LAMENESS AND PAIN IN DAIRY COWS

by

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DVM, University of Ljubljana, Slovenia, 1998

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(ANIMAL SCIENCE)

THE UNIVERSITY OF BRITISH COLUMBIA

December, 2005

ABSTRACT

Pain associated with the injuries of hoof and surrounding tissues is an important cause of lameness. However, this condition can also be caused by non-painful changes of the locomotion system. The objective of this study was to detect the attributes of impaired gait that are associated with pain. Gait was evaluated using a subjective scoring system and objective kinematic measures, while putative pain was controlled with the known analgesic, ketoprofen. Lactating Holstein cows (n=27), diagnosed with lameness were divided into 3 groups. Each group was assigned a different dose of ketoprofen (0, 1.5, and 3.0 mg/ kg BM). The experimental period lasted 9 d and was divided into 3 phases: before, during and after treatment, each phase lasting for 3 d. During treatment cows were treated with the IV injections of assigned dose of ketoprofen. On each day of the experiment the cows' gait was recorded and video clips were analyzed using both subjective and objective methods. Subjective assessment of gait included evaluation of overall gait score and gait attributes including head bob, tracking-up, back arch and reluctance to bear weight. Kinematic measurements included stride length, maximum stride height, stride duration and hoof speed. Results of subjective gait assessment showed an improvement in overall gait score and tracking-up in cows receiving the analgesic. Kinematic analysis of gait revealed time trends for all kinematic measurements throughout the experimental period hindering the analysis of the analgesic effects. In conclusion, pain affected lameness attributes, specifically overall gait score and tracking-up. Kinematic methods may provide a sensitive method of detecting other changes in gait.

(Keywords: lameness, pain, cattle, ketoprofen, gait scoring)

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BM	Body Mass
DIM	Days in Milk
HS	Hoof Strike
IV	Intra-Venous
NAHMS	National Animal Health Monitoring System
NRS	Numerical Rating Score
NSAID	Non-Steroid Anti-Inflammatory Drug
SD	Standard Deviation
SEM	Standard Error of the Mean
TMR	Total Mixed Ration
TO	Toe Off
VAS	Visual Analogue Scale

ACKNOWLEDGEMENTS

I would like to thank the members of my supervisory committee Dr. Marina von Keyserlingk and Dr. Dan Weary from the UBC Animal Welfare Program, and Dr. David Sanderson from the UBC School of Human Kinetics for their support, guidance, and encouragement.

Many thanks to Frances Flower for all her help with setting up my experiment, recruiting the cows, the training for gait scoring and kinematic analysis and much appreciated feedback throughout this project, and to Trevor De Vries for his help with statistical analysis.

I am grateful for all the support and help from the staff of UBC Dairy Education and Research Centre in Agassiz, BC. Thank you for your patience with a city slicker like I am, and sorry again for the unfortunate incidence of "letting the cows run free", although they probably enjoyed it. I greatly appreciate the assistance of Tomasz Grudnik and Julie Woodward during the experiment.

This project was made possible with the financial support by the Natural Sciences and Engineering Research Council of Canada (NSERC) through the Industrial Research Chair in Animal Welfare, and by contributions from the Dairy Farmers of Canada, the BC Dairy Foundation, the BC SPCA, members of the BC Veterinary Medical Association, and many others listed on the website of the Animal Welfare Program (http://www.landfood.ubc.ca/animalwelfare/index.htm). Ketoprofen was generously supplied by Merial Canada Inc. I would also like to thank Ad futura - Science and Education Foundation of the Republic of Slovenia for their financial support and UBC's

Faculty of Land and Food Systems and Faculty of Graduate Studies for the James A. Shelford Memorial Scholarship and the International Partial Tuition Scholarship.

I owe great thanks to many of my fellow students in the Animal Welfare Program. Thank you for numerous trips to the local Starbucks and sharing with me the misery of Vancouver's winter weather under the coil heaters. This is also in recognition of Crew Point 05 (including Nuni) and its "big time facilitations". Never cease fighting for the truth!

I thank my family and friends on the other side of the planet for their struggle in trying to understand what project I am currently running and when I am finally coming home; *Dragi vsi, hvala vam za trud, da bi razumeli, s čim se v bistvu ukvarjam. Opravičujem se za nenehno odlašanje vrnitve domov. Ta projekt mi je vzel nekaj več časa, kot sem pričakoval, a sem prepričan, da je vreden truda.*

And finally, thank you Vancouver. These two and a half years have been a great adventure that I will never forget.

Mitja

CO-AUTHORSHIP STATEMENT

The study was designed collaboratively by Mitja Sedlbauer, Dr. Dan Weary, Dr. Marina von Keyserlingk, Dr. David Sanderson and Frances Flower. Mitja Sedlbauer performed the experiment with the aid of the assistants, analyzed all data and prepared the manuscript under the guidance of Dr. Weary, Dr. von Keyserlingk, and Dr. Sanderson.

Chapter I

General introduction

Welfare and Economic Concerns

The importance of lameness in dairy cattle has been increasingly recognized in the last two decades (Kelton et al., 1998; Rushen, 2001) and is now considered one of the most urgent health and welfare problems of dairy cattle as well as one of the most significant economic issues for the dairy industry (Whitaker et al., 2000; Gröhn et al., 2003).

Pain associated with lameness clearly decreases the welfare of cows (Whay et al., 1997, 1998; O'Callaghan, 2002). Some of the first evidence establishing that claw lesions, such as sole ulcer and sole haemorrhages, and lameness are associated with pain was provided by Whay et al. (1997). Using decreased nociceptive thresholds as indicators of chronic pain, these authors reported that different claw lesions cause pain of different intensities (Whay et al., 1998). Pain likely influences both individual and social behaviour of affected animals. For example, lame cows have reduced daily activity levels (O'Callaghan et al., 2003), spending more time lying and less time feeding (Galindo and Broom 2002). Moreover, lame cows are less likely to start social interactions with other cows, although they are as likely to be subjected to aggressive behaviour by other animals, as sound cows (Galindo and Broom 2002).

