses of lame-sound (lame minus sound) limb were analyzed using linear mixed models with lame leg as the experimental unit in JMP (JMP 15.1, SAS Institute Inc. Cary, NC). Lame leg nested in a treatment group and T0 VLS were designated as a random effect, with treatment, time, treatment by time interaction, and replicate designated as fixed effects. VLS was dichotomized into binomial yes/no, and were analyzed using a Chi-Squared Test in JMP. KGA outcome responses of lame-sound limb were analyzed using linear mixed models with lame leg as the experimental unit in JMP (JMP 15.1, SAS Institute Inc. Cary, NC). Lame leg nested in a treatment group and time zero (T0) VLS were designated as a random effect, with treatment, time, treatment by time interaction, and replicate designated as fixed effects. VLS was dichotomized into binomial yes/no, and was analyzed using a Chi-Squared Test in JMP. KGA outcome responses of lame-sound limb were analyzed using linear mixed models with lame leg as the experimental unit in JMP (JMP 15.1, SAS Institute Inc. Cary, NC). Lame leg nested in treatment group and replicate were designated as a random effect, with time, treatment, and treatment by time interaction, designated as fixed effects.

F-tests were utilized for testing the significance of main effects and interactions. Pairwise comparisons on response variables with significant overall differences were performed using the Tukey Honestly Significant Difference (HSD) test. Planned contrasts include comparing drug (firocoxib vs. meloxicam vs. transdermal flunixin) and dose levels. Statistical significance was determined at a priori at $P \leq 0.05$. Data are presented as least square means, or as a count. Results are reported as least square means with a 95% confidence interval.

Results

Forty-four crossbred castrated meat type goats, between nine-twelve mo of age and with an average body weight of 35 kg (25 kg - 43 kg) were enrolled. Prior to enrollment, all animals were found to be free of lameness, as determined by a trained individual (AL) using a visual lameness scoring (VLS) system adapted from Deeming et al., (2018).

Infrared Thermography (IRT) (ºC)

Differences between IRT average temperatures from lame-sound limbs did differ by timepoint ($P \leq 0.0001$) and by treatment ($P \leq 0.0001$) and did not have a significant treatment by time interaction ($P = 0.45$). These data are summarized in Table 4.1.

Mechanical Nociception Threshold (MNT) (kg'F)

MNT differences between lame-sound limb did differ by treatment ($P \leq 0.0001$) and timepoint ($P \leq 0.0001$) but did not have a significant treatment by time interaction ($P = 0.20$). These data are summarized in Table 4.1 and represented graphically in Figure 4.2.

Plasma Cortisol (CORT)

Plasma cortisol differed by timepoint ($P \leq 0.0001$) but did not have a significant treatment ($P = 0.86$) or a treatment by time interaction ($P = 0.79$). These data are summarized in Table 4.1.

Plasma Substance P (Sub P)

Plasma Substance P did not differ by timepoint ($P = 0.99$), treatment ($P = 0.13$) or treatment by timepoint interaction ($P = 0.89$). These data are summarized in Table 4.1.

Kinetic Gait Analysis (KGA)

Kinetic Gait Analysis outcomes are shown in Table 4.1.

Data is represented graphically in Figure 4.3.

Stance Time (s) (Lame-Sound Limb)

The lame limb and sound limb stance time did not have a significant treatment by timepoint interaction ($P = 0.92$) but did differ by timepoint ($P = 0.0022$) and by treatment ($P = 0.0002$).

Stride Length (cm) (Lame-Sound Limb)

The lame limb and sound limb stride length did not have a significant treatment by timepoint interaction ($P = 0.99$) but did differ by timepoint ($P \leq 0.0001$) and by treatment ($P \leq 0.0001$).

Force (kg) (Lame-Sound Limb)

The lame limb and sound limb force did not have a significant treatment by timepoint interaction ($P = 0.99$) but did differ by timepoint ($P \leq 0.0001$) and by treatment ($P \leq 0.0001$).

Impulse (kg x s) (Lame-Sound Limb)

The lame limb and sound limb impulse did not differ by timepoint ($P = 0.88$), treatment ($P = 0.34$), or treatment by timepoint interaction ($P = 0.56$).

Contact pressure (kg/cm^2) (Lame-Sound Limb)

The lame limb and sound limb contact pressure did not have a significant treatment by timepoint interaction ($P = 0.99$) but did differ by timepoint ($P = 0.0039$) and by treatment ($P = 0.032$).

Gait Distance (cm) (Lame-Sound Limb)

Gait distance change from baseline values (-24h) did not differ by timepoint ($P = 0.72$), treatment ($P = 0.16$), or treatment by timepoint interaction ($P = 0.11$).

Gait Velocity (kg x s) (Lame-Sound Limb)

Gait velocity change from baseline values (-24h) did not differ by timepoint ($P = 0.14$), treatment ($P = 0.099$), or treatment by timepoint interaction ($P = 0.99$).

Visual Lameness Scoring (VLS)

A ChiSq Whole Model Fit was reported at ($P \leq 0.0001$) with a ChiSq value (Lack of Fit) of ($P = 0.04$). Visual Lameness Scores (VLS) did differ by timepoint ($P \leq 0.0001$) and by treatment ($P \leq 0.0001$) but did not have a significant treatment by timepoint interaction ($P = 0.91$). These data are represented graphically in Figure 4.4.

Visual Analogue Scoring (VAS)

Visual Analogue Scores (VAS) did differ by timepoint ($P \leq 0.0001$) and by treatment ($P \leq 0.0001$) but did not have a significant treatment by timepoint interaction ($P = 0.06$).

These data are represented graphically in Figure 4.4.

Discussion

Data from this study suggests that goats with amphotericin B-induced lameness experience measurable analgesia after administration of transdermal flunixin or oral meloxicam. In contrast, oral firocoxib showed limited effectiveness and may not be a reliable option to mitigate pain in goats with amphotericin B-induced lameness. The primary objective of this study was to compare the analgesic properties of oral firocoxib, oral meloxicam, and transdermal flunixin at three varying dosages to a positive and negative control after induced lameness in crossbred meat type goats. Doses for each drug were chosen from a combination of published research conducted in cattle and sheep, and from current treatment plans utilized by licensed veterinarians at Kansas State Veterinary Health Center for caprine patients.

Amphotericin B has been previously used as a repeatable, reliable model for lameness research in animal species like horses and cattle (Kotschwar et al., 2009; Coetzee et al., 2014). Only two other studies have validated this lameness model in goats (Weeder et al., 2023; Reppert et al., 2019). In this study, peak lameness was observed 1-3d post lameness induction with amphotericin B. Lameness completely resolved in all animals on study by 7d. Data from this study suggests that an intra articular joint injection of amphotericin B is a reliable model to induce lameness in crossbred meat type goats. Goats in this study showed no lasting symptoms at the study conclusion after being returned to pasture.

While NSAIDs such as firocoxib, meloxicam, and flunixin analgesic effects have been investigated before in varied species and lameness models, their analgesic effects can vary. In a urate crystal UC lameness model, oral firocoxib at a dose of 5.0mg/kg provided effective analgesia in dogs (Drag et al., 2007). Results from this study showed that oral firocoxib had little

to no analgesic effect in goats with induced lameness. Oral meloxicam at a dose of 0.5mg/kg with or without gabapentin reduced lameness severity in beef calves with chemical lameness (Coetzee et al., 2014). Meaningful differences in VLS and cortisol were observed in lactating dairy cattle with induced lameness given oral meloxicam at a dose of 1.0mg/kg when compared to a placebo (Warner et al., 2021). Results from this study suggested that goats received little to some analgesic effects from oral meloxicam after induced lameness with amphotericin B. The most efficacious dosage for this oral meloxicam in our study was 2.0 mg/kg.

Flunixin transdermal at 3.3mg/kg provides effective analgesia in cattle with induced lameness (Kleinhenz et al., 2019). Flunixin IV at a dose of 1.0mg/kg was efficacious in providing analgesia to dairy steers with induced amp B lameness (Schulz et al., 2011). Horses given flunixin IV 1.1mg/kg alone or in combination with phenylbutazone did not see effective pain mitigation after lameness induction (Foreman et al., 2011). Goats administered transdermal flunixin after induced lameness in our study showed the most analgesic effect. Transdermal flunixin at 3.3 mg/kg or 5.0 mg/kg were the most effective dosages overall when compared to other drugs and dosages in this study.

This study is the first to evaluate three different NSAIDs at three different dosages in a goat lameness model using multiple outcome measurements. Pain responses from individuals in this study implemented both physiological and behavioral parameters, including IRT, MNT, blood plasma cortisol, blood plasma substance P, VAS, VLS, and KGA.

Results from this study suggest that effective treatments for goats with lameness-associated pain may include transdermal flunixin at either 3.3mg/kg or 5.0mg/kg. The transdermal formulation of flunixin also possesses a long half-life, is reported to work systemically faster than oral analgesic options and is a non-specific inhibitor of COX-1 and

COX-2 (Reppert et al., 2019; Health MA, 2025). In previous studies, transdermal flunixin has shown pain mitigation efficacy for lameness in cattle (Kleinhenz et al., 2016) castration in goats and piglets (Graves et al., 2020; Lopez-Soriano et al., 2023) transportation stress and BRD pain in cattle (Martin et al., 2022; Van Engen et al., 2014) improved pregnancy rates in embryo transfer cows (Kasimanickam et al., 2019), and reduced pain expression and improved milk yield in early postpartum dairy cows (Schmitt et al., 2023a; Schmitt et al., 2023b). Further comprehensive pharmacokinetic data is needed to refine flunixin transdermal dosages in caprine patients experiencing pain from lameness conditions.

Limitations of this study include a small number of animals per treatment group, evaluation of only meat type goat breeds, variation between washout time periods of left hind induction and right hind induction (2 – 4 wk intervals), and weather variation due to length of study (June 2022-November 2022). Behavior and grimace scoring were evaluated during this study but were not ready for analysis at the time of publication. As behavior is an important part of pain evaluation, current conclusions are limited to the available outcome measures from this study. Another limitation of this study is not completing analysis of renal function or monitoring for gastric ulceration, particularly for higher dosages for firocoxib, meloxicam, and flunixin transdermal. No adverse gastric or renal functions were noted amongst goats in this study.

The use of firocoxib, meloxicam, or flunixin transdermal is considered extra label use, but is approved under AMDUCA in the US. The AMDUCA Act of 1994 states “Under the provisions of 21 CFR 530, the FDA recognizes the professional judgment of veterinarians and permits the extra label use of drugs by veterinarians under certain conditions. Extralabel use of drugs may only take place within the scope of a valid VCPR. Extralabel use is limited to circumstances when the health of an animal is threatened or suffering or death may result from

failure to treat" (FDA, 2024). Veterinarians prescribing any of these NSAIDs to caprine patients should contact the Food Animal Residue Avoidance Database (FARAD) for the latest estimates for meat withdrawal intervals.

Future research aims include establishing the pharmacokinetics of the drug regimens used in this study, validating amphotericin B lameness induction models in dairy goat breeds, and evaluation of both behavior and grimace scoring data for a more thorough comprehension of pain. Lameness continues to be an animal welfare and economic issue in livestock production, particularly as the U.S. goat population grows. Results from this study suggest that optimal treatments for goats with lameness associated pain may include transdermal flunixin at either 3.3 mg/kg or 5.0 mg/kg (FTD-LOW OR FTD-HIGH). Further pharmacokinetic research is warranted to optimize dosing strategies for meat type goats experiencing pain from lameness conditions.

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Figures

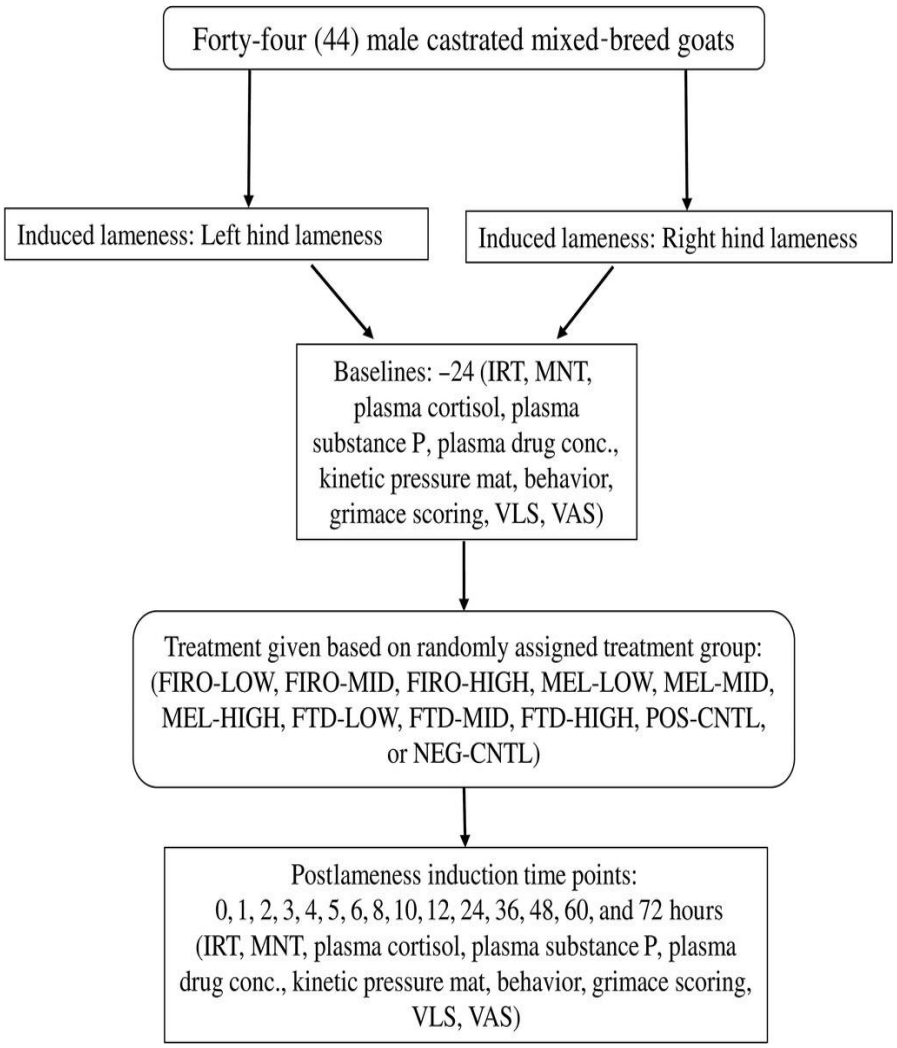

Figure 4.1 Flow chart of study events for evaluating analgesic effects of NSAIDs in meat-type goats with amphotericin B-induced lameness. The study was conducted from June to December 2022, and involved administration of firocoxib, meloxicam, or transdermal flunixin at varying dosages following induction of lameness by intra-articular amphotericin B injection. Goats were evaluated with behavioral and physiological pain assessments over a 7d period post-lameness induction.

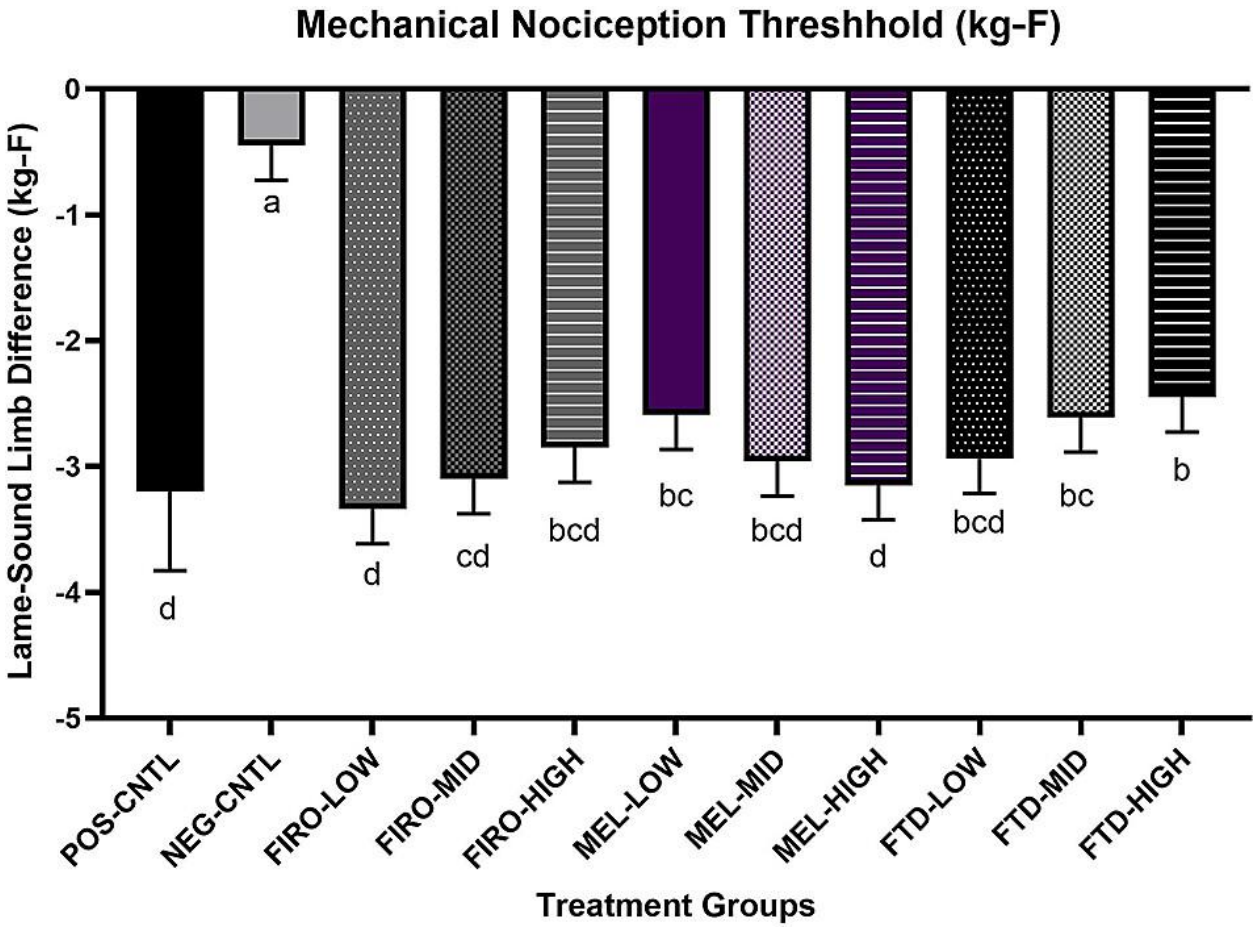

Figure 4.2 Mechanical Nociception Threshold (MNT) overall mean (± SEM) in meat type goats following analgesic treatment or no treatment after induced lameness with amphotericin B or no lameness induction. This study evaluated the analgesic efficacy of oral firocoxib (0.5, 1.0, 2.0 mg/kg), oral meloxicam (1.0, 2.0, 3.0 mg/kg), and transdermal flunixin (3.3, 4.2, 5.0 mg/kg) compared to positive and negative controls. MNT assesses the pressure threshold required to elicit a nociceptive response, indicating pain sensitivity.

