Behavioural changes caused by lameness and lameness treatment in dairy cows housed in automatic milking systems in the UK

by

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Abstract

Lameness is a detrimental problem for the welfare of cows and farmer profits. It is described as an abnormal gait caused by pain in the affected limb. Lameness causes changes in cow behaviour, which have direct impact on milk production as cows reduce their overall activity. Little information is available on how lameness treatment affects cow behaviour. New technologies to measure behaviour can help to study the effect of lameness and lameness treatment on dairy cow behaviour. The main aim of this thesis was to investigate behavioural changes caused by lameness and lameness treatment in cows housed in automatic milking systems; in addition it aimed to investigate the effect of claw horn lesions on the likelihood of recovery from lameness.

The effect of lameness on dairy cows visits to an automatic milking system was investigated using a case-control study. Lame cows were matched to non-lame controls and the number of visits was compared. It was observed that lame cows visited the automatic milking system less in comparison to non-lame cows in particular between 00:00 and 05:59.

The effect of lameness on rumination time was investigated in a longitudinal study. Cows were mobility scored once a week and rumination time averaged across the 2 days after mobility score. Lame cows ruminated 8 minutes/day less than non-lame cows.

The effect of lameness on the milking visits and rumination time of newly lame cows was investigated in a longitudinal study. The behaviour of newly lame cows selected for a randomised clinical trial was compared to the behaviour of matched non-lame control animals. Newly lame cows did not show any reduction in rumination time or milking visits in comparison to non-lame animals.

The effect of lameness treatment on dairy cow behaviour was investigated in a randomised clinical trial. Newly lame cows were randomly allocated to one of four treatments; these cows were matched to non-lame control cows. Cows treated with a therapeutic trim and a foot block increased their lying time in the five days after treatment in comparison to non-lame cows. The increase in lying was distributed throughout the day. None of the treatments caused changes in the number of milking visits per day after treatment. Lame cows treated with trim and NSAID showed a reduction in their rumination time (-59 minutes/day) during the 5 days after treatment in comparison to non-lame cows.

The effect of claw horn lesion type on the likelihood of recovery from lameness was investigated using photographs taken before lameness hoof trimming was applied. The results showed that cows with white line haemorrhage and those that were more severely lame at the time of treatment were less likely to recover at 2 weeks after hoof trimming was applied. In addition, recovery was positively associated with the size of the white line haemorrhage. This is likely to link to the fact that size of the lesion was significantly related to lesion severity. In this study it was observed that milder lesions were bigger in size than severe lesions, for all lesion types. Finally it is noteworthy that differences in treatment success between different operators were observed.

The present thesis demonstrated that lameness and lameness treatment affected cow behaviour, and may delay recovery. Early recognition and prompt intervention should be encouraged in order to reduce the behavioural changes caused by lameness and improve the recovery rates from disease.

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Dedications

I dedicate this thesis to:

My grandparents who taught me to love and respect animals:

“Animals are so intelligent; they only need to speak like us”. (*Los animalitos son tan inteligentes, solo les falta hablar)*

“If they could talk to us, who knows what they will reply to us”. (*Si nos pudieran hablar, que no nos dirian?)*

Petronila Ibanez Pacheco 1919 – 2011

All the animals that have been with me throughout my life, they taught me that they were more than fluffiness, in particular to Mush, Foxi and Fluffy who passed away during my PhD time.

Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Nottingham. The work is original, except where indicated by special reference in the text, and no part of the thesis has been submitted for any other academic award. Any views expressed in the thesis are those of the author.

