EFFICACY OF FLUNIXIN MEGLUMINE IN THE AMELIORATION OF LAMENESS IN AN AMPHOTERICIN B INDUCED TRANSIENT SYNOVITIS ARTHRITIS MODEL IN DAIRY STEERS

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Abstract

Lameness in cattle is a common cause of pain however there are no approved cattle analgesic drugs. Flunixin meglumine, the only non-steroidal anti-inflammatory drug approved for use in adult dairy cattle, is labeled for pyrexia associated with bovine respiratory disease, endotoxemia, acute mastitis and associated inflammation. There is currently a lack of objective data regarding the analgesic efficacy of flunixin meglumine in cattle.

The objectives of this study were to characterize an amphotericin B-induced lameness model and to ascertain the analgesic effects of flunixin meglumine using multimodal assessment. We hypothesized that flunixin meglumine would provide analgesia as evidenced by increased activity levels as well as increased exerted force and contact area on the affected limb in flunixin treated steers.

Amphotericin B-induced synovitis arthritis was induced in the distal interphalangeal joint of 10 dairy steers. The cattle were randomly allocated between a treatment and a control group. The treatment steers received flunixin meglumine at the time of arthritis induction and at 12 hours post-induction. Accelerometric, gait, pressure mat, vital parameter and plasma cortisol data were gathered in the pre and post-induction phases. The data were analyzed using linear mixed models with treatment and time designated as fixed effects.

Induction of amphotericin B arthritis produced a moderate, transient lameness. Control steers were more than twice as likely to be lame as flunixin meglumine treated steers using visual lameness assessment (92.2% ± 8.1 versus 40.7% ± 2.5) ($P<0.03$). Flunixin meglumine treated steers placed significantly greater force and contact area on the affected foot. Control steers also placed significantly greater force, impulse and contact area on the paired claw as compared to control steers. Flunixin treated steers spent considerably less time in recumbency than their control counterparts, particularly in the immediate post-induction time period.

This is one of the first studies to document the character of an amphotericin B-induced synovitis arthritis model in cattle as well as to document analgesic efficacy of a nonsteroidal anti-inflammatory drug in an induced lameness model. Flunixin meglumine was efficacious in providing analgesia in an amphotericin B-induced lameness model in dairy steers.

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Dedication

This thesis is dedicated to my wonderful family and friends. Their support, love and wisdom have been instrumental in creating this thesis. I am blessed.

CHAPTER 1 - Literature Review

Lameness

Although a significant amount of the current veterinary literature has been dedicated to lameness in cattle, there is much that remains to be elucidated, particularly with regard to how cattle alter behavior, gait, body posture and weight-bearing due to lameness-associated pain. The objectives of this review are to provide an overview of the pertinent published literature regarding lameness, pain and analgesia in cattle as well as to provide a literary background for the thesis project analyzing the analgesic effects of flunixin meglumine using an amphotericin-B induced synovitis lameness model in dairy steers.

Prevalence

Lameness is one of the most costly and recalcitrant issues within the dairy and beef industries. Although many risk factors in cattle have been identified [1]and preventative measures initiated, the high prevalence of lameness in cattle continues to be problematic. In fact, it is suggested that the overall prevalence of lameness in dairy cattle in the United States is increasing. A recent National Animal Health Monitoring System dairy study found that lameness of cattle increased from 10.5% in 1996 to 14.0% in 2007[2]. Producer insensitivity to lameness-associated pain and distress, poor detection techniques, increasing herd size coupled with labor issues are likely to blame for this trend[3] as cows are allowed to progress from either subclinical or acute lameness to chronic lameness without adequate intervention or triage. Recent prevalence estimates of clinical lameness in high production dairy cows range from 13.2% to 24.6% [4,5]. Disorders such as white line separation, solar ulceration, interdigital necrobacillosis and digital dermatitis have been shown to affect up to 69% of dairy cattle in the United Kingdom[1] , with a large number of these cattle presenting with subclinical lameness. Although there is little published regarding the prevalence of lameness in beef cattle, lameness can be a significant problem in cow-calf herds resulting in involuntary culling as well as genetic and economic loss [6]. A study in Nebraska feedlot calves found that lameness contributed to 16% of all health related concerns and 5% of the death loss [7].

In a recent audit of United States market cows and bulls, it was found that 30% of cull animals were lame at the time of slaughter[2]. Of these animals, 4% were graded as significantly or severely lame with locomotion scores of 4 to 5/5. The audit shows a dramatic increase in the number of lame cull dairy cattle presented for slaughter, from 23% in 1994 to 49% in 2007. In the same audit the number of lame beef cull animals presented for slaughter has increased as well, with 16% of cull beef cows lame and 31% of cull beef bulls lame in 2007 as compared to 11% and 27% in 1994. Although arthritis is only one of many conditions resulting in lameness, it continues to be one of the top causes of meat trim waste with 6% of market cows or bulls affected with an arthritic joint at the time of slaughter[2].

Economic Loss

The bulk of economic waste associated with lameness is due to production losses rather than treatment associated cost[8]. In the dairy industry, these production losses are encompassed by decreased milk yield, increased calving to conception intervals as well as an increased risk of involuntary culling [9-14]. In the dairy industry lameness remains second only to mastitis in terms of herd productivity losses [15]. Bicalho et al. (2008) found that lameness associated milk losses in dairy cattle were between 314 and 424 kilograms per cow per 305 day lactation[12]. Although economic loss estimates vary dramatically from study to study, a cost of up to $627 per case of sole ulceration has been reported[8].

Etiology and Risk Factors

The causes of lameness are numerous [16]. Multiple factors including genetics, nutrition, immune status, management, housing as well as the presence or absence of infectious agents, increase the susceptibility of cattle to specific hoof and claw pathology[17]. More than 88% of lameness can be localized to the foot or claw, with 85% of foot lameness found in the lateral claw of the hindlimb[8]. Common underlying claw or foot conditions leading to lameness in cattle include laminitis, digital dermatitis, pododermatitis circumscripta, interdigital phlegmon and septic arthritis of the digits. A recent study by Barker et al. (2009) documented risk factors for disorders such as white line disease, sole ulcers and digital dermatitis. Risk factors for sole ulcer development included parity (≥ 4), the use of lime in free stalls, use of roads or concrete paths in between the parlor and pasture as well as use of sparse bedding for greater than 4 months. Risk factors for white line disease included expanding herd size, increased parity, nightly housing for pastured cattle and solid grooved concrete flooring. Cows housed on solid grooved concrete flooring were also at a greater risk for developing digital dermatitis. The risk of digital dermatitis was lessened in cows at least 6 months postpartum [18].

Identification of Lameness and Associated Pain

Lameness is one of the most common causes of distress in dairy cattle and can result in significant pain and debilitation [19]. It is considered one of the most reliable animal-based indicators of dairy cattle welfare [3]. For herdsmen, owners and veterinarians alike, identification and quantification of lameness-associated pain and distress in cattle can be challenging. As a species where it is advantageous to mask pain due to potential predators, cattle are often considered "stoic" in nature [20]. While physiologically they respond similarly to humans with regard to pain, they cannot self-report or quantify pain, making assessment of pain and response to therapy difficult to ascertain [20, 21].

Assessment of Pain

Behavior is often used to evaluate pain in animals[22] and can give good "indices of the duration and the differentiation phases of pain experience[23]." However in recent surveys of private practice veterinarians, veterinarians differed in opinion regarding what behaviors were indicators of pain in cattle. In one practitioner survey, commonly suggested signs of pain by respondents included anorexia, vocalization, bruxism, depression and abnormal posture however there was not significant agreement between the survey respondents as to what signs entailed pain in cattle [24]. When specific lesions were assigned a pain score by respondents in another practitioner survey, lameness lesions such as sole ulceration and digital necrobacillosis were consistently scored as the most painful lesions. These lameness lesions were deemed more painful than cesarean section, calf castration and mastitis based off of the personal experience of the survey respondents[25]. The association of lameness with significant pain is likely due to a variety of factors; however the visible nature of lameness may be more prominent than behavioral changes associated with other disorders such as mastitis. Establishing a link between behavioral signs and severity of pain can be difficult. Even mild cases of mastitis can be associated with hyperalgesia and significant pain as evidenced by increased hock to hock distance [26, 27].

Lameness Behavior

Lameness is associated with altered behavior however there have been few studies of to document the behavioral effects of lameness in dairy cattle [28]. Normal dairy cattle spend a significant amount of time lying down, with the average dairy cow spending between 9 and13 hours in recumbency each day depending on environment, pregnancy status, lactation number and health among other factors[28-30]. If deprived of recumbency time, cattle will prioritize lying down to recoup lost time lying down at the cost of other activities such as eating [29]. Lame cows enter the parlor later than sound cows and shift weight more often than sound cows during milking[31]. On pasture, lame cows spend less time grazing, more time lying down and more time ruminating than their sound counterparts. This leads to decreased consumption as evidenced by a reduced grass bite rate. These behavioral changes are particularly profound in severely lame cattle[31].

The severity of lameness and type of stall dictate when and for how long a lame cow will lie down. Singh et al (1993) found that there was a difference in distribution of lying time between non-lame and lame cows in cubicles. Although the recumbency periods were roughly the same in duration, non-lame cattle spent more time in a recumbent position during the night, while recumbency in lame cattle was more evenly distributed throughout a 24 hour period. The lame cows were noted to assume abnormal postures while recumbent within cubicles[30]. In a study by Cook et al (2004) there was no difference in the total number of lying and standing episodes between non-lame and lame cattle, however lame cattle spent more time standing in matted stalls than sand stalls in between recumbency periods. In cows with mild lameness, cows stood 2.32 hours longer in matted stalls than in sand stalls. Moderately lame cows stood 4.31 hours longer in matted stalls than in sand stalls. This was likely due to a number of factors influencing a cow's decision to lie down, including ease of getting up and down, stall design, the potential to slip and the desire to allot a certain amount of time towards recumbency [28].

Although the inherent need to lie down appears to drive recumbency rates in cattle, the recumbency behavior of cattle is complex, with many factors affecting the duration and time frame of recumbency. In particular, the presence of lameness significantly alters recumbency behavior in cattle, making percentage recumbency over time a potentially potent marker for lameness.

Visual Lameness Assessment

Painful limb or claw lesions can result in altered gait and body posture in cattle [32]. Visual assessment of how cattle walk or even stand [33] has been used extensively to diagnose lameness in cattle both in the field and in the research setting. While basic visual lameness assessment differentiates between sound and lame cattle without the use of a scoring system, visual locomotion scoring systems link specific gait and posture attributes to degrees of lameness using a numerical or sliding scale. Visual locomotion scoring is the current gold standard of lameness detection in cattle and is valued for its feasibility of use [34], its non-invasiveness and low expense. It can be incorporated into a herd health management program, allowing the veterinarian, herdsman or farmer the ability to track lameness incidence and prevalence within the herd [35].

There are many locomotion scoring systems described within the literature. While no system yields complete accuracy, repeatability or sensitivity, each system has assets that may prove useful depending on the data and type of analysis desired [35]. The most basic scoring systems are numerical rating scale systems which differentiate the gait between lame and non-lame cattle using simple descriptors linked to a graduated scale such as that described by Whay (1997)[36], Tranter and Morris (1991)[37] and Wells (1993)[4]. In these scoring systems, a 0 or 1 is allocated as non-lame. The severity of lameness or associated gait abnormalities increases with the numerical score, with scores of 4 or 5 corresponding to severe lameness. The simplicity of the scoring systems allows for ease of use however specific descriptors are often minimal allowing for potential variation in interpretation [38]. Visual analogue scale scoring systems use a sliding scale with endpoints of soundness and severe lameness (Figure 1.1). Visual analogue scoring allows for increased sensitivity but may result in reduced agreement between observers scoring the same cattle [35, 39]. More verbally descriptive locomotion scoring systems such as those developed by Mason and Leaver (1998)[40] (Table 1.1) and Sprecher (1997)[41] (Table 1.2) have linked specific gait and posture attributes such as curvature of the spine, asymmetry of gait and recumbency to degree of lameness. These detailed descriptors allow for detection of more subtle abnormalities in gait and posture and can be of value in a research setting where high resolution is desired [34]. Whay and Main (1999) compared three of the most popular scoring systems, including the Mason and Leaver locomotion scoring system, a 6 point Numerical Rating Scale system and the Visual Analogue Scale. The Mason and Leaver locomotion scoring system was found to be most valuable for the detection of subtle gait abnormalities leading to lameness, with half of the scale dedicated to gait changes associated with subclinical lameness. The Mason and Leaver scoring system was initially developed to evaluate how diet and hoof growth affected pre-lameness gait. With a 9 point scale, it can be a challenging tool to learn and implement in the field. The 6 point Numerical Rating Scale was found to be more useful for detecting clinical lameness, with a significant portion of the scale dedicated to clinical lameness. The Visual Analogue Scale offered increased sensitivity due to its continuous scale, but there was tendency to underestimate lameness, particularly in clinically severe cases [35].