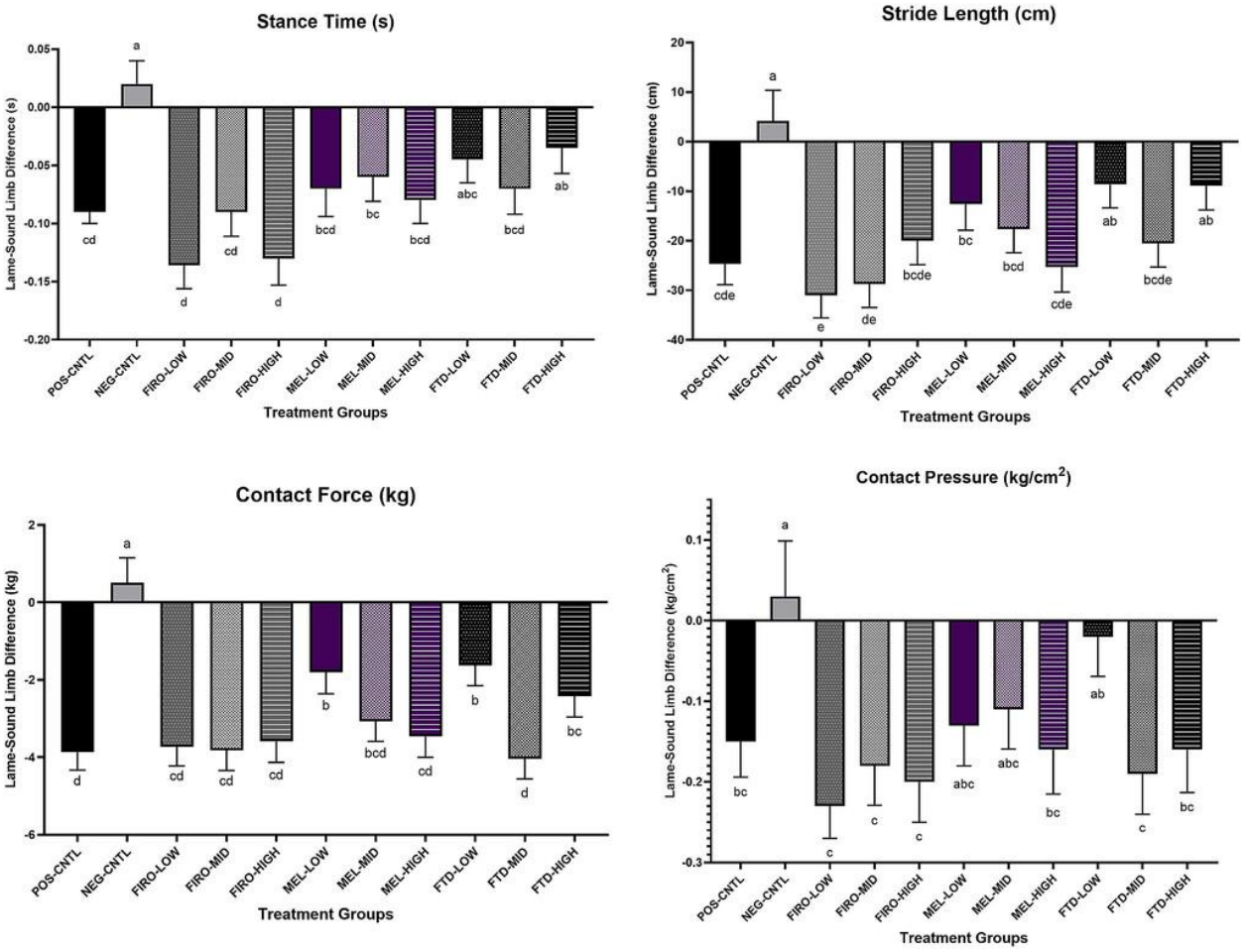

Figure 4.3 Kinetic Gait Analysis (KGA) overall mean (± SEM) parameters in meat type goats following analgesic treatment or no treatment after induced lameness with amphotericin B or no lameness induction. This study evaluated the analgesic efficacy of oral firocoxib (0.5, 1.0, 2.0 mg/kg), oral meloxicam (1.0, 2.0, 3.0 mg/kg), and transdermal flunixin (3.3, 4.2, 5.0 mg/kg) compared to positive and negative controls. KGA parameters measure gait changes related to limb loading and symmetry, reflecting pain-associated gait alterations.

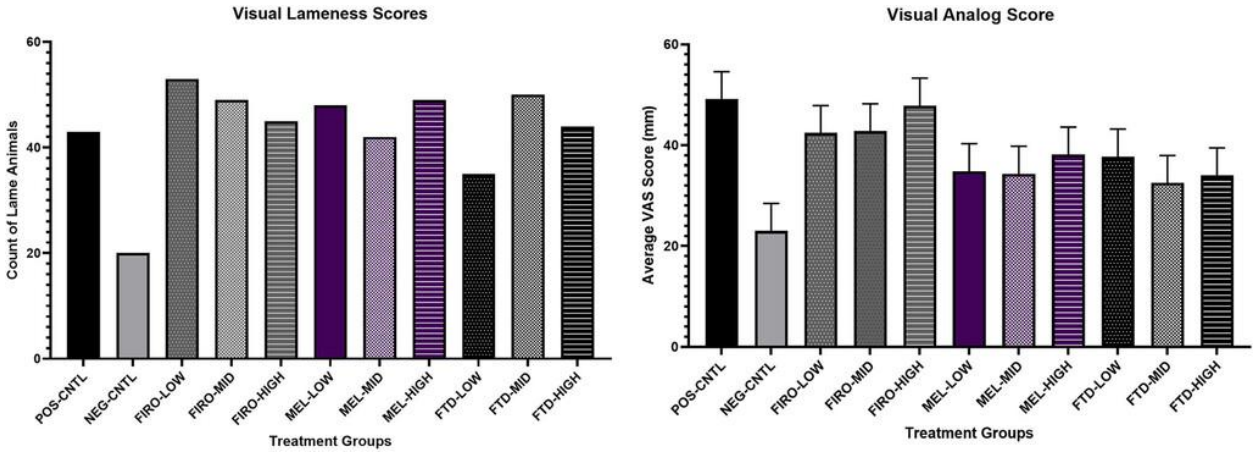

Figure 4.4 Count of lame goats with a Visual Lameness Score (VLS) ≥ 2 and mean visual Analogue Score (VAS) per treatment group following amphotericin B-induced lameness or no lameness induction. This study evaluated the analgesic efficacy of oral firocoxib (0.5, 1.0, 2.0 mg/kg), oral meloxicam (1.0, 2.0, 3.0 mg/kg), and transdermal flunixin (3.3, 4.2, 5.0 mg/kg) compared to positive and negative controls. VLS and VAS are behavioral pain assessments used to score severity of lameness and pain intensity, respectively.

Tables

Table 4.1 Overall mean differences (95% CI) between hind limbs for significant treatment effects on infrared thermography (IRT), mechanical nociception threshold (MNT), and kinetic gait parameters in goats with amphotericin B–induced lameness.

	IRT (°C)	**MNT (kg'F)**	**Stance Time (s)**	**Stride length (cm)**	**Force (kg)**	**Contact Pressure (kg/cm²)**
Time P-Value (*P*)	≤ 0.0001	≤ 0.0001	= 0.92	≤ 0.0001	≤ 0.0001	= 0.0039
Treatment P-Value (*P*)	≤ 0.0001	≤ 0.0001	= 0.0002	≤ 0.0001	≤ 0.0001	= 0.0324
Trt*Time P-Value (*P*)	= 0.45	= 0.20	= 0.92	= 0.99	= 0.99	= 0.99
POS-CNTL (Amp B)	4.84 (3.59-6.09)	-3.20 (-3.77-(-2.64)	-0.10 (-0.13-(-0.06)	-24.64 (-33.05-(-16.23)]	-3.86 (-4.79-(-2.94)	-0.15 (-0.24-(-0.06)
NEG-CNTL (Saline)	0.17 (-1.09-1.44)	-0.45 (-1.02-0.11)	0.02 (-0.03-0.0004)	4.22 (-7.93-16.33)	0.51 (-0.74-1.77)	0.03 (-0.10-0.17)
FIRO-LOW (0.5 mg/kg)	3.12 (1.88-4.35)	-3.34 (-3.90-(-2.77)	-0.14 (-0.17-(-0.09)	-30.97 (-40.02-(-21.92)	-3.73 (-4.70-(-2.75)	-0.23 (-0.32-(-0.14)
FIRO-MID (1.0 mg/kg)	4.59 (3.34-5.83)	-3.10 (-3.66-(-2.53)	-0.10 (-0.14-(-0.05)	-28.73 (-38.01-(-19.45)	-3.82 (-4.86-(-2.79)	-0.18 (-0.28-(-0.08)
FIRO-HIGH (2.0 mg/kg)	3.71 (2.45-4.96)	-2.85 (-3.17-(-2.01)	-0.13 (-0.18-(-0.08)	-19.92 (-29.46-(-10.52)	-3.59 (-4.65-(-2.52)	-0.20 (-0.30-(-0.14)
MEL-LOW (1.0 mg/kg)	3.85 (2.55-5.15)	-2.59 (-3.17-(-2.01)	-0.03 (-0.12-(-0.03)	-12.59 (-22.96-(-2.21)	-1.80 (-2.91-(-0.69)	-0.13 (-0.24-(-0.02)
MEL-MID (2.0 mg/kg)	4.82 (3.52-6.12)	-2.96 (-3.54-(-2.38)	-0.06 (-0.10-(-0.02)	-17.66 (-26.96-(-8.36)	-3.07 (-4.10-(-2.04)	-0.11 -0.21-(-0.02)
MEL-HIGH (3.0 mg/kg)	4.33 (3.06-5.60	-3.15 (-3.73-(-2.58)	-0.08 (-0.13-(-0.04)	-25.30 (-35.23-(-15.37)	-3.46 (-4.53-(-2.38)	-0.17 (-0.027-(-0.05)
FTD-LOW	4.33	-2.94	-0.04	-8.60	-1.63	-0.02

(3.3 mg/kg)	(3.08-5.58)	(-3.51-(-2.37)	(-0.08-(-0.0001)	(-17.96-0.76)	(-2.65-(-0.60)	(-0.12-0.06)
FTD-MID (4.2 mg/kg)	4.83 (3.58-6.07)	-2.61 (-3.17-(-2.04)	-0.08 (-0.12-(-0.03)	-20.52 (-29.91-(-11.13)	-4.04 (-5.08-(-3.01)	-0.19 (-0.29-(-0.09)
FTD-HIGH (5.0 mg/kg)	3.57 (2.33-4.80)	-2.45 (-3.01-(-1.89)	-0.03 (-0.07-0.008)	-8.86 (-18.51-0.78)	-2.42 (-3.49-(-1.36)	-0.17 -0.27-(-0.06)

References

A, M. K. (2002). Non-steroidal anti-inflammatory analgesics: a review of current practice. In. Journal of Veterinary Emergency & Critical Care.

Celozzi, S., Battini, M., Prato-Previde, E., & Mattiello, S. (2022). Humans and Goats: Improving Knowledge for a Better Relationship. Animals (Basel), 12(6). https://doi.org/10.3390/ani12060774

Christodoulopoulos, G. (2009). Foot lameness in dairy goats. Res Vet Sci, 86(2), 281-284. https://doi.org/10.1016/j.rvsc.2008.07.013

Coetzee, J. F. (2013). Assessment and management of pain associated with castration in cattle. Vet Clin North Am Food Anim Pract, 29(1), 75-101. https://doi.org/10.1016/j.cvfa.2012.11.002

Corporation, W. (2024). Oasis Cartridges and 96-Well Plates Care and Use Manual. In.

Crosby-Durrani, H. E., Clegg, S. R., Singer, E., Angell, J. W., Evans, N. J., Carter, S. D.,Duncan, J. S. (2016). Severe Foot Lesions in Dairy Goats Associated with Digital Dermatitis Treponemes. J Comp Pathol, 154(4), 283-296. https://doi.org/10.1016/j.jcpa.2016.04.001

Deeming, L. E., Beausoleil, N. J., Stafford, K. J., Webster, J. R., & Zobel, G. (2018). Technical note: The development of a reliable 5-point gait scoring system for use in dairy goats. J Dairy Sci, 101(5), 4491-4497. https://doi.org/10.3168/jds.2017-13950

Distel, M., Mueller, C., & Bluhmki, E. (1995). Side Effects of Anti-Inflammatory Drugs IV: Global Analysis of Gastrointestinal Safety of a New NSAID, Meloxicam. In (pp. 183-193).

Distel, M., Mueller, C., Bluhmki, E., & Fries, J. (1996). Safety of meloxicam: a global analysis of clinical trials. Br J Rheumatol, 35 Suppl 1, 68-77. https://doi.org/10.1093/rheumatology/35.suppl_1.68

Drag, M., Kunkle, B. N., Romano, D., & Hanson, P. D. (2007). Firocoxib efficacy preventing urate-induced synovitis, pain, and inflammation in dogs. Vet Ther, 8(1), 41-50.

Drew, M. L. (1998). Update on the Animal Medicinal Use Clarification Act of 1994 Regulations for Wildlife Veterinarians. In (pp. 163-167). 1998 Proceedings AAZV and AAWV Joint Conference.

Dubeuf, J.-P., Genis, J. C., Morand-Fehr, P., & Ruiz Morales, F. d. A. (2023). The contribution of goats in the future of redesigning of livestock activities and value chains. In (Vol. 227). Small Ruminant Research: Science Direcy.

FDA. (2024). Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). In.

FDA, U. (2018). Bioanalytical Method Validation: Guidance for Industry. In.

Foreman, J. H., & Ruemmler, R. (2011). Phenylbutazone and flunixin meglumine used singly or in combination in experimental lameness in horses. Equine Vet J Suppl (40), 12-17. https://doi.org/10.1111/j.2042-3306.2011.00485.x

Geyer, R. E. (1997). Extralabel Drug Use and Compounding in Veterinary Medicine. 52(3), 291-295.

Haahr, M., & Svend, H. (1999-2025). Random Number Generator. In.

Health, M. A. (2024). Banamine Transdermal (flunixin transdermal solution).

Hill, N. P., Murphy, P. E., Nelson, A. J., Mouttotou, N., Green, L. E., & Morgan, K. L. (1997). Lameness and foot lesions in adult British dairy goats. Vet Rec, 141(16), 412-416. https://doi.org/10.1136/vr.141.16.412

Ingvast-Larsson, C., Högberg, M., Mengistu, U., Olsén, L., Bondesson, U., & Olsson, K. (2011). Pharmacokinetics of meloxicam in adult goats and its analgesic effect in disbudded kids. J Vet Pharmacol Ther, 34(1), 64-69. https://doi.org/10.1111/j.1365-2885.2010.01195.x

Kasimanickam, R., Kasimanickam, V., Gold, J., Moore, D., Kastelic, J. P., Pyrdek, D., & Ratzburg, K. (2019). Injectable or transdermal flunixin meglumine improves pregnancy rates in embryo transfer recipient beef cows without altering returns to estrus. Theriogenology, 140, 8-17. https://doi.org/10.1016/j.theriogenology.2019.08.011

Kotschwar, J. L., Coetzee, J. F., Anderson, D. E., Gehring, R., KuKanich, B., & Apley, M. D. (2009). Analgesic efficacy of sodium salicylate in an amphotericin B-induced bovine synovitis-arthritis model. J Dairy Sci, 92(8), 3731-3743. https://doi.org/10.3168/jds.2009-2058

Lees, P., & Taylor, P. M. (1991). Pharmacodynamics and pharmacokinetics of flunixin in the cat. Br Vet J, 147(4), 298-305. https://doi.org/10.1016/0007-1935(91)90002-5

Lopez-Soriano, M., Merenda, V. R., Anderson, S., Trindade, P. H. E., Leidig, M. S., Messenger, K.,Pairis-Garcia, M. D. (2023). Efficacy of inguinal buffered lidocaine and intranasal flunixin meglumine on mitigating physiological and behavioral responses to pain in castrated piglets. Front Pain Res (Lausanne), 4, 1156873. https://doi.org/10.3389/fpain.2023.1156873

Marshall, T. S. (2009). Abomasal ulceration and tympany of calves. Vet Clin North Am Food Anim Pract, 25(1), 209-220, viii. https://doi.org/10.1016/j.cvfa.2008.10.010

Martin, M., Kleinhenz, M., Kleinhenz, K., Reppert, E., Blasi, D., Parks, T.,Coetzee, J. (2020). Comparison of the effect of tildipirosin administered alone or in combination with transdermal flunixin on the performance, health, activity, and well-being of transported

feedlot calves on arrival at the feedlot. Transl Anim Sci, 4(1), 452-459. https://doi.org/10.1093/tas/txaa005

McIlwraith, C. W., Fessler, J. F., Blevins, W. E., Page, E. H., Rebar, A. H., Van Sickle, D. C., & Coppoc, G. L. (1979). Experimentally induced arthritis of the equine carpus: clinical determinations. Am J Vet Res, 40(1), 11-20.

Nalon, E., & Stevenson, P. (2019). Addressing Lameness in Farmed Animals: An Urgent Need to Achieve Compliance with EU Animal Welfare Law. Animals (Basel), 9(8). https://doi.org/10.3390/ani9080576

Rangel-Nava, A., Ramírez-Uribe, J. M., Recillas-Morales, S., Ibancovichi-Camarillo, J. A., Venebra-Muñoz, A., & Sánchez-Aparicio, P. (2019). Pharmacological Regulation in the USA and Pharmacokinetics Parameters of Firocoxib, a Highly Selective Cox-2, by Pain Management in Horses. J Equine Vet Sci, 77, 36-42. https://doi.org/10.1016/j.jevs.2019.02.007

Reppert, E. J., Kleinhenz, M. D., Viscardi, A., Montgomery, S. R., Crane, A. R., & Coetzee, J. F. (2020). Development and evaluation of two different lameness models in meat goats, a pilot study. Transl Anim Sci, 4(4), txaa193. https://doi.org/10.1093/tas/txaa193

Schmitt, R., Pieper, L., Borchardt, S., Swinkels, J. M., Gelfert, C. C., & Staufenbiel, R. (2023a). Effects of a single transdermal administration of flunixin meglumine in early postpartum Holstein Friesian dairy cows: Part 1. Inflammatory and metabolic markers, uterine health, and indicators of pain. J Dairy Sci, 106(1), 624-640. https://doi.org/10.3168/jds.2021-20555

Schmitt, R., Pieper, L., Borchardt, S., Swinkels, J. M., Gelfert, C. C., & Staufenbiel, R. (2023b). Effects of a single transdermal administration of flunixin meglumine in early postpartum Holstein Friesian dairy cows: Part 2. Milk yield, culling risk, and reproductive performance. J Dairy Sci, 106(1), 641-652. https://doi.org/10.3168/jds.2022-21995

Stuart, A. K., Kukanich, B., Caixeta, L. S., Coetzee, J. F., & Barrell, E. A. (2019). Pharmacokinetics and bioavailability of oral firocoxib in adult, mixed-breed goats. Journal of veterinary pharmacology and therapeutics.