Signed: …………………………………

Date: ……………………………………

Publications

Chapter 2 and 3

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Chapter 2

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List of Abbreviations

AMS	Automatic Milking System
BW	Body Weight
CI	Confident Interval
DD	Digital Dermatitis
Coef	Coefficient
DEFRA	Department for Environment, Food and Rural Affairs
DHEA	Dehydroepiandrosterone
DIM	Days in Milk
DMI	Dry Matter Intake
Freq	Frequency
HHE	Heel horn erosion
ID	Identification
IQR	Inter Quartile Range
kg	Kilogram
l	Litre
MCMC	Markov chain Monte Carlo
mg	Milligrams
NSAID	Non-Steroidal Anti-inflammatory Drug
OR	Odds Ratio
P-value	Significant value
RCT	Randomised Clinical Trial
SH	Sole Haemorrhage
SU	Sole Ulcer
TMR	Total mixed ration
UK	United Kingdom
USA	United States of America
WLD	White Line Disease

Love of animals is a universal impulse, a common ground on which all of us may meet. By loving and understanding animals, perhaps we humans shall come to understand each other

Louis J. Camuti

(1894 - 1981. The first American veterinarian specialist feline practitioner).

The animals of the world exist for their own reasons. They were not made for humans any more than black people were made for white, or women created for men.

Alice Walker

(Author of 'The Colour Purple')

Chapter 1

Literature Review

1.1 Lameness – A background

Lameness is defined as an abnormal gait, cows change the load on the affected limb in order to reduce pain (Scott, 1989). This causes a reduction in locomotion and cows show restless behaviour, particularly at milking switching the weight of the affected leg towards the healthy foot (Rushen et al., 2010). Lameness is one of the most important animal welfare problems in the dairy industry with at least four in ten cows becoming lame every lactation (DEFRA/ADAS, 2007; Flower et al., 2008; Barker et al., 2010; Rushen et al., 2010). In 2012, there were 1.8 million producing cows in the UK and 23 million in the European union (DairyCo, 2013).

1.1.1 Normal gait

The cow walks using a diagonal and ipsilateral limb gait during which a level spine is maintained (Phillips, 2002; Flower et al., 2005; Greenough, 2007). This gait presents 3 distinct phases in each stride. Support and thrust of the limb characterizes the weight-bearing phase, which enables the load of the body to move over the limb. The protraction and retraction phases are considered as part of the swinging phase. These phases allow retraction of the limb from the ground and extension of it in order to reach the ground again (Phillips, 2002; Greenough, 2007).

The use of kinematics in the study of gait has provided information about the dairy cow's normal gait and weight bearing. Flower et al. (2005) found that healthy cows presented a mean stride duration of 1.26 seconds with a mean stride length of 139.5 cm. Dairy cows applied more weight to their front legs (51-53%) than to their back legs (47-49%) when standing (Chapinal et al., 2009a). When walking, cows applied 55-60% of their weight to the forelimbs in order to support and steer the load (Phillips, 2002).

Van der Tol et al. (2002) showed that the medial claw of the forelimbs and lateral claw of the hind limbs bear more weight when cows were standing (Van der Tol et al., 2002). Further, these authors found that at the individual claw surface, the sole area of the claws is under greater pressure when standing; in the front limbs the pressure concentrates at the front of the sole and in the hind limbs at the back of the sole. Differences have been observed in the weight load on the claws of the hind limbs, the medial claws bear more weight before calving and whilst the overload switches to the lateral claw after calving (Ossent et al., 1987). It is suggested that the load distribution at the claw and limb level may be affected by the stage of pregnancy (Schmid et al., 2009).

When walking, forces applied to the claws increase (in comparison to when standing) and load differences between claws also increase (Van der Tol et al., 2003). In the forelimbs the loading forces were distributed almost evenly between claws. In the hind limbs loading forces showed an uneven distribution with more force applied over the lateral claw than the medial claw (Van der Tol et al., 2003). Schmid et al. (2009) observed, using high-speed cinematography in cows walking on a treadmill, that the lateral claw landed before the medial claw in both fore and hind limbs.

1.1.2 Lameness gait

Pain in a limb causes changes to the gait. In cows, changes to their gait are dependent upon whether a hind or forelimb is affected. The position of the head and an uneven back when walking may indicate whether a fore or a hind limb is affected (Greenough, 2007). Lame cows try to lean away from the affected limb or modify their gait in order to reduce the load on the affected limb (Scott, 1989). Cows suffering with claw horn lesions showed shortened strides and a slow walk; and increased the pressure for a longer period on the remaining healthy limbs (Flower et al., 2005). The extent of change to the stride and weight bearing are relative to the severity of the lesions (Flower et al., 2005). Acceleration forces on the affected limb also change (Scott, 1989), particularly when lameness occurs on the hind limbs (Pastell et al., 2009; Chapinal et al., 2011).