The impact of lameness on the economy of dairy production is threefold. Firstly, numerous studies have quantified the effect of lameness on milk production. One study, reported that total mean estimated reduction in milk yield per lactation was 360 kg (Green et al., 2002). Lame cows have been reported to produce 0.5- 1.5 kg less milk per day than cows that have not been diagnosed as lame (Warnick et al., 2001). Secondly, a commonly overlooked complication of lameness is delayed ovarian cyclicity. Garbarino et al. (2004) found that lame cows had 3.5 times greater odds of exhibiting delayed ovarian cyclicity than cows not affected by lameness (sound animals). Further, median interval from calving to first luteal phase was longer in lame and moderately lame cows (36 and 32 d, respectively), when compared to sound cows (29 d) (Garbarino et al., 2004). Lastly, lameness has been cited as one of the primary factors contributing to involuntary culling of lactating dairy cattle. Whitaker et al. (2000) performed an analysis of involuntary culling on 340 farms in southern England and found that on average 1.7% (range 0-5%) of the herd is culled annually due to lameness. Booth et al. (2004) measured survival as the time between calving and death or sale and found that cows diagnosed with lameness during the first half of lactation had a hazard ratio 2 times that of sound cows, regardless of the cause of lameness.

Epidemiology

Incidence and prevalence of lameness vary greatly in herds within and between countries (e.g. Clarkson et al., 1996; Cook, 2003). In addition to geographical variability, seasonal differences in incidence and prevalence of lameness are also evident (Wells et al., 1993; Clarkson et al., 1996; Murray et al., 1996; Whitaker et al., 2000; Cook, 2003). Herds observed in the study by Cook et al. (2003) were divided into quartiles. The 25% of herds having the least amount of lameness had a prevalence of lameness lower than 15%. On this basis, Nordlund et al. (2004) suggested 15% as the lowest achievable prevalence for confined dairy cattle but cautioned that this target may vary with different management systems (i.e. loose-housed, tie-stall, access to pasture) applied on different farms (Nordlund et al., 2004).

Greenough et al. (1981) estimated that the incidence of lameness in UK varied from 5 to 50%. Recently the US based National Animal Health Monitoring System (2003), reported an annual incidence of lameness of (mean ± SEM) 20.4% ± 1% in dairy cows and 8.1% ± 0.7% in bred heifers. Clarkson et al. (1996) reported the mean annual incidence of lameness across 37 dairy farms in 5 regions of Great Britain to be 54.3% with a mean prevalence of 20.6%. Interestingly, the mean incidence and prevalence for 37 farms were reported to be higher in winter (31.7% and 25.0%, respectively) than in summer (22.9% and 18.6%, respectively). A considerably lower annual incidence of lameness (23.7%) of dairy cattle in Great Britain was reported by Whitaker et al. (2000). However, similar to that reported by Clarkson (1996), there was an uneven distribution between winter and summer (13.7% and 10.0%, respectively) in this study.

A recent study performed in Wisconsin, USA reported seasonal differences in the prevalence of clinical lameness at (mean ± SD) 21.1% ± 10.5% in the summer and 23.9% ± 10.7% in the winter (Cook, 2003). Wells et al. (1993) also studied the mid-western region of the US but found a lower prevalence of lameness in lactating cows; 13.7% in winter and 16.7% in spring. Factors contributing to this discrepancy may include differences in farm management, a general increase in lameness among dairy cattle, and improved methods of detecting lameness. Increasing herd size may also be a factor, as large herd size has previously been implicated as a risk factor for lameness (Whitaker et al., 2000).

Seasonal differences in incidence and prevalence may be due to differences in management of cattle during winter and summer, especially in herds that are kept on pasture during the summer (Murray et al., 1996; Whitaker et al., 2000). An interesting issue in interpretation of seasonal differences is that lameness often develops some time after hooves have been injured (Murray et al., 1996) indicating that lameness caused during one period (e.g. winter), may be expressed in the other.

Aetiology

Greenough et al. (1981) define lameness as a clinical sign of disease or abnormality of the musculo-skeletal system. According to these authors, lameness can develop as a voluntary effort to reduce pain due to injury (supporting limb lameness and swinging limb lameness) or as an involuntary (mechanical) impairment of gait due to damaged muscles, ligaments or nerves. Moreover, it is estimated that in 90% of cases, lameness is caused by inflammation or injury of the distal parts of the limb (i.e. hoof and skin in digital area) (Webster, 1987; O'Callaghan, 2002).

Several hoof and skin pathologies are mentioned as causes of lameness in dairy cattle. Greenough et al. (1981) classify diseases of the distal parts of the limb in two groups: a) diseases of the digital skin and subcutis; including digital and interdigital dermatitis, interdigital necrobacillosis, verucose dermatitis, interdigital skin hyperplasia and injuries and, b) diseases of horn and sensitive *laminae*; including lesions of horn wall (horn fissures), sole ulcer, traumatic pododermatitis, white line disease and heel erosion. However, lameness in dairy cattle is most often attributed to the presence of sole ulcers, white line lesions, sole haemorrhages and digital dermatitis (Murray et al., 1996).

Diagnostics

Several methods of detecting and measuring severity of lameness in dairy cattle are currently in use. The fact that pain is one of the causes of lameness in dairy cattle, poses a specific problem in diagnostics of this condition. The stoical nature of *Bovidae* has been described as an evolved survival strategy from the wild enabling species of prey to mask any signs of pain and discomfort and thereby avoiding the attention of predators (O'Callaghan, 2002). Pain in herd animals is therefore often difficult to detect before the causal condition has progressed to the stage when the inherent mechanisms to compensate for the pain have been exhausted. The importance of skill in diagnosis of lameness in dairy cattle was illustrated by Whay et al. (2002), who found that farmers failed to identify three cases of lameness out of four as identified by a trained observer.

Hoof Health Inspection. Based on the assumption that a vast majority of lameness cases are caused by injuries of the distal parts of the limb (O'Callaghan, 2002; Webster, 1987), hoof health inspections (Greenough & Vermunt, 1991) are used to detect the presence and severity of injuries. Injuries of the corium result in sole haemorrhages. Under normal conditions of hoof growth and wear these become visible 8 to 10 weeks after the injury has occurred (Bergsten and Frank, 1996; Lischer and Ossent, 2000). This method, therefore reflects damage inflicted weeks or months before the examination and thus fails to offer accurate insight to the present status of the hoof. However, in the case of faster developing conditions of the distal limb, such as digital and inter-digital dermatitis, hoof inspection can give an accurate insight into possible causes of lameness. Another disadvantage of hoof health inspection is that not all lame cows have visible hoof injuries (Winkler and Willen, 2001), suggesting again that the delayed expression of hoof injuries is an important problem in diagnostics of lameness. Further, the cause of lameness is not unique to the hoof since it can also be caused by injuries of more proximal parts of legs or even other parts of the locomotor system.

Locomotion Assessment. Severity of lameness can also be assessed using direct observations of a cow's gait (e.g. Manson & Leaver, 1988; Sprecher et al., 1997). These methods typically employ observations of behaviours and measurements of gait attributes associated with impaired gait of injured cows. These techniques can be divided into subjective and objective methods.