Locomotion scoring systems have been used extensively to evaluate production losses associated with different degrees of lameness. Sprecher's locomotion scoring system was one of the first to document how locomotion scores could be linked to production performance. In contrast to the Mason and Leaver scoring system, the focus of the Sprecher locomotion scoring system was clinical lameness rather than subclinical lameness. Sprecher et al. (1997) found that cows with locomotion scores greater than 2/5 were significantly more likely to have extended intervals from calving to first service and to conception than cows with scores less than or equal to 2/5. These cattle were also 8.4 times more likely to be culled than cows with scores less than or equal to 2/5 [41]. Bicalho published a study in 2007 comparing visual locomotion scores to the risk of pregnancy. A 5 point numerical scale was used, where non-lame cows were assigned a score of 1 and severely lame cows were assigned a score of 5. It was found that cows with a visual locomotion score of greater than or equal to 3 were 15% less likely to be pregnant than their herd counterparts with locomotion scores of less than 3. Cows during were visual locomotion scores of greater than or equal to 4 were 24% less likely to be pregnant than cattle with scores of less than 4[42]. Other production parameters such as milk yield have been linked to degrees of lameness using visual locomotion scoring. Hernandez et al. (2005) used a 6 point locomotion scoring system to evaluate lameness in cows the first 100 days of lactation. Milk yield was significantly decreased during the first 100 days of lactation in lame cows (locomotion score >4/6), as compared to moderately lame and sound cows (locomotion score $\leq$ 4/6)[10]. These findings reveal the value of visual lameness assessment in a production setting as several production parameters are closely linked to the degree of lameness. When certain thresholds are established, valuable management information can be elucidated from the locomotion scores.

Although visual locomotion scoring has been successfully linked to multiple production parameters and it remains the only feasible method of lameness detection available on a wide scale, its merit as a lameness detection technique continues to be a source of debate due to inherent subjectivity[38]. Subjectivity can lead to poor intra and inter-observer agreement with regard to scoring. In a clinical setting poor correlation can lead to undetected lameness with subsequent production losses. In a research setting, poor observer agreement can result in increased error within a study. Agreement between observers can vary depending on professional experience. While locomotion scoring agreement between two trained professionals evaluating high production dairy cattle has been reported to be up to 92% , researchers such as Espejo have found little intra and inter-agreement between observers in lameness detection. Lack of agreement is particularly evident between observers when there is a difference in professional training. Wells et al. (1993) and Espejo et al. (2006) determined the prevalence of lame dairy cows in high production herds to be 3 times that of the prevalence estimated by farm personal[4, 5]. Using a 9 point scale, Engel et al. (2003) found that the probability of exact agreement between trained professionals and untrained personal varied between 25% and 47 %. If one numerical score difference was allowed between trained professionals and untrained personal scoring, the probability of agreement between the two parties was increased to 80% agreement. Whether this is sufficient agreement depends on the underlying purpose of scoring the cattle. In particular, 80% agreement may not be sufficient in a research setting where the goal is to reduce error while in the clinical setting it may be sufficient enough to identify and treat most suspect cattle. The percentage increase in agreement between scores was variably increased if the untrained personal in Engel's study were provided training to visually appraise lameness using the specified 9 point scale[44]. Although this leads to the conclusion that training provides little improvement in correlation between observers, the 9 point scale is quite complex, and this must be taken into account as significant experience is needed to feel comfortable with the scoring system. It would be advantageous to consider other, more simplified scoring systems when performing a study of this nature.

Channon et al. (2009) found that the Mason and Leaver scoring system was highly variable with regard to observer agreement unless the locomotion score scale was simplified into lame or sound scoring. The observer agreement was significantly increased when a threshold of lame (locomotion score ≥3) or not lame (locomotion score <3) was used[38]. Agreement between observers was affected by the degree of lameness, with subtle lameness leading to reduced correlation.

Bicalho et al. (2007) found that the sensitivity and specificity of detecting painful claw lesions in dairy cattle using locomotion scoring to be 67.2% and 84.6%, respectively[42]. In this particular study, visual identification of the underlying lesion and assessment of pain using applied pressure were used as a gold standard. Poor sensitivity in detecting painful limb lesions may be due to gait variation and differences in lesion pain. In addition, innate differences in posture and gait between individuals due to physical characteristics such as udder fill and conformation can potentially affect the sensitivity and specificity of lameness detection using locomotion scoring[45]. This resultant gait variation can lead to poor correlation between the identified distal limb lesion and the associated locomotion score. In particular, lesions such as white line disease and solar hemorrhage/bruising often do not correlate with locomotion score, suggesting that the potential pain associated with these lesions has already been resolved or is not severe enough to result in a change in gait. In one study, only 48% of the variation in gait could be linked to the identified underlying lameness condition [36]. However, in a more recent study by Flower et al. (2006), 92% of cows were correctly classified as to the presence of sole ulcers. In this study a Numerical Rating Score system explained 73% of the gait variation seen in cattle with sole ulceration[43]. It is likely that the success of visual lameness scoring is affected not only by the particular underlying lesion but is also affected by the case definition used to classify the underlying lameness lesions as well as the individual gait variation inherent in cattle.

Visual lameness assessment continues to be an important tool in lameness detection both in the field and in the research setting, particularly because there are no other techniques that are as versatile or as easily implemented. As there are a wide variety of scoring systems available, it is imperative that the proper scoring system be chosen for the goals of lameness detection. Although subjectivity is inherent in visually appraisal of lameness, identifying and correcting for factors that may lead to decreased sensitivity and observer agreement such as poor training, complex, improperly understood scoring systems and improperly defined lameness lesions may help improve success in detecting lameness.

Objective Analysis

Although visual locomotion scoring remains an important tool in both the research and field setting, more objective methods of detecting lameness are desired as visual lameness detection can be subjective in nature. Objective analysis of gait typically requires some type of quantification of the associated kinematic movements and kinetic forces involved in locomotion.

Kinematic assessment most commonly utilizes videography to document the movement of limbs in a manner that can be analyzed using computer software. Temporal (stride duration, limb coordination patterns), linear (stride length) and angular (displacements, velocities, and accelerations) data are computated[46]. Kinetic analysis of cattle has been used to document reduced joint angulation in indoor housed dairy cattle[47]and to calculate spatial requirements for recumbent Holstein cattle[48]. With regard to locomotion and lameness, Flower et al. (2005) found that cattle with sole ulcers walk more slowly, have shorter stride lengths and spent more than twice as much time during their gait cycle with three limbs in contact with the floor[49]. Flooring can also affect the kinematic variables associated with gait. Softer, higher friction floor surfaces improve the gait of cattle as evidenced by longer stride lengths, higher maximum stride height, more stride overlap, less time with 3 hooves in contact with the ground at the same time, and increased velocity of locomotion. In cattle with sole ulceration the stride height is diminished as compared to cows without sole ulceration[50]. These findings support the use of kinematic analysis in cattle gait assessment however its use is likely limited to the research setting due to the logistics of data acquisition and analysis.

During the act of locomotion ground reaction forces are produced in transverse, longitudinal and vertical planes. These forces are exerted at the claw-floor interface and can be measured using force plate technology. When an animal steps on a force plate, the resultant strain is converted to an electric signal which is analyzed using computer software. Variables such as the ground reaction forces, impulse, center of pressure and the time at peak force can be quantified[46].

In cattle, the stance phase can be broken down into several phases or moments. These have been classified as the heel strike moment, the maximum breaking moment, the midstance moment, the maximum propulsive moment and the push off moment. The heel strike moment occurs when the foot is first placed on the ground and weight is transferred onto the respective limb. At this point the vertical component of the ground reaction forces has reached 30% of its peak. The maximum breaking moment is the deceleration phase of stance. This is where the longitudinal component of the ground reaction forces is at its minimal value. At the midstance moment, the previous deceleration of the limb changes to propulsion, resulting in a longitudinal ground reaction force of zero. The leg is in a vertical position, resulting in a peak vertical force. As the body is propelled forward during the maximum propulsive moment, the longitudinal force reaches its peak. At push off moment, the vertical ground reaction force is decreased to 30% of its peak value. This is when the foot has almost left the ground surface[51].

Pressure mat technology calculates pressure and other variables such as stride length, impulse (Figure 1.2), the pressure integral, contact area and force distribution. Variables such as the impulse and pressure integral are computed to take into account changes in momentum that can affect the force measurements. Pressure sensors with a known area are imbedded into a mat that the animal stands on or walks across. These pressure sensors measure vertical ground reaction forces produced during locomotion. As pressure is a function of force per unit area, the pressures produced during a foot fall can be calculated from the vertical ground reaction forces produced during the footfall. The vertical ground reaction forces are spread over the contact area of the claw. The resultant pressure corresponds to the local compression of underlying tissue and horn [51]. Specialized software programs create a color gradated digitized image corresponding to the calculated pressures. Variables related to vertical force as well as those that can be determined from the digital image, i.e. stride length and contact area, can be calculated.

Force distribution, pressure and contact area are important variables in bovine claw health and a significant amount of research has been undertaken in the past 20 years to determine what constitutes normal and abnormal values for these variables. As up to 85% of hind limb lameness is localized to the lateral claw, there has been speculation that overburdening of the lateral claw may predispose cattle to lameness[8]. Early work by Scott (1987) suggested that there was little difference in force, contact area and applied pressure between digits. However, continued research suggests that there is in fact a significant difference in force distribution as well as a difference in pressure between claws at the claw- ground interface in normal cows[52]. As has been determined by Ossent (1987) and others, the age of the cow as well as the hoof studied, i.e. front or rear limb, can affect how force and other variables are balanced between claws[59].

During standing as determined by both force plate and pressure mat analysis, the medial claws of the front limbs and the lateral claws of the hind limbs of adult cows are typically subjected the highest loads, with up to 80% of the total force on a hind limb placed on the lateral claw. The soles of these loadbearing claws are subject to high pressures, with the posterior portion of the front limb medial claws and the apical portion of the hind limb lateral claws bearing the peak pressures [53]. Van der Tol et al. (2002) speculates that the localization of peak pressures to the apical portion of the lateral hind limb claws and the posterior portion of the medial claw is due to the positioning of the center of body mass of the cow. In adult cows it is cranial to the hind limbs and caudal to the front limbs. In walking cows, van der Tol et al. (2004) found that the vertical ground reaction forces were equal in the front limb claws. However in the hind limb, the posterior portion of the lateral claw was most burdened during heel strike. This shifted toward the medial claw until both claws bore equal force at the push-off moment of the stance phase of locomotion. In contrast to the above mentioned studies, Scott (1988) found that walking heifer calves bore weight primarily on the hoof wall rather than the sole. The difference in support of the claw is likely due to differences in housing, maturity of the study animals and whether trimming of the claws was performed. In particular, Scott's 1988 study was performed using untrimmed heifer calves housed on straw while van der Tol's 2002 study was performed using recently trimmed, mature cows housed on slatted concrete flooring[51, 54].

Preventative or functional claw trimming can change how pressure and force are distributed at the level of the foot and sole. Calvalho et al. (2005) found that the pressure distribution under the medial sole, heel bulb and toe was minimally changed with balanced claw trimming, with a slight shifting of pressure towards the medial sole[8]. However, in another study functional claw trimming was found to reduce the total pressure load of the lateral hind limb claw by 24% with a resultant increase in total pressure of 50% on the medial hind limb claw. The posterior portion of the claw obtained a pressure reduction of 42% while the anterior portion of the claw remained unchanged with regard to pressure. In these cattle the center of gravity was shifted forward toward the toe region and away from where sole ulcers tend to develop. The effect of functional claw trimming was visible for roughly 26 weeks, leading to recommendations that functional claw trimming should be undertaken every 4 months in herds with a high prevalence of claw disease [55]. Van der Tol et al. (2004) used preventative trimming to decrease lateral claw total force by 10% and increase medial claw total force by 10%, with a total gain of 12.5 cm2 in contact area. However the claws remained subjected to localized high peak pressures even after preventative trimming[56]. These findings suggest that while claw trimming does significantly alter pressure and force, the high peak pressures implicated for sole ulcer development may still be present.

Claw or foot discomfort can lead to altered weight distribution in cattle. Weight distribution can be accomplished through the shifting of weight onto the ipsilateral claw or through the shifting of weight onto another limb. Uncomfortable surfaces have also been shown to lead to altered weight distribution however cattle have a limited ability to shift weight from their hind limbs to front limbs during standing [57].

Early force plate research revealed that standing cattle with mild undiagnosed hind limb foot discomfort were apt to shift weight onto the lateral hind limb claw from the medial claw [58], suggesting an underlying medial claw lesion. Gait and weight distribution have been evaluated in the periparturient period as cows appear to be at a higher risk of developing hind limb foot pathology after parturition[59]. In first calf heifers the ground reaction forces were found to be relatively equal in the hind limb claws before parturition. After parturition, the largest ground reaction forces were noted in the hind limb lateral claws. This change shift in load was attributed to the maturation of the heifers rather than a result of pregnancy, with increasing age leading to increased lateral claw load bearing. It was found that heifers that prematurely developed lateral claw load bearing before parturition were more likely to develop sole ulcers after parturition, suggesting that chronic overloading of the lateral claw leads to an increased risk of claw pathology [59]. Scott (1988) also found that changes in vertical ground reaction force and pressure at the level of the foot in pregnant cows were not attributable to weight distribution as a result of carrying a calf. Although body weight is increased in pregnant cattle, it is equally distributed and the resultant increased pressure measurements are shared between all 4 limbs, thus not increasing the risk of hoof pathology [54, 60].