Lameness is more likely to occur in the hind limbs (Clarkson et al., 1996). Neveux et al. (2006) observed that when cows had an uncomfortable surface placed under their front limbs, they were capable of redistributing the load to the hind limbs. This did not occur when the uncomfortable surface was placed under the hind limbs, in this case cows distributed some of the load to the contralateral hoof but not to the front hooves (Neveux et al., 2006). These findings showed that cows are not able to transfer the load from the back feet to the front feet but they can transfer load from the front to the back. The musculoskeletal anatomy of the forelimbs and hind limbs play an important role in the development of lameness in the hind limbs and the mitigation of the load impact on the fore limbs (Toussaint-Raven et al., 1985; Schmid et al., 2009).

1.1.3 Bovine foot anatomy

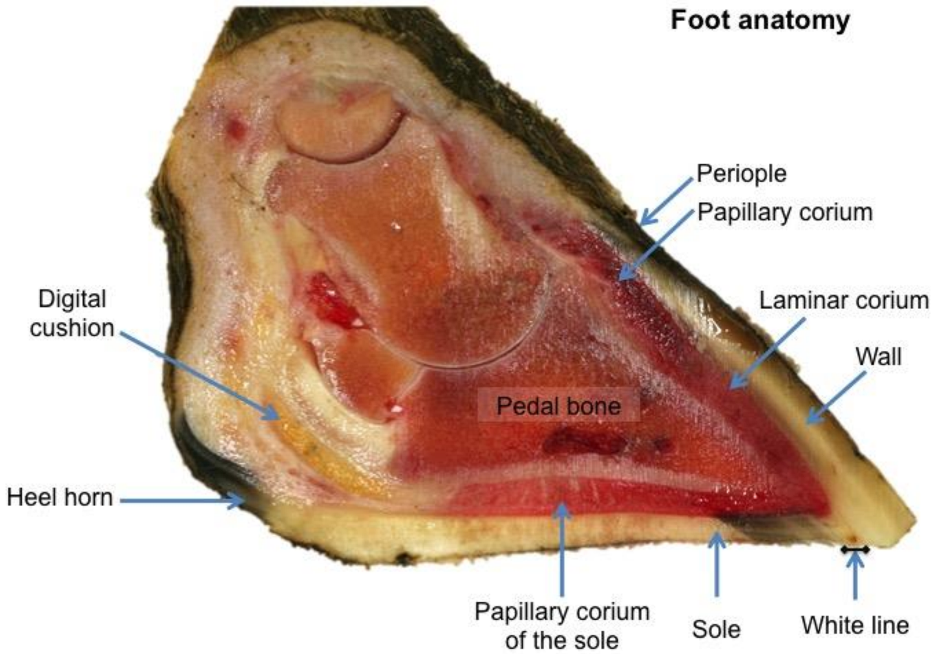

Figure 1.1 Anatomical description of the bovine foot (Picture courtesy of Reuben Newsome).

The bovine foot has a claw or hoof that has five principal structures (Figure 1.1): periople, wall, sole, white line and bulb or heel (Greenough, 2007; Blowey, 2008). These structures develop from the epidermis and their function is to protect the inner structures of the claw (e.g. corium). The periople ring is the new horn rich in water content and it is located just below the coronary band. At the bottom of the claw is the sole area and it is separated from the wall by the white line. These two latter structures do not have the same strength as the wall, and are the areas more prone to lesions (Blowey, 2008). The corium gives support to the foot and provides the nutrients required by the sole and white line and the other inner structures. Direct damage to the corium (e.g. overload pressure) can cause bleeding and pain. The digital cushion at the heel is made of fat and elastic tissue and it has shock absorbing properties (Raber et al., 2004; Greenough, 2007; Blowey, 2008). The digital cushion lies between the pedal bone and the corium and is thought to provide support and flexibility to the claw (Greenough, 2007).