Gait scoring, often referred to as locomotion scoring, is a commonly used umbrella term describing subjective techniques. Subjective techniques currently available typically rate lameness on a numerical scale according to presence and intensity of behaviours associated with lameness. The first scoring system developed by Manson and Leaver (1988) in identifying lame dairy cattle, employs a numeric rating score (NRS) – a scale of nine points (1-5, including half-scores), where scores greater than 3 represent clinical lameness. An obvious drawback of this system is the lack of clear definitions of observed behaviours making it difficult to score cows consistently. Specific behaviours observed in this system are presence of abduction and adduction, difficulty in turning, rising and walking. More elaborate definitions of lameness are offered in later studies. Retaining the principle of NRS these newer systems focus on behaviours such as vertical head movement, length of stride and tracking-up (Tranter and Morris, 1991; Whay et al., 1997), asymmetry of gait (Wells et al., 1993) and arched back (Sprecher et al., 1997). The most recent system mentioned in the relevant literature is described by Flower and Weary (2005). In addition to an overall NRS (Table 1.1), these authors independently evaluated six distinct behaviours (Table 1.2) using a visual analogue scale (VAS). VAS scores are recorded on a straight line where a written description of the extremes of the observed behaviour (min, max) is provided at each end of the line. The observer evaluates the behaviour by marking a point along the line that represents the intensity relative to two extremes (Welsh et al., 1993). Rather than assigning discrete scores as with NRS values, the VAS provides continuous data.

Table 1. 1. A numerical rating scale (NRS) for walking dairy cows (from Flower and Weary, 2005)

NRS	Description	Behavioural criteria
1.0	Smooth and fluid movement	Flat back Steady head carriage Hind hooves land on or front of fore-hooves (track-up) Joints flex freely Symmetrical gait All legs bear weight equally
2.0	Imperfect locomotion but ability to move freely not diminished	Flat or mildly arched back Steady head carriage Hind hooves do not track-up perfectly Joints slightly stiff Slightly asymmetric gait All legs bear weight equally
3.0	Capable of locomotion but ability to move freely is compromised	Arched back Steady head carriage Hind hooves do not track up Joints show signs of stiffness Asymmetric gait Slight limp can be discerned
4.0	Ability to move freely is obviously diminished	Obvious arched back Head bobs slightly Hind hooves do not track up Joints are stiff and strides are hesitant Asymmetric gait Reluctant to bear weight on at least one limb but still uses that limb in locomotion
5.0	Ability to move is severely restricted and subject must be vigorously encouraged to move	Extremely arched back Obvious arched back Poor tracking-up with short strides Obvious joint stiffness (lack of joint flexion with very hesitant and deliberate strides) Asymmetric gait Inability to bear weight on one or more limbs

Table 1. 2. Description of six behaviours associated with lameness, scored on a visual analogue scale (VAS) (from Flower and Weary, 2005)

Behaviour	Endpoints of VAS	
	Min	Max
Back arch	Flat or concave spine	Convex arch between the withers and tail base
Head bob	Steady and even head carriage	Pronounced, uneven head movement
Tracking-up	Hind hoof falls in imprint left by front hoof	Hind hoof falls short of imprint left by the front hoof
Joint flexion	Subject flexes and extends limbs through the normal range of motion	Limited flexion and extension resulting in stiffness
Asymmetric gait	Rhythmic 4-beat hoof placement	Arrhythmic hoof placement
Reluctance to bear weight	Bears weight equally over all legs	Uneven weight bearing among legs

The lack of clearly defined standards used during lameness scoring may contribute to the low repeatability of these scores, including high within-observer variation and low accuracy (Keegan et al., 1998; O'Callaghan et al., 2003). Analysis of within- and between-observer agreement is normally used to assess reliability of measurements acquired with subjective scoring systems (e.g. Keegan et al., 1998; Wells et al., 1993, Winkler and Willen, 2001). For example, Keegan et al. (1998) used a kappa coefficient to assess agreement within and between observers. Kappa coefficient is defined as an index of agreement between two ratings, corrected for chance. The coefficient takes values from 0 (agreement purely by chance) to 1 (perfect agreement) (Everitt, 1998). With the use of continuous variables, such as VAS (Welsh et al., 1993; Flower and Weary, 2005), a more appropriate tool for analysis of agreement is the calculation of coefficients of determination (R^2) between repeated observations by the same observer (within observer) or observations of multiple observers (between observers).

Subjective methods rely greatly on skill of observers (Keegan et al., 1998). They are also affected by subjective evaluation of behaviours. Given the shortcomings with subjective lameness assessments, there is a need to develop alternative, more measurable and objective diagnostic methods for lameness in dairy cattle.

Objective methods measure the physical properties of locomotion and express them in mechanical units. Biomechanics is a scientific discipline that studies biological systems using the methods of mechanical engineering. Two branches of biomechanics are commonly used in gait analysis: kinetics and kinematics. Kinetics involves quantifying forces, moments and masses involved in locomotion, whereas kinematics involves quantifying the motion (Whittle, 1996).

Recently kinematic methods have been adopted from horse locomotion analysis and introduced to analysis of cattle locomotion (Herlin & Derevemo, 1997; Ceballos et al., 2004; Flower et al., 2005). The cow's locomotion is recorded with high-speed video and then digitized to allow computer analysis of movements using specialized software. Measures include minimal and maximal joint angles during walking (Herlin and Derevemo, 1997) and stride attributes such as stride length, height, duration, speed and acceleration. A study by Flower et al. (2005) compared the gait characteristics of healthy and lame dairy cows using the kinematic method and found that strides of sound cows were faster, longer and had shorter durations than the strides of cows diagnosed with sole ulcers.

It is important to note that the kinematic study of gait in cattle is still at an early stage of development. Although it is premature to discuss the issues of applicability of this method in every day practice, it appears that kinematic methods offer a promising approach for use as a research tool for studying lameness in dairy cattle.

Pain and Lameness

Analgesics and anaesthetics can be used in assessment of pain. If an abnormal behaviour, such as lameness, of an animal normalizes when treated with analgesics but returns with the cessation of analgesic treatment, we can infer that the abnormal behaviour was caused by pain (Rutherford, 2002). Methodologically this idea presents challenges on several levels.

Lameness may not be caused exclusively by pain (Greenough et al., 1981). Gait impairment may be both voluntary (supporting and swinging limb lameness) and involuntary (mechanical lameness). In such a case an analgesic treatment would not be fully effective as mechanical impairments of the musculo-skeletal or nervous system would still be causing lameness. Even if lameness was due solely to pain, the analgesics may not be potent enough to alleviate the pain, resulting in the persistence of lameness. In both cases the researcher may be tempted to conclude erroneously that lameness was caused solely by non-painful impairment of the locomotor system. However, the pain might have existed as a primary or secondary cause, but was either too strong or was only one of the reasons for lameness, which therefore could not be mitigated by the analgesic.