In cattle with lameness, the force distribution over the lame and contralateral non-lame limb can vary significantly. Scott (1989) found that in lame cattle there was a difference in applied forces between the lame and non-lame contralateral limb, typically with redistribution of the force onto the contralateral non-lame limb. While the maximum vertical force was typically reduced in the lame limb, adaptation of the horizontal forces (i.e. acceleration or deceleration of the foot) in lieu of this occurred in some cattle. Changes in the horizontal forces (i.e. acceleration or deceleration) applied to the contralateral non-lame limb were also seen, likely as a means to further mitigate force on the lame limb[61].

One of the inherent weaknesses of kinetic analysis of gait in large animals is the variability in ground reaction forces produced by individual animals when walking. This variability is significantly increased in lame animals, making it difficult to differentiate lame from non-lame cattle using a simple limb movement variable such as vertical ground reaction force[61, 62]. To overcome these irregularities, lameness detection models have been created to analyze multiple limb movement variables such as peak ground reaction force, impulse, stance time, average ground reaction time, step size and energy transfer from the limb to the ground. These logistic regression models are fashioned to predict the probability of a cow being lame or non-lame. Additional mathematical transformation of the limb movement variables has resulted in increased success in predicting lame and sound cattle[62, 63]. These models emphasize the importance of a multimodal approach to lameness and pain detection in cattle.

Accelerometry has been used extensively by medical professionals and researchers to evaluate metabolic energy expenditures, physical activities and response to treatment in human and animal patients. The technology provides objective and quantitative analysis of the functional ability of patients in a remote and noninvasive manner. Change of acceleration due to both gravity and body movement is used to differentiate between the active and resting state in humans as well as determine body positioning [64, 65]. Static activity such as positioning of the body is determined by the orientation of specified body parts to the gravitational field. During dynamic activity, the orientation of the specified part changes over time, resulting in a varied acceleration signal as compared to the acceleration of gravity [66]. Accelerometry has proven to be an objective and reliable tool for gait analysis in animals. In horse it has been used to characterize the forces involve in the hoof-ground interaction during the stance phase as well as to detect front and hind limb lameness[67-69]. In canines, the gait impairments present in a type of muscular dystrophy were characterized using triaxial accelerometry [70]. Accelerometry has also been used to determine activity levels in normal canines as well as canines in the post-operative recovery phase. A study by Culp et al. (2009) found that dogs undergoing laparascopic ovariohysterectomy were significantly more active after surgery than dogs undergoing a traditional ventromedian celiotomy approach ovariohysterectomy [71]. This study shows how accelerometry can be used to judge the efficacy of techniques or treatment modalities through the evaluation of body posture and activity levels. In cattle accelerometry has been used to quantify how lame cattle allocate time to various activities or body postures[118]. A pilot study using triaxial accelerometry found that lame feeder calves spent more time lying down that matched controls [72]. Accelerometry promises to be an important tool in lameness detection and evaluation of treatment modalities in cattle.

Accurate detection of lameness is important both in the clinical setting and in the research setting. Tools such as accelerometry and pressure mat analysis offer the benefit of increased objectivity in lameness detection as compared to visual lameness assessment. However it is likely that a multimodal approach to lameness detection, with use of multiple diagnostic tools, will outperform any single diagnostic modality.

Analgesia and Pain

Analgesic intervention is important for both welfare and economic implications of lameness, however there are currently no drugs specifically approved for analgesic use in cattle in the United States. Although lack of access to approved cattle analgesic drugs likely limits analgesic intervention in cattle practice, inadequate treatment of pain in cattle is also the result of factors including lack of ability or unwillingness to recognize pain, lack of knowledge regarding pain mechanisms, unfamiliarity with available drugs and cost of the analgesic treatment including associated milk and slaughter withhold losses [24, 25, 73-75]. Because of the chronic nature of most lameness, many cattle experience lameness of significant duration and pain without any analgesic intervention[76]. While some cases of lameness are acute in nature and respond readily to therapy, a significant number of lameness lesions are chronic in nature and can result in an allodynic and hyperalgesic state where the nociceptive threshold of pain is significantly reduced even after the lesion itself has resolved. In painful lameness lesions with the potential to become chronic conditions, early analgesic intervention may prevent subsequent secondary hyperalgesia and potential "windup"[32]. Efficacy of analgesic drugs is likely reduced in cattle with hyperalgesia however non-steroidal anti-inflammatory drugs have been shown to be potent antihyperalgesic and analgesic mediators [77].

Several studies have documented the effects of local anesthesia and nonsteroidal anti-inflammatory medications on natural acquired lameness in cattle [78-80]. Lidocaine injected into the heel bulb of lame cattle with undiagnosed lameness was found to result in minimal improvement in locomotion numerical rating scores although weight distribution was shared more equally between the limbs[78]. In cows with hyperalgesia due to lower limb lesions, ketoprofen administration significantly increased the mechanical nociceptive threshold; however there was little change in the locomotion score[79]. Ketoprofen administered to lame cattle with naturally acquired lesions was also shown to result in minimal improvement in locomotion numerical rating score[80]. A recent study by Kotschwar et al. (2009) found no improvement in visual lameness scores in dairy steers with amphotericin-B induced lameness after administration of sodium salicylate[81]. The minimal change in lame cattle locomotion scores in analgesic studies has been attributed to several potential factors, including inability to determine the cause of the underlying gait abnormalities and poor non-steroidal efficacy in ameliorating lameness associated pain[80]. The efficacy of the drugs may also be reduced in hyperalgesic cattle as neural pathways of pain may be altered.

Castration[82], dehorning[23], flank laparotomy[823, 84], thermal threshold studies[85] are some of the reported bovine models of induced pain. Of these models, castration and dehorning procedures in cattle are by far the most utilized pain models in bovine published literature, primarily due to the fact that they are commonly used in routine bovine veterinary practice to improve animal welfare, handler safety and meat quality. The procedures are simple and inexpensive to perform, and they offer the advantage of a known time onset of pain as well as a high degree of reproducibility among a study group. Their use as an induced pain model has been justified as a way to promote further research into pain mechanisms and treatment options with the goal of promoting welfare improvement for a large number of animals[21, 86]. In pain studies in cattle that use dehorning or castrating models, pain is often evaluated in a multimodal approach, using behavioral, physiologic and production measurements. Plasma cortisol measurements are typically compared to behavioral changes such as head and tail shaking, ear flicking and decreased rumination as seen in dehorning models [87]. As a stress-induced hormone, cortisol is just one part of a complex physiologic response to stress. When the brain perceives stress, corticotrophin releasing hormone is released from the hypothalamus. This stimulates release of adrenocorticotropic hormone from the anterior pituitary gland. The adrenocorticotropic hormone subsequently stimulates release of glucocorticoids such as cortisol from the adrenal gland [88]. While plasma cortisol measurements have been the most extensively used assessment tool of pain-induced stress in acute bovine pain models such as dehorning [23], the relationship between stress and plasma cortisol concentration is not always linear [89]. In addition to other factors, handling-induced stress must be differentiated from pain-induced stress.

In the dehorning or castration model of pain, the initial peak in plasma cortisol concentration is likely due to impulse barrage of the affected nociceptors from pain as well as stress associated with the dehorning or castration event. This initial plasma cortisol peak eventually reaches a plateau and then declines, likely correlating to the presence and decline of inflammatory-related pain [90]. Abnormal frequency of normal behaviors such as head shaking or ear flicking in dehorned calves typically correspond to this spike and subsequent plateau in plasma or serum cortisol concentration[87] however different procedures and techniques produce different behavioral responses and peak cortisol concentrations. In particular, when comparing various castration techniques, the Burdizzo castration technique produces a smaller rise in cortisol concentration as compared to surgical castration or banding [91].

Physiologic measurements such as heart rate, respiratory rate and temperature have also been utilized in research to assess pain. Catecholamine release via activation of the sympathetic and hypothalamo-pituitary-adrenocortical axis can result in elevated vital parameters such as heart rate, respiratory rate and temperature. These parameters can be viable markers of pain as has been shown with dehorning where high heart rates are correlated to pain, however the short duration of elevation combined with the stress of the procedure and fear of people can cloud assessment of pain[21,86, 92,93].

In pain models such as dehorning and castration, preemptive analgesic intervention through the administration of local anesthesia and non-steroidal anti-inflammatory drugs, has been studied extensively to determine effectiveness of each towards the prevention or alleviation of acute pain based on behavioral, physiologic and production measurements[23,82,90-92,94,97,98].

Local anesthetics such as lidocaine are commonly used in practice to provide anesthesia to surgical sites. Local or regional anesthesia results in blockage of nerve sodium channels with resultant limitation of signal depolarization and conduction. When local anesthesia such as lidocaine or bupivicaine is preemptively administered for dehorning, disbudding or castration procedures, the initial peak concentration of cortisol is typically dampened or eliminated completely during the time the drug is actively working[23, 90]. As the signal blockade of the local anesthetic wears off, the cortisol response is not diminished but rather delayed as compared to control animals. This spike in cortisol concentrations can be delayed past the point that the cortisol concentration would normally return to pre-treatment levels in calves not receiving local anesthesia[90]. It is suspected that local anesthesia prevents acclimatization of the hypothalamo-pituitary-adrenocortical axis to the initial noxious insult, thus mitigating important negative feedback mechanisms and resulting in post-analgesic spikes in cortisol undampened by the time course of the injury[90]. In dehorned calves not receiving local anesthesia, the initial noxious stimulus leads to corticosteroid release which likely acts in an anti-inflammatory manner, mitigating to some degree subsequent inflammation [23, 90].

One of the most studied non-steroidal anti-inflammatory drugs in acute and chronic pain research in cattle has been ketoprofen. Although it is reported to have both central and peripheral action in producing analgesia[23], its mechanism of action is primarily as a nonspecific cyclooxygenase enzyme inhibitor. Although various studies differ in the degree of dampening of the cortisol response after dehorning or castration, the administration of preemptive ketoprofen reduces post-procedure cortisol levels, particularly when combined with lidocaine anesthesia. McMeekan et al. (1998) found post-dehorning cortisol levels to be similar to pre-dehorning cortisol levels when preemptive local anesthesia was combined with administration of ketoprofen[90]. This was similar to findings in castrated calves where lidocaine and ketoprofen were administered preemptively[82, 91]. It appears that the delayed cortisol rise normally seen in calves receiving solely lidocaine local anesthesia is impeded or completely prevented by the administration of ketoprofen in addition to the lidocaine. As this cortisol response is likely due to inflammation, the ketoprofen appears to be mitigating inflammation and thus pain. This is supported with reduced post-operative pain-induced behavioral abnormalities in hot-iron dehorned calves receiving ketoprofen [94]. However a study by Milligan et al. (2004) found that young calves being dehorned either did not benefit or did not respond to the same degree as older calves after ketoprofen administration. Although post-dehorning cortisol concentrations were slightly reduced after the administration of ketoprofen, in young calves (2- 14 days of age) the pain associated behaviors were unaffected[95]. The differences in cortisol and behavior response to preemptive non-steroidal anti-inflammatory drugs and local anesthetic administration before noxious stimuli are likely the result of multiple factors such as variation in study parameters, age of calves, difference in techniques, and lack of individual parameters that are unequivically correlated with pain. Although the research suggests the potential alleviation of pain when local anesthesia is combined with a non-steroidal anti-inflammatory drug, further research is needed to identify specific markers of pain that can be assessed to document the efficacy of these analgesics.

Flunixin meglumine is the only nonsteroidal anti-inflammatory drug currently labeled for use in beef and lactating dairy cattle in the United States. Its label indications include pyrexia associated with respiratory disease, endotoxemia, acute mastitis as well as inflammation associated with the aforementioned disorders[96]. While it is not labeled for analgesic use, it does appear to have analgesic properties stemming from the prevention of arachadonic acid metabolite production through nonspecific cyclooxygenase enzyme inhibition[97]. The reduction of these metabolites, particularly prostaglandins, leads to prevention of peripheral nociceptor stimulation in damaged tissues [23, 97, 98].

Multimodal therapy involves the incorporation of multiple classes of analgesics for reduction of pain. This can be important in chronic, hyperalgesic states where the pain is multifactorial and complex. The different receptors and mechanism of action of different drugs can be used to target different regions along the pain pathway. Continuous extradural analgesia using a combination of methadone, ketamine and bupivacaine was reported to alleviate distal limb pain in a cow with severe hyperalgesia[99].

The above synopsis of pain research in cattle yields conflicting conclusions regarding the effectiveness of analgesic intervention. Although analgesic therapy seems intuitively useful towards the amelioration of pain in cattle, concrete evidence is lacking for effective clinical efficacy, particularly in lame cattle. This likely stems from the fact that pain is a complex phenomenon that is often poorly understood. Measurable indices such as cortisol do not necessarily correlate with nociception or the animal's interpretation of the noxious insult. This gives credence to the discovery and development of other, more relevant measurable indices of pain to evaluate the efficacy of nonsteroidal anti-inflammatory drugs such as flunixin meglumine.