USDA. (2024). Sheep and Goats (January 2024). In USDA, National Agricultural Statistics Service: National Agriculture Statistics Service (NASS), Agricultural Statistics Board.

Van Engen, N. K., Stock, M. L., Engelken, T., Vann, R. C., Wulf, L. W., Karriker, L. A.,Coetzee, J. F. (2014). Impact of oral meloxicam on circulating physiological biomarkers of stress and inflammation in beef steers after long-distance transportation. J Anim Sci, 92(2), 498-510. https://doi.org/10.2527/jas.2013-6857

Warner, R., Kleinhenz, M. D., Ydstie, J. A., Schleining, J. A., Wulf, L. W., Coetzee, J. F., & Gorden, P. J. (2021). Randomized controlled trial comparison of analgesic drugs for control of pain associated with induced lameness in lactating dairy cattle. J Dairy Sci, 104(2), 2040-2055. https://doi.org/10.3168/jds.2020-18563

Weeder, M. M., Kleinhenz, M. D., Reppert, E. J., Fritz, B. R., Viscardi, A. V., Montgomery, S. R.,Coetzee, J. F. (2023). Optimal lameness induction model development using amphotericin B in meat goats. Transl Anim Sci, 7(1), txad105. https://doi.org/10.1093/tas/txad105

Weeder, M. M., Kleinhenz, M. D., Reppert, E. J., Weaver, L. F., Johnson, B. T., Leslie, A. A.,Coetzee, J. F. (2024). Comparison of firocoxib and meloxicam for pain mitigation in goats undergoing surgical castration. J Am Vet Med Assoc, 262(4), 498-505. https://doi.org/10.2460/javma.23.10.0575

Chapter 5 - Pharmacokinetic parameters of oral firocoxib, oral meloxicam, and transdermal flunixin in meat type goats undergoing an amphotericin B lameness model

Pharmacokinetics of NSAIDs in meat type goats

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Abstract

The objective of this study was to evaluate and compare the pharmacokinetics of oral firocoxib, oral meloxicam, and transdermal flunixin (TD) in forty-four (44) adult, male castrated, crossbred goats. Pharmacokinetic (PK) analysis was performed for each goat in each phase using non-compartmental methods with descriptive statistics reported. Mean plasma half-life ($T_{1/2}$ (h)) for oral (PO) administration of firocoxib at varying dosages in meat type castrated goats were reported at 0.5mg/kg, 1.0mg/kg, and 2.0mg/kg were 9.1 (range 6.9-13.4), 10.2 (range 6.4-14.7) and 9.2 (range 6.8-12.8). For oral meloxicam, mean plasma $T_{1/2}$ at doses of 1.0 mg/kg, 2.0 mg/kg, and 3.0 mg/kg were 13.3 (range: 10.1–22.3), 13.1 (range: 12–24), and 11.7 (range: 8.3–20.5) hours, respectively. Transdermal flunixin showed mean plasma $T_{1/2}$ of 16.5 (range: 10.7–62) at 3.3 mg/kg, 22.0 (range: 16.6–67.4) at 4.2 mg/kg, and 17.8 (range: 7.4–56.3) at 5.0 mg/kg. These results highlight significant variability in drug disposition and suggest that further research is warranted to optimize dosing regimens for oral firocoxib, oral meloxicam, and transdermal flunixin in goats. Results emphasize the need for species-specific dosing strategies and suggest that further pharmacokinetic and pharmacodynamic studies are necessary to optimize NSAID use in goats.

Keywords: firocoxib, flunixin, goats, meloxicam, pharmacokinetics

Introduction

Firocoxib, meloxicam, and flunixin are non-steroidal anti-inflammatory drugs (NSAIDs) commonly used for their anti-inflammatory and analgesic properties in veterinary species. Currently, there are no analgesic drugs specifically approved by the U.S. Food and Drug Administration (FDA) for use in goats. However, under the Animal Medicinal Drug Use Clarification Act of 1994, veterinarians may prescribe NSAIDs for goats when a valid veterinary client-patient relationship exists (FDA, 2024).

In the United States, firocoxib and meloxicam are approved for use in horses and small animals, and both are available in oral formulations, making them accessible and practical for field use. Flunixin is available in both injectable and transdermal (pour-on) forms. The transdermal formulation of flunixin is FDA-approved in cattle for the treatment of pyrexia associated with bovine respiratory disease and mastitis, and for pain control associated with interdigital phlegmon (foot rot) (Merck Animal Health, 2024).

In goats, published pharmacokinetic data for firocoxib, meloxicam, and transdermal flunixin are limited to single-dose, descriptive studies (Stuart et al., 2019; Ingvast-Larrson et al., 2011; Karademir et al., 2016; Reppert et al., 2019). Due to the absence of species-specific data, veterinarians often extrapolate dosage, administration routes, and treatment protocols from other livestock species. However, goats have unique metabolic and physiological characteristics that can greatly differ from species like cattle and sheep. This highlights the need for targeted pharmacokinetic research in goats to inform evidence-based treatment decisions.

The objective of this study was to characterize and compare pharmacokinetics of oral firocoxib, oral meloxicam, and flunixin transdermal at three varying dosages for each drug in castrated male meat-type goats with experimentally induced lameness.

Materials and Methods

Ethical Approval

This study was approved by the Institutional Animal Care and Use Committee at Kansas State University (Protocol #4387).

Experimental Animals

Forty-four castrated, Spanish-influenced crossbred meat-type goats between 9 to 12 months of age, with an average body weight of 35 kg (range: 25–43 kg), were enrolled in the study. Prior to enrollment, all animals were evaluated and deemed free of illness or injury by a licensed veterinarian.

Housing and Husbandry

Goats were individually housed (with visual access to conspecifics) in raised pens within an enclosed barn at the Kansas State Sheep and Meat Goat Unit. Animals were provided ad libitum access to water and fed brome hay twice daily throughout the study.

Treatment Administration

Nine different treatments were implemented in this study. Firocoxib (57mg tablets; Equioxx Tablets; Boehringer Ingelheim Animal Health, Duluth, GA), meloxicam (15mg tablets; Zydus Pharmaceuticals Inc., Pennington, NJ), and flunixin transdermal (50mg/mL; Banamine Transdermal, Merck Animal Health, Madison, NJ) were each given at three different dosages. Each drug-dosage combination was considered a treatment, totaling nine treatments.

Goats in this trial were simultaneously enrolled in a concurrent study investigating the analgesic effects of each treatment on goats with experimentally induced lameness. Each goat received an intra-articular joint injected into the left hind limb and right hind limb with a dose of amphotericin B (5mg/0.25mL). A fourteen-day washout period (14d) occurred between lameness

induced in the left hind limb and the right hind limb. This lameness induction model was chosen based on previous published literature (Weeder et al., 2023). Each goat received two treatments during this crossover design trial. One treatment was given in the first phase, a washout period occurred, and then each goat was given a second treatment in phase two. At least 14 days were allowed for a washout between treatments. PO treatments were administered in a gelatin capsule using a balling gun, while TD treatments were applied with a syringe directly on the skin. PO of firocoxib and meloxicam treatments were rounded to the nearest ½ tablet based on dosage calculations. The treatments used were:

- **FIRO-LOW (n = 8):** Firocoxib tablet at 0.5 mg/kg
- **FIRO-MID (n = 8):** Firocoxib tablet at 1.0 mg/kg
- **FIRO-HIGH (n = 8):** Firocoxib tablet at 2.0 mg/kg,
- **MEL-LOW (n = 8)**: Meloxicam tablet at 1.0 mg/kg,
- **MEL-MID (n = 8):** Meloxicam tablet at 2.0 mg/kg.
- **MEL-HIGH (n = 8):** Meloxicam tablet at 3.0 mg/kg,
- **FLU-LOW (n = 8):** Flunixin transdermal at 3.3 mg/kg,
- **FLU-MID (n = 8):** Flunixin transdermal at 4.2 mg/kg, and
- **FLU-HIGH (n = 8):** Flunixin transdermal at 5.0 mg/kg.

Oral formulations of firocoxib and meloxicam were chosen over parenteral routes due to ease of administration and practicality from a producer standpoint. Low dosages for each drug were chosen based on previous research or approvals in adult cattle, calves, and sheep (US FDA 2017; Stock et al., 2013; Stock et al., 2015). Mid and high range dosages were chosen with the knowledge that goats metabolize drug at a higher rate than calves, cattle, and sheep, which insinuated higher drug dosages for investigation (Myers et al., 2021). Dosages were chosen

based on current knowledge and experience of a board-certified veterinary pharmacologist (MDK). Control goats (n=8) were utilized for the concurrent lameness trial that occurred during this study but were not utilized for pharmacokinetic analysis. This contributed to the overall animal numbers (Weeder et al., 2025).

Plasma Sample Collection

Intravenous jugular catheters (16G X 7.5 cm, Mila International Inc., Florence, KY, US) were placed aseptically by a licensed veterinarian 12h prior to start of study in either the left or right jugular vein. Catheters were flushed prior to and after sample collection using heparinized saline at a rate of 10iu per 1mL (Medefil, Glendale Heights, IL, US) to maintain catheter patency. After the 24h timepoint, catheters were removed, and samples were collected via jugular venipuncture (alternating left and right sides for animal comfort). Blood samples were collected at the following timepoints: -24, 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, 60, and 72h. Approximately 3mL of blood was collected at each timepoint and placed into lithium heparin vacutainer tubes (BD Medical, Franklin Lakes, NJ, US). Blood samples were centrifuged at 1500 g for 15min after collection. Separated plasma was pipetted into 2mL cryovials (VWR International, Radnor PA, US) and stored at –80 C.

Determination of Plasma Drug Concentrations: UHPLC-MS/MS

Chemicals and Materials

Analytical standards of meloxicam, meloxicam-d3, flunixin, flunixin-d3, and firocoxib were purchased from the Cayman Chemical Company (Ann Arbor, MA), while firocoxib-d6 was purchased from Alfa Chemistry (Holbrook, NY). Optima LC-MS grade acetonitrile, formic acid, methanol, water, and HPLC grade phosphoric acid were provided by Fisher Chemical (Ottawa, Ontario, CA). The LC-MS grade dimethyl sulfoxide was supplied by Thermo Scientific

(Waltham, MA). Untreated, blank goat plasma in lithium heparin was obtained from Lampire Biological Laboratories (Pipersville, PA). Oasis PRiME HLB 96 well µElution plates were purchased from Waters Corporation (Milford, MA).

UHPLC-MS/MS Sample Preparation and Extraction

Plasma samples were thawed at room temperature (23°C) prior to analysis start. A mixed analyte stock standard solution containing firocoxib (FIRO), meloxicam (MEL), and flunixin (FTD) was prepared at 3.00E+02, 10.0, and 10.0 µg/mL, respectively, in dimethyl sulfoxide (DMSO) and stored at -20°C. A mixed internal standard (IS) stock solution containing firocoxib-d6 (FIRO-d6), meloxicam-d3 (MEL-d3), and flunixin-d3 (FTD-d3) was prepared at 40.0, 10.0, and 10.0 µg/mL, respectively, in DMSO and stored at -20°C.

The total volumes of the prepared study, daily mixed calibration, and daily mixed quality control (QC) samples were 300.0 µL each. All samples consisted of goat plasma, mixed IS solution, and either aqueous phosphoric acid (4%, v/v) diluent— for study samples— or mixed analyte standard in aqueous phosphoric acid (4%, v/v) —for daily mixed calibration and QC samples. Specifically, the daily mixed calibration and QC samples included 100.0 µL of blank goat plasma, while the study samples included the goat plasma of interest at identical volumes. All samples were spiked with mixed IS solution and diluted appropriately with phosphoric acid (4%, v/v aq) to contain 3.00E+02, 75.0, and 75.0 ng/mL of FIRO-d6, MEL-d3, and FTD-d3, respectively. The daily mixed calibration samples produced calibration curves for FIRO, MEL, and FTD by appropriate phosphoric acid (4%, v/v aq) dilutions to the mixed analyte stock. The FIRO calibration curves were prepared at the following concentrations: 0.750, 1.50, 3.00, 7.50, 15.0, 30.0, 75.0, 150, 300, 750, 1500, 2250, and 3000 ng/mL. The MEL calibration curves were prepared at the following concentrations: 0.0250, 0.0500, 0.100, 0.250, 0.500, 1.00, 2.50, 5.00,

10.0, 25.0, 50.0, 75.0, and 100 ng/mL. The FTD calibration curves were prepared at the following concentrations: 0.0250, 0.0500, 0.100, 0.250, 0.500, 1.00, 2.50, 5.00, 10.0, 25.0, 50.0, 75.0, and 100 ng/mL. The standard concentrations for the daily mixed calibration samples were established to three significant figures. The daily mixed QC samples contained FIRO, MEL, and FTD mixed analyte stock diluted appropriately by phosphoric acid (4%, v/v aq). The QC concentrations of FIRO were 1.50, 30.0, 300, and 2250 ng/mL. The QC concentrations of MEL were 0.0500, 1.00, 10.0, and 75.0 ng/mL. The QC concentrations of FTD were 0.0500, 1.00, 10.0, and 75.0 ng/mL. The standard concentrations for the daily mixed QC samples were established to three significant figures. Study samples that exceeded the method's linear calibration range were diluted as needed with 4% (v/v) aqueous phosphoric acid before extraction and reanalysis. All samples were centrifuged at 1300 × g for 20 minutes at 20°C using a Thermo Scientific Sorvall ST 16R centrifuge.

Using a positive pressure manifold, the supernatants were subjected to reversed-phase solid phase extraction (RP-SPE) with Oasis PRiME HLB µElution plates (Waters Corporation, 2024) according to the protocol in Supplemental Table A.1.

UHPLC-MS/MS Instrumentation

The simultaneous quantitation of FIRO, MEL, and FTD in caprine plasma was performed on a Waters Acquity Ultra Performance Liquid Chromatography H-Class PLUS-tandem Xevo TQ-S Mass Spectrometry instrument. The chromatographic conditions and gradient elution program are described in Tables 5 and 6, respectively. The mass spectrometric source conditions and multiple reaction monitoring (MRM) detection parameters are reported in Tables 7 and 8, respectively. Quantitative data were acquired and processed using TargetLynx operating software.

Method Validation

The developed UHPLC-MS/MS method to simultaneously quantitate FIRO, MEL, and FTD in caprine plasma adhered to the FDA's *Bioanalytical Method Validation Guidance for* Industry report (FDA, U.S., 2018). The calibration curves, with logarithmic axis transformation, ranged from [1.50E+00, 5.00E-02, 5.00E-02] to [3.00E+03, 1.00E+02, 1.00E+02] in ng/mL for FIRO, MEL, FTD, respectively, and exhibited coefficients of determination (R^2) consistently greater than 0.990. Due to daily mixed QC accuracy and reproducibility, the limits of quantitation (LOQ) were 1.50, 1.00, and 1.00 ng/mL for FIRO, MEL, and FTD, respectively. The method was validated by an analysis of the daily mixed QC samples; the average percent recovery, average intra-day precision – percent relative standard deviation (% RSD) – and inter-day precision (% RSD) results are in Supplemental Table A.5.

Pharmacokinetic Analysis

Pharmacokinetic analysis for each goat was performed using computer software (PK Solver). For each goat, the plasma drug concentrations over time were analyzed using noncompartmental analysis with uniform weighting based on the statistical moment theory (Gibaldi & Perrier, 1982). The individual animal pharmacokinetics were determined for each drug (firocoxib, meloxicam or flunixin transdermal) and dose level (Low, Mid, High). The terminal rate constant (λ_z) was determined by linear regression using the last 3-5 time points of the log plasma concentration-time curve. The terminal rate constant (λ_z) was used to calculate the terminal half-life ($T_{½}$). The area under the plasma concentration drug curve (AUC) was calculated using the trapezoidal method. To determine $AUC_{0-\infty}$, the last measurable drug concentration was extrapolated to infinity. The time to maximum concentrations (T_{max}) and maximum

concentrations (C_{max}) were determined by visual inspection of the data. Descriptive statistics are reported.

Results

No adverse physiological or behavioral effects were noted in animals enrolled on trial given firocoxib, meloxicam, or transdermal flunixin at the tested dosages.

Pharmacokinetic parameters for each of the three dosages of PO firocoxib, PO meloxicam, and TD flunixin following single administration in meat-type castrated goats are summarized as mean values with corresponding ranges in Table 5.1. Mean plasma concentrations over time for firocoxib dosages are shown in Figure 5.1, for meloxicam dosages in Figure 5.2, and for flunixin transdermal dosages in Figure 5.3.

Method validation results for LC-MS analysis for each drug are summarized in Table 5.2.

Discussion

This study is the first to describe the pharmacokinetics of PO firocoxib, PO meloxicam, and TD flunixin at varying dosages in healthy, adult, castrated male crossbred goats with experimentally induced lameness. No adverse behavioral or physiological effects were observed following drug administration in any animal on study.

Goats are known to metabolize many drugs more rapidly than cattle, sheep, and other livestock (Myers et al., 2021), necessitating species-specific pharmacokinetic data to guide appropriate dosing. This study addressed this gap by evaluating multiple dosages of three commonly used NSAIDs.

The lack of any FDA approved analgesic for goats presents a significant challenge to effective pain management in this species. The only approved analgesic for any livestock species in the U.S. is TD flunixin at 3.3 mg/kg in cattle (FDA, 2017), which was chosen as the low dose in this study. Firocoxib and meloxicam, although widely available and routinely prescribed in small animals and horses, both remain unapproved for use in goats. However, these drugs can be legally used under AMDUCA when a valid veterinary client-patient relationship exits.

The pharmacokinetics of firocoxib in goats have only been minimally described in previous studies. Stuart et al. (2019) reported a T½ of 21.51 hours following 0.5 mg/kg oral administration, compared to our mean T½ of 9.1 hours at the same dosage. This shorter half-life in our study may reflect differences in sex (male vs. female), age, breed, or physiological condition (i.e., lameness model), all of which can influence drug disposition.

To our knowledge, no previous studies have reported firocoxib pharmacokinetics in goats at 1.0 or 2.0 mg/kg doses. Additionally, there are no published data on firocoxib's pharmacodynamic

parameters in goats, such as IC_{50} or IC_{80} for prostaglandin inhibition. This gap in literature limits the ability to link pharmacokinetics with expected analgesic efficacy.

Previous studies have evaluated meloxicam in goats at lower doses (typically 0.5 mg/kg), reporting T½ values ranging from 10.7 to 11.8 hours (Bublitz et al., 2019; Karademir et al., 2016; Ingvast-Larsson et al., 2011). Our results showed similar half-lives at higher doses (2.0–3.0 mg/kg), suggesting consistent elimination kinetics across a range of oral dosages. However, to date, no IC_{50} or IC_{80} values have been reported for meloxicam in goats, limiting conclusions regarding effective plasma concentrations or appropriate dosing intervals. Further pharmacodynamic studies are warranted.

Multiple studies have examined transdermal flunixin in goats at 3.3 mg/kg, with highly variable reported T½ values: Reppert et al. (2019) reported 43.12 h, Enoch B de S Jr et al. (2022) reported 21.63 h, and Graves et al. (2020) reported 7.65 h. Our mean T½ at 3.3 mg/kg (16.5 h) was most consistent with the findings of Graves et al. (2020), indicating moderate variability in absorption and elimination.