Literature on assessment of lameness by means of controlling pain is scarce. Owens et al. (1995) employed a combination of subjective and objective methods to compare the effects of ketoprofen and phenylbutazone on chronic hoof pain and lameness in horses. They found that ketoprofen at 1.65 times the recommended dose significantly reduced chronic pain and lameness when compared to phenylbutazone. In a second study, behavioural observations were used to assess the effect of carprofen on lameness in broiler chicken (McGeown et al., 1999). This study found that lame birds were able to cross obstacles on their way to the feeder more quickly when they had been treated with analgesics.

Analgesics. Pain can be treated with narcotic and non-narcotic analgesics. Clinical use of both is limited by their properties. Opioids, as representatives of narcotic analgesics, are, in general, fairly potent analgesics. Their adverse effects, however, commonly include tolerance, dependence, respiratory depression, euphoria and sedation. Their sedation potency could also cause ataxia and general changes in gait. The most common members of the non-narcotic group of analgesics are non-steroidal anti-inflammatory drugs (NSAIDs). These have no significant effect on the central nervous system, they are less potent but can cause adverse effects such as gastro-intestinal upset.

The use of pain medication for lameness in dairy cattle has several drawbacks. The most important of these is that analgesics do not cure the cause of lameness but only offer short-term relief from pain. The application of analgesics often requires milk and meat withdrawal, affecting the economy of dairy farming. There are also practical and animal welfare concerns about administering analgesics since the majority of analgesics for use in cattle are designed to be administered intra-muscularly or intra-venuously.

Although it has been more than 100 years since the first NSAID compound, acetylsalicilic acid, was registered under the name Aspirin®, its exact mechanism of action was not fully elucidated until recently. NSAIDs act as inhibitors of enzymatic production of prostaglandins and other eicosanoids, which are produced after cell injury. Prostaglandins are important mediators of pain and inflammation that contribute to development of fever and lead to sensitization of nerve fibres (Christoph & Buschmann, 2002; Sunshine & Olson, 1994). Thus, NSAIDs act peripherally – at the origin of the pain -

and actually prevent pain from developing. It has been suggested that NSAIDs also have an effect on the neural system at the spinal level (Malmberg & Yaksh, 1992) but NSAIDs, do not bind to narcotic receptors.

Ketoprofen is widely used in human and veterinary medicine as a treatment of both chronic and acute pain (Christoph & Buschmann, 2002; Alkatheeri et al., 1999). Ketoprofen (2-(3- benzoylphenyl) - propionic acid) belongs to the class of arylpropionic acids of NSAIDS. It is a chiral compound, appearing as two enantiomers, namely R(-)- and S(+)-ketoprofen (Christoph & Buschmann, 2002; Igarza et al., 2004).

Pharmacokinetic properties of ketoprofen differ broadly when used in different species. For example, elimination half life is reported to be 25 min in horses (Jaussand et al., 1993) and 4.16 h in camels (Alkatheeri et al., 1999). In dairy cattle one study found the elimination half-life to be 29.6 min (De Graves et al., 1996). It has also been shown that pharmacokinetic properties of ketoprofen in dairy cattle significantly differ between animals in different physiological states (i.e. gestation, lactation, newborns) (Igarza et al., 2004).

Although pain control alone may not be the best treatment of lameness in every day practice, there is a potential for its use in the research of lameness. Experimental application of analgesics may help us explain how pain affects gait of injured cows.

Objectives

The objective of this study was to estimate what attributes of lameness in dairy cattle are caused by pain. For this purpose lame cows were treated with a mild analgesic and their gait was observed before, during and after application of the drug.

The expected changes in gait during the analgesic treatment would be limited to those caused by pain (i.e. voluntary rather than mechanical). The NSAID, ketoprofen, used in this study has a mild analgesic action. Therefore, it was reasonable to expect that gait attributes, caused by the most painful injuries, would not change due to the use of this analgesic.

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Chapter II

Lameness and Pain in Dairy Cows

INTRODUCTION

The importance of lameness in dairy cattle and its impact on production and animal welfare has been increasingly recognized over the last two decades (Kelton et al., 1998; Rushen, 2001). Caused mostly by injuries and inflammation of the distal parts of legs (Webster, 1987; O'Callaghan, 2002), lameness can have an effect on both individual and social behaviour of affected animals (Galindo and Broom, 2002; O'Callaghan et al., 2003). Lameness can also result in reduced milk production (e.g. Warnick et al., 2001; Green et al., 2002), delayed reproduction (Garbarino et al., 2004) and involuntary culling of lactating dairy cattle (Whitaker et al., 2000; Gröhn et al., 2003). Reports on incidence vary from 20% to 55% (Greenough et al., 1981; Tranter and Morris, 1991; Clarkson et al., 1996; Whitaker et al., 2000; NAHMS, 2003) and measures of prevalence vary from 10% to 30% in herds around the world (Wells et al., 1993; Clarkson et al., 1996; Whitaker et al., 2000; Cook, 2003; Tadich et al., 2005).

Lameness can be assessed using visual observations of a cow's gait (e.g. Manson & Leaver, 1988; Sprecher et al., 1997) or analyzed using objective methods, such as biomechanical techniques (e.g. van der Tol, 2004; Flower et al., 2005). Subjective methods typically rate lameness with a NRS according to presence and intensity of behaviours typically associated with lameness, such as abduction and adduction, difficulty in turning, rising and walking (Manson and Leaver, 1988), head movement, asymmetry of gait (Wells et al., 1993), length of stride and tracking-up (Tranter and Morris, 1991; Whay et al., 1997), and arched back (Sprecher et al., 1997). In addition to the overall gait score (NRS) based on simultaneous evaluation of several gait attributes, each attribute can be assessed separately using a VAS (Flower and Weary, 2005). A VAS consists of a straight line with extremes of the condition labelled at either end. The observer simply marks the line at the position corresponding to his/her evaluation of the condition (Welsh et al., 1993).

In contrast, objective methods measure the physical properties of locomotion and express these in mechanical units (m, s, kg). Kinematic methods of locomotion analysis used in cattle have been adopted from horse locomotion analysis (Herlin & Derevemo, 1997; Ceballos et al., 2004; Flower et al., 2005), capturing cow gait on film and using motion analysis software to calculate certain aspects of gait such as minimal and maximal joint angles during walking (Herlin and Derevemo, 1997) or stride attributes such as stride length, height, duration, speed and acceleration. A recent study using these kinematic techniques found that strides of healthy cows were faster and longer than those of cows with sole ulcers (Flower et al., 2005).