Lameness Models

Naturally acquired lesions lameness lesions in cattle have been used to evaluate pain and the efficacy of various treatments for lameness associated pain. Studies have shown that it is possible to scale the severity of the underlying disease process leading to lameness in cattle through visual lameness assessment, nociceptive threshold tests and examination [32], making it a potentially sensitive model of pain. Dyer et al. (2007) objectively assessed claw pain of dairy cows through the use of hoof testers with a built in pressure gauge. A pain index was calculated and compared to locomotion scores. While there was a strong effect of lateral claw pain on ipsilateral limb locomotion scores, subclinical pain as well as potential undiagnosed upper limb lameness and variability in gait reduced the strength of the correlation[100]. Flower et al. (2008) evaluated the effectiveness of ketoprofen on improving gait in lame cattle. Ketoprofen was found to modulate gait minimally as evidenced by visual lameness assessment, however it could not be ruled out that factors other than claw pain were responsible for the alterations in gait [80]. These studies highlight that much remains to be learned regarding limb locomotion, especially with regard to gait variation between cows. Gait variation can be due to a variety of underlying causes such as differences in lesion pain as well as differences in physical characteristics such as udder fill and conformation. In particular, a poor case definition for the underlying lesion can lead to erroneous conclusions regarding the cause of the lameness associated pain. This can lead to low correlation between the identified distal limb lesion and the associated locomotion score. While the acquisition of cattle with naturally acquired lesions avoids the ethical concerns of induction of pain in bovine research, it can be difficult to completely characterize and source and severity of the lesions involved.

The lack of correlation between naturally acquired lesions, lameness and resultant pain underscores the importance of validated, reliable and reproducible lameness models of pain in cattle. While current studies using models of pain such as dehorning or castration typically rely on behavioral observation and physiologic measurements for pain assessment [21], a lameness model also allows for measurement of physical variables that can potentially be linked to pain.

The ideal lameness inducing agent creates a lameness model with a pain scale that is reliable, sensitive and valid. For pharmaceutical investigation, this ideal model would be transient in nature, would not cause permanent damage to structures involved, would be moderate in lameness severity and would have sufficient duration for evaluation of the chosen drug. Many different arthritis inducing protocols have been published in the veterinary literature, and include such agents as E. coli, Staphylococcus aureus, lipolysaccharide and amphotericin B[107-117]. Most of these models have potential systemic side effects, cause severe joint damage or are too short in duration to evaluate the efficacy of most therapeutic agents. Previously reported induced lameness models of pain in cattle include oligosaccharide induced laminitis[101] and bacterial induced arthritis[102, 103]. In other species such as horses, there are many induced arthritis models. Induction of infectious arthritis using agents such as E.coli[104]and Staphylococcus aureus[105] in horses has been used to evaluate treatment of septic joints. Lipopolysaccharide endotoxin lameness models[106] have been used extensively in equine research to evaluate synovitis arthritis lameness. While these induction agents provide a predictable onset and course of clinical lameness, they can be associated with significant discomfort, joint pathology and systemic signs of illness.

Although no induction agent is ideal, the amphotericin B-induced synovitis arthritis model has several advantages in that it produces a moderate intensity of lameness, it is transient in nature with minimal articular changes, it lacks significant systemic side effects and it creates an arthritis of medium length duration. Amphotericin B, a polyene antibiotic, has been used since the late 1970’s as an aseptic transient synovitis lameness model in horses [107-117]. Intra-articular injection of polyene antibiotics such as amphotercin B result in disruption of lysosomes and release of inflammatory mediators leading to synovitis [116]. In 2009 Kotschwar et al. reported the use of an amphotericin B induced transient synovitis arthritis model to evaluate the effects of sodium salicylate on the resultant lameness in cattle[81]. This model was found to produce a predictable and moderate synovitis arthritis that was transient in duration.

Conclusions

Lameness continues to be a costly and prevalent condition in the cattle industry and remains a significant welfare concern. Identification of lameness, triage and prevention are key in solving this problem however there remains much to learn regarding the best method of lameness detection, the most efficacious treatments of lameness as well as effective prevention measures.

Identification of lameness and lameness associated pain can be hampered by the stoic nature of cattle. Physiologic measurements can be valuable in detecting acute pain but the results can be confounded by the stress response of the animal. Behavioral indicators of pain associated with lameness can be subtle and interpretation can be subjective however it has been found that lameness significantly alters recumbency behavior, making the monitoring of recumbency a potentially potent tool of lameness analysis. Subjective tools such as visual lameness assessment continue to be valuable for the detection of lameness however more objective tools such as force plate, pressure mat and accelerometry may offer increased sensitivity, particularly in the research setting. Regardless of the technique or tool used to assess lameness and lameness associated pain, it is likely that a multimodal approach will yield the best results.

Although there are few analgesic drugs approved for use in cattle, there has been a significant amount of published literature focused on pain management in cattle. Unfortunately much of this literature has found little to support the use of common analgesics such as nonsteroidal anti-inflammatory drugs in lameness pain alleviation. The lack of efficacy of nonsteroidal anti-inflammatory drugs in these studies is likely multifactorial, but development of valid, reproducible models of pain as well as objective tools of assessment may help better define the efficacy of analgesic therapy as well as elucidate the unknowns regarding lameness, pain and analgesia in cattle.

Figures and Tables

Figure 1.1 Visual Analogue Scale

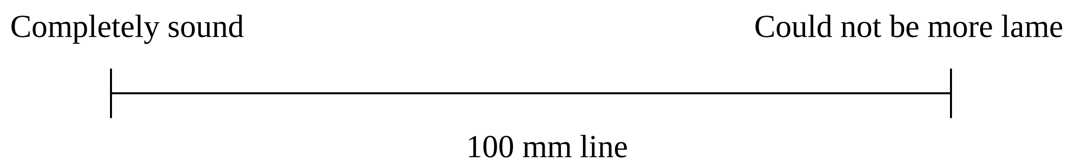


Figure 1.2 Impulse

$$F\Delta t = m\Delta v$$

Where $F\Delta t$ is the change in force over time (impulse) and $m\Delta v$ is the change in momentum.

Table 1.1 Assessment criteria of the Mason and Leaver lameness scoring system

Lameness Score	Assessment Criteria
1	Minimal abduction/adduction, no unevenness of gait, no tenderness
1.5	Slight abduction/ adduction, no unevenness or tenderness
2.0	Abduction/ adduction present, uneven gait, perhaps tender
2.5	Abduction/ adduction present, uneven gait, tenderness of feet
3.0	Slight lameness, not affecting behavior
3.5	Obvious lameness, some difficulty turning, not effecting behavior
4.0	Obvious lameness, difficulty in turning, behavior pattern affected
4.5	Some difficulty in rising, difficulty in walking, behavior pattern affected
5.0	Extreme difficulty in rising, difficulty in walking, adverse effects on behavior pattern

Table adapted from Mason and Leaver (1988)

Table 1.2 Assessment criteria of the Sprecher lameness scoring system

Lameness Score	Clinical Description	Assessment Criteria
1	Normal	The cow stands and walks with a level-back posture. Her gait is normal.
2	Mildly lame	The cow stands with a level-back posture but develops an arched-back posture while walking. Her gait remains normal.
3	Moderately lame	An arched-back posture is evident both while standing and walking. Her gait is affected and is best described as short-striding with one or more limbs.
4	Lame	An arched-back posture is always evident and gait is best described as one deliberate step at a time. The cow favors one or more limbs/feet.
5	Severely lame	The cow additionally demonstrates an inability or extreme reluctance to bear weight on one or more of her limbs/feet.

Table adapted from Sprecher et al. (1997)

CHAPTER 2 - Amphotericin B-Induced Synovitis Arthritis Study

Introduction

Lameness is a common cause of pain, distress and debilitation in cattle leading to economic loss across all sectors of the cattle industry[19]. In the dairy industry lameness remains second only to mastitis in terms of herd productivity losses[15]. These losses are encompassed by decreased milk yield, increased calving to conception rates as well as an increased risk of involuntary culling [9-14]. Although many risk factors in cattle have been identified [1]and preventative measures initiated, the high prevalence of lameness in cattle continues to be problematic. This translates into a significant animal welfare issue as lameness prevalence is one of the most reliable animal-based indicators of dairy cattle welfare[3].

There are few options for effective alleviation of lameness associated pain in dairy cattle. This is in part due to a lack of approved analgesic drugs. Cost, convenience, labor constraints and difficulty in recognizing pain have also limited the use of analgesic intervention in painful cattle. Subsequently, analgesic therapies are not provided to many cattle with painful lameness pathologies or to cattle undergoing potentially painful surgical or medical procedures[76].

Concerns over the welfare of lame dairy cattle are juxtaposed against concerns regarding food safety and drug residues with the use of pharmaceutical intervention. As food safety regulations become more stringent with regard to off-label use of drugs in food producing animals, it is imperative that action be taken on the part of the cattle industry to assure pharmaceutical analgesic options are available for cattle, with particular importance placed on maintaining appropriate welfare standards.

Currently flunixin meglumine, a nonspecific cyclooxygenase inhibitor, is the only Food and Drug Administration approved nonsteroidal anti-inflammatory drug available for use in adult dairy cattle. Flunixin meglumine is not currently approved for analgesic use. Label indications for flunixin meglumine include pyrexia associated with bovine respiratory disease, endotoxemia, acute mastitis and inflammation associated with the aforementioned disorders[96]. Procurement of analgesic labeling for flunixin meglumine and other similar nonsteroidal anti-inflammatory drugs is difficult due to a lack of objective data regarding efficacy[79]. Objectively documenting the efficacy of flunixin meglumine for lameness associated pain would provide a potentially viable treatment option for lameness and would open additional avenues for pain research.

As a species where overt displays of illness may lead to predation in the wild, cattle have gained a reputation for their “stoic” demeanor in the presence of pain. However their physiologic and anatomic similarities to other mammals far outweighs minor differences, making it likely that they are capable of feeling pain in a manner similar to other mammals [20, 21]even if they do not respond in a like manner. The stoic demeanor of cattle can lead to difficulties in quantifying pain. “Current assessments of pain are often based on extremely limited scales or single parameters. However, pain is a complex multidimensional phenomenon and the responses of animals to it are also complex[21].”

Although there are a number of models available for pain research in cattle, the ability to extract validated, reliable and reproducible data is limited in most of the current models. Studies have shown that it is possible to scale disease severity for lameness in cattle [32] making it a potentially sensitive model of pain. A lameness model allows for multiple methods of assessment, including objective assessment of gait using tools such as pressure mat analysis. A lameness model would also be one of the most externally valid options for assessing analgesic efficacy in cattle experiencing lameness induced pain.

The ideal lameness inducing agent creates a lameness scale that is reliable, sensitive and valid[21]. For pharmaceutical investigation, this model would be transient in nature, would not cause permanent damage to joint structures, would be moderate in lameness severity and would have duration sufficient for evaluation of the chosen drug. Although no induction agent is ideal, the amphotericin B-induced synovitis arthritis model embodies many of the aforementioned characteristics.

Amphotericin B, a polyene antibiotic, has been used since the late 1970’s as an aseptic transient synovitis lameness model in horses [107-117]. Intra-articular injection of polyene antibiotics such as amphotercin B result in disruption of lysosomes and release of inflammatory mediators leading to synovitis [117]. In 2009 Kotschwar et al. reported the use of an amphotericin B induced transient synovitis arthritis model to evaluate the effects of sodium salicylate on the resultant lameness in cattle[81]. It was found to produce a predictable and moderate synovitis arthritis that was transient in duration.

The objectives of this study were to characterize lameness induced by an amphotericin B induced synovitis arthritis pain model and to determine the analgesic effects of flunixin meglumine in cattle using an amphotericin B induced synovitis-arthritis pain model and multimodal analysis.. We hypothesized that flunixin meglumine would provide analgesia for lameness associated pain in cattle.

Experimental Design

All experimental procedures in this study were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC) under the supervision of the University Veterinarian (Protocol #2591).

Ten Holstein steers were obtained from local dairy steer suppliers. Inclusion criteria included no lameness or musculoskeletal abnormalities upon visual gait analysis and physical examination.

The study was conducted in two blocks (n=6 steers and n=4 steers, respectively) based on availability of cattle and facilities. The cattle were randomly allocated to either a treatment or a control group based on a Latin square approach at the start of each block. The principle investigator was blinded toward the allocation of steers into treatment groups until after completion of the study. An overview of the study schedule is presented in Figure 2.1.

The steers were housed in individual stalls (3.7 m^2) with access to water and brome hay ad libidum. This was supplemented with a balanced beef feedlot diet composed of cracked corn, whole oats, whole milo, dry distiller’s grain, and protein/vitamin/mineral supplement. Fans were used to provide ventilation and fly control was present. At the time of procurement the steers were allowed a minimum of 3 days of acclimatization to their surroundings, the research facilities and the equipment. Twice daily the steers were walked 15 minutes at a steady pace back and forth through the pressure mat testing area to reduce the amount of gait disruption during the trial.