1.1.4 Aetiology of lameness

Among the four most common causes for lameness in the UK are claw horn lesions, white line disease (WLD), sole ulcer (SU) and haemorrhage (SH), and infectious diseases such as digital dermatitis (DD) and interdigital necrobacillosis (Hedges et al., 2001; Green et al., 2002; Blowey, 2005; Barker et al., 2009).

1.1.4.1 Infectious diseases

The most common infectious foot disease is digital dermatitis (DD), also known as Mortellaro disease. DD was first reported in Italy in 1974, and since then the disease has been found in many parts of the world. Various treponema species are the causal organism of this digital infection, which mainly affects the hind feet (Blowey, 1992b; Archer et al., 2010a). Treponemas spp associated with such infection have been isolated from the gastrointestinal tract (Evans et al., 2012). However, the contagious cycle of this disease has not been clearly identified. Initial appearance of DD is as an acute inflammation of the skin in the heel area of the foot. The skin becomes inflamed and granulation tissue might be present, which is prone to bleeding. The infected area may appear as an active ulcer which may then spread further around the dew claws or interdigital space (Greenough, 2007).

A further cause of infectious lameness is foul in the foot, which is a multibacterial infection, described for the first time in 1854. *Fusobacterium necrophorum* is the main pathogen isolated, but it may occur in association with other bacteria such as *Bacteroides melaninogenicus* (Greenough, 2007). Foul in the foot starts as an acute necrotic infection in the interdigital space (Archer et al., 2010a). Claws are separated due to an edenomatous swelling and later a mild necrotic exudation appears in between the claws. This infection can spread rapidly to the axial borders of the claw when untreated (Alban et al., 1995). This painful condition causes fever, reduction in feed intake and drop in milk production (Greenough, 2007).

Footbaths containing antibiotics and disinfectant solutions, and parenteral antibiotics are recommended for the treatment of infectious diseases in the hoof. Although some concerns have been raised regarding the type of products that can be used for footbaths (Nuss, 2006). In general, it is recommended that footbaths are located in an accessible area for the cows and long enough for cows to step in twice on each hoof, water should be high enough to cover the coronary band. Footbaths should be easy to clean and kept maintained with an adequate concentration of the chemical product (Nuss, 2006). Cows hooves should be clean before they enter the footbath and allowed to dry afterwards (Laven and Logue, 2006). It has been recommended to change the footbath every day or after 200 cows have used it. For individual cases, studies have found better healing rates with the use of bandages containing tetracycline hydrochloride powder (Cutler et al., 2013) with less painful responses and better clinical outcomes with salicylic acid bandages (Schultz and Capion, 2013).

1.1.4.2 Claw horn lesions

The most common claw horn lesions are sole ulcers (SU), white line disease (WLD) and sole haemorrhage (SH; Figure 1.2) (Bicalho et al., 2007; Cramer et al., 2008; Archer et al., 2010a), with the lateral claw of the hind limbs being often affected (Ahrens et al., 2011). Barker et al. (2009) reported an average of 43% of SU and 36% of WLD on cows treated by farmers. Meanwhile, Leach et al. (2012) observed between 70% to 100% SH on cows treated by the researcher. SU is observed as a protruding mass after the removal of damaged sole horn, causing exposure of the underlying corium that appears as granulated tissue. SH is caused by the diffusion of blood through the tubules in the sole horn and it is described as a bruise in the horn seen as red or purple stains. WLD is caused by a breakdown in the horn between the sole and the wall, it is observed as haemorrhage or as damage at this point due to the presence of foreign bodies (Blowey, 1992b; Greenough, 2007; Archer et al., 2010a).