This variability across studies and within our own data may reflect differences in drug application (e.g., grooming behavior, environmental contamination, or improper placement), as well as individual variation in hair coat thickness, skin characteristics, or metabolic rate. The experimental lameness model may also have influenced drug disposition, though the extent of that effect is unknown.

Reppert et al. (2019) showed that PGE_2 concentrations decreased by 100% after IV administration of flunixin, but only by 50% after transdermal application. They also reported an IC_{80} of 0.28 μg/mL for PGE_2 inhibition, providing a pharmacodynamic benchmark for efficacy.

In our study, plasma concentrations of flunixin fell below the reported IC_{80} between the 12 and 24 hour timepoints across all dosages. This suggests that twice-daily administration may be necessary to maintain therapeutic concentrations in goats, which differs from current recommendations in cattle.

Results suggest that dose linearity was observed between low and mid-range doses for all three NSAIDs but not between mid and high doses. For example, firocoxib at 2.0 mg/kg and meloxicam at 3.0 mg/kg did not exhibit proportionally increased plasma concentrations. Possible explanations include saturation of gastrointestinal absorption mechanisms at higher doses or increased pre-systemic metabolism leading to reduced bioavailability.

Based on both the pharmacokinetic data and findings from the concurrent analgesia study (Weeder et al., 2025), transdermal flunixin at 3.3 or 5.0 mg/kg, and oral meloxicam at 2.0 mg/kg, are the most promising options for pain management in goats with lameness.

While flunixin TD showed the most prolonged plasma half-life and mean residence time, its plasma concentration fell below the reported IC_{80} within 24 hours, suggesting a shorter-than-expected duration of effect. However, its FDA approval in cattle and ease of application make it a practical and regulatory-compliant option for off-label use in goats.

Meloxicam at 2.0 mg/kg maintained consistent pharmacokinetic properties across animals and represents another viable alternative. However, due to the absence of pharmacodynamic targets in goats, clear recommendations on dosing intervals remain speculative.

This study utilized a novel, high-throughput UHPLC-MS/MS method that allows for simultaneous quantification of firocoxib, meloxicam, and flunixin in caprine plasma. This method required minimal plasma volume (100 µL), enabled rapid turnaround (~10 minutes/sample), and was validated for sensitivity, precision, and accuracy. Compared to prior

studies that used single-analyte assays, this method improves efficiency and preserves sample volume, making it well-suited for future caprine pharmacology research. This method also holds potential for expansion in additional analytes.

Limitations of this study include the absence of IV administration for bioavailability calculation, limited demographic diversity (i.e., only young, male, meat-type goats), and a focus solely on oral and transdermal administration routes.

Future studies should evaluate these drugs in female, dairy, and older goats; explore intravenous administration to calculate absolute bioavailability; and incorporate pharmacodynamic assessments such as COX inhibition or prostaglandin levels. Such research is critical to establishing evidence-based dosing regimens for effective, safe, and legal analgesic use in caprine species.

Although additional research is needed, this study provides foundational pharmacokinetic data for three widely accessible NSAIDs in goats. Our findings support the potential utility of flunixin transdermal and meloxicam oral formulations, while also identifying variability in drug disposition that must be addressed in future studies. The validated analytical method and multi-dose approach represent significant strengths of this study and provide a platform for continued advancement in caprine analgesia.

Author Contributions:

M.D.K., E.J.R., P.D., and J.F.C. contributed to study design. M.M.W., R.T., M.B., A.K.C., A.A.N. and B.R.F. performed the experiments. C.T.C, K.K., R.T., M.B., and M.M.W performed data analysis. M.M.W composed the manuscript with help from M.D.K., E.J.R., and R.T. All authors have read and approved the final document.

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The data supporting this study's findings are available from the corresponding authors upon reasonable request.

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The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to. The authors confirm that they have adhered to US standards for the protection of animals used for scientific research.

References

C, F., J, D., B, Y., K, C., J, M., & A, B. (2020). Ex vivo COX-1 and COX-2 inhibition in equine blood by phenylbutazone, flunixin meglumine, meloxicam, and firocoxib: Informing clinical NSAID selection. In. BEVA.

Corporation, W. (2024). Oasis Cartridges and 96-Well Plates Care and Use Manual. In.
de la Puente, R., Diez, R., Diez, M. J., Fernandez, N., Sahagun, A. M., Rodriguez, J. M.,

Lopez, C. (2024). Pharmacokinetics of Meloxicam in Different Animal Species: A Comprehensive Review. *Veterinary Sciences*, *11*(11). https://doi.org/10.3390/vetsci11110519

FDA. (2024). Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). In.

FDA, U. S. (2018). Bioanalytical Method Validation: Guidance for Industry. In.

Graves, M. T., Schneider, L., Cox, S., Caldwell, M., Krawczel, P., Lee, A., & Lear, A. (2020). Evaluation of the pharmacokinetics and efficacy of transdermal flunixin for pain mitigation following castration in goats. *Transl Anim Sci*, *4*(4), txaa198. https://doi.org/10.1093/tas/txaa198

Health, M. A. (2024). *Banamine Transdermal (flunixin transdermal solution)*.

Ingelheim, B. (2025). *METACAM (meloxicam) Brand Products*.

Königsson, K., Törneke, K., Engeland, I. V., Odensvik, K., & Kindahl, H. (2003). Pharmacokinetics and pharmacodynamic effects of flunixin after intravenous, intramuscular, and oral administration to dairy goats. *Acta Vet Scand*, *44*(3-4), 153-159. https://doi.org/10.1186/1751-0147-44-153

M, B. C., A, M. D., Travis, M., R, F. V., Thomas, H., & E, B. R. (2019). Comparitive plasma and urine concentrations of flunixin and meloxicam in goats. In (Vol. Volume 174, pp. 40-46). Small Ruminant Research.

Myers, M. J., Howard, K. D., & Kawalek, J. C. (2021). Pharmacokinetic comparison of six anthelmintics in sheep, goats, and cattle. *J Vet Pharmacol Ther*, *44*(1), 58-67. https://doi.org/10.1111/jvp.12897

Shukla, M., Singh, G., Sindhura, B. G., Telang, A. G., Rao, G. S., & Malik, J. K. (2007). Comparative plasma pharmacokinetics of meloxicam in sheep and goats following intravenous administration. *Comp Biochem Physiol C Toxicol Pharmacol*, *145*(4), 528-532. https://doi.org/10.1016/j.cbpc.2007.01.020

Stock, M. L., Coetzee, J. F., KuKanich, B., & Smith, B. I. (2013). Pharmacokinetics of intravenously and orally administered meloxicam in sheep. *Am J Vet Res*, *74*(5), 779-783. https://doi.org/10.2460/ajvr.74.5.779

Stock, M. L., Gehring, R., Barth, L. A., Wulf, L. W., & Coetzee, J. F. (2014). Pharmacokinetics of firocoxib in preweaned calves after oral and intravenous administration. *J Vet Pharmacol Ther*, *37*(5), 457-463. https://doi.org/10.1111/jvp.12124

US, FDA. (2017). Freedom of information summary original new animal drug application Banamine Transdermal. In. US FDA CVM.

Weeder, M. M., Kleinhenz, M. D., Reppert, E. J., Curtis, A. K., Viscardi, A. V., Leslie, A.,Coetzee, J. F. (2025). Comparison of multiple drug regimens for reduction of synovitis-induced lameness pain in meat-type goats. *Am J Vet Res*, 1-10. https://doi.org/10.2460/ajvr.25.04.0134

Young, B. K., Yu, J., & Hye, H. Y. (2022). Simultaneous Liquid Chromatography Tandem Mass Spectrometric Determination of 35 Prohibited Substances in Equine Plasma for Doping Control. In (Vol. 13, pp. 158-165): Mass Spectrometry Letters.

Figures

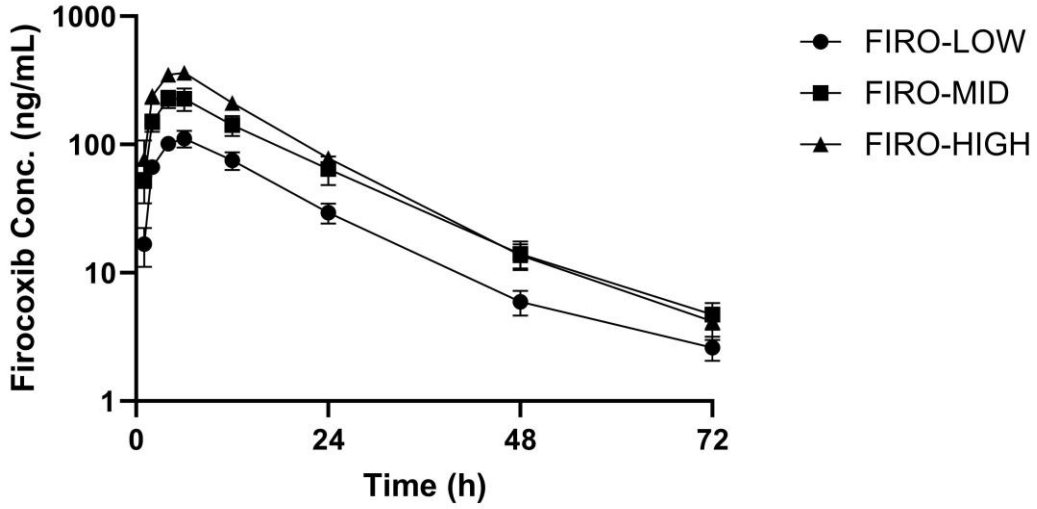

Figure 5.1. Plasma concentrations of firocoxib in goats following oral administration at three dosages. Goats were administered oral firocoxib at 0.5 mg/kg (FIRO-LOW), 1.0 mg/kg (FIRO-MID), or 2.0 mg/kg (FIRO-HIGH) after induction of amphotericin B–induced lameness. Plasma drug concentrations were measured over time to assess pharmacokinetic profiles and systemic exposure.

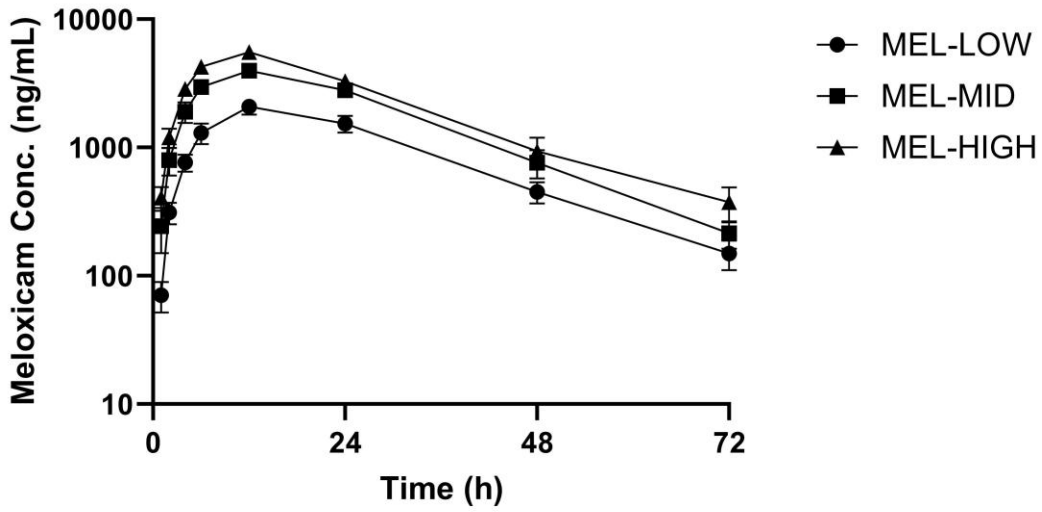

Figure 5.2. Plasma concentrations of meloxicam in goats following oral administration at three dosages. Goats were administered oral meloxicam at 1.0 mg/kg (MEL-LOW), 2.0 mg/kg (MEL-MID), or 3.0 mg/kg (MEL-HIGH) after induction of amphotericin B–induced lameness. Plasma drug concentrations were measured over time to evaluate absorption and systemic exposure.

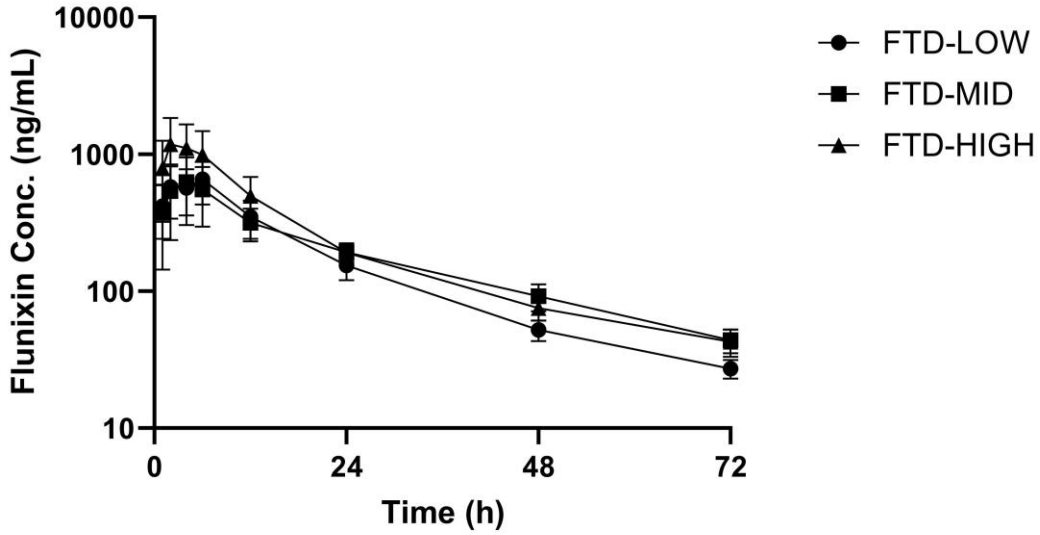

Figure 5.3. Plasma concentrations of transdermal flunixin in goats following application at three dosages. Goats received transdermal flunixin at 3.3 mg/kg (FTD-LOW), 4.2 mg/kg (FTD-MID), or 5.0 mg/kg (FTD-HIGH) after induction of amphotericin B–induced lameness. Plasma drug concentrations were measured over time to determine pharmacokinetic behavior and potential analgesic exposure.

Tables

Table 5.1. Pharmacokinetic outcome parameters of firocoxib, meloxicam, and flunixin for goats with amphotericin-B induced lameness and then analgesic treatment. Treatments modeled included oral (PO) firocoxib at 0.5mg/kg (n=8), PO firocoxib at 1.0mg/kg (n=8), PO firocoxib at 2.0mg/kg (n=8), PO meloxicam at 1.0mg/kg (n=8), PO meloxicam at 2.0mg/kg (n=8), PO meloxicam at 3.0mg/kg (n=8), transdermal (TD) flunixin at 3.3mg/kg (n=8), TD flunixin at 4.2mg/kg (n=8), and TD flunixin at 5.0mg/kg (n=8).

Parameter[1]	Unit	FIRO		
		0.5 mg/kg (n=8)	1.0 mg/kg (n=8)	2.0 mg/kg (n=8)
$T_{1/2}$	h	9.1 6.9 – 13.4	10.2 6.4 – 14.7	9.2 6.8 – 12.8
T_{max}	h	5.4 4 – 6	4.7 4 – 6	4.9 4 – 6
C_{max}	ng/mL	106.7 65.8 – 205.4	200.7 140.2 – 531.8	381.5 331.2 – 431.7
$AUC_{0-\infty}$	ng/mL*h	1,948.1 898 – 3,790	4,102.7 2,747 – 10,458	6,237.8 5,018 – 7,954
AUC Extrapolated	%	0.97 0.90 – 0.99	0.98 0.96 – 0.99	0.99 0.98 – 1.00
$MRT_{0-\infty}$	h	14.9 11.6 – 20.7	16.0 11.2 – 21.6	13.9 9.9 – 19.0

Parameter[1]	Unit	MEL		
		1.0 mg/kg (n=8)	2.0 mg/kg (n=8)	3.0 mg/kg (n=8)
$T_{1/2}$	h	13.3 10.1 – 22.3	12.2 9.8 – 15.4	11.7 8.3 – 20.5

T_{max}	h	13.3 12 – 24	13.1 12 -24	11.0 6 - 12
C_{max}	ng/mL	2040.3 1431.4 – 3709.1	3891. 7 2762.5 – 5240.9	5511.7 3707.5 – 7611.5
$AUC_{0-\infty}$	ng/mL*h	66,547 43,029 – 108,659	122,035 79,737 – 211,993	160,916 99,684 – 262,293
AUC Extrapolated	%	0.95 0.89 – 0.99	0.97 0.94 – 0.99	0.96 0.89 – 1.00
$MRT_{0-\infty}$	h	26.3 19.9 – 38.5	23.8 20.4 – 30.4	22.6 14.7 – 35.4

Parameter[1]	Unit	FTD		
		3.3 mg/kg (n=8)	4.2 mg/kg (n=8)	5.0 mg/kg (n=8)
$T_{1/2}$	h	16.5 10.7 – 62.0	22.0 16.6 – 67.4	17.8 7.4 – 56.3
T_{max}	h	6.4 2 – 24	10.5 4 – 24	5.1 2 – 24
C_{max}	ng/mL	407.6 63.9 – 2085.7	436.7 103.9 – 1654.8	600.7 108.1 – 5602.4
$AUC_{0-\infty}$	ng/mL*h	11,699.2 6,759 – 32,184	14,813.7 9,218 – 24,691	18,182.4 11,244 – 61,127
AUC Extrapolated	%	0.84 0.51 – 0.98	0.83 0.51 – 0.98	0.81 0.57 – 0.99
$MRT_{0-\infty}$	h	26.4 12.0 – 97.1	32.0 11.3 – 100.8	29.0 8.9 – 85.9

[1] *$T_{1/2}$ is elimination half-life, T_{max} is time to Cmax,* C_{max} *is maximum plasma concentration,*

$AUC_{0-\infty}$ is percent of the AUC extrapolated, AUC is extrapolated area under the curve extrapolated to infinity, $MRT_{0-\infty}$ is mean residence time extrapolated to infinity.

Harmonic means are presented for $T_{1/2}$. *All other means are geometric means.*

Table 5.2. Method validation results based on daily mixed QC sample analysis. Analytes evaluated were meloxicam (MEL), flunixin (FTD), and firocoxib (FIRO).

Analyte	Nominal concentration (ng/mL)	Average recovery (%), n = 3	Average intra-day precision (% RSD), n = 3	Inter-day precision (% RSD), n = 5
MEL	1.00E+00	9.22E+01	1.98E+00	9.00E+00
	1.00E+01	9.83E+01	1.90E+00	3.47E+00
	7.50E+01	9.87E+01	1.47E+00	2.06E+00
FTD	1.00E+00	9.50E+01	2.34E+00	6.57E+00
	1.00E+01	9.71E+01	2.44E+00	3.37E+00
	7.50E+01	1.00E+02	1.73E+00	2.45E+00
FIRO	1.50E+00	9.74E+01	3.71E+00	9.38E+00
	3.00E+01	1.01E+02	5.29E+00	8.27E+00
	3.00E+02	1.00E+02	2.03E+00	3.23E+00
	2.25E+03	9.80E+01	3.24E+00	4.72E+00

References

A, M. K. (2002). Non-steroidal anti-inflammatory analgesics: a review of current practice. In. Journal of Veterinary Emergency & Critical Care.