The deviations in gait seen in lame cattle are in part likely due to the pain associated with injuries on the feet and legs (Whay et al., 1998). However, much of the variation we see in both objective and subjective measures of gait could be due to factors other than pain, including damage in locomotor system (muscles, ligaments, nerves) (Greenough et al., 1981), natural variation among cows, and physical constraints such as a distended udder. The obvious method of determining which gait attributes are specifically associated with pain is to observe the effect of an analgesic on behaviour of the animal with impaired gait (Rutherford, 2002). Unfortunately, to date there has been little work published on this issue. Existing studies performed on horses and broilers employ subjective and objective diagnostic approaches to evaluate the potency of analgesics (Owens et al., 1995; McGeown et al., 1999) but fail to focus specifically on the gait characteristics associated with pain.

Non-steroidal anti-inflammatory drugs (NSAIDs), such as ketoprofen, are generally less potent than narcotic analgesics. However, they have little or no effect on the central nervous system and are thus less likely to cause sedation and tranquilization as occurs with opioids (Friedrichs, 2002). The distribution half-life ($t_{1/2\alpha}$) of ketoprofen in dairy cattle is about 4 min (De Graves et al., 1996), and elimination half-life ($t_{1/2\beta}$) is approximately 30 min after IV application (De Graves et al., 1996; Igarza et al., 2004). Thus, approx. 12 min (3x $t_{1/2\alpha}$) after the application, the drug has been distributed throughout the body and 30 min after the application 50% of the drug has been eliminated in urine. Although this information suggests that ketoprofen in cattle acts extremely rapidly, another study was able to show that ketoprofen persisted in the areas of inflammation for much longer than expected. Concentrations of ketoprofen in inflammatory exudates were measurable for as long as 9 hours after IV injection (Landoni et al. 1995). Ketoprofen has been shown to be effective in dairy cattle in controlling pain after surgical procedures, such as dehorning (Milligan et al., 2004) and castration (Ting et al., 2003) of calves.

The aim of this study was to detect gait attributes of lameness in dairy cattle that are due to pain, comparing subjective and objective diagnostic methods. Pain was controlled with ketoprofen, a mild analgesic, which has previously been shown to have an analgesic action on dairy cattle.

MATERIALS AND METHODS

Cows

The experiment was performed at The University's *Dairy Education and Research Centre* in Agassiz, BC. Twenty seven lactating Holstein dairy cows [(mean ± SD) BM: 691 ± 65 kg; parity: 4.1 ± 1.6; DIM: 139 ± 68 d] were used in this study. Cows were selected from our 240-cow milking herd based on an initial gait score (see Flower and Weary, 2005) of 3 or greater (on the scale from 1 to 5). Average gait score on the first day of trial was 3.4 ± 0.56 (range: 3 to 4.5).

Housing and Management

Animals were fed a TMR formulated to meet the nutrient requirements appropriate for their stage of lactation as per the recommendations provided by NRC (2001). Animals were fed and milked twice daily at approximately 0500 and 1600 h. Water was available *ad libitum* in self-filling water troughs. All animals were cared for according to the standards of the Canadian Council on Animal Care (1993) and a protocol approved by the UBC Animal Care Committee.

Treatments

Each animal was assigned to a dose of 0, 1.5, or 3 mg/kg BM of ketoprofen (Anafen®, Merial Canada Inc.), such that the three treatment groups were balanced according to initial gait score, BM, parity and DIM. The experiment lasted for 9 d: 3 d pre-treatment (before), 3 d treatment (during) and 3 d post-treatment (after). On treatment days each animal received an IV injection of 30 ml of saline solution with the assigned concentration of ketoprofen and administered by a trained veterinarian.

Video Recording and Data Acquisition

To habituate animals to the experimental procedure cows were subjected to all aspects of the experimental procedure (with the exception of aversive haltering procedures associated with the IV injections into the jugular vein due to concerns regarding animal welfare) for 7 d prior to the data collection period.

Reflective marker bands were attached proximally to the metacarpo-phalangeal and metatarso-phalangeal joints of each cow while in the milking parlour for the duration of the habituation period and the experimental phase of the study (Flower et al., 2005). Upon leaving the milking parlour, cows were moved to a pen containing head locks and restrained for up to 1 h during the before and after treatment phases and up to 2 h when the ketoprofen was administered on the assigned treatment days. Based on the pharmacokinetic features of ketoprofen for dairy cattle, the drug was administered intravenously 15 to 20 min before recordings. This was achieved so that the treatment was injected to cows in groups of 7. After 15 to 20 min cows were moved in a calm manner to the alley where the video recordings were undertaken. The same treatment protocol was performed for all cows in the experiment. Cows walked individually down a 1.2 m wide and 40 m long alley. One side of the alley consisted of a solid black wall that provided contrast for digitizing video recordings and the other side consisted of metal rail fencing material.

While walking along the alley, cows were recorded using a high-speed (60 frames /s) video camera (Panasonic AG-456UP, Matsushita Electric). The camera was placed 10 metres from the walking path and the axis of the lens was orthogonal to the plane of motion, facing the black wall providing a frame width of 7 m. A 500W light was attached below the camera and directed at the wall. At the beginning of each recording session the alley was cleaned with automatic scrapers. To calibrate the computer program used for digitization and locomotion analysis, a 1m ruler with 0.05 m reflective markers on both ends was held in front of the camera in the middle of the walking path at the beginning of each recording session.

Video recordings were digitized using Peak Motus (version 3.2, Peak Performance Technologies Inc., Englewood, CO). Two sets of events were recorded for each hoof: hoof strike (HS; defined as when hoof touches the ground at the beginning of the stance phase) and toe off (TO; defined as when the hoof leaves the ground at the beginning of the swing phase). These two events defined swing and stance phases, which allowed for the measurement of stride length (mm), maximum stride height (mm), stride duration (s), and hoof speed (m/s).

The subjective assessment of gait included NRS with detailed descriptions (Table 1.1), as well as 4 individually evaluated gait attributes (back arch, head bob, tracking-up and reluctance to bear weight) using a VAS. The NRS was based on a 9-point scale (1-5, including half-integer scores), whereby 1 represented sound gait and 5 represented severely lame. Half integer scores were allocated if the gait of an animal exceeded requirements of a particular score, but did not meet the requirements of the next successive score.

Specific gait attributes were scored directly on a computer screen using a VAS by positioning the cursor on a 5 cm scale. Brief descriptions of extreme intensities of observed behaviours were provided at the ends of the scales (Table 2.1). According to the position of the indicator on the scale a continuous numerical value from 0 – 5 was calculated, representing the severity of the observed behaviour. Videos were scored using a VCR and a colour TV. The observer was blinded for days of the experiment and treatment group of the cows. Each video clip was observed at least 10 times: twice for each of the 4 gait attributes and twice for the NRS.