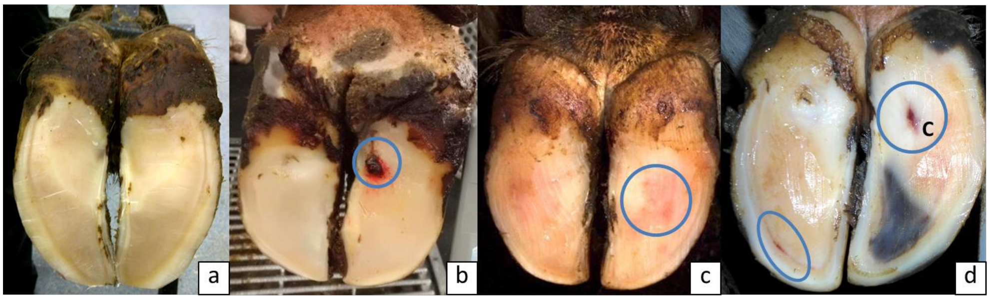

Figure 1.2 Healthy horn (a) and claw horn lesions: sole ulcer (b), sole haemorrhage (c and d-marked as C) and white line disease (d-at the bottom of the claw). Lesions in each picture identified with a ○ (blue circle) (Pictures by Giuliana Miguel & Heather Thomas).

SU and SH have been classified according to their appearance following a numerical scale or using severity categories (Leach et al., 1998; Sogstad et al., 2005) (Table 6.1); further description of SU is dependent on the amount of corium exposed and whether infection is present (Leach et al., 1998; Sogstad et al., 2005) (Table 6.1). WLD can be classified similarly to SH (Leach et al., 1998), or divided into two types: white line haemorrhage and separation, each with independent categories (Sogstad et al., 2005) (Table 1.1).

The cause of claw horn lesions is still unclear. Findings from multidisciplinary research indicates that these lesions may develop due to a combination of environmental and cow factors; which vary dependent upon the type of lesions (Barker et al., 2009). In the case of SU and WLD, studies associate their appearance with late pregnancy physiology (Raber et al., 2004), digital cushion thickness and body condition (Green et al., 2014), with the latter two causes being of high hereditability (Oberbauer et al., 2013; Oikonomou et al., 2014). Laminitis caused by ruminal acidosis has been associated with the development of claw horn lesions, though no clear associations have been observed (Lean et al., 2013). Hardness of claw horn has also been associated with the presences of lesions, as claws become softer after prolonged exposure to wet conditions (Borderas et al., 2004).

Table 1.1 Definitions of severity scores for claw horn lesions in dairy cows.

Author & year	Lesion	Definition
Leach et al. (1998)	Haemorrhage (sole or white line)	Diffuse red or yellow in horn
		Stronger red colouration
		Deep dense red
		Port colouration
		Red raw, possibly fresh blood
	Sole ulcer	Corium exposed
		Severe sole ulcer-major loss of horn
		Infected sole ulcer
Sogstad et al. (2005)	Haemorrhages of the white line	Mild: Slight haemorrhagic discoloration
		Moderate: Haemorrhage on a single spot or several superficial haemorrhages covering >20% of the white line
		Severe: Profound haemorrhage on a single spot or extensive haemorrhagic discoloration covering >50% of white line
	Haemorrhages of the sole	Mild: Slight haemorrhagic discoloration
		Moderate: Haemorrhage on a single spot or several superficial haemorrhages covering >20% of the sole surface
		Severe: Profound haemorrhage on a single spot or extensive haemorrhagic discoloration covering >50% of the sole surface
	Sole ulcer	Mild: Exposed, unaffected corium
		Moderate: Granulation tissue, necrosis, purulent exudates and separation of the sole horn
		As score 2 with additional affection of the deeper structures of the claw
	White-line fissure	Fissure, which disappear with deep cut beneath normal trimming level
		Deep fissure perforating next to the corium of sole or wall
		Corium is affected with purulent exudates, eventually with necrosis, granulation tissue and separation of the wall and/or sole

Digital circulation, horn and digital cushion composition might be affected by physiological and hormonal changes around parturition and the beginning of lactation (Leach et al., 1997; Logue et al., 2004). Histological changes around calving can cause weakness in the suspensory and supportive apparatus, generating an overload of the third phalanx over the digital cushion (Raber et al., 2004). Tarlton et al. (2002) observed that before calving there was an increase in the levels of an enzyme “hoofase”, which may increase the flexibility of the connective tissue supporting the third phalanx within the hoof capsule .