Adcock, S. J. J., & Tucker, C. B. (2018). The effect of disbudding age on healing and pain sensitivity in dairy calves. *J Dairy Sci, 101*(11), 10361-10373. https://doi.org/10.3168/jds.2018-14987

Allen, C., Hopewell, S., & Prentice, A. (2005). Non-steroidal anti-inflammatory drugs for pain in women with endometriosis. *Cochrane Database Syst Rev (*4), CD004753. https://doi.org/10.1002/14651858.CD004753.pub2

Allen, K. A., Coetzee, J. F., Edwards-Callaway, L. N., Glynn, H., Dockweiler, J., KuKanich, B.,Bergamasco, L. (2013). The effect of timing of oral meloxicam administration on physiological responses in calves after cautery dehorning with local anesthesia. *J Dairy Sci, 96*(8), 5194-5205. https://doi.org/10.3168/jds.2012-6251

Alvarez, L., Adcock, S. J. J., & Tucker, C. B. (2019). Sensitivity and wound healing after hot-iron disbudding in goat kids. *J Dairy Sci, 102*(11), 10152-10162. https://doi.org/10.3168/jds.2018-16062

Alvarez, L., De Luna, J. B., Gamboa, D., Reyes, M., Sánchez, A., Terrazas, A.,Galindo, F. (2015). Cortisol and pain-related behavior in disbudded goat kids with and without cornual nerve block. *Physiol Behav, 138*, 58-61. https://doi.org/10.1016/j.physbeh.2014.10.026

Alvarez, L., Nava, R. A., Ramírez, A., Ramírez, E., & Gutiérrez, J. (2009). Physiological and behavioural alterations in disbudded goat kids with and without local anaesthesia. *Applied Animal Behaviour Science, 117*(3), 190-196. https://doi.org/https://doi.org/10.1016/j.applanim.2009.01.001

Arifah, A. K., Landoni, M. F., & Lees, P. (2003). Pharmacodynamics, chiral pharmacokinetics, and PK-PD modelling of ketoprofen in the goat. *J Vet Pharmacol Ther, 26*(2), 139-150. https://doi.org/10.1046/j.1365-2885.2003.00455.x

Bach, A., Dinarés, M., Devant, M., & Carré, X. (2007). Associations between lameness and production, feeding and milking attendance of Holstein cows milked with an automatic milking system. *J Dairy Res, 74*(1), 40-46. https://doi.org/10.1017/S0022029906002184

Bain, M. (2024). Animal Welfare. In *Welfare of Livestock/Production Animals*. Merck Veterinary Manual.

Baynes, R. E., Dedonder, K., Kissell, L., Mzyk, D., Marmulak, T., Smith, G.,Riviere, J. E. (2016). Health concerns and management of select veterinary drug residues. *Food Chem Toxicol, 88*, 112-122. https://doi.org/10.1016/j.fct.2015.12.020

Beausoleil, N. J., & Mellor, D. J. (2015). Advantages and limitations of the Five Domains model for assessing welfare impacts associated with vertebrate pest control. *N Z Vet J*, *63*(1), 37-43. https://doi.org/10.1080/00480169.2014.956832

Berg, J. N., Maas, J. P., Paterson, J. A., Krause, G. F., & Davis, L. E. (1984). Efficacy of ethylenediamine dihydriodide as an agent to prevent experimentally induced bovine foot rot. *Am J Vet Res*, *45*(6), 1073-1078.

Beyene, T. (2016). Veterinary Drug Residues in Food-animal Products: Its Risk Factors and Potential Effects on Public Health. In (Vol. 7:1). Journal of Veterinary Science and Technology.

Bickell, E. G. (2023). The Animal Welfare Act: Background and Selected Issues. In.

Blackie, N., Amory, J., Bleach, E., & Scaife, J. (2011). The effect of lameness on lying behaviour of zero grazed Holstein dairy cattle. *Applied Animal Behaviour Science*, *134*(3), 85-91. https://doi.org/https://doi.org/10.1016/j.applanim.2011.08.004

Brambell, R. (1965). Report of the technical committee to enquire into the welfare of animals kept under intensive livestock husbandry systems. In. London: Her Majesty's Stationary Office.

Broom, D. M. (1991). Animal welfare: concepts and measurement. *J Anim Sci*, *69*(10), 4167-4175. https://doi.org/10.2527/1991.69104167x

Browning, H. (2020). The Natural Behavior Debate: Two Conceptions of Animal Welfare. *J Appl Anim Welf Sci*, *23*(3), 325-337. https://doi.org/10.1080/10888705.2019.1672552

Brusin, V., Ceballos, M. C., Trindade, P. H. E., Rocha Góis, K. C., Conde, G., Tessarine Barbosa, V.,Paranhos da Costa, M. J. R. (2022). Flunixin Meglumine Is Superior to Meloxicam for Providing Analgesia after Surgical Castration in 2-Month-Old Goats. *Animals (Basel)*, *12*(23). https://doi.org/10.3390/ani12233437

Burnett, T. A., Madureira, A. M. L., Silper, B. F., Tahmasbi, A., Nadalin, A., Veira, D. M., & Cerri, R. L. A. (2015). Relationship of concentrations of cortisol in hair with health, biomarkers in blood, and reproductive status in dairy cows. *Journal of Dairy Science*, *98*(7), 4414-4426. https://doi.org/https://doi.org/10.3168/jds.2014-8871

Bushnell, M. C., Ceko, M., & Low, L. A. (2013). Cognitive and emotional control of pain and its disruption in chronic pain. *Nat Rev Neurosci*, *14*(7), 502-511. https://doi.org/10.1038/nrn3516

Byrne, D. T., Berry, D. P., Esmonde, H., McGovern, F., Creighton, P., & McHugh, N. (2019). Infrared thermography as a tool to detect hoof lesions in sheep. *Transl Anim Sci*, *3*(1), 577-588. https://doi.org/10.1093/tas/txy132

C, F., J, D., B, Y., K, C., J, M., & A, B. (2020). Ex vivo COX-1 and COX-2 inhibition in equine blood by phenylbutazone, flunixin meglumine, meloxicam, and firocoxib: Informing clinical NSAID selection. In. BEVA.

Califf, R. M. (2018). Biomarker definitions and their applications. *Exp Biol Med (Maywood), 243*(3), 213-221. https://doi.org/10.1177/1535370217750088

Carr, D. B., & Goudas, L. C. (1999). Acute pain. *Lancet, 353*(9169), 2051-2058. https://doi.org/10.1016/S0140-6736(99)03313-9

Carter, B. S., & Brunkhorst, J. (2017). Neonatal pain management. *Semin Perinatol, 41*(2), 111-116. https://doi.org/10.1053/j.semperi.2016.11.001

Castro-Costa, A., Caja, G., Salama, A. A. K., Rovai, M., Flores, C., & Aguiló, J. (2014). Thermographic variation of the udder of dairy ewes in early lactation and following an Escherichia coli endotoxin intramammary challenge in late lactation. *Journal of Dairy Science, 97*(3), 1377-1387. https://doi.org/https://doi.org/10.3168/jds.2013-6978

Całkosiński, I., Dobrzyński, M., Rosińczuk, J., Dudek, K., Chrószcz, A., Fita, K., & Dymarek, R. (2015). The use of infrared thermography as a rapid, quantitative, and noninvasive method for evaluation of inflammation response in different anatomical regions of rats. *Biomed Res Int, 2015*, 972535. https://doi.org/10.1155/2015/972535

Celozzi, S., Battini, M., Prato-Previde, E., & Mattiello, S. (2022). Humans and Goats: Improving Knowledge for a Better Relationship. *Animals (Basel), 12*(6). https://doi.org/10.3390/ani12060774

Chamberlain, D. B. (1999). Babies Don't Feel Pain: A Century of Denial in Medicine. In (Vol. 1, pp. 145-168). Journal of Prenatal and Perinatal Psychology & Health 14.

Chapinal, N., de Passillé, A. M., & Rushen, J. (2009). Weight distribution and gait in dairy cattle are affected by milking and late pregnancy. *J Dairy Sci, 92*(2), 581-588. https://doi.org/10.3168/jds.2008-1533

Chou, R., & Huffman, L. H. (2007). Nonpharmacologic Therapies for Acute and Chronic Low Back Pain: A Review of the Evidence for an American Pain Society/American College of Physicians Clinical Practice Guideline. *Annals of Internal Medicine, 147*, 492-504.

Christodoulopoulos, G. (2009). Foot lameness in dairy goats. *Res Vet Sci, 86*(2), 281-284. https://doi.org/10.1016/j.rvsc.2008.07.013

Church, J. S., Hegadoren, P. R., Paetkau, M. J., Miller, C. C., Regev-Shoshani, G., Schaefer, A. L., & Schwartzkopf-Genswein, K. S. (2014). Influence of environmental factors on infrared eye temperature measurements in cattle. *Res Vet Sci, 96*(1), 220-226. https://doi.org/10.1016/j.rvsc.2013.11.006

Coetzee, J. F. (2011). A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the United States. *Applied Animal Behaviour Science, 135*, 192-213.

Coetzee, J. F. (2013). Assessment and management of pain associated with castration in cattle. *Vet Clin North Am Food Anim Pract, 29*(1), 75-101. https://doi.org/10.1016/j.cvfa.2012.11.002

Coetzee, J. F., KuKanich, B., Mosher, R., & Allen, P. S. (2009). Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. *Vet Ther, 10*(4), E1-8.

Coetzee, J. F., Lubbers, B. V., Toerber, S. E., Gehring, R., Thomson, D. U., White, B. J., & Apley, M. D. (2007). Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. In (Vol. 69:6). American Veterinary Medical Association.

Coetzee, J. F., Lubbers, B. V., Toerber, S. E., Gehring, R., Thomson, D. U., White, B. J., & Apley, M. D. (2008). Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. *Am J Vet Res*, *69*(6), 751-762. https://doi.org/10.2460/ajvr.69.6.751

Coetzee, J. F., Mosher, R. A., Anderson, D. E., Robert, B., Kohake, L. E., Gehring, R.,Wang, C. (2014a). Impact of oral meloxicam administered alone or in combination with gabapentin on experimentally induced lameness in beef calves. *J Anim Sci*, *92*(2), 816-829. https://doi.org/10.2527/jas.2013-6999

Coetzee, J. F., Mosher, R. A., Anderson, D. E., Robert, B., Kohake, L. E., Gehring, R.,Wang, C. (2014b). Impact of oral meloxicam administered alone or in combination with gabapentin on experimentally induced lameness in beef calves. *J Anim Sci*, *92*(2), 816-829. https://doi.org/10.2527/jas.2013-6999

Coetzee, J. F., Nutsch, A. L., Barbur, L. A., & Bradburn, R. M. (2010). A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. *BMC Vet Res*, *6*, 12. https://doi.org/10.1186/1746-6148-6-12

Coetzee, J. F., Shearer, J. K., Stock, M. L., Kleinhenz, M. D., & van Amstel, S. R. (2017). An Update on the Assessment and Management of Pain Associated with Lameness in Cattle. *Vet Clin North Am Food Anim Pract, 33*(2), 389-411. https://doi.org/10.1016/j.cvfa.2017.02.009

Coetzee, J. F., Sidhu, P. K., Seagen, J., Schieber, T., Kleinhenz, K., Kleinhenz, M. D.,Lechtenberg, K. (2019). Transmammary delivery of firocoxib to piglets reduces stress and improves average daily gain after castration, tail docking, and teeth clipping1. *J Anim Sci*, *97*(7), 2750-2768. https://doi.org/10.1093/jas/skz143

Cook, M. (2023). Urinary Calculi of Small Ruminats. In. Veterinary Clinics of North America: Food Animal Practice.

Cooper, C., Chapurlat, R., Al-Daghri, N., Herrero-Beaumont, G., Bruyère, O., Rannou, F.,Reginster, J. Y. (2019). Safety of Oral Non-Selective Non-Steroidal Anti-Inflammatory Drugs in Osteoarthritis: What Does the Literature Say? *Drugs Aging*, *36*(Suppl 1), 15-24. https://doi.org/10.1007/s40266-019-00660-1

Corporation, W. (2024). Oasis Cartridges and 96-Well Plates Care and Use Manual. In.

Crawford, P. E., Hamer, K., Lovatt, F., Behnke, M. C., & Robinson, P. A. (2023). Improving analgesia provision for sheep: An analysis of farm medicine records and attitudes towards pain relief on sheep farms in Northern Ireland. *Vet Rec Open*, *10*(2), e75. https://doi.org/10.1002/vro2.75

Crosby-Durrani, H. E., Clegg, S. R., Singer, E., Angell, J. W., Evans, N. J., Carter, S. D.,Duncan, J. S. (2016). Severe Foot Lesions in Dairy Goats Associated with Digital Dermatitis Treponemes. *J Comp Pathol*, *154*(4), 283-296. https://doi.org/10.1016/j.jcpa.2016.04.001

Cuthbertson, H., Tarr, G., Loudon, K., Lomax, S., White, P., McGreevy, P.,González, L. A. (2020). Using infrared thermography on farm of origin to predict meat quality and physiological response in cattle (Bos Taurus) exposed to transport and marketing. *Meat Science*, *169*, 108173. https://doi.org/https://doi.org/10.1016/j.meatsci.2020.108173

D., F. (2003). Assessing Animal Welfare at the Farm and Group Level: The Interplay of Science and Values. In (Vol. 12 (4), pp. 433-443). Animal Welfare.

Danneman, P. J. (1997). Chapter 6 - Monitoring of Analgesia. In D. F. Kohn, S. K. Wixson, W. J. White, & G. John Benson (Eds.), *Anesthesia and Analgesia in Laboratory Animals* (pp. 83-103). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012417570-9/50009-X

Dawkins, M. S. (2008). The Science of Animal Suffering. *Ethology*, *114*(10), 937-945. https://doi.org/https://doi.org/10.1111/j.1439-0310.2008.01557.x

De Heus, P., Van Oossanen, G., Van Dierendonck, M. C., & Back, W. (2010). A Pressure Algometer Is a Useful Tool to Objectively Monitor the Effect of Diagnostic Palpation by a Physiotherapist in Warmblood Horses. *Journal of Equine Veterinary Science*, *30*(6), 310-321. https://doi.org/https://doi.org/10.1016/j.jevs.2010.04.010

de la Puente, R., Diez, R., Diez, M. J., Fernandez, N., Sahagun, A. M., Rodriguez, J. M.,Lopez, C. (2024). Pharmacokinetics of Meloxicam in Different Animal Species: A Comprehensive Review. *Veterinary Sciences*, *11*(11). https://doi.org/10.3390/vetsci11110519

Deeming, L. E., Beausoleil, N. J., Stafford, K. J., Webster, J. R., & Zobel, G. (2018). Technical note: The development of a reliable 5-point gait scoring system for use in dairy goats. *J Dairy Sci*, *101*(5), 4491-4497. https://doi.org/10.3168/jds.2017-13950

DeVane, C. L. (2001). Substance P: A New Era, a New Role. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *21*(9), 1061-1069. https://doi.org/https://doi.org/10.1592/phco.21.13.1061.34612

DeVon, H. A., Piano, M. R., Rosenfeld, A. G., & Hoppensteadt, D. A. (2014). The association of pain with protein inflammatory biomarkers: a review of the literature. *Nurs Res*, *63*(1), 51-62. https://doi.org/10.1097/NNR.0000000000000013

Distel, M., Mueller, C., & Bluhmki, E. (1995). Side Effects of Anti-Inflammatory Drugs IV: Global Analysis of Gastrointestinal Safety of a New NSAID, Meloxicam. In (pp. 183-193).

Distel, M., Mueller, C., Bluhmki, E., & Fries, J. (1996). Safety of meloxicam: a global analysis of clinical trials. *Br J Rheumatol*, *35 Suppl 1*, 68-77. https://doi.org/10.1093/rheumatology/35.suppl_1.68

DM, M. (2013). Obstructive urolithiasis in ruminants-A review. In G. MA (Ed.), (pp. 233-238). Vet World.

Drag, M., Kunkle, B. N., Romano, D., & Hanson, P. D. (2007). Firocoxib efficacy preventing urate-induced synovitis, pain, and inflammation in dogs. *Vet Ther*, *8*(1), 41-50.

Drew, M. L. (1998). Update on the Animal Medicinal Use Clarification Act of 1994 Regulations for Wildlife Veterinarians. In (pp. 163-167). 1998 Proceedings AAZV and AAWV Joint Conference.

Dubeuf, J.-P., Genis, J. C., Morand-Fehr, P., & Ruiz Morales, F. d. A. (2023). The contribution of goats in the future of redesigning of livestock activities and value chains. In (Vol. 227). Small Ruminant Research: Science Direcy.

E, D. C., F, D., D, L., P, S., S, B., E, C., M, M. (2023). Welfare assessment of horses: the AWIN approach. In: Cambridge University Press.

E, M. J., W, P. S., & B, C. D. (2008). *Small Animal Clinical Pharmacology, Second Edition*. Saunders Ltd. https://doi.org/https://doi.org/10.1016/B978-0-7020-2858-8.X5001-5

E.M., W., G., G., & A.M., N. (1993). Comparison of a visual analogue scale and a numerical rating scale for assessment of lameness, using sheep as a model. In.

Eriksson, H. K., Daros, R. R., von Keyserlingk, M. A. G., & Weary, D. M. (2020). Effects of case definition and assessment frequency on lameness incidence estimates. *Journal of Dairy Science*, *103*(1), 638-648. https://doi.org/https://doi.org/10.3168/jds.2019-16426

Evelyn, M. (2024). Overview of Lameness in Goats. In. Merck Veterinary Manual.

FAO. (2006). World agriculture: towards 2030/2050. In. Global Perspective Studies Unit.

Farghal, M., Pajor, E., Luna, S. P. L., Pang, D., Windeyer, M. C., & Ceballos, M. C. (2024). Development of the calf grimace scale for pain and stress assessment in castrated Angus beef calves. *Sci Rep*, *14*(1), 25620. https://doi.org/10.1038/s41598-024-77147-6

FAWC, F. A. W. C. (2009). Animal Welfare in Great Britain: Past, Present, and Future. In. FAWC. 17 Smith Square, London.

FDA. (1994). Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). In: In: FDA (ed.) No.21 CFR 530.

FDA. (2015). The FDA's Guidance for Industry #207: Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food Producing Animals: Marker Residue Depletion Studies to Establish Product Withdrawal Periods. In: FDA.

FDA. (2024). Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). In.

FDA, U. (2018). Bioanalytical Method Validation: Guidance for Industry. In.