At arrival commercially manufactured remote sensor units (motes; SENSR, Elkader, IA) were affixed to the lateral aspect of the left rear limb of the steers just proximal to the fetlock using a protective plastic housing and canvas straps. Triaxial acceleration measurements based on body positioning and gravity were acquired using 3 and 5 second Epochs. Data were aggregated on an hourly basis starting one day before induction of arthritis (day 0) and extending through completion of the study (day 3) and subsequently analyzed to access percent time lying down.

Lameness scoring was performed using the 5 point scale published by Anderson et al. (Table 2.1)[6]. A visual lameness score was assigned to each steer immediately prior to induction of arthritis (t=0) as well as at 6, 12, 24, 30, 36, 48, 54 and 60 hours post-induction to visually describe the gait of the steers and document the degree of lameness produced by the model. Each lameness score was determined by watching the steer walk a minimum of 20 meters in a straight line, turn, and walk 20 meters back to the starting point. All lameness examinations were performed on even, non-sloped concrete floors free of obstructions and debris. Each steer was assigned visual lameness scores by two separate investigators trained in lameness detection. The scores were averaged at the time of assessment. The steers were unmarked as to treatment although the small study size made recognition of individual steers more likely, particularly from time point to time point.

If at any time a steer became grade 4 out of 4 lame or remained grade 3 out of 4 lame for longer than 48 hours during the study, the steer was removed from the study group and appropriate therapy was instituted to alleviate pain. Potential therapy rescue measures included administration of flunixin meglumine (2.2 mg/kg intravenously once daily, Schering-Plough Animal Health Corp., Kenilworth, NJ), morphine sulfate (0.1 mg/kg subcutaneously once daily), lavage of the joint with a balanced isotonic solution, and intra-articular injection of 100 mg of 2% lidocaine.

Physical examination of the steers was performed every 12 hours starting immediately prior to induction of arthritis and continued until the end of the trial (t=60 hours). Vital parameters including heart rate, respiratory rate and temperature were recorded for analysis.

A pressure mat (Tekscan Hugemat 5400 XL; Tekscan, South Boston, MA) was placed in a designated alleyway with cattle panels placed parallel along each side of the mat extending 3 meters proximal and distal to the mat to allow for ease of funneling cattle onto the mat. The pressure mat measured a total of .9 meters by 2.4 meters with 1.5 sensors per square centimeter. A 5 millimeter thick rubber mat, extending beyond the length of the pressure mat, was placed over the pressure mat to assure normal ambulation and traction by hiding the mat from view and providing a consistent walking surface. Research grade software (Tekscan) allowed for real-time recording of the stance phase of stride as well the simultaneous recording of multiple foot falls.

Video synchronization was used to assist in the determination of hoof placement on the mat. Characterization of weight bearing and force distribution of the hind limbs during locomotion was accomplished through calculation of duration of stride, force, impulse, contact area, pressure and integral measurements using Tekscan software. Readings were taken immediately prior to induction of arthritis (t=0) as well as at 6, 12, 24, 30, 36, 48, 54 and 60 hours post-induction.

Jugular blood samples were obtained immediately prior to induction of arthritis (t=0) as well as at 6, 12, 24, 30, 36, 48, 54 and 60 hours post-induction using a 3.8 centimeter 18 gauge needle and 10 milliliter syringe. The steers were restrained in a cattle chute. Halters were placed on steers' heads and were securely fastened to the side of the chute to allow for safe access to the jugular furrow of the neck. The blood samples were collected in EDTA (BD Diagnostics, Franklin Lakes, NJ) tubes and then stored on ice and centrifuged at 1,600 x g for 15 minutes at 0°C. Plasma was removed and frozen in cryovials at -40°C.

Plasma cortisol concentrations were determined by a solid-phase competitive chemiluminescent enzyme immunoassay (Immulite 1000 Cortisol, DPS, CA) as described and validated by Coetzee et al. (2007) [121]. A sample volume of 100 µL was used in each assay well. The reported calibration range for the assay is 28 to 1,380 nmol/L with an analytical sensitivity of 5.5 nmol/L.

All 10 steers received a distal interphalangeal joint intra-articular injection in the left rear limb lateral claw at (T=0) to induce a transient synovitis arthritis. The intra-articular injections were performed by the same investigator in order to minimize technique variability. The cattle from each block (n= 6 and n=4) were randomly allocated between a treatment group and a control group via Latin Square block allocation to ensure balanced treatment groups. The treatment steers (n=5) received flunixin meglumine (1 milligram per kilogram intravenously) at the time of arthritis induction (T= 0) and at 12 hours (T=12) post-induction of arthritis. The control steers (n=5) did not receive non-steroidal anti-inflammatory drugs at these times. While the control steers did not receive a placebo injection, they were handled in a similar manner to the treatment steers. The principle investigator was blind to the treatments.

At time 0, the steers were placed in a cattle chute and the distal left limb was secured using a rope. The left hind lateral pastern region was clipped with a No. 40 blade proximal to the dorsal coronary band. The site was sterilely prepared using a five minute period of alternating povidone iodine scrub and 70% isopropyl alcohol disinfection. Using sterile technique, an 18 gauge 3.8 centimeter needle was placed one centimeter proximal to the coronary band and one centimeter abaxial to the tendon of the long digital extensor muscle. The needle was angled distally toward the sole into the left hind limb lateral distal interphalangeal joint of the lateral digit of each steer. Twenty milligrams of amphotericin B (2 ml of a 10 mg/ml solution) (X-Gen Pharmaceuticals, Inc, Big Flats, NY) was administered. The intra-articular location of the injection was confirmed by aspiration of joint fluid, ease of injection of the amphotericin B solution, and positive pressure return of fluid into the syringe.

Visual lameness scores were evaulated using SAS software (SAS Institute, INC, Cary, NC, USA). The lameness scores were transformed into binary variables and the proportional odds of lameness were calculated using random effects for repeated measures (time, steer and replicate group). The binary breakpoint was set between a locomotion score of 0 and locomotion scores greater than 0 to differentiate between non-lame and lame cattle.

Generalized linear models were developed using JMP 5.1.2 analytical software (SAS Institute, INC, Cary, NC, USA) to evaluate accelerometric data, serum cortisol concentrations, vital parameter data (heart rate, respiratory rate, core body temperature) and pressure mat variables (force, impulse, contact area, pressure, pressure integral, peak force, and peak pressure) for the affected limb, contralateral limb, affected claw and paired claw. Stance duration was calculated in a similar matter for the affected limb and contralateral limb. P-values < 0.05 were considered significant. Generalized linear models included fixed effects of treatment and time (post-treatment), and random effects to account for lack of independence of individual measurements related to replicate group and repeated measures in individuals. In addition, the serum cortisol measurements were analyzed with the baseline cortisol ($t=0$) as a covariate in the model. With regard to time as an effect, although pre-induction values were not directly compared to post-induction values, the pressure mat variables were evaluated over the whole study period, allowing for some inference regarding pre and post-induction differences in values. The different effects were tested for interactions.

The impulse and pressure integral were calculated to take into account variation in force or pressure due alterations in gait speed as lame animals typically ambulate at a slower pace than sound counterparts. The impulse and integral are the integral values of the force or pressure with respect to time. The formulas for calculation of impulse and the pressure integral are outlined in Figure 2.2.

Results

The 10 steers enrolled in the study ranged in age from approximately 6 months to 15 months, with body weights between 220 - 564 kilograms as measured within 30 days of the trial. Environmental conditions during the study ranged from an average of 72 to 79.5 degrees Fahrenheit and 40 to 63.6% humidity, with one block conducted in a completely climate controlled environment and the second occurring in a semi-controlled climate. No statistically significant differences ($p<0.05$) were found between the two blocks (replicates) of steers with regard to pressure mat variables, physiologic parameters, accelerometry values or lameness scores.

Rescue measures were initiated for one steer (control group) due to lameness that did not taper in severity or resolve by the end of the trial (LS=3 for greater than 48 hours). These measures included administration of flunixin meglumine (1.1 mg/kg IV), Lactated Ringers Solution lavage of the joint and administration of intra-articular sodium ceftiofur (150 milligrams). The steer subsequently recovered with no noted long term deficits.

Visual Lameness Score

All the steers (n=10) became visibly lame within the first 6-12 hours of the study. While mild variation existed in the grade of lameness between individual steers in each treatment group, there was a consistent peak in severity that occurred between 6 and 12 hours and subsequently dissipated over the course of the study, with most steers visually scored as normal by the end of the study (Figure 2.3).

Although the odds of lameness were not significantly different at any time point during the trial for the study steers as a whole, there was a statistically significant effect of treatment present. Control steers had increased probability of having a lameness score greater than zero at any time point during the study as compared to flunixin meglumine treated steers, 92.2% ± 8.1 versus 40.7% ± 2.5, respectively ($p<0.03$). In this model, time was considered a random effect. No interactions were tested.

Pressure mat

There were no significant treatment and time interactions ($p<0.05$) with regard to pressure mat values thus only main effects (time, treatment) will be reported. Although stance duration (affected limb, contralateral limb) as well as force, peak force, impulse, contact area , pressure, peak pressure, pressure integral were calculated for the affected limb, contralateral limb, affected claw and paired claw only statistically significant values or values approaching statistical significance will be reported (Table 2.2; Figures 2.4- 2.8).

Treatment effect

Steers receiving flunixin meglumine exerted statistically greater maximum force ($p<0.03$) and mean force ($p<0.05$) on the affected limb during the stance phase as compared with control steers. The flunixin treated steers also had a statistical trend of increased impulse ($p<0.06$) on the affected limb during the stance phase as compared with control steers. Flunixin treated steers had statistically significant increases in mean contact area ($p < 0.04$) of the affected foot during the stance phase as compared with control steers. The maximum area of the contralateral foot during the stance phase was statistically greater ($p<0.04$) in the flunixin treated steers as compared to the control steers. Steers receiving flunixin meglumine exerted statistically greater ($p<0.02$) maximum force and mean force ($p< 0.005$) on the paired claw (to the affected claw) during the stance phase as compared to control steers. The impulse ($p< 0.03$) exerted during the stance phase was significantly greater for the paired claw (to the affected claw) in flunixin treated steers as compared to the control steers. The maximum contact area ($p< 0.02$) and mean contact area ($p<0.004$) of the paired claw (to the affected claw) during the stance phase were significantly greater in flunixin treated steers as compared to the control steers. The mean peak force ($p<0.06$) in the paired claw (to the affected claw) during the stance phase trended towards being statistically greater in flunixin treated steers as compared to control steers. Table 2.2

There were no statistically significant impulse, peak force, pressure, peak pressure or integral values with regard to flunixin treatment and the affected limb. There were no statistically significant force, peak force, impulse, pressure, peak pressure or integral values with regard to flunixin treatment and the contralateral limb. There were no statistically significant peak force, pressure, peak pressure or integral values with regard to flunixin treatment and the paired claw (to the affected claw). There were no statistically significant force, peak force, impulse, area, pressure, peak pressure or integral values with regard to flunixin treatment and the affected claw. There was no statistically significant effect of treatment with regard to stance duration.

Time effect

Although pre-induction values and post-induction values were not directly compared, there was a statistically significant difference in the integral values ($p<0.04$) exerted on the paired claw (to the affected claw) in the study steers over the course of the study. This was represented by a peak in the integral value at 6 hours post-induction, with values returning to normal over the course of the study. There was also a statistical trend of differences in maximum force ($p<0.053$) and maximum peak force ($p<0.06$) exerted on the paired claw (to the affected claw) as well as integral ($p<0.08$) exerted on the contralateral foot over the course of the study in the study steers. These maximum force and maximum peak force values tended to vary over the course of the study with no apparent pattern. Figures 2.5-2.6

There were no statistically significant differences in force, peak force, impulse, contact area, pressure or integral values over time in the affected limb of the study steers. There were no statistically significant differences in force, peak force, impulse, contact area, pressure or integral values over time in the contralateral foot of the study steers. There were no statistically significant differences in force, peak force, impulse, contact area or pressure values over time in the paired claw (to the affected claw). There were no statistically significant differences in force, peak force, impulse, contact area, pressure or integral values over time in the affected claw of the study steers.

The duration of the stance phase in the affected limb trended toward statistical difference over time ($p<0.07$) in the study steers. The duration of the stance phase of the contralateral limb was statistically different over time ($p<0.002$) in the study steers. Figures 2.7 and 2.8

Vital Parameters

The heart rate ($p<0.01$), respiratory rate ($p<0.0003$) and the rectal temperature ($p<0.0004$) of the study steers were statistically different over time ($p<0.013$). Of these physiologic parameters, the heart rate was visibly elevated at 12 hours post-induction of arthritis, with both the respiratory rate and rectal temperature increasing slightly in value over the course of the study (Figure 2.9) in the study steers.

Accelerometric Analysis

Accelerometry results were similar between the two blocks of cattle. For accelerometric data, the fixed effects of treatment and trial day were found to have an interaction ($p<0.0001$).