Lower body condition score (<2.5), particularly during and after parturition, is linked with a higher risk of lameness (Hoedemaker et al., 2009). Further a positive association between digital cushion thickness and body condition score has also been observed, with cows having thinner digital cushion more likely to experience claw horn lesions (Bicalho et al., 2009). Raber et al. (2006) observed that the structure of the digital cushion changes as the cow ages, from a looser connective structure to a tissue high in fat that then becomes collagenous connective tissue when cows are approximately in their 3rd parity. The digital cushion became thinner as days in milk progressed, reaching its minimum thickness at 120 days after parturition (Bicalho et al., 2009). Cows were more likely to suffer with claw horn lesions when in their first parity (Raber et al., 2004). SH, SU and WLD diagnosis is more likely to occur from 0-2 months after parturition and SU and WLD diagnosis from 2-4 months after parturition (Green et al., 2014). A strong genetic correlation has been reported between digital cushion thickness and SU and WLD (Oikonomou et al., 2014). Thus poor body condition in conjunction with environmental risk factors can generate pressure on the claw causing the appearance of claw horn lesions (Tarlton et al., 2002; Knott et al., 2007).

Treatment of these lesions varies according to severity. Foot trimming is the first recommendation, usually applying the Dutch Five Step method, which allows for relief of pain and helps with the recovery of the hoof tissue. Foot trimming aims to reduce the localised pressure at the sole; distributing the weight on to the strongest parts of the claw (e.g. the wall) (Blowey, 1992a; Van der Tol et al., 2004). This method consists of a routine foot trimming, the toe length is trimmed up to 7.5cm approx. of length, matching both claws; this is followed by creating a dish at the axial sole. If any of the claws have lesions, a further corrective trimming is performed either to reduce height or to remove any damaged hoof and to clear lesions. This method helps improving gait smoothness and rhythmicity, as reduces the load on the affected claw (Tanida et al., 2011).

In addition to foot trimming, anecdotal advice suggests the application of a block to the healthy claw of the affected foot. This allows some alleviation of pressure to the affected claw giving time for it to heal and promotes the comfort of the cow (Shearer et al., 2013). There is little research on the efficacy of the application of foot blocks on the recovery rate of lame cows. Pyman (1997) investigated the effects of bandages, plastic shoes and foot blocks on the treatment of claw horn lesions. He found that more cows in the block groups (wooden 65.8% and rubber 76.2%) were not visually lame by day 7 than in the bandage group (32.3%). Wehrle et al. (2000) observed that cows treated with 3 different types of blocks (wooden, rubber and wedge shaped) were not visually lame by day 4. There are no studies looking at the effect of the use of blocks on the healing rate of the claw horn lesions. Although there are strict recommendations on how to apply blocks to avoid further damage to the hoof and the welfare of the cow (Shearer et al., 2013).

It is accepted that lameness is a painful condition and the use of analgesics can help in the healing process and improve the welfare of cows (Huxley and Whay, 2006; Flower et al., 2008; Chapinal et al., 2010c). Flower et al. (2008) studied the effect of the non-steroidal anti-inflammatory drug (NSAID) Ketoprofen on the locomotion score of lame cows, comparing locomotion score before and after treatment. Animals were treated with saline solution and one of the following dosages: 0.0, 0.3, 1.5 and 3.0 mg/kg of body weight (BW). Animals given Ketoprofen at higher doses (1.5 or 3.0 mg/kg of BW) showed significant improvement in locomotion score; on the other hand, animals treated with saline solution or Ketoprofen at 0.3 mg/kg of BW showed little or no improvement in gait. Laven et al. (2008) treated lame cows with four treatments: foot trimming, foot trimming + NSAID (Tolfenamic acid), and the previous two plus a block; treatments were randomly applied. The study did not find any significant difference between treatments in locomotion score.