Fitzpatrick, F. A. (2004). Cyclooxygenase enzymes: regulation and function. *Curr Pharm Des*, *10*(6), 577-588. https://doi.org/10.2174/1381612043453144

Flower, F. C., & Weary, D. M. (2006). Effect of hoof pathologies on subjective assessments of dairy cow gait. *J Dairy Sci*, *89*(1), 139-146. https://doi.org/10.3168/jds.S0022-0302(06)72077-X

Foreman, J. H., & Ruemmler, R. (2011). Phenylbutazone and flunixin meglumine used singly or in combination in experimental lameness in horses. *Equine Vet J Suppl (*40), 12-17. https://doi.org/10.1111/j.2042-3306.2011.00485.x

Fortier, L. A., Gregg, A. J., Erb, H. N., & Fubini, S. L. (2004). Caprine obstructive urolithiasis: requirement for 2nd surgical intervention and mortality after percutaneous tube cystostomy, surgical tube cystostomy, or urinary bladder marsupialization. *Vet Surg*, *33*(6), 661-667. https://doi.org/10.1111/j.1532-950X.2004.04089.x

Fraccaro, E., Coetzee, J. F., Odore, R., Edwards-Callaway, L. N., Kukanich, B., Badino, P.,Bergamasco, L. (2013). A study to compare circulating flunixin, meloxicam and gabapentin concentrations with prostaglandin E_2 levels in calves undergoing dehorning. *Res Vet Sci*, *95*(1), 204-211. https://doi.org/10.1016/j.rvsc.2013.01.018

Fraser, D., Weary, D. M., Pajor, E. A., & Milligan, B. N. (1997). A Scientific Conception of Animal Welfare that Reflects Ethical Concerns. In (Vol. 6, pp. 187-205). Animal welfare.

Galindo, F., & Broom, D. M. (2002). Effects of lameness of dairy cows. *J Appl Anim Welf Sci*, *5*(3), 193-201. https://doi.org/10.1207/S15327604JAWS0503_03

Gallelli, L., Colosimo, M., Pirritano, D., Ferraro, M., De Fazio, S., Marigliano, N. M., & De Sarro, G. (2007). Retrospective evaluation of adverse drug reactions induced by

nonsteroidal anti-inflammatory drugs. *Clin Drug Investig*, *27*(2), 115-122. https://doi.org/10.2165/00044011-200727020-00004

Gao, H.-M., & Hong, J.-S. (2008). Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. *Trends in immunology*, *29 8*, 357-365.

Geyer, R. E. (1997). Extralabel Drug Use and Compounding in Veterinary Medicine. *52*(3), 291-295.

Glew, A., Aviad, A. D., Keister, D. M., & Meo, N. J. (1996). Use of ketoprofen as an antipyretic in cats. *Can Vet J*, *37*(4), 222-225.

Glynn, H. D., Coetzee, J. F., Edwards-Callaway, L. N., Dockweiler, J. C., Allen, K. A., Lubbers, B.,KuKanich, B. (2013). The pharmacokinetics and effects of meloxicam, gabapentin, and flunixin in postweaning dairy calves following dehorning with local anesthesia. *J Vet Pharmacol Ther*, *36*(6), 550-561. https://doi.org/10.1111/jvp.12042

Gobbi, F. P., Di Filippo, P. A., Mello, L. M., Lemos, G. B., Martins, C. B., Albernaz, A. P., & Quirino, C. R. (2020). Effects of Flunixin Meglumine, Firocoxib, and Meloxicam in Equines After Castration. *J Equine Vet Sci*, *94*, 103229. https://doi.org/10.1016/j.jevs.2020.103229

Gofur, M. R. Effect of testosterone on penile and urethral development in Black Bengal goat. In (Vol. 17, Issue 2, pp. 133-139). Bangl. J. Vet Med.

Graves, M. T., Schneider, L., Cox, S., Caldwell, M., Krawczel, P., Lee, A., & Lear, A. (2020). Evaluation of the pharmacokinetics and efficacy of transdermal flunixin for pain mitigation following castration in goats. *Transl Anim Sci*, *4*(4), txaa198. https://doi.org/10.1093/tas/txaa198

Guesgen, M. J., Beausoleil, N. J., Leach, M., Minot, E. O., Stewart, M., & Stafford, K. J. (2016). Coding and quantification of a facial expression for pain in lambs. *Behav Processes*, *132*, 49-56. https://doi.org/10.1016/j.beproc.2016.09.010

Haahr, M., & Svend, H. (1999-2025). Random Number Generator. In.

Haefeli, M., & Elfering, A. (2006). Pain assessment. *Eur Spine J*, *15 Suppl 1*(Suppl 1), S17-24. https://doi.org/10.1007/s00586-005-1044-x

Hansen, B., & Hardie, E. (1993). Prescription and use of analgesics in dogs and cats in a veterinary teaching hospital: 258 cases (1983-1989). *J Am Vet Med Assoc*, *202*(9), 1485-1494.

Harrison, R. (1964). Animal Machines. In: Vincent Stuart Ltd.

Hart, S., Merkel, R., & Gipson, T. (2019). Current Situation and Future Prospects of the US Goat Industry. In. AgEcon.

Haussler, K. K. (2020). Pressure Algometry for the Detection of Mechanical Nociceptive Thresholds in Horses. *Animals (Basel), 10*(12). https://doi.org/10.3390/ani10122195

Haussler, K. K., Behre, T. H., & Hill, A. E. (2008). Mechanical nociceptive thresholds within the pastern region of Tennessee Walking Horses. *Equine Vet J, 40*(5), 455-459. https://doi.org/10.2746/042516408X278166

Health, M. A. (2024). *Banamine Transdermal (flunixin transdermal solution)*.

Hempstead, M. N., Shearer, J. K., Sutherland, M. A., Fowler, J. L., Smith, J. S., Smith, J. D.,Plummer, P. J. (2020). Cautery Disbudding Iron Application Time and Brain Injury in Goat Kids: A Pilot Study. *Front Vet Sci*, *7*, 568750. https://doi.org/10.3389/fvets.2020.568750

Hempstead, M. N., Waas, J. R., Stewart, M., Cave, V. M., & Sutherland, M. A. (2018a). Evaluation of alternatives to cautery disbudding of dairy goat kids using physiological measures of immediate and longer-term pain. *J Dairy Sci*, *101*(6), 5374-5387. https://doi.org/10.3168/jds.2017-13814

Hempstead, M. N., Waas, J. R., Stewart, M., Dowling, S. K., Cave, V. M., Lowe, G. L., & Sutherland, M. A. (2018b). Effect of isoflurane alone or in combination with meloxicam on the behavior and physiology of goat kids following cautery disbudding. *J Dairy Sci*, *101*(4), 3193-3204. https://doi.org/10.3168/jds.2017-13507

Hemsworth, P. H. (2007). Ethical stockmanship. *Aust Vet J, 85*(5), 194-200. https://doi.org/10.1111/j.1751-0813.2007.00112.x

Herzberg, D. E., & Bustamante, H. A. (2021). Animals models of chronic pain. Are naturally occuring diseases a potential model for translational research? In. Austral journal of veterinary sciences.

Hewson, C. J., Dohoo, I. R., Lemke, K. A., & Barkema, H. W. (2007). Factors affecting Canadian veterinarians' use of analgesics when dehorning beef and dairy calves. *Can Vet J, 48*(11), 1129-1136.

Hill, N. P., Murphy, P. E., Nelson, A. J., Mouttotou, N., Green, L. E., & Morgan, K. L. (1997). Lameness and foot lesions in adult British dairy goats. *Vet Rec*, *141*(16), 412-416. https://doi.org/10.1136/vr.141.16.412

Hood, D. E., & Tarrant, P. J. V. (1981). The Problem of Dark-Cutting in Beef. Current Topics in Veterinary Medicine and Animal Science,

Hopster, H., van der Werf, J. T., & Blokhuis, H. J. (1998). Stress enhanced reduction in peripheral blood lymphocyte numbers in dairy cows during endotoxin-induced mastitis. *Vet Immunol Immunopathol*, *66*(1), 83-97. https://doi.org/10.1016/s0165-2427(98)00189-5

Huber, J., Arnholdt, T., Möstl, E., Gelfert, C. C., & Drillich, M. (2013). Pain management with flunixin meglumine at dehorning of calves. *J Dairy Sci*, *96*(1), 132-140. https://doi.org/10.3168/jds.2012-5483

Hudson, J. T., Slater, M. R., Taylor, L., Scott, H. M., & Kerwin, S. C. (2004). Assessing repeatability and validity of a visual analogue scale questionnaire for use in assessing pain and lameness in dogs. *Am J Vet Res*, *65*(12), 1634-1643. https://doi.org/10.2460/ajvr.2004.65.1634

Hulka, B. S., Wilcosky, T. C., & Griffith, J. D. (1990). Biological markers in epidemiology. In.

Huxley, J. N. (2006). Current attitudes of cattle practitioners to pain and the use of analgesics in cattle. In. VetRecord.

Häger, C., Biernot, S., Buettner, M., Glage, S., Keubler, L. M., Held, N.,Bleich, A. (2017). The Sheep Grimace Scale as an indicator of post-operative distress and pain in laboratory sheep. *PLoS One*, *12*(4), e0175839. https://doi.org/10.1371/journal.pone.0175839

Idris, M., Uddin, J., Sullivan, M., McNeill, D. M., & Phillips, C. J. C. (2021). Non-Invasive Physiological Indicators of Heat Stress in Cattle. *Animals*, *11*(1). https://doi.org/10.3390/ani11010071

Ingelheim, B. (2025a). Equioxx (firocoxib). In.

Ingelheim, B. (2025b). *METACAM (meloxicam) Brand Products*.

Ingelheim, B. (2025c). *PREVICOX (firocoxib) for Oesteoarthrits in Dogs*.

Ingvast-Larsson, C., Högberg, M., Mengistu, U., Olsén, L., Bondesson, U., & Olsson, K. (2011). Pharmacokinetics of meloxicam in adult goats and its analgesic effect in disbudded kids. *J Vet Pharmacol Ther*, *34*(1), 64-69. https://doi.org/10.1111/j.1365-2885.2010.01195.x

Ishihara, A., Reed, S. M., Rajala-Schultz, P. J., Robertson, J. T., & Bertone, A. L. (2009). Use of kinetic gait analysis for detection, quantification, and differentiation of hind limb lameness and spinal ataxia in horses. *J Am Vet Med Assoc*, *234*(5), 644-651. https://doi.org/10.2460/javma.234.5.644

Ito, K., von Keyserlingk, M. A., Leblanc, S. J., & Weary, D. M. (2010). Lying behavior as an indicator of lameness in dairy cows. *J Dairy Sci*, *93*(8), 3553-3560. https://doi.org/10.3168/jds.2009-2951

J, D. F., S, J. B., & J, M. A. (2017). *Pharmacology and Therapeutics for Dentistry*. Mosby.

Johnston, C. H., Whittaker, A. L., Franklin, S. H., & Hutchinson, M. R. (2022). The Neuroimmune Interface and Chronic Pain Through the Lens of Production Animals. *Front Neurosci*, *16*, 887042. https://doi.org/10.3389/fnins.2022.887042

Joy, A., Taheri, S., Dunshea, F. R., Leury, B. J., DiGiacomo, K., Osei-Amponsah, R.,Chauhan, S. S. (2022). Non-invasive measure of heat stress in sheep using machine learning techniques and infrared thermography. *Small Ruminant Research, 207*, 106592. https://doi.org/https://doi.org/10.1016/j.smallrumres.2021.106592

Kaler, J., Mitsch, J., Vázquez-Diosdado, J. A., Bollard, N., Dottorini, T., & Ellis, K. A. (2020). Automated detection of lameness in sheep using machine learning approaches: novel insights into behavioural differences among lame and non-lame sheep. *R Soc Open Sci*, *7*(1), 190824. https://doi.org/10.1098/rsos.190824

Karademir, U., Akin, I., Erdogan, H., Ural, K., & Asici, G. S. (2016). Effect of Ketoprofen on acute phase protein concentrations in goats undergoing castration. *BMC Vet Res*, *12*(1), 123. https://doi.org/10.1186/s12917-016-0748-y

Karademir, U., Erdogan, H., Boyacioglu, M., Kum, C., Sekkin, S., & Bilgen, M. (2016). Pharmacokinetics of meloxicam in adult goats: a comparative study of subcutaneous, oral, and intravenous administration. *N Z Vet J, 64*(3), 165-168. https://doi.org/10.1080/00480169.2015.1124811

Kasimanickam, R., Kasimanickam, V., Gold, J., Moore, D., Kastelic, J. P., Pyrdek, D., & Ratzburg, K. (2019). Injectable or transdermal flunixin meglumine improves pregnancy rates in embryo transfer recipient beef cows without altering returns to estrus. *Theriogenology, 140*, 8-17. https://doi.org/10.1016/j.theriogenology.2019.08.011

Kirker-Head, C. A., Chandna, V. K., Agarwal, R. K., Morris, E. A., Tidwell, A., O'Callaghan, M. W.,Kumar, M. S. (2000). Concentrations of substance P and prostaglandin E2 in synovial fluid of normal and abnormal joints of horses. *Am J Vet Res*, *61*(6), 714-718. https://doi.org/10.2460/ajvr.2000.61.714

Kleinhenz, M. D., Gorden, P. J., Burchard, M., Ydstie, J. A., & Coetzee, J. F. (2019). Rapid Communication: Use of pressure mat gait analysis in measuring pain following normal parturition in dairy cows. *J Anim Sci*, *97*(2), 846-850. https://doi.org/10.1093/jas/sky450

Kleinhenz, M. D., Gorden, P. J., Smith, J. S., Schleining, J. A., Kleinhenz, K. E., Juarez, J. R.,Coetzee, J. F. (2019). Effects of transdermal flunixin meglumine on experimentally induced lameness in adult dairy cattle. *J Dairy Sci*, *102*(7), 6418-6430. https://doi.org/10.3168/jds.2018-15091

Kleinhenz, M. D., Van Engen, N. K., Gorden, P. J., Ji, J., Walsh, P., & Coetzee, J. F. (2017). Effects of transdermal flunixin meglumine on pain biomarkers at dehorning in calves. *J Anim Sci*, *95*(5), 1993-2000. https://doi.org/10.2527/jas.2016.1138

Kleinhenz, M. D., Van Engen, N. K., Gorden, P. J., Kleinhenz, K. E., Kukanich, B., Rajewski, S. M.,Coetzee, J. F. (2018). The impact of pain on the pharmacokinetics of transdermal flunixin meglumine administered at the time of cautery dehorning in Holstein calves. *Vet Anaesth Analg*, *45*(6), 849-857. https://doi.org/10.1016/j.vaa.2018.06.008

Kleinhenz, M. D., Van Engen, N. K., Gorden, P. J., KuKanich, B., Rajewski, S. M., Walsh, P., & Coetzee, J. F. (2016). The pharmacokinetics of transdermal flunixin meglumine in Holstein calves. *J Vet Pharmacol Ther*, *39*(6), 612-615. https://doi.org/10.1111/jvp.12314

Kleinhenz, M. D., Viscardi, A. V., & Coetzee, J. F. (2021). Invited Review: On-farm pain management of food production animals. *Applied Animal Science*, *37*(1), 77-87. https://doi.org/https://doi.org/10.15232/aas.2020-02106

Knych, H. K., Arthur, R. M., Gretler, S. R., McKemie, D. S., Goldin, S., & Kass, P. H. (2021). Pharmacokinetics of transdermal flunixin meglumine and effects on biomarkers of inflammation in horses. *J Vet Pharmacol Ther*, *44*(5), 745-753. https://doi.org/10.1111/jvp.12993

Kongara, K., Purchas, G., Dukkipati, V., Venkatachalam, D., Ward, N., Hunt, H., & Speed, D. (2023). Pharmacokinetics and effect on renal function and average daily gain in lambs after castration and tail docking, of firocoxib and meloxicam. *N Z Vet J*, *71*(6), 306-314. https://doi.org/10.1080/00480169.2023.2232337

Kongara, K., Singh, P., Venkatachalam, D., & Chambers, J. P. (2023). Pain Assessment in Goat Kids: Focus on Disbudding. *Animals (Basel)*, *13*(24). https://doi.org/10.3390/ani13243814

Korelidou, V., Grbovic, Z., Pavlovic, D., Simovic, I., Panic, M., Temenos, A., & Gelasakis, A. I. (2025). Clinical Assessment of Dairy Goats' Udder Health Using Infrared Thermography. *Animals (Basel)*, *15*(5). https://doi.org/10.3390/ani15050658

Kotschwar, J. L., Coetzee, J. F., Anderson, D. E., Gehring, R., KuKanich, B., & Apley, M. D. (2009). Analgesic efficacy of sodium salicylate in an amphotericin B-induced bovine synovitis-arthritis model. *J Dairy Sci*, *92*(8), 3731-3743. https://doi.org/10.3168/jds.2009-2058

Kvaternick, V., Pollmeier, M., Fischer, J., & Hanson, P. D. (2007). Pharmacokinetics and metabolism of orally administered firocoxib, a novel second generation coxib, in horses. *J Vet Pharmacol Ther*, *30*(3), 208-217. https://doi.org/10.1111/j.1365-2885.2007.00840.x

Kyrou, I., Chrousos, G. P., & Tsigos, C. (2006). Stress, visceral obesity, and metabolic complications. *Ann N Y Acad Sci*, *1083*, 77-110. https://doi.org/10.1196/annals.1367.008

Königsson, K., Törneke, K., Engeland, I. V., Odensvik, K., & Kindahl, H. (2003). Pharmacokinetics and pharmacodynamic effects of flunixin after intravenous, intramuscular, and oral administration to dairy goats. *Acta Vet Scand*, *44*(3-4), 153-159. https://doi.org/10.1186/1751-0147-44-153

Langford, D. J., Bailey, A. L., Chanda, M. L., Clarke, S. E., Drummond, T. E., Echols, S.,Mogil, J. S. (2010). Coding of facial expressions of pain in the laboratory mouse. *Nat Methods*, *7*(6), 447-449. https://doi.org/10.1038/nmeth.1455

Leach, M. C., Klaus, K., Miller, A. L., Scotto di Perrotolo, M., Sotocinal, S. G., & Flecknell, P. A. (2012). The assessment of post-vasectomy pain in mice using behaviour and the Mouse Grimace Scale. *PLoS One*, *7*(4), e35656. https://doi.org/10.1371/journal.pone.0035656

Lees, P., & Taylor, P. M. (1991). Pharmacodynamics and pharmacokinetics of flunixin in the cat. *Br Vet J*, *147*(4), 298-305. https://doi.org/10.1016/0007-1935(91)90002-5

Lees, P., & Toutain, P. L. (2012). The role of pharmacokinetics in veterinary drug residues. *Drug Test Anal*, *4 Suppl 1*, 34-39. https://doi.org/10.1002/dta.1374

Livingston, A. (2000). Mechanism of Action of Nonsteriodal Anti-Inflammatory Drugs. In (Vol. 30). The Veterinary Clinics of North America.

Livingston, A. (2010). Pain and Analgesia in Domestic Animals. In F. Cunningham, J. Elliott, & P. Lees (Eds.), *Comparative and Veterinary Pharmacology* (pp. 159-189). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-10324-7_7

Lizarraga, I., & Chambers, J. P. (2012). Use of analgesic drugs for pain management in sheep. *N Z Vet J*, *60*(2), 87-94. https://doi.org/10.1080/00480169.2011.642772

Loeser, J. D., & Melzack, R. (1999). Pain: an overview. *The Lancet*, *353*, 1607-1609.