Trial day *treatment interactions were statistically significant for multiple days and treatment combinations. Flunixin treated steers spent a greater percentage of time standing after lameness induction than the control calves in the initial post-induction period. The difference in activity level between the flunixin treated group and the control steers slowly diminished over the course of the study. Figure 2.10

Cortisol Analysis

The plasma cortisol levels ($p < 0.03$) were significantly different over the study period in regardless of treatment. The initial post-induction cortisol level (t=6) was elevated compared to any other post-induction time point. No interactions were present between time and treatment. Numerically the control steers had a consistently higher plasma cortisol concentration as compared to the flunixin treated steers however there was no statistically significant difference found between the treatment groups ($p < 0.13$). Figure 2.11

Discussion

This is one of the first studies to document the magnitude and duration of amphotericin B-induced synovitis arthritis in cattle as well as to evaluate the analgesic efficacy of a cyclooxygenase inhibitor in an induced lameness model. In 2009 Kotschwar et al. reported the use of an amphotericin B-induced transient arthritis model to evaluate the effects of sodium salicylate[81]. The amphotericin B-induced lameness model was found to produce a predictable and moderate synovitis arthritis that was transient in duration. However sodium salicylate was found to have minimal impact on the reduction of the induced lameness.

In this study, amphotericin B was used in a similar manner to the Kotschwar study to create a transient synovitis arthritis model for assessment of flunixin meglumine, a nonsteroidal anti-inflammatory drug. This current study found the amphotericin B-induced synovitis model to provide a controllable and sustained, but transient painful insult. The lameness peaked between 6 and 12 hours post-induction in this current study as judged by visual lameness assessment, with relatively quick resolution of lameness in both the control and flunixin treated steers. While the severity and duration of lameness in the control steers were similar to the findings in Kotschwar's study, they were significantly less than that described in the equine literature, with equine studies documenting moderate to severe lameness (grade 3-4/5) of 3 days to 2 weeks in duration[107-117]. This noted decrease in severity and duration of lameness in cattle may be due to several reasons, including that only one injection of amphotericin B was administered as opposed to several injections in the majority of the equine studies. The selected joint may also affect the severity and duration of lameness as amphotericin B was injected in the distal interphalangeal joint rather than one of the carpal or tarsal joints. And cattle can transfer a significant amount of weight from one claw to another which likely decreases pain associated lameness in the affected claw[58,59]. These findings are likely an indication of species difference with regard to severity of synovitis and clinically apparent signs of lameness and thus pain.

This study documented by the quick return to normal locomotion scores in the control steers over the course of several days. This is advantageous as the amphotericin B model allows for evaluation of intervention measures for a prolonged pain insult not possible with more acute pain models such as castration and dehorning. The moderate severity of lameness coupled with the transient nature of the synovitis is advantageous for the welfare concerns of research cattle as the cattle return to soundness quickly. The duration provides ample time to determine the efficacy of preemptive and interstudy flunixin meglumine administration in amelioration of lameness as the half life flunixin meglumine in cattle is approximately 5.2 hours[119].

Previous studies have evaluated the effect of cyclooxygenase inhibitors such as ketoprofen in naturally acquired lameness[80,79]. Ketoprofen was found to modulate gait minimally, however it could not be ruled out that factors other than claw pain were responsible for the alterations in gait[80]. The studies were conducted in cattle with lameness of various etiologies, thus limiting interpretation of initial degree of pain and response to the course of drug therapy. While the acquisition of cattle with naturally acquired lesions circumvents the ethical concerns of induction of pain in bovine research, it can be difficult to completely characterize and source and severity of the lesions involved. The lack of correlation between naturally acquired lesions and lameness resolution underscores the importance of a validated, reliable and reproducible model of pain in cattle such as the amphotericin B-induced synovitis model.

Visual lameness assessment successfully delineated between the flunixin meglumine treated steers and control steers. It was found that the control steers were more than twice as likely to be lame as flunixin treated steers at any time point during study. The dramatic difference between the control steers and the flunixin treated steers with regard to odds of lameness was likely influenced by the preemptive administration of flunixin meglumine in this study. It is likely that sole post-induction administration of flunixin meglumine would not have diminished the inflammatory cascade to the same degree as preemptive administration of flunixin meglumine, leading to a less dramatic difference between the two groups. Future research will be necessary to confirm this.

After induction of arthritis, the flunixin treated steers ambulated differently than control steers as revealed by pressure mat analysis. This was manifested primarily through increased force and contact area in the affected limb, with a trend of increased impulse. In a similar pattern, the paired claw (same limb as the affected claw) had increased force, impulse, contact area as well as a trend of increased peak force values. These findings suggest that flunixin meglumine administration was successful in the blunting of inflammatory pain associated with the synovitis arthritis through inhibition of prostaglandin production. Flunixin treated steers were less painful on the affected limb than their control counterparts, and this was evidenced by the shifting of the above mentioned values (force, impulse, contact area, and peak force) toward affected limb, albeit primarily to the paired claw. This suggests that while the flunixin steers were more comfortable bearing weight on the affected limb, they did have some residual discomfort was present in the affected claw, leading to increases in contact area and shifting of force onto the paired claw.

Flunixin treated steers were also noted to have increased contralateral foot contact area. This finding does not make intuitive sense as one would expect decreased contact area in the contralateral limb as compared to control steers if flunixin was providing some analgesic relief in the affect limb. However what we know about the driving forces of gait and force distribution in cattle is still rudimentary. Weight shifting has been shown to occur in cattle with sensitive or painful claws [58,59] but lame cattle can alter gait not only by the reduction of vertical ground forces in affected limbs but also through the modification of horizontal acceleratory and deceleratory forces[61]. More simply, force and associated limb acceleration can be altered in multiple planes to provide pain relief in cattle. It is likely that the force and contact area distribution in these steers is dynamic between multiple limbs. As only the rear limbs were assessed in this study, it is possible that the front limbs were contributing to these changes as well. Increased comfort in the flunixin treated steers may have led to redistribution of weight onto the rear limbs.

Although the pre-induction and post-induction values measured in this study were not directly compared, several inferences could be made based on the timeline of arthritis induction and the general trends of the data. Regardless of whether or not the steers received flunixin meglumine, there were changes in the integral values over the course of the study in the paired claw (the same limb as the affected claw). These changes were consistent with the time course of the synovitis arthritis as evidenced by a significant increase in the integral of the paired claw noted at the predicted peak of lameness six hours post-induction of arthritis. As the integral is a function of pressure over time to account for variation in acceleration, this increase is to be expected in a steer that is bearing a significant amount of weight or force on a small surface area as is seen when animals are "toe touching lame." Trends in the maximum force and maximum peak force were also noted in the paired claw. Although the associations are more tenuous in nature, the maximum peak force did tend to increase toward the end of the study, indicating that the steers were likely placing increased amounts of force on the paired claw as they became more comfortable. These findings were true of both the control and treatment groups, suggesting that although flunixin administration appeared to mitigate some of the pain associated with the synovitis arthritis, it did not completely eliminate the pain as evidenced by the similar trends in both groups of steers over the time period of the study.

The duration of the stance phase of both limbs varied over time. In the affected limb, a trend towards increased stance duration was noted, particularly at the time of peak lameness (T=6). The duration of the stance phase tended to decline over the course of the study, with the duration approaching pre-induction times towards the conclusion of the study. In the same manner, the contralateral limb stance duration was significantly increased in the initial post-induction phase of the study (T=12) at which point the duration steadily declined until it approached pre-induction levels at the end of the study. These findings support the transient nature of the lameness produced by the amphotericin B synovitis arthritis model. The increased duration of stance is consistent with a decreased overall locomotion speed as was anecdotally noted after induction of arthritis. Decreased locomotion speed has been correlated with lameness [120].

Physiologic variables such as heart rate, respiratory rate and temperature are often used to assess pain in the research and clinical setting. While physiologic parameters can be viable markers of pain as was shown in one castration study [92] where high heart rates were correlated to pain, these parameters are typically most accurate in the assessment of acute pain. In this study the heart rate, respiratory rate and the rectal temperature of the study steers were statistically different over the time course of the study. Of these parameters the heart rate was statistically elevated at 12 hours post-induction of arthritis. The respiratory rate and rectal temperature in the study steers had general trends of increased values as the study progressed. Based on these findings, it is likely that the noxious event of arthritis induction, pain and perhaps the stress of the procedure led to increased heart rates in the initial post-induction period. Further interpretation of these findings is difficult due to the fact that the stress of the procedure or noxious event combined with a fear of the people involved can make interpretation of elevated parameters such as heart rate difficult [21, 93]. Heart rate may be an indicator of acute pain however further research would be necessary to confirm this. Factors such as the ambient temperature and humidity can affect parameters such as heart rate, respiratory rate and core body temperature which may explain the rise in respiratory rate and rectal temperatures over the course of the study. The chronicity of the tissue insult can also lead to less predictable physiologic sympathetic and hypothalamo-pituitary-adrenocortical axis responses.

As a stress-induced hormone, cortisol is just one part of a complex physiologic response to stress. When the brain perceives stress the hypothalamo-pituitary-adrenocortical axis is activated, leading to glucocorticoid release from the adrenal glands [88]. While plasma cortisol measurements have been the most extensively used assessment tool of pain-induced stress in acute bovine pain models such as dehorning [23], the relationship between stress and plasma cortisol concentration is not always linear [89]. In addition to other factors, handling induced stress must be differentiated from pain-induced stress. In this study, the plasma cortisol levels were significantly different over the time course of the study. The increase in plasma cortisol levels noted at 6 hours post-induction of arthritis may correspond to either psychological distress, pain-induced stress or a combination thereof. Although the control steers had consistently higher cortisol levels after induction of arthritis, there was no significant difference between the treatment groups. This suggests that either flunixin meglumine was ineffective at alleviating pain-induced stress or that plasma cortisol analysis was an insensitive tool of pain assessment in this model. Based on the other findings of the study, it is likely that the initial spike in plasma cortisol levels is consistent with pain however further research will be needed to ascertain the significance of cortisol measurements in the amphotericin B-induced lameness model.

The accelerometry results in this study characterized the behavioral manifestations of amphotericin B induced synovitis arthritis lameness in steers. In this study there was a significant interaction between time and treatment with regard to the activity levels of the study steers. In particular, the flunixin treated steers spent significantly less time in recumbency than their control counterparts during the early post-induction phase of the trial. As the trial progressed, the difference in activity level between the flunixin treated group and the control steers slowly diminished. This is to be expected as the amphotericin B lameness model appeared to peak in severity 6-12 hours after induction of arthritis and flunixin meglumine was administered at 0 and 12 hours. As a non-specific prostaglandin inhibitor it is expected that flunixin meglumine would exhibit its full effect at the peak of the model's pain and inflammatory cascade 6 to 12 hours after induction.

Based on the pressure mat, gait and accelerometric analysis findings, the amphotericin B induced synovitis arthritis model produced a moderate but transient lameness. Flunixin meglumine proves to be efficacious in providing analgesia in an amphotericin B induced lameness model in dairy steers. Future research is warranted into researching the dose dependent effects analgesic effects of flunixin meglumine. Eventual crossover into clinical populations will be needed to prove the efficacy of flunixin meglumine in providing effective analgesia to cattle with naturally acquired lameness.

Figures and Tables

Table 2.1 Assessment Criteria for the Anderson Lameness Scoring System for Cattle

Lameness Score	Assessment Criteria
0	Normal gait
1	Mild: walks easily, readily; bears full weight on foot and limb but has an observable gait alteration; stands on all four limbs; line of back bone normal
2	Moderate: reluctant to walk and bear weight but does use the limb to ambulate; short weight-bearing phase of stride; rests the affected limb when standing; increased periods of recumbency, may see arching of back bone
3	Severe: reluctant to stand; refuses to walk without stimulus, non-weight-bearing on affected limb; " hoops" over limb rather than bear weight; does not use limb when standing and lies down most of the time; backbone arched with caudoventral tip to pelvis
4	Catastrophe: recumbent; unable to rise; humane euthanasia often indicated

Table adapted from Anderson et al. (2001)

Table 2.2 Effect of treatment (flunixin meglumine) on pressure mat variables

Limb/Claw							
Arthritic limb	**Treatment group**	**Maximum force LS Means**	**Mean force LS Means**	**Impulse LS Means**	**Mean area LS Means**		
	Control	82.7	52.3	37.1	28.9		
	Flunixin	103.3	67.2	56.1	36.2		
	p value	0.03	0.05	0.06	0.04		
	Std. error	14.5	14.5	14.5	14.5		
Paired claw	**Treatment group**	**Maximum force LS Means**	**Mean force LS Means**	**Impulse LS Means**	**Maximum area LS Means**	**Mean area LS Means**	**Mean peak force LS Means**
	Control	38.9	20.5	14.6	18.6	11.6	15.0
	Flunixin	49.1	30.4	25.3	23.3	16.2	18.7
	p value	0.02	0.005	0.03	0.02	0.004	0.06
	Std. error	10.1	6.3	6.9	3.0	2.2	3.2
Contralateral limb	**Treatment group**	**Maximum area**					
	Control	45.2					
	Flunixin	50.1					
	p value	0.04					
	Std. error	8.4					

Generalized linear models included fixed effects of treatment, time and random effects to account for lack of independence of individual measurements related to replicate (steer grouping) and repeated measures on individuals. P<0.05 is considered statistically significant.