Table 2.1. Description of 4 gait attributes associated with lameness, scored on a visual analogue scale (VAS) (adopted from Flower and Weary, 2005)

Behaviour	**Endpoints of VAS**	
	Min	**Max**
Back arch	Flat or concave spine	Convex arch between the withers and tail base
Head bob	Steady and even head carriage	Pronounced, uneven head movement
Tracking-up	Hind hoof falls in imprint left by front hoof	Hind hoof falls short of imprint left by the front hoof
Reluctance to bear weight	Bears weight equally over all legs	Uneven weight bearing among legs

One third of video material randomly selected from the whole material was observed twice by the same observer to test the repeatability. Two days were allowed between two observations. To test repeatability the second set of observations was regressed against the first set and coefficient of determination (R^2) was calculated. Repeatability of observations was the greatest for back arch and tracking-up (R^2= 0.86), followed by NRS and reluctance to bear weight (R^2= 0.78), and head bob (R^2= 0.75).

Statistical analysis

Observations on multiple days were averaged to form one value per cow per phase. These values were then compared across the two baseline phases (before vs. after). Variables with significantly different baseline values were dropped from the analysis. This exclusion was based on the following assumptions: 1) severity of pain related behaviour decreases during the analgesic treatment and returns to baseline with cessation of the treatment, and 2) pharmacokinetic properties of ketoprofen do not support the possibility of carry-over effect lasting longer than 24 hours after injection. All other variables were averaged across the baseline phases. The means of during phase were then subtracted from those of the baseline phases, to calculate a difference value for each cow. The resulting difference scores were then regressed against the dose of the analgesic in order to test for a linear relationship between the two variables. All statistical analyses were performed using general linear model procedure of SAS (Proc GLM; SAS Inst. Inc., Cary, NC). Sample size (n) was reduced due to exclusion of the outliers by 4 for analysis of subjective data (n= 23), and by 1 for analysis of kinematic data (n= 26).

Subjective measures

With the exception of back arch, comparison of the subjective measures taken before treatment with those obtained after treatment phase revealed no differences, allowing us to pool the values from these two phases to create a baseline value for use in subsequent comparisons (Fig 2.1). Due to a significant increase in severity of back arch in the post treatment phase compared to the pre treatment phase (*P<0.01*), this variable was excluded from further analysis.

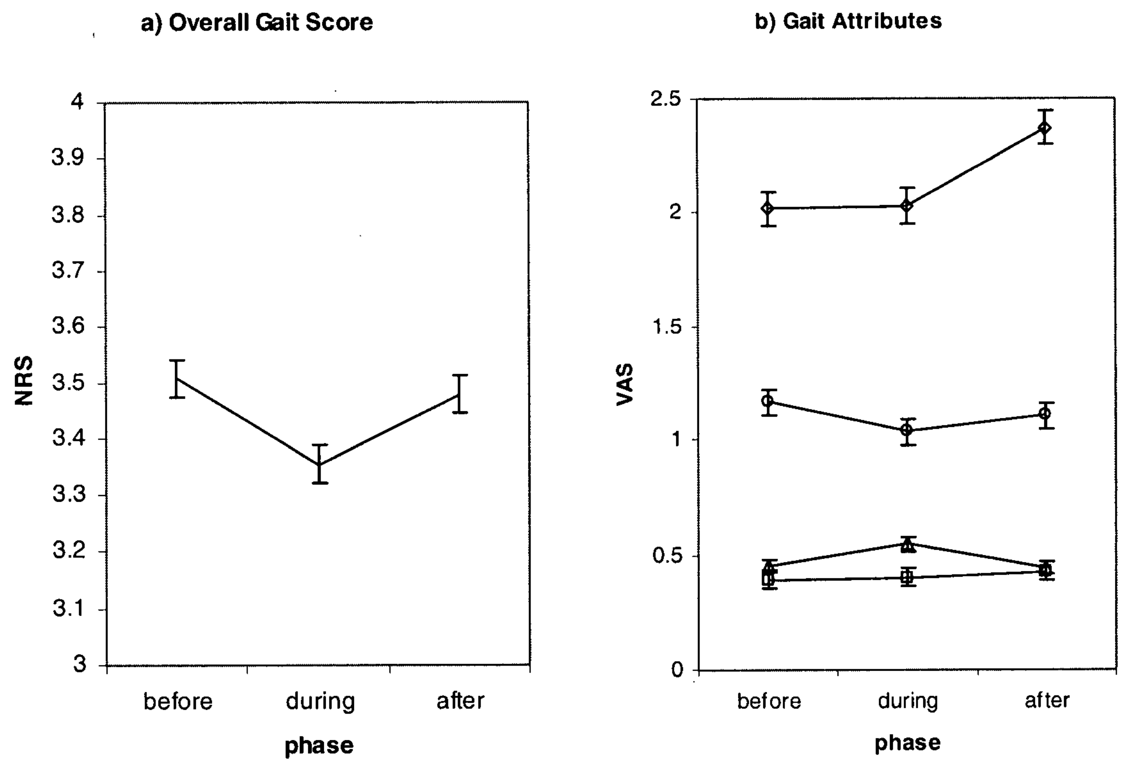

Figure 2.1. Subjective variables. Average values in phases before, during and after treatment (± SEM) for a) overall gait score and b) gait attributes: back arch (◇), reluctance to bear weight (○), tracking-up (△) and head bob (□). (n= 23)

As expected, those animals given saline during the treatment phase (i.e. the 0 mg/ kg BM ketoprofen) showed no improvement in NRS; whereas, those animals given either dose of ketoprofen showed improvements in NRS (Fig. 2.1a). There was a positive linear response in gait score with increasing dose of analgesic administered (R^2=0.27; 22 df; *P*= 0.011) with those cows receiving the highest dose realizing the greatest benefits in terms of improved NRS (Fig. 2.2a). Cows receiving an intermediate dose (1.5 mg/kg) improved by approximately 0.13, while those receiving the highest dose (3 mg/kg) improved by approximately 0.3.