Lopez-Soriano, M., Merenda, V. R., Anderson, S., Trindade, P. H. E., Leidig, M. S., Messenger, K.,Pairis-Garcia, M. D. (2023). Efficacy of inguinal buffered lidocaine and intranasal flunixin meglumine on mitigating physiological and behavioral responses to pain in castrated piglets. *Front Pain Res (Lausanne)*, *4*, 1156873. https://doi.org/10.3389/fpain.2023.1156873

Lopez-Soriano, M., Rocha Merenda, V., Esteves Trindade, P. H., Loureiro Luna, S. P., & Pairis-Garcia, M. D. (2022). Efficacy of transdermal flunixin in mitigating castration pain in piglets. *Front Pain Res (Lausanne)*, *3*, 1056492. https://doi.org/10.3389/fpain.2022.1056492

Love, E. J., Murrell, J., & Whay, H. R. (2011). Thermal and mechanical nociceptive threshold testing in horses: a review. *Veterinary Anaesthesia and Analgesia*, *38*(1), 3-14. https://doi.org/10.1111/j.1467-2995.2010.00580.x

Low, P., Jaak, P., Diana, R., David, E., Van, S. B., & Christof, K. (2012). The Cambridge Declaration on Consciousness. In. Francis Crick Memorial Conference on Consciousness in Human and non-Human Animals: University of Cambridge.

M, B. C., A, M. D., Travis, M., R, F. V., Thomas, H., & E, B. R. (2019). Comparitive plasma and urine concentrations of flunixin and meloxicam in goats. In (Vol. Volume 174, pp. 40-46). Small Ruminant Research.

M.D., K. (2018). The impact of transdermal flunixin meglumine on biomarkers of pain in calves when administered at the time of surgical castration without local anesthesia. In V. E. N. K. S. J. S. G. P. J. J. J. W. C. P. S.C.B (Ed.), (Vol. 212, pp. 1-6). Livestock Science.

Macmillan, K., Colazo, M. G., & Cook, N. J. (2019). Evaluation of infrared thermography compared to rectal temperature to identify illness in early postpartum dairy cows. *Research in Veterinary Science, 125*, 315-322. https://doi.org/https://doi.org/10.1016/j.rvsc.2019.07.017

Maldague, X. P. V. (2001). Theory and Practice of Infrared Technology for Nondestructive Testing.

Marchi, A., Vellucci, R., Mameli, S., Rita Piredda, A., & Finco, G. (2009). Pain biomarkers. *Clin Drug Investig, 29 Suppl 1*, 41-46. https://doi.org/10.2165/0044011-200929001-00006

Marshall, T. S. (2009). Abomasal ulceration and tympany of calves. *Vet Clin North Am Food Anim Pract, 25*(1), 209-220, viii. https://doi.org/10.1016/j.cvfa.2008.10.010

Martin, M., Kleinhenz, M., Kleinhenz, K., Reppert, E., Blasi, D., Parks, T.,Coetzee, J. (2020). Comparison of the effect of tildipirosin administered alone or in combination with transdermal flunixin on the performance, health, activity, and well-being of transported feedlot calves on arrival at the feedlot. *Transl Anim Sci*, *4*(1), 452-459. https://doi.org/10.1093/tas/txaa005

Martin, M. S., Kleinhenz, M. D., Edwards-Callaway, L. N., Engle, T. E., Guimaraes, O., Schafer, D. W.,Coetzee, J. F. (2022a). The effect of breed, sex, and oral meloxicam administration on pain biomarkers following hot-iron branding in Hereford and Angus calves. *J Anim Sci*, *100*(3). https://doi.org/10.1093/jas/skac038

Martin, M. S., Kleinhenz, M. D., Viscardi, A. V., Curtis, A. K., Johnson, B. T., Montgomery, S. R.,Coetzee, J. F. (2022b). Effect of bupivacaine liposome suspension administered as a cornual nerve block on indicators of pain and distress during and after cautery dehorning in dairy calves. *Journal of Dairy Science*, *105*(2), 1603-1617. https://doi.org/https://doi.org/10.3168/jds.2021-21004

Martin, M. S., Kleinhenz, M. D., Viscardi, A. V., Curtis, A. K., Johnson, B. T., Montgomery, S. R.,Coetzee, J. F. (2022c). Effect of bupivacaine liposome suspension administered as a local anesthetic block on indicators of pain and distress during and after surgical castration in dairy calves. *J Anim Sci, 100*(1). https://doi.org/10.1093/jas/skab378

Martin, M. S., Kleinhenz, M. D., Viscardi, A. V., Montgomery, S. R., Cull, C. A., Seagren, J. E.,Coetzee, J. F. (2022d). Comparison of lidocaine alone or in combination with a local nerve block of ethanol, bupivacaine liposome suspension, or oral meloxicam to extend analgesia after scoop dehorning in Holstein calves. *JDS Commun, 3*(3), 189-194. https://doi.org/10.3168/jdsc.2021-0178

Martin, M. S., Kleinhenz, M. D., White, B. J., Johnson, B. T., Montgomery, S. R., Curtis, A. K.,Coetzee, J. F. (2022e). Assessment of pain associated with bovine respiratory disease

and its mitigation with flunixin meglumine in cattle with induced bacterial pneumonia. *J Anim Sci*, *100*(2). https://doi.org/10.1093/jas/skab373

May, A. (2008). Chronic pain may change the structure of the brain. *Pain*, *137*(1), 7-15. https://doi.org/10.1016/j.pain.2008.02.034

McCarberg, B. (2023). Acute Pain in Perspective. *J Fam Pract*, *72*(6 Suppl), S7-S12. https://doi.org/10.12788/jfp.0617

McElveen, J. V. (1951). Changes in Production During World War II by Size of Farm. In. AgEcon Search.

McIlwraith, C. W., Fessler, J. F., Blevins, W. E., Page, E. H., Rebar, A. H., Van Sickle, D. C., & Coppoc, G. L. (1979). Experimentally induced arthritis of the equine carpus: clinical determinations. *Am J Vet Res*, *40*(1), 11-20.

McLean, A. K., Falt, T., Abdelfattah, E. M., Middlebrooks, B., Gretler, S., Spier, S.,Knych, H. K. (2023). Transdermal Flunixin Meglumine as a Pain Relief in Donkeys: A Pharmacokinetics Pilot Study. *Metabolites*, *13*(7). https://doi.org/10.3390/metabo13070776

McLennan, K. M., Rebelo, C. J. B., Corke, M. J., Holmes, M. A., Leach, M. C., & Constantino-Casas, F. (2016). Development of a facial expression scale using footrot and mastitis as models of pain in sheep. *Applied Animal Behaviour Science*, *176*, 19-26. https://doi.org/https://doi.org/10.1016/j.applanim.2016.01.007

Meira, E. B. d. S., Wiloch, E. E., Nixon, E., Yeatts, J. L., Sheela, F. F., Smith, G. W., & Baynes, R. E. (2022). The pharmacokinetics of transdermal flunixin in lactating dairy goats. *Journal of Dairy Science*, *105*(1), 549-559. https://doi.org/https://doi.org/10.3168/jds.2021-20460

Mellor, D. J. (2016). Updating Animal Welfare Thinking: Moving beyond the "Five Freedoms" towards "A Life Worth Living". *Animals (Basel)*, *6*(3). https://doi.org/10.3390/ani6030021

Mellor, D. J., & Beausoleil, N. J. (2017). Equine Welfare during Exercise: An Evaluation of Breathing, Breathlessness and Bridles. *Animals (Basel)*, *7*(6). https://doi.org/10.3390/ani7060041

Mellor, D. J., E., P.-K., & K.J., S. (2009). The Sciences of Animal Welfare. In: Oxford UK.

Mellor, D. J., & Littin, K. E. (2004). - Using science to support ethical decisions promoting humane livestock slaughter and vertebrate pest control. - *13*, - S132.

Mendl, M., Burman, O. H. P., Parker, R. M. A., & Paul, E. S. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Applied Animal Behaviour Science*, *118*(3), 161-181. https://doi.org/https://doi.org/10.1016/j.applanim.2009.02.023

Merskey, H. (1994). Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. Prepared by the International Association for the Study of Pain, Subcommittee on Taxonomy. *Pain. Supplement, 3,* S1-226.

Molaei, M. M., Mostafavi, A., Kheirandish, R., Azari, O., & Shaddel, M. (2015). Study of disbudding goat kids following injection of clove oil essence in horn bud region. *Vet Res Forum, 6*(1), 17-22.

Muley, M. M., Krustev, E., & McDougall, J. J. (2016). Preclinical Assessment of Inflammatory Pain. *CNS Neurosci Ther, 22*(2), 88-101. https://doi.org/10.1111/cns.12486

Musser J.M, B., K.L, A., & K.L., T. (1998). Pharmacokinetic parameters and milk concentrations of ketoprofen after administration as a single intravenous bolus dose to lactating goats. In (Vol. 21, pp. 358-363). J. vet. Pharmacol. Therap.

Myers, M. J., Howard, K. D., & Kawalek, J. C. (2021). Pharmacokinetic comparison of six anthelmintics in sheep, goats, and cattle. *J Vet Pharmacol Ther, 44*(1), 58-67. https://doi.org/10.1111/jvp.12897

N., C. (2001). Information Point: Visual Analogue Scale (VAS). In (Vol. 10). Journal of Clinical Nursing: Blackwell Science Ltd.

N., G., S., G., A., A., S., A., & R., M. (2016). A comparison of visual analog scale and Wong Baker facial pain scale for pain measurement in post lower caesarean section case. In. International Journal of Reproduction, Contraception, Obstetrics, and Gynecology.

Nahm, F. S. (2013). Infrared thermography in pain medicine. *Korean J Pain, 26*(3), 219-222.

Nalon, E., & Stevenson, P. (2019). Addressing Lameness in Farmed Animals: An Urgent Need to Achieve Compliance with EU Animal Welfare Law. *Animals (Basel), 9*(8). https://doi.org/10.3390/ani9080576

Oehm, A. W., Knubben-Schweizer, G., Rieger, A., Stoll, A., & Hartnack, S. (2019). A systematic review and meta-analyses of risk factors associated with lameness in dairy cows. *BMC Vet Res, 15*(1), 346. https://doi.org/10.1186/s12917-019-2095-2

Ohl, F., & van der Staay, F. J. (2012). Animal welfare: at the interface between science and society. *Vet J, 192*(1), 13-19. https://doi.org/10.1016/j.tvjl.2011.05.019

OIE. (2008). Terrestrial Animal Health Code 2008: Introduction to the recommendations for animal welfare. In (p. 1-2): World Organization for Animal Health (OIE).

Payne, M. A., Craigmill, A. L., Riviere, J. E., Baynes, R. E., Webb, A. I., & Sundlof, S. F. (1999). The Food Animal Residue Avoidance Databank (Farad): Past, Present and Future. *Veterinary Clinics of North America: Food Animal Practice, 15*(1), 75-88. https://doi.org/https://doi.org/10.1016/S0749-0720(15)30208-5

Pelfort, X., Torres-Claramunt, R., Sánchez-Soler, J. F., Hinarejos, P., Leal-Blanquet, J., Valverde, D., & Monllau, J. C. (2015). Pressure algometry is a useful tool to quantify pain in the medial part of the knee: an intra- and inter-reliability study in healthy subjects. *Orthop Traumatol Surg Res*, *101*(5), 559-563. https://doi.org/10.1016/j.otsr.2015.03.016

Prunier, A., Mounier, L., Le Neindre, P., Leterrier, C., Mormède, P., Paulmier, V.,Guatteo, R. (2013). Identifying and monitoring pain in farm animals: a review. *Animal*, *7*(6), 998-1010. https://doi.org/10.1017/S1751731112002406

Pérez de Diego, A. C., Sánchez-Cordón, P. J., Pedrera, M., Martínez-López, B., Gómez-Villamandos, J. C., & Sánchez-Vizcaíno, J. M. (2013). The use of infrared thermography as a non-invasive method for fever detection in sheep infected with bluetongue virus. *The Veterinary Journal*, *198*(1), 182-186. https://doi.org/https://doi.org/10.1016/j.tvjl.2013.07.013

Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S.,Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain*, *161*(9), 1976-1982. https://doi.org/10.1097/j.pain.0000000000001939

Ramirez-Garcialuna, J. L., Rangel-Berridi, K., Bartlett, R., Fraser, R. D., & Martínez-Jiménez, M. A. (2022). Use of Infrared Thermal Imaging for Assessing Acute Inflammatory Changes: A Case Series. *Cureus*, *14*.

Rana, M. S., Lee, S. Y., Kang, H. J., & Hur, S. J. (2019). Reducing Veterinary Drug Residues in Animal Products: A Review. *Food Sci Anim Resour*, *39*(5), 687-703. https://doi.org/10.5851/kosfa.2019.e65

Rangel-Nava, A., Ramírez-Uribe, J. M., Recillas-Morales, S., Ibancovichi-Camarillo, J. A., Venebra-Muñoz, A., & Sánchez-Aparicio, P. (2019). Pharmacological Regulation in the USA and Pharmacokinetics Parameters of Firocoxib, a Highly Selective Cox-2, by Pain Management in Horses. *J Equine Vet Sci*, *77*, 36-42. https://doi.org/10.1016/j.jevs.2019.02.007

Rault, J.-L., Lay, D. C., & Marchant, J. N. (2011). Castration induced pain in pigs and other livestock. *Applied Animal Behaviour Science*, *135*(3), 214-225. https://doi.org/https://doi.org/10.1016/j.applanim.2011.10.017

Rault, J. L., Hintze, S., Camerlink, I., & Yee, J. R. (2020). Positive Welfare and the Like: Distinct Views and a Proposed Framework. *Front Vet Sci*, *7*, 370. https://doi.org/10.3389/fvets.2020.00370

Reeves, P. T. (2010). Drug residues. *Handb Exp Pharmacol*(199), 265-290. https://doi.org/10.1007/978-3-642-10324-7_11

Regan, T. (1983). The Case for Animal Rights. In: University of California Press.

Reppert, E. J., Kleinhenz, M. D., Montgomery, S. R., Bornheim, H. N., Magnin, G., Sidhu, P. K.,Coetzee, J. F. (2019a). Pharmacokinetics and pharmacodynamics of intravenous and transdermal flunixin meglumine in meat goats. *J Vet Pharmacol Ther, 42*(3), 309-317. https://doi.org/10.1111/jvp.12756

Reppert, E. J., Kleinhenz, M. D., Montgomery, S. R., Heiman, J., Sura, A., Bornheim, H. N.,Coetzee, J. F. (2019b). Pharmacokinetics and pharmacodynamics of intravenous and transdermal flunixin meglumine in alpacas. *J Vet Pharmacol Ther, 42*(5), 572-579. https://doi.org/10.1111/jvp.12800

Reppert, E. J., Kleinhenz, M. D., Viscardi, A., Montgomery, S. R., Crane, A. R., & Coetzee, J. F. (2020). Development and evaluation of two different lameness models in meat goats, a pilot study. *Transl Anim Sci, 4*(4), txaa193. https://doi.org/10.1093/tas/txaa193

Reppert, E. J., Streeter, R. N., Simpson, K. M., & Taylor, J. D. (2016). Retrograde catheterization of the urinary bladder in healthy male goats by use of angiographic catheters. *Am J Vet Res, 77*(11), 1295-1299. https://doi.org/10.2460/ajvr.77.11.1295

Rifkin, R. E., Grzeskowiak, R. M., Mulon, P. Y., Adair, H. S., Biris, A. S., Dhar, M., & Anderson, D. E. (2019). Use of a pressure-sensing walkway system for biometric assessment of gait characteristics in goats. *PLoS One, 14*(10), e0223771. https://doi.org/10.1371/journal.pone.0223771

Riviere, J. E., Craigmill, A. L., & Sundlof, S. F. (1986). Food Animal Residue Avoidance Databank (FARAD): An Automated Pharmacologic Databank for Drug and Chemical Residue Avoidance. *J Food Prot, 49*(10), 826-830. https://doi.org/10.4315/0362-028X-49.10.826

Robertson, S. A. (2001). Analgesia and Analgesic Techniques. *Veterinary Clinics of North America: Exotic Animal Practice, 4*(1), 1-18. https://doi.org/https://doi.org/10.1016/S1094-9194(17)30047-6

Rokka, M., Eerola, S., Perttilä, U., Rossow, L., Venäläinen, E., Valkonen, E.,Peltonen, K. (2005). The residue levels of narasin in eggs of laying hens fed with unmedicated and medicated feed. *Mol Nutr Food Res, 49*(1), 38-42. https://doi.org/10.1002/mnfr.200400042

Rosier, E. M., Iadarola, M. J., & Coghill, R. C. (2002). Reproducibility of pain measurement and pain perception. *Pain, 98*(1-2), 205-216. https://doi.org/10.1016/s0304-3959(02)00048-9

Ross, M., & Dyson, S. (2011). *Diagnosis and Management of Lameness in the Horse*. https://doi.org/10.1016/C2009-0-50774-X

Roth, J. A. (1985). Animal Stress: Cortisol as Mediator of Stress-Associated Immunosuppression in Cattle. In (pp. 225-243).

Rushen, J. (2008). Farm animal welfare since the Brambell report. *Applied Animal Behaviour Science, 113*(4), 277-278. https://doi.org/https://doi.org/10.1016/j.applanim.2008.01.019

S.M., M., J.A., R., K., M., Jack, M., Ison, S., Bunger, L., & Dwyer, C. M. (2010). Development and validation of on-farm behavioural scoring systems to assess birth assistance and lamb vigor. In. Cambridge Core.

Sanford, S. E. (1989). Ontario. Meningoencephalitis caused by thermal disbudding in goat kids. *Can Vet J*, *30*(10), 832.

Schmitt, R., Pieper, L., Borchardt, S., Swinkels, J. M., Gelfert, C. C., & Staufenbiel, R. (2023). Effects of a single transdermal administration of flunixin meglumine in early postpartum Holstein Friesian dairy cows: Part 1. Inflammatory and metabolic markers, uterine health, and indicators of pain. *J Dairy Sci*, *106*(1), 624-640. https://doi.org/10.3168/jds.2021-20555

Schmitt, R., Pieper, L., Borchardt, S., Swinkels, J. M., Gelfert, C. C., & Staufenbiel, R. (2023b). Effects of a single transdermal administration of flunixin meglumine in early postpartum Holstein Friesian dairy cows: Part 2. Milk yield, culling risk, and reproductive performance. J Dairy Sci, 106(1), 641-652. https://doi.org/10.3168/jds.2022-21995

Schulz, K. L., Anderson, D. E., Coetzee, J. F., White, B. J., & Miesner, M. D. (2011). Effect of flunixin meglumine on the amelioration of lameness in dairy steers with amphotericin B-induced transient synovitis-arthritis. *Am J Vet Res*, *72*(11), 1431-1438. https://doi.org/10.2460/ajvr.72.11.1431

Schwartzkopf-Genswein, K. S., Fierheller, E. E., Caulkett, N. A., Janzen, E. D., Pajor, E. A., Gonzalez, L. A., & Moya, D. (2012). Achieving pain control for routine management procedures in North American beef cattle. In (Vol. 2, pp. 52-58). Animal Frontiers.