Figure 2.1 Overview of study

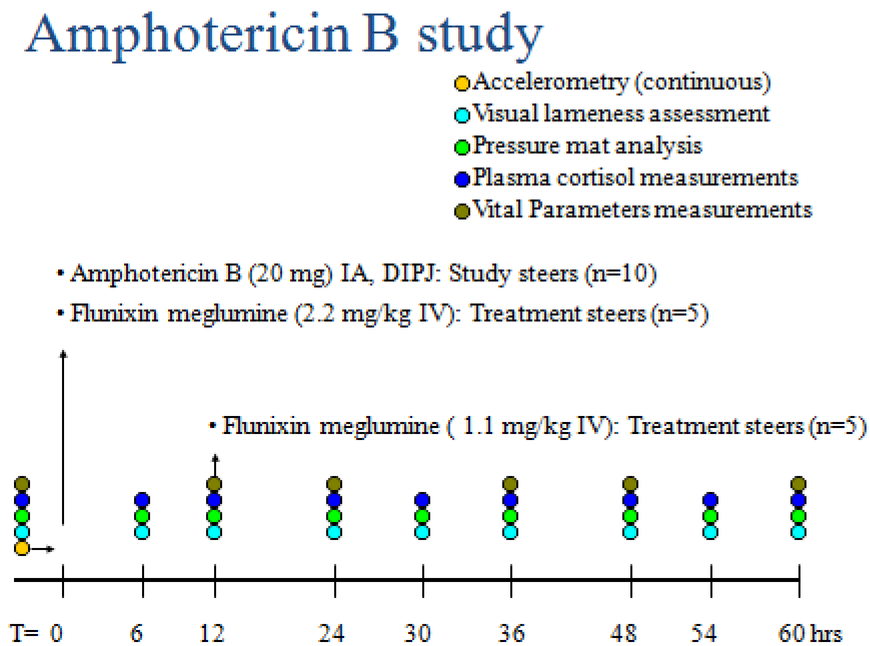


Figure 2.2 Impulse and integral calculation

Impulse: The integral of force with respect to time

$$F\Delta t = m\Delta v$$

Where $F\Delta t$ is the change in force over time (impulse) and $m\Delta v$ is the change in momentum

Integral: The integral of pressure with respect to time

$$P\Delta t$$

Where $P \Delta t$ is the change in pressure over time

Figure 2.3 Average Visual Lameness Scores of Flunixin Treated steers versus Control steers during trial

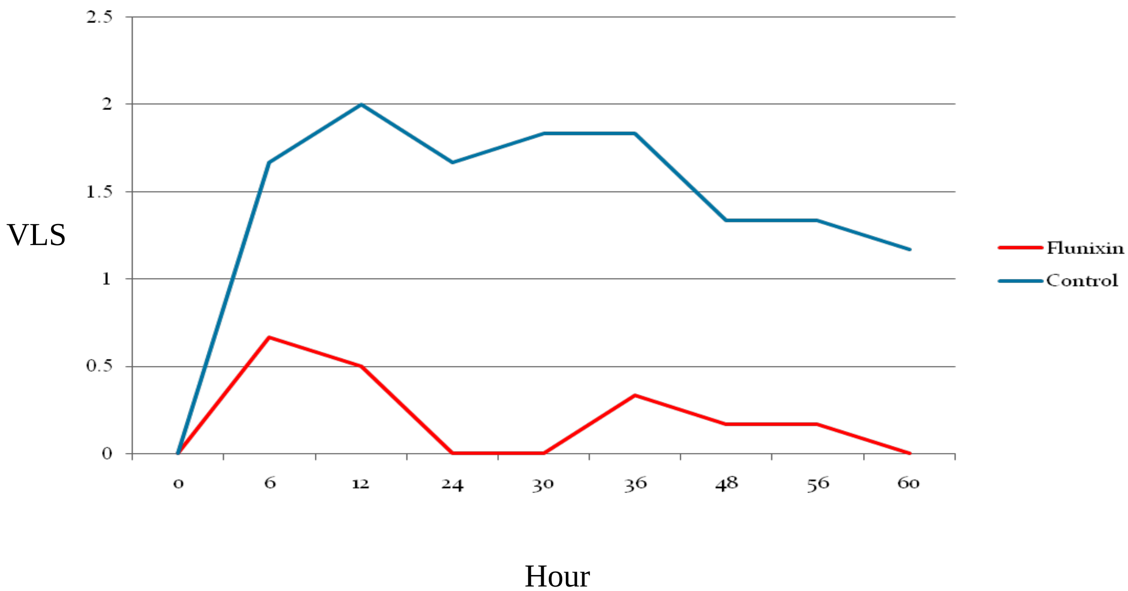


Figure 2.4 The integral LS means of the paired claw in the study steers over time