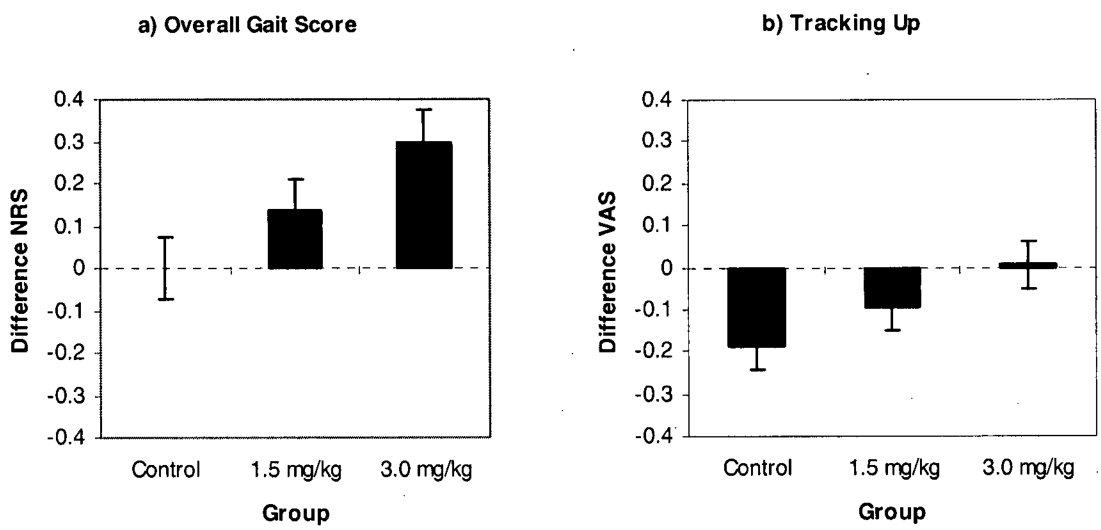

Figure 2.2. Mean difference (± SEM) in overall gait score (a) and tracking-up (b). Differences are between the average of the base line phases (before and after treatment) and the treatment phase (n = 23).

Administration of the ketoprofen did not improve tracking-up but rather prevented this attribute from worsening. In the control and 0.15 mg ketoprofen dose groups cows declined 0.19 and 0.10 but the cows receiving the highest dose of ketoprofen remained stable in their tracking-up, resulting in a linear relationship between dose and changes in severity of tracking-up (R^2=0.24; 22df; P= 0.01).

Head bob and reluctance to bear weight showed no significant change with dose of the analgesic.

Kinematic data

All kinematic gait variables differed before and after the treatment period (Fig. 2.3.). Although unexpected, we observed a consistent change over time in each of the kinematic variables measured in the current study. For example, the average stride length was (mean ± SEM) 1490 ± 5.8 mm before treatment and then decreased by 2.9% (44 mm) and 2.4% (36 mm) during and after treatment, respectively (Fig. 2.3a). Average maximum stride height was 86 ± 0.6 mm before treatment, decreased by 2.3% (2 mm) during treatment and declined a further 4.6% (4 mm) in the after treatment period (Fig. 2.3b). Stride duration (Fig. 2.3c) and hoof speed (Fig. 2.3d) were similarly affected with stride duration decreasing and hoof speed increasing over the course of the three study periods.

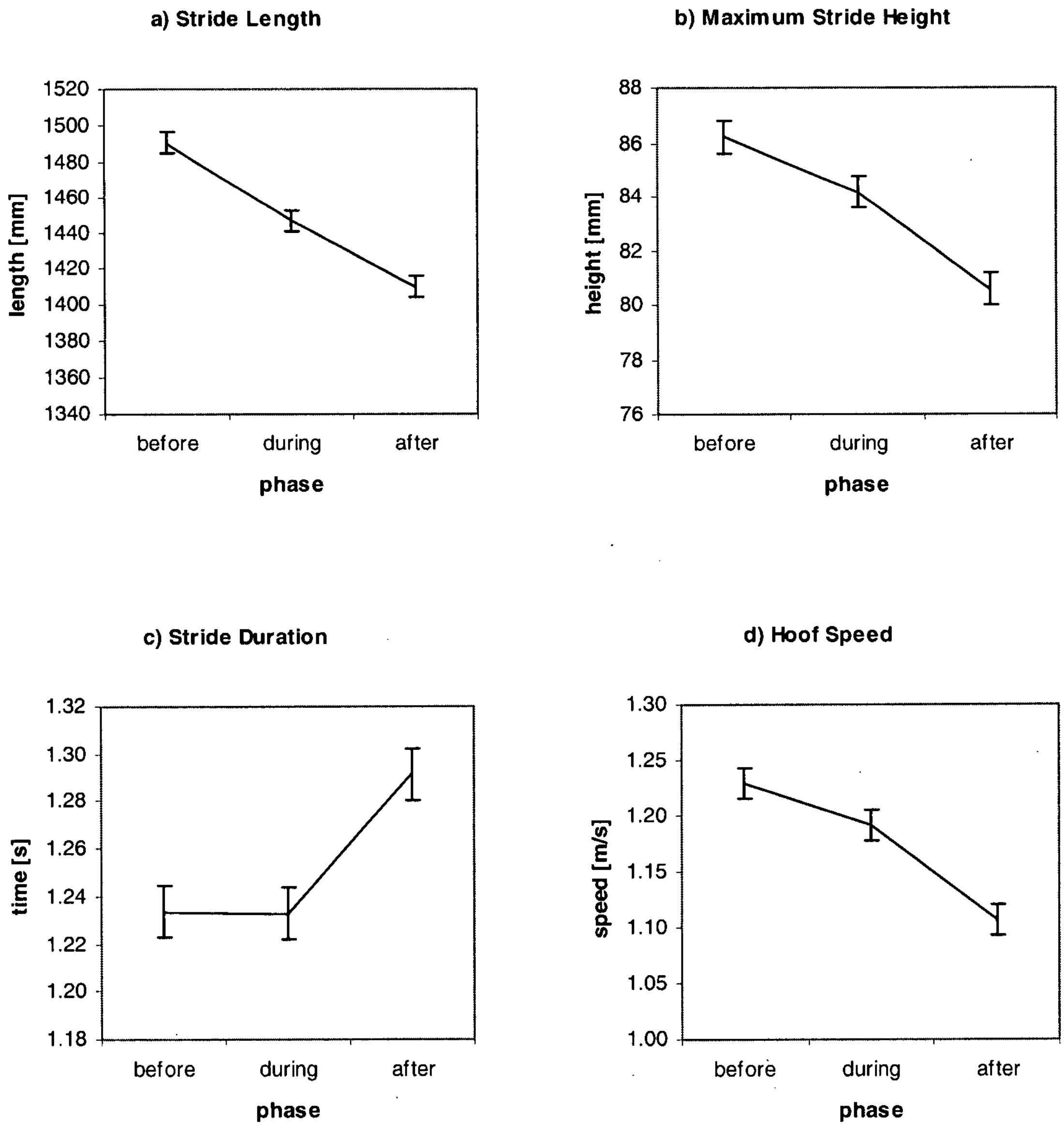

Figure 2.3. Average values in phases before, during, and after treatment (± SEM) for stride length (a), maximum stride height (b), stride duration (c) and hoof speed (d) as determined using kinematic analyses (n=26).