Searle, D., Dart, A. J., Dart, C. M., & Hodgson, D. R. (1999). Equine castration: review of anatomy, approaches, techniques, and complications in normal, cryptorchid and monorchid horses. *Aust Vet J*, *77*(7), 428-434. https://doi.org/10.1111/j.1751-0813.1999.tb12083.x

Sherwin, C. M. (2001). Can invertebrates suffer? Or, how robust is argument-by-analogy? *Animal Welfare*, *10*(Suppl), S103-S118. https://doi.org/10.1017/S0962728600023551

Shukla, M., Singh, G., Sindhura, B. G., Telang, A. G., Rao, G. S., & Malik, J. K. (2007). Comparative plasma pharmacokinetics of meloxicam in sheep and goats following intravenous administration. *Comp Biochem Physiol C Toxicol Pharmacol*, *145*(4), 528-532. https://doi.org/10.1016/j.cbpc.2007.01.020

Singer, P. (1975). Animal Liberation: A New Ethics for Our Treatment of Animals. In: HarperCollins.

Sischo, W. M., Norman, H. S., & Kiernan, N. E. (1999). Use of the Food Animal Residue Avoidance Databank (FARAD). *J Am Vet Med Assoc*, *214*(3), 344-350.

Smith, E. S., & Lewin, G. R. (2009). Nociceptors: a phylogenetic view. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*, *195*(12), 1089-1106. https://doi.org/10.1007/s00359-009-0482-z

Sneddon, L. U., Elwood, R. W., Adamo, S. A., & Leach, M. C. (2014). Defining and assessing animal pain. *Animal Behaviour*, *97*, 201-212. https://doi.org/10.1016/j.anbehav.2014.09.007

Snijdelaar, D. G., Dirksen, R., Slappendel, R., & Crul, B. J. P. (2000). Substance P. *European Journal of Pain*, *4*.

Sotocinal, S. G., Sorge, R. E., Zaloum, A., Tuttle, A. H., Martin, L. J., Wieskopf, J. S.,Mogil, J. S. (2011). The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain*, *7*, 55. https://doi.org/10.1186/1744-8069-7-55

Sprecher, D. J., Hostetler, D. E., & Kaneene, J. B. (1997). A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. *Theriogenology*, *47*(6), 1179-1187. https://doi.org/https://doi.org/10.1016/S0093-691X(97)00098-8

Steagall, P. V., Bustamante, H., Johnson, C. B., & Turner, P. V. (2021). Pain Management in Farm Animals: Focus on Cattle, Sheep and Pigs. *Animals (Basel)*, *11*(6). https://doi.org/10.3390/ani11061483

Stevens, B. J., & Franck, L. (1995). Special needs of preterm infants in the management of pain and discomfort. *J Obstet Gynecol Neonatal Nurs*, *24*(9), 856-862. https://doi.org/10.1111/j.1552-6909.1995.tb02571.x

Stewart, M., Stookey, J. M., Stafford, K. J., Tucker, C. B., Rogers, A. R., Dowling, S. K.,Webster, J. R. (2009). Effects of local anesthetic and a nonsteroidal antiinflammatory drug on pain responses of dairy calves to hot-iron dehorning. *J Dairy Sci*, *92*(4), 1512-1519. https://doi.org/10.3168/jds.2008-1578

Stock, M. L., Coetzee, J. F., KuKanich, B., & Smith, B. I. (2013). Pharmacokinetics of intravenously and orally administered meloxicam in sheep. *Am J Vet Res*, *74*(5), 779-783. https://doi.org/10.2460/ajvr.74.5.779

Stock, M. L., Gehring, R., Barth, L. A., Wulf, L. W., & Coetzee, J. F. (2014). Pharmacokinetics of firocoxib in preweaned calves after oral and intravenous administration. *J Vet Pharmacol Ther*, *37*(5), 457-463. https://doi.org/10.1111/jvp.12124

Stokes, J. E., Leach, K. A., Main, D. C. J., & Whay, H. R. (2012). An investigation into the use of infrared thermography (IRT) as a rapid diagnostic tool for foot lesions in dairy cattle. *The Veterinary Journal*, *193*(3), 674-678. https://doi.org/https://doi.org/10.1016/j.tvjl.2012.06.052

Stuart, A. K., KuKanich, B., Caixeta, L. S., Coetzee, J. F., & Barrell, E. A. (2019). Pharmacokinetics and bioavailability of oral firocoxib in adult, mixed-breed goats. *J Vet Pharmacol Ther*, *42*(6), 640-646. https://doi.org/10.1111/jvp.12795

Sundlof, S. F., Craigmill, A. L., & Riviere, J. E. (1991). Use of the food animal residue avoidance databank. *J Am Vet Med Assoc*, *198*(5), 816-819.

Sutherland, M. A., Davis, B. L., Brooks, T. A., & Coetzee, J. F. (2012). The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia. *J Anim Sci*, *90*(7), 2211-2221. https://doi.org/10.2527/jas.2011-4260

Sutherland, M. A., Huddart, F. J., & Stewart, M. (2019). Short communication: Evaluation of the efficacy of novel disbudding methods for dairy calves. *J Dairy Sci*, *102*(1), 666-671. https://doi.org/10.3168/jds.2018-15230

Sutherland, M. A., Worth, G. M., Dowling, S. K., Lowe, G. L., Cave, V. M., & Stewart, M. (2020). Evaluation of infrared thermography as a non-invasive method of measuring the autonomic nervous response in sheep. *PLoS One*, *15*(5), e0233558. https://doi.org/10.1371/journal.pone.0233558

Suvas, S. (2017). Role of Substance P Neuropeptide in Inflammation, Wound Healing, and Tissue Homeostasis. *The Journal of Immunology*, *199*(5), 1543-1552. https://doi.org/10.4049/jimmunol.1601751

T, N. (2017). Castration of male livestock and the potential of immunocastration to improve animal welfare and production traits: Invited Review. In L. H (Ed.), (Vol. 47 (6), pp. 731-742). South African Journal of Animal Science.

T., P., M.F., S. C., R., M., L., C., B., F., & M., R. (2018). Comparison of visual lameness scores to gait asymmetry in racing Thoroughbreds during trot in-hand. In: British Equine Veterinary Association.

Tannenbaum, J., & Bennett, B. T. (2015). Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. *J Am Assoc Lab Anim Sci*, *54*(2), 120-132.

Tapper, K. R., Johnson, A. K., Karriker, L. A., Stalder, K. J., Parsons, R. L., Wang, C., & Millman, S. T. (2013). Pressure algometry and thermal sensitivity for assessing pain sensitivity and effects of flunixin meglumine and sodium salicylate in a transient lameness model in sows. *Livestock Science*, *157*(1), 245-253. https://doi.org/https://doi.org/10.1016/j.livsci.2013.07.017

Tekeli, I. O., Turk, E., Durna Corum, D., Corum, O., & Uney, K. (2020). Effect of castration procedure on the pharmacokinetics of meloxicam in goat kids. *J Vet Pharmacol Ther*, *43*(5), 429-434. https://doi.org/10.1111/jvp.12872

Thompson, K. G., Bateman, R. S., & Morris, P. J. (2005). Cerebral infarction and meningoencephalitis following hot-iron disbudding of goat kids. *N Z Vet J*, *53*(5), 368-370. https://doi.org/10.1080/00480169.2005.36578

Thomsen, P. T., Munksgaard, L., & Tøgersen, F. A. (2008). Evaluation of a Lameness Scoring System for Dairy Cows. *Journal of Dairy Science, 91*(1), 119-126. https://doi.org/https://doi.org/10.3168/jds.2007-0496

Tracey, W. D. (2017). Nociception. *Curr Biol, 27*(4), R129-R133. https://doi.org/10.1016/j.cub.2017.01.037

Treede, R. D., Rief, W., Barke, A., Aziz, Q., Bennett, M. I., Benoliel, R.,Wang, S. J. (2015). A classification of chronic pain for ICD-11. *Pain, 156*(6), 1003-1007. https://doi.org/10.1097/j.pain.0000000000000160

Tschoner, T., Mueller, K. R., Zablotski, Y., & Feist, M. (2024). Pain Assessment in Cattle by Use of Numerical Rating and Visual Analogue Scales-A Systematic Review and Meta-Analysis. *Animals (Basel), 14*(2). https://doi.org/10.3390/ani14020351

Tuchscherer, M., Kanitz, E., Puppe, B., Tuchscherer, A., & Stabenow, B. (2004). Effects of postnatal social isolation on hormonal and immune responses of pigs to an acute endotoxin challenge. *Physiol Behav, 82*(2-3), 503-511. https://doi.org/10.1016/j.physbeh.2004.04.056

US, FDA. (2017). Freedom of information summary original new animal drug application Banamine Transdermal. In. US FDA CVM.

Usamentiaga, R., Venegas, P., Guerediaga, J., Vega, L., Molleda, J., & Bulnes, F. G. (2014). Infrared Thermography for Temperature Measurement and Non-Destructive Testing. *Sensors (Basel, Switzerland), 14*, 12305 - 12348.

USDA. (2010). Goat 2009, Part I: Reference of Goat Management Practices in the United States. In. Fort Collins, CO: 2009 USDA-APHIS-VS, CEAH.

USDA. (2024). Sheep and Goats (January 2024). In *USDA, National Agricultural Statistics Service*: National Agriculture Statistics Service (NASS), Agricultural Statistics Board.

USDA. (2025). Animal Production. In.

van der Staay, F. J., Goerlich, V. C., Meijboom, F. L., & Arndt, S. S. (2025). Animal welfare definitions, frameworks, and assessment tools: Advancing the measurement and laying the foundation for improved animal welfare through a three-step approach. *Anim Welf, 34*, e30. https://doi.org/10.1017/awf.2025.23

Van Engen, N. K., Stock, M. L., Engelken, T., Vann, R. C., Wulf, L. W., Karriker, L. A.,Coetzee, J. F. (2014). Impact of oral meloxicam on circulating physiological biomarkers of stress and inflammation in beef steers after long-distance transportation. *J Anim Sci, 92*(2), 498-510. https://doi.org/10.2527/jas.2013-6857

Vane, J. R., & Botting, R. M. (1998). Anti-inflammatory drugs and their mechanism of action. *Inflamm Res, 47 Suppl 2*, S78-87. https://doi.org/10.1007/s000110050284

Viscardi, A. V., Reppert, E. J., Kleinhenz, M. D., Wise, P., Lin, Z., Montgomery, S.,Coetzee, J. F. (2021). Analgesic Comparison of Flunixin Meglumine or Meloxicam for Soft-Tissue Surgery in Sheep: A Pilot Study. *Animals (Basel), 11*(2). https://doi.org/10.3390/ani11020423

Viscardi, A. V., & Turner, P. V. (2018). Use of Meloxicam or Ketoprofen for Piglet Pain Control Following Surgical Castration. *Front Vet Sci, 5*, 299. https://doi.org/10.3389/fvets.2018.00299

Visser, E. M., Menke, E. S., & van Loon, J. P. (2019). Pressure algometry for assessment of abdominal wall sensitivity in horses after ventral midline coeliotomy. *Vet Anaesth Analg, 46*(6), 820-828. https://doi.org/10.1016/j.vaa.2019.03.008

Voogt, A. M., Ursinus, D. T. H., Sijim, M., & Bongers, J. H. (2023). From the Five Freedoms to a more holistic perspective on animal welfare in the Dutch Animals Act. In. Frontiers in Animal Science.

Warner, R., Kleinhenz, M. D., Ydstie, J. A., Schleining, J. A., Wulf, L. W., Coetzee, J. F., & Gorden, P. J. (2021). Randomized controlled trial comparison of analgesic drugs for control of pain associated with induced lameness in lactating dairy cattle. *J Dairy Sci, 104*(2), 2040-2055. https://doi.org/10.3168/jds.2020-18563

Weary, D. M., Niel, L., Flower, F. C., & Fraser, D. (2006). Identifying and preventing pain in animals. *Applied Animal Behaviour Science, 100*(1), 64-76. https://doi.org/https://doi.org/10.1016/j.applanim.2006.04.013

Webster, H. B., Morin, D., Jarrell, V., Shipley, C., Brown, L., Green, A.,Constable, P. D. (2013). Effects of local anesthesia and flunixin meglumine on the acute cortisol response, behavior, and performance of young dairy calves undergoing surgical castration. *J Dairy Sci, 96*(10), 6285-6300. https://doi.org/10.3168/jds.2012-6238

Webster, J. (2016). Animal Welfare: Freedoms, Dominions and "A Life Worth Living". *Animals (Basel), 6*(6). https://doi.org/10.3390/ani6060035

Weeder, M. M., Kleinhenz, M. D., Reppert, E. J., Curtis, A. K., Viscardi, A. V., Leslie, A.,Coetzee, J. F. (2025). Comparison of multiple drug regimens for reduction of synovitis-induced lameness pain in meat-type goats. *Am J Vet Res*, 1-10. https://doi.org/10.2460/ajvr.25.04.0134

Weeder, M. M., Kleinhenz, M. D., Reppert, E. J., Fritz, B. R., Viscardi, A. V., Montgomery, S. R.,Coetzee, J. F. (2023). Optimal lameness induction model development using amphotericin B in meat goats. *Transl Anim Sci, 7*(1), txad105. https://doi.org/10.1093/tas/txad105

Weeder, M. M., Kleinhenz, M. D., Reppert, E. J., Weaver, L. F., Johnson, B. T., Leslie, A. A.,Coetzee, J. F. (2024). Comparison of firocoxib and meloxicam for pain mitigation in goats undergoing surgical castration. *J Am Vet Med Assoc, 262*(4), 498-505. https://doi.org/10.2460/javma.23.10.0575

Welsh, E. M., & Nolan, A. M. (1995). Effect of flunixin meglumine on the thresholds to mechanical stimulation in healthy and lame sheep. *Research in Veterinary Science*, *58*(1), 61-66. https://doi.org/https://doi.org/10.1016/0034-5288(95)90090-X

Wilson, J. E., Chandrasekharan, N. V., Westover, K. D., Eager, K. B., & Simmons, D. L. (2004). Determination of expression of cyclooxygenase-1 and -2 isozymes in canine tissues and their differential sensitivity to nonsteroidal anti-inflammatory drugs. *Am J Vet Res*, *65*(6), 810-818. https://doi.org/10.2460/ajvr.2004.65.810

WMS., R., & RL., B. (1959 (as reprinted 1992).). The principles of humane experimental technique. In. Wheathampstead (UK) Universities Federation of Animal Welfare.

Xie, W. L., Chipman, J. G., Robertson, D. L., Erikson, R. L., & Simmons, D. L. (1991). Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci U S A*, *88*(7), 2692-2696. https://doi.org/10.1073/pnas.88.7.2692

Yeates, J. W. (2012). Brain-pain: Do animals with higher cognitive capacities feel more pain? Insights for species selection in scientific experiments. In (p. 25-46): Graue Reihe.

Yeates, J. W., & Main, D. C. J. (2008). Assessment of positive welfare: A review. *The Veterinary Journal*, *175*(3), 293-300. https://doi.org/https://doi.org/10.1016/j.tvjl.2007.05.009

Young, B. K., Yu, J., & Hye, H. Y. (2022). Simultaneous Liquid Chromatography Tandem Mass Spectrometric Determination of 35 Prohibited Substances in Equine Plasma for Doping Control. In (Vol. 13, pp. 158-165): Mass Spectrometry Letters.

Zaninelli, M., Redaelli, V., Luzi, F., Bronzo, V., Mitchell, M., Dell'Orto, V.,Savoini, G. (2018). First Evaluation of Infrared Thermography as a Tool for the Monitoring of Udder Health Status in Farms of Dairy Cows. *Sensors (Basel)*, *18*(3). https://doi.org/10.3390/s18030862

Zetner, K., W.D., R., J., W., P., K., & I., S. (1996). Pain relief after dental treatment in cats. In (Vol. 77, pp. 678-682): Praktische Tierarzt.

Appendix A - Pharmacokinetic parameters of oral firocoxib, oral meloxicam, and transdermal flunixin in meat type goats undergoing an amphotericin B lameness model

Supplemental Table A. 1. Solid phase extraction protocol facilitated by Oasis hydrophilic-lipophilic sorbent and positive pressure assistance.

Step #	Strategy type	Strategy details	Volume (µL)
1	Sample introduction	Pre-treated sample	300.0
2	Hydrophilic wash	Water: methanol (90:10, v/v)	300.0
3	Organic elution	Acetonitrile: methanol (90:10, v/v)	50.00
4	Direct eluate dilution	Formic acid in water (0.1%, v/v)	50.00

Supplemental Table A. 2. Chromatographic conditions for the Waters Acquity Ultra Performance Liquid Chromatography H-Class PLUS system.

Autosampler temperature (°C)	5.0
Injection volume (µL)	2.00
Column	Acquity UPLC C18 HSS T3 (100 Å, 1.8 µm, 2.1 x 50 mm)
Pre-column	Acquity UPLC C18 HSS T3 VanGuard (100 Å, 1.8 µm, 2.1 x 5 mm)
Column temperature (°C)	40.0
Mobile phase	A: Formic acid in water (0.1%, v/v)
	B: Formic acid in acetonitrile (0.1%, v/v)

Supplemental Table A. 3. Gradient elution program with the mobile phase composed of 0.1%, v/v formic acid in water (A) and 0.1%, v/v formic acid in acetonitrile (B). [OBJ]

Time (min.)	Mobile phase (%)		Flow rate (mL/min.)
	A	B	
0.00	95.0	5.00	0.400
4.00	5.00	95.0	
5.00	5.00	95.0	
5.01	95.0	5.00	
7.00	95.0	5.00	

Supplemental Table A. 4. Mass spectrometric source conditions for the Waters Xevo TQ-S tandem Mass Spectrometry system.

Capillary voltage (kV)	3.30
Source temperature (°C)	150
Desolvation -nitrogen- gas flow (L/hr.)	800
Desolvation temperature (°C)	200
Cone -nitrogen- gas flow (L/hr.)	150
Collision -argon- gas flow (mL/min.)	0.15

Supplemental Table A. 5. Targeted MRM detection parameters for collision-induced fragmentation. Analytes were meloxicam (MEL), meloxicam-d3 (MEL-d3), flunixin (FTD), flunixin-d3 (FTD-d3), firocoxib (FIRO), and firocoxib-d6 (FIRO-d6).

Compound	Precursor ion (m/z)	Product ion (m/z)	Transition classification	Cone energy (V)	Collision energy (V)	Electrospray ionization mode
MEL	351.9851	115.0434	Quantifier	48	20	Positive
		140.9734	Qualifier		18	
MEL-d3	354.9851	140.9764	Quantifier	44	20	
		187.0535	Qualifier		14	
FTD	297.0166	264.0135	Quantifier	6	32	
		279.0269	Qualifier		22	
FTD-d3	300.0127	112.1246	Qualifier	30	46	
		264.0180	Quantifier		32	
FIRO	337.0489	129.9968	Qualifier	24	30	
		283.0431	Quantifier		8	
FIRO-d6	343.0889	135.8492	Qualifier	26	28	
		289.0823	Quantifier		8	