1.1.4.3 Other causes of lameness

Leg injuries constitute another cause of lameness. These injuries are observed on protruding areas of the leg (e.g. joints). Lesions observed can range from hair loss and skin ulceration up to swollen joints (Weary and Taszkun, 2000; Regula et al., 2004; Kielland et al., 2009; Potterton et al., 2011). The most common areas for this pathology are the carpal, fetlock, tarsal, hip and stifle joints (Weary and Taszkun, 2000), with a greater proportion of lesions observed at the carpal and tarsal joints (Wechsler et al., 2000; Haskell et al., 2006).

The tarsal joint or hock is under constant friction and pressure when a cow lies down and stands up. Environmental factors may promote inflammation or damage to the skin causing pain (Weary and Taszkun, 2000; Wechsler et al., 2000; Potterton et al., 2011; Andreasen and Forkman, 2012). Hair loss, ulceration and swelling may develop independently to one another and may be affected differently by risk factors in the environment (Potterton et al., 2011).

1.1.5 Prevalence of lameness

Lameness is a common problem throughout the world. Studies on the prevalence of lameness have been conducted around the world with the percentage of cows affected being similar. In the UK the prevalence of lame cows is between 30% (range 24 to 40%) (Reader et al., 2011), and 36.8% (0-79.2%) (Barker et al., 2010). In Europe, lameness prevalence was observed to be between 13.8% and 28.7% in Spain (Bach et al., 2007; Pérez-Cabal and Alenda, 2014), 31% (9 to 64%) in Czech Republic (Sarova et al., 2011) and an average of 34% between Austria and Germany (Dippel et al., 2009). In the USA, the prevalence observed was 24.6% ranging between 3.3 to 57.3% (Espejo et al., 2006). In South America, a very similar figure has been observed, a study in Chile observed a lameness prevalence of 32% in big farms and 28% in smaller farms (Tadich et al., 2010). A recent study in China found a 31% prevalence of clinical lameness, though the prevalence was negatively associated with farm size and positively associated with age of the barn (Chapinal et al., 2014).

Lameness prevalence findings can be influenced by the mobility score, environmental factors such as housing system or farm management, and the time of the year when the observation was carried out (Dippel et al., 2009). For example in Spain, Bach et al. (2007) carried out a longitudinal study on one farm over a period of 8 months, and observed a 28.7% lameness prevalence. This study diagnosed lameness using a mobility scoring system (scale 1-5). On the other hand, Pérez-Cabal and Alenda (2014) found a 13.8% lameness prevalence. This latter study was carried out in 23 herds over a period of six years and lameness was diagnosed as an abnormal gait only.

1.1.6 Risk factors for lameness

There are many risk factors associated with lameness, the most important identified were housing and management practices (Barker et al., 2007; Olmos et al., 2009; Barker et al., 2010). In the case of infectious diseases (e.g. DD), the type of floor used in sheds and level of hygiene were identified as the main risk factors. Barker et al. (2009) observed that cows housed on solid grooved concrete floors are at higher risk of developing DD than those cows housed on solid non-grooved concrete floors. Somers et al. (2005) found that cows housed under poor hygiene and increased moisture were at higher risk of suffering with DD. Recent findings support the theory that cows may develop tolerance or immunity to DD infections as they get older (Somers et al., 2005; Barker et al., 2009; Green et al., 2014).

In the case of claw horn lesions, as previously discussed, physiological changes around calving in conjunction with management practices may increase the risk to developing claw horn lesions (Webster, 2002; Blowey, 2005; Cook and Nordlund, 2009). Proudfoot et al. (2010) observed that cows diagnosed with sole haemorrhage and sole ulcers in mid lactation increased their standing time 2 weeks before and up to 24hrs after calving in comparison to cows without lesions. Increased standing time, particularly on hard surfaces, can increase the pressure of the third phalanx over the corium, which over time increases the risk of sole ulcers developing (Lischer Ch et al., 2002). Hard tracks (e.g. concrete) also increase the rate of wear of the claw horn on the sole making this thin and more likely to develop SU (Barker et al., 2009). Yards and alleyways with grooved concrete floor may also increase the risk of developing WLD; over time this type of floor may become slippery increasing the