DISCUSSION

The overall improvement in NRS when cows received ketoprofen, together with the linear relationship between analgesic dose and the extent of improvement, illustrated the effect of pain on lameness in dairy cattle. To our knowledge this is the first demonstration of dose dependent changes in cattle lameness in response to treatment with analgesics.

Of the specific behaviours scored, only tracking-up also showed a dose response with ketoprofen, but this was largely driven by those cows on the control treatment worsening during treatment phase. The IV administration of the ketoprofen required restraint for approximately 1 h longer for some cows prior to the administration of the ketoprofen during the treatment phase than either the before or after phases. This increase in forced standing time may have increased any pain due to pre-existing hoof injuries, worsening tracking-up. No effect of analgesic treatment was observed for head bob, back arch, and reluctance to bear weight, suggesting that these variables are less useful indicators of pain due to lameness than either overall gait score or tracking-up.

Although these dose effects were clear for NRS and tracking-up, the magnitude of the effects was modest. For example, the difference in gait score between baseline and treatment periods was less than half a point, even at the highest dose of analgesic, a difference similar to the resolution of the scoring system. Our ability to detect these relatively subtle changes in gait is limited by the reliability of the assessment method. Martin and Bateson (1993) argued that clear and unambiguous definitions of behaviour could improve the reliability of the scores. For example, Garner et al. (2002) found that consistency of subjective gait scores for lame broilers improved when the scoring system provided observers with more detailed descriptions of the criteria. Thus, any improvements in gait scoring methods, such as clearer definitions of the behaviours, may improve our ability to detect changes in gait due to pain.

The modest effect of ketoprofen on gait may also have been due to the pain underlying lameness being too strong for the potency of this analgesic. Further research using stronger analgesics or local anaesthesia of the lower limb is recommended, although appropriate controls will be required to rule out systemic effects on behaviour.

Development and healing of hoof injuries are fairly long processes (Kempson and Logue, 1993; Lischer et al., 2002). A study by Green et al. (2002) found that milk production of lame cows was reduced for up to 4 months before and 5 months after diagnosis of lameness, suggesting that cows may be experiencing pain and altering their gait for months. These long-term changes in gait would likely cause hypertrophy of certain muscle groups (due to intensive use) and concurrent atrophy of the others (due to disuse), processes that would require weeks to reverse after the pain is removed. In other situations locomotor structures may also be permanently damaged (e.g. injuries of tendons and ligaments). In both types of cases we expect little response to short-term treatment with analgesics.

This experiment was designed to demonstrate the effects of the analgesic treatment on gait attributes, but the test of treatment required that behaviour was similar before and after treatment. Unfortunately, this condition was not met for any of the four kinematic variables. These differences between the time periods may have been due to cows not being sufficiently habituated to the experimental protocol prior to the experiment. Comparing the average of values across all three phases suggests that the gait was becoming increasingly relaxed, as indicated by shorter and lower strides, increased duration of strides and decreased hoof speed. Flower et al. (2005) allowed a longer habituation period (4 wk), but did not describe how gait changed over this period or their 7-d experimental period. Thus more work is required to describe how these gait measures vary over time and in response to habitation. What is clear from the current results is that the kinematic methods can detect relatively subtle changes in gait, suggesting that the technique may be useful in future lameness research.

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Chapter III

General Conclusion

The aim of this study was to detect gait attributes of lameness in dairy cattle that are associated with pain using a subjective gait scoring method and an objective, kinematic diagnostic method. It has been suggested that behavioural changes that are caused by pain will diminish during analgesic treatment but will return once the analgesic treatment is terminated (Rutherford 2002). This experiment was designed to test this prediction by observing various gait and stride attributes before, during and following treatment with a mild systemic analgesic, ketoprofen.

The results of the subjective gait analysis indicated that ketoprofen was able to improve the overall gait of lame cows as well as decrease severity of tracking-up. However, all other gait attributes were unaffected by treatment, indicating that either the pain was too great for the NSAID to provide any pain relief or that the behavioural changes associated with locomotion were not driven by pain. Future research should therefore investigate the use of stronger analgesics or local anaesthesia of the lower limb to determine if greater levels of pain relief result in improved locomotion in lame dairy cows. This information will allow researchers to understand the interaction between pain and lameness and possibly evaluate the extent of pain associated with different gait attributes.

Although the results of this research indicate that ketoprofen appears to cause some decrease in the pain associated with lameness, this method of decreasing the severity of lameness in dairy cows should not be used as the sole treatment of this condition. Rather, researchers should use this information to better understand which behaviours are associated with pain and to emphasise these behaviours in subjective gait scoring systems used on farm by producers. Such improvements will hopefully allow for earlier detection of lame animals. Concurrently, research efforts must also continue to determine risk factors contributing to lameness with the overall goal to prevent lameness.

A large part of this thesis focused on the use of kinematic gait analysis as a means of objectively assessing whether treatment of lameness with ketoprofen would cause any subtle changes in stride attributes, such as stride length or stride height. The results obtained in the present study were inconclusive. Despite one week of habituation, cows continued to decrease their walking speed throughout the course of the study indicating that they were not sufficiently habituated to the experimental protocol. Unfortunately, I was not able to test this prior to treatment phase and thus strongly recommend that future research of this nature incorporate longer habituation phases.

Acquisition of kinematic data by the means of video recordings is a complex procedure with substantial space and time requirements due to factors like fixing and cleaning the reflective markers, placement of camera, and analysis of video clips. This poses a question of applicability of this method to every-day use. Future studies should look into the possibilities of applying other, potentially more practical tools of acquisition of the data. For example, an ultrasonic technique proposed by Zejie et al., (1996) makes use of wireless ultrasonic transmitters, each one with a unique frequency, which are attached to the subject. The emitting signals are picked up by three receivers and locations of the transmitters calculated. Although to my knowledge this technology has not been applied to dairy cattle as a means of identifying changes in locomotion, it could possibly offer insights into the three-dimensional trajectory of all four hooves, allowing a more profound analysis of gait than does the two-dimensional video-based technique used in the present study.

Development of more convenient kinematic techniques for gait analysis in dairy cattle could ultimately lead to implementation of continuous gait monitoring as a daily routine on dairy farms. Incorporation of objective techniques such as kinematic gait analysis into existent elements of dairy farms such as scales, possibly in combination with the equipment for kinetic analysis of gait, as suggested by Neveux (2005) would greatly improve detection of lameness in dairy cattle.

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