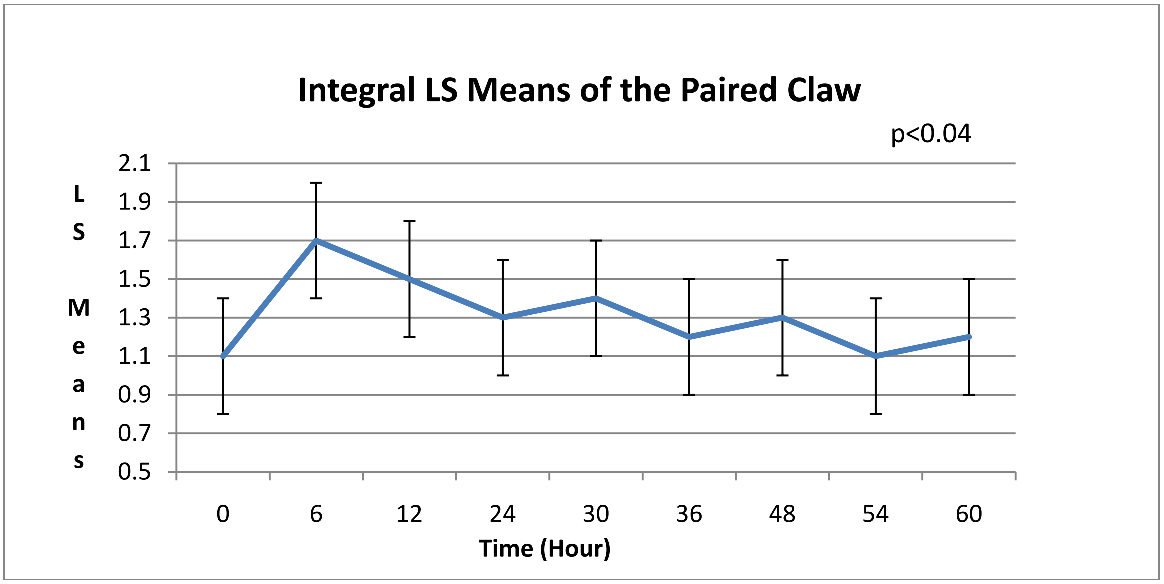


Figure 2.5 Maximum force LS means of the paired claw in study steers over time

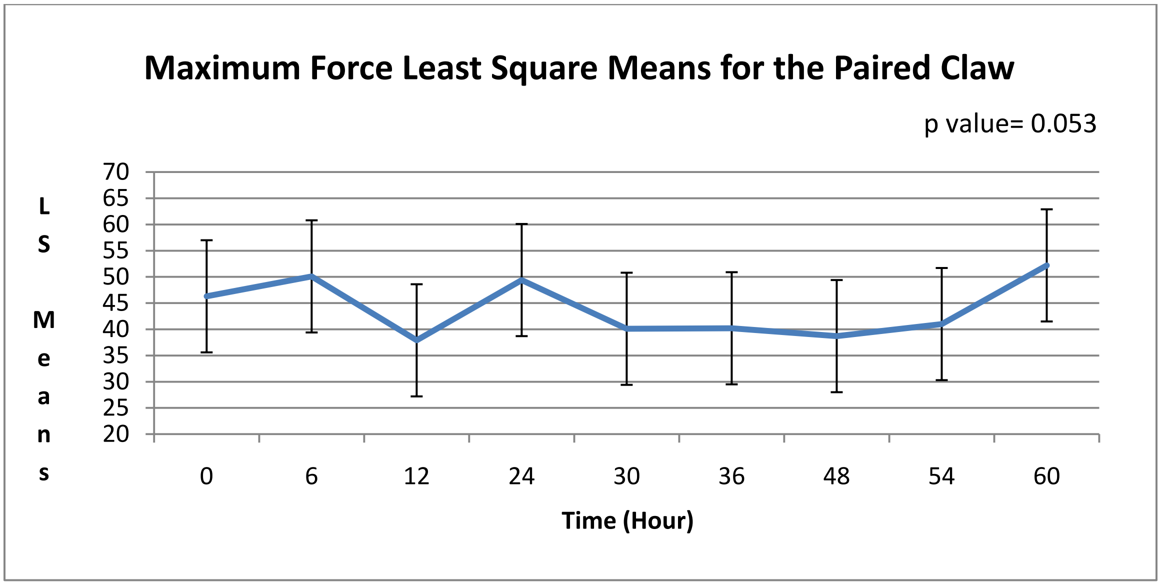


Figure 2.6 Maximum peak force of the paired claw in study steers

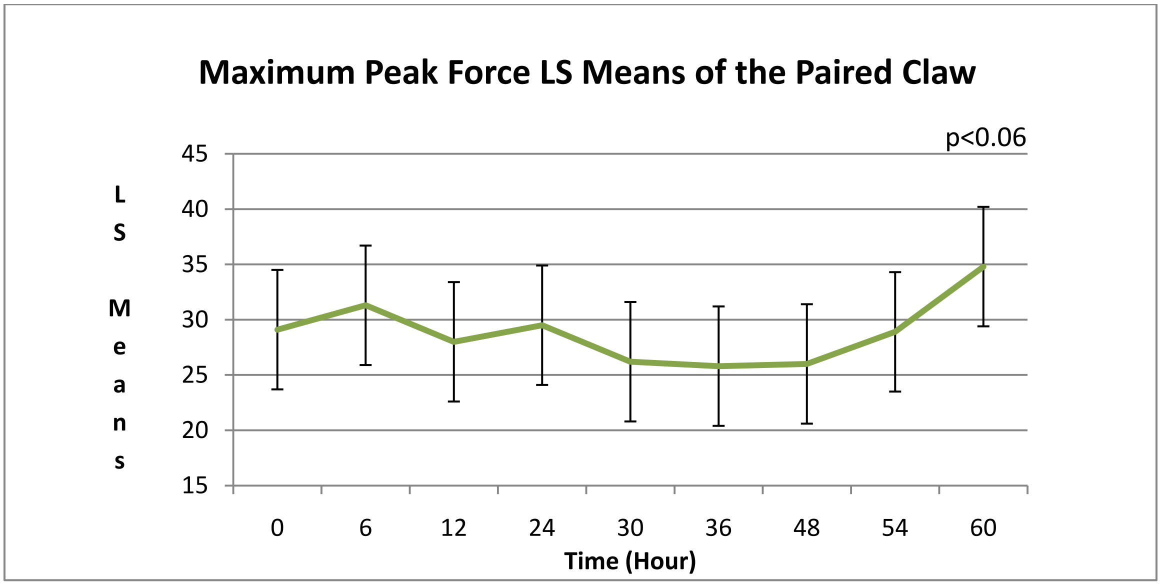


Figure 2.7 Stance duration of the affected limb in study steers

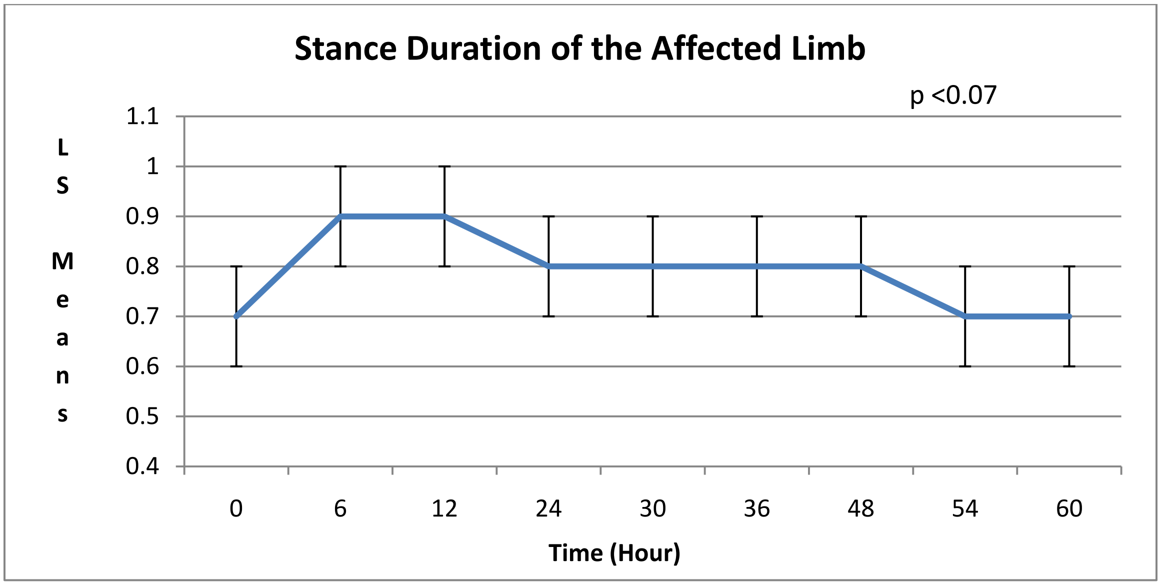


Figure 2.8 Stance duration of the contralateral limb in study steers

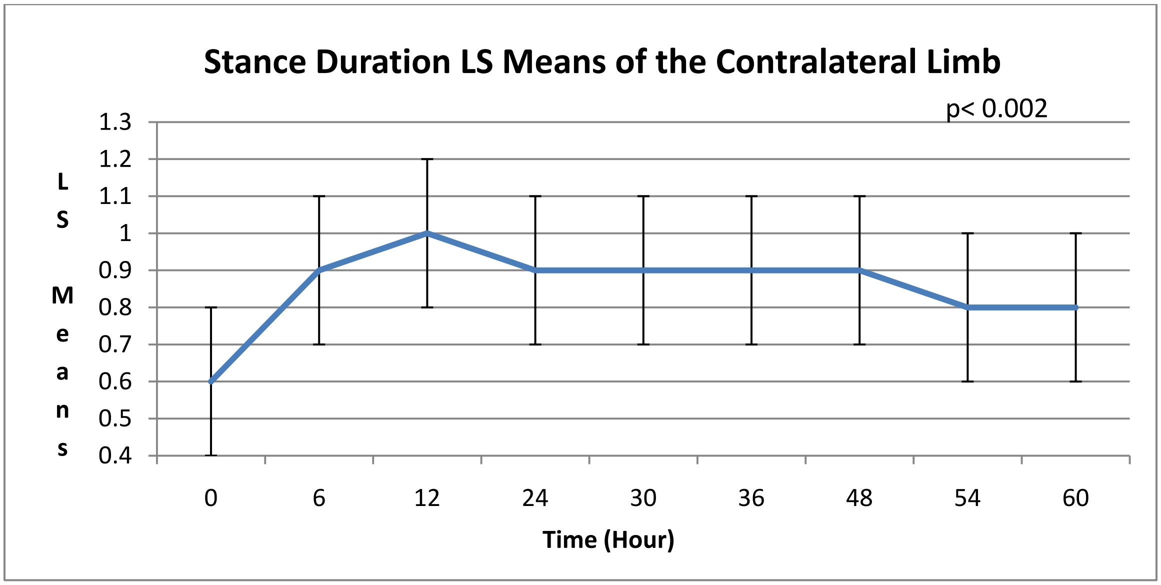
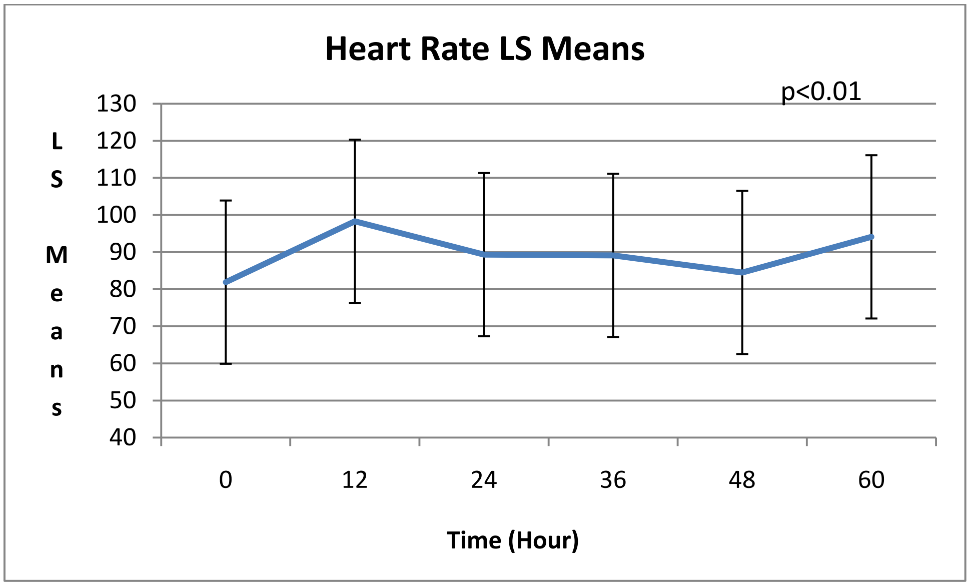
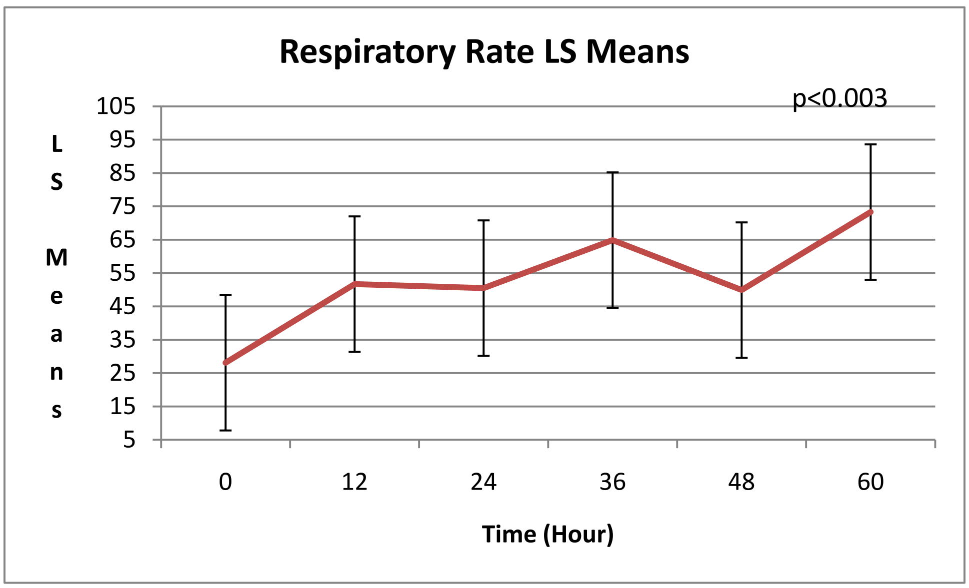


Figure 2.9 Time-dependent physiologic variables: Heart rate, respiratory rate, rectal temperature LS means over time





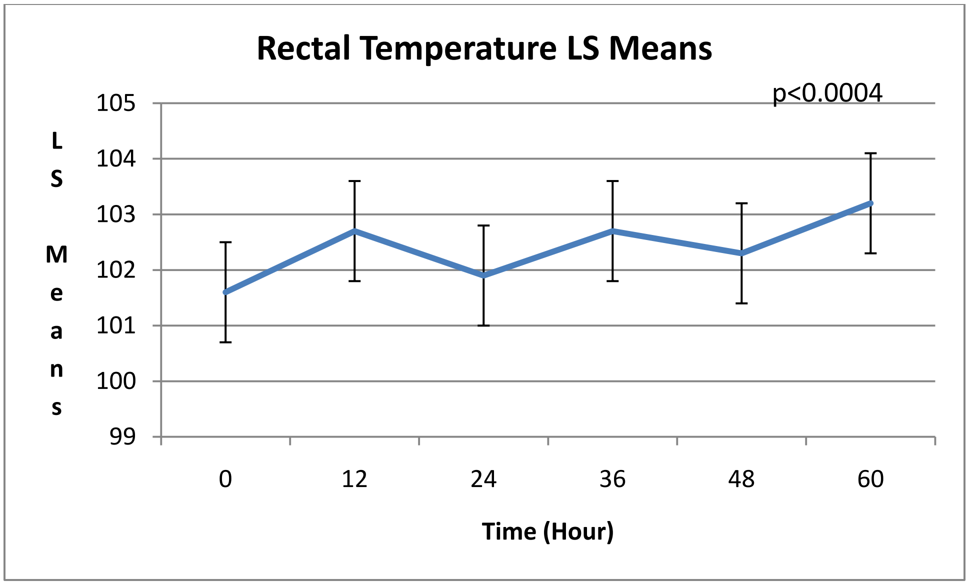

Rectal Temperature LS Means
p<0.0004
LS Means
105
104
103
102
101
100
99
0
12
24
36
48
60
Time (Hour)

Figure 2.10 Percentage recumbency based on trial day* treatment interactions using accelerometry in study steers

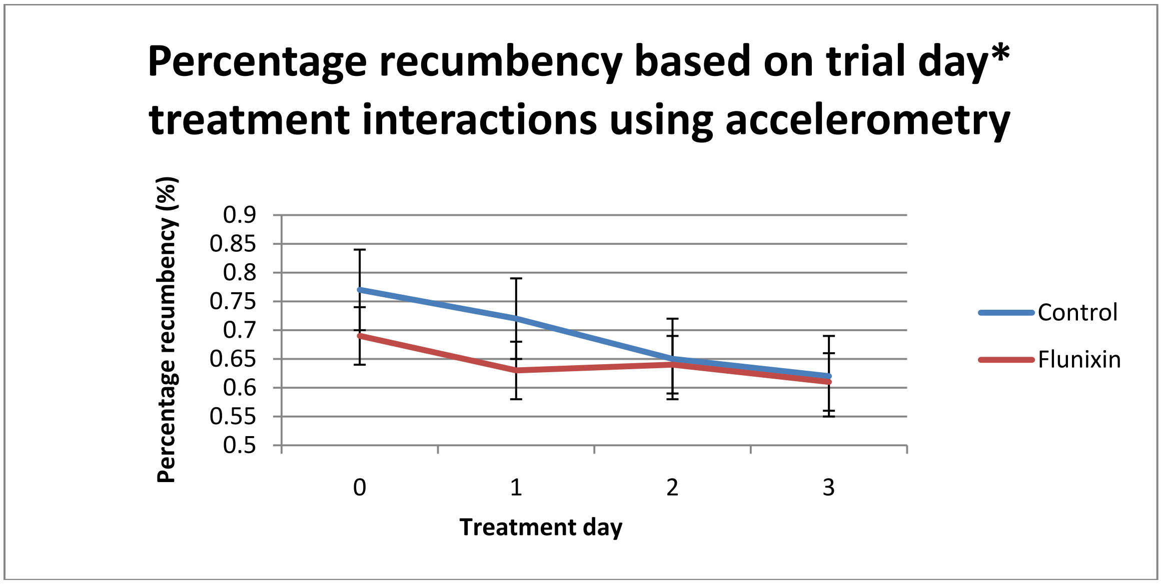
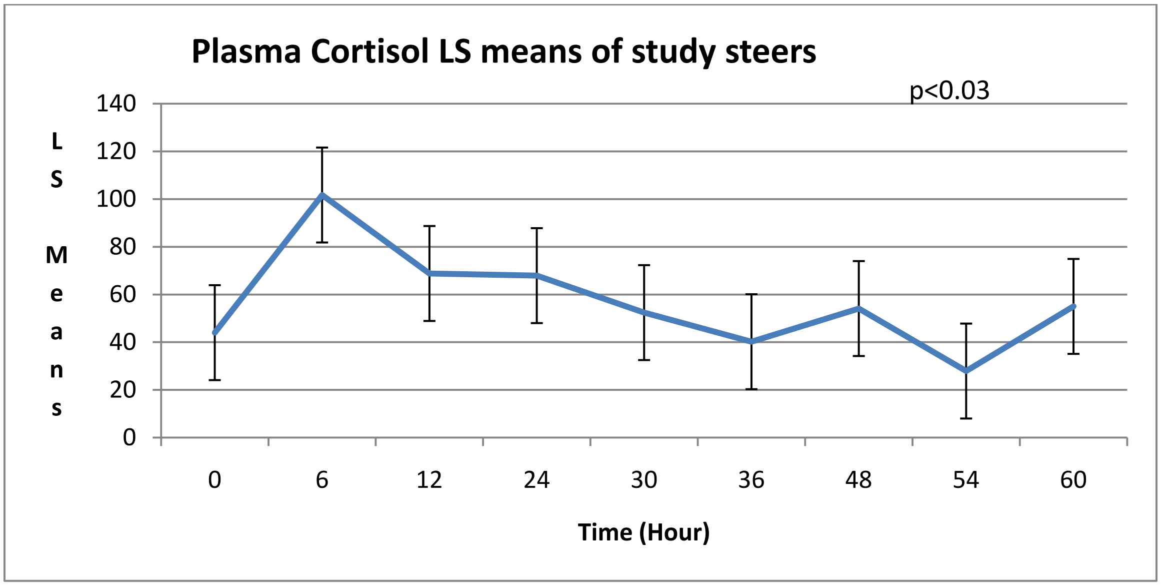


Figure 2.11 Plasma cortisol LS means over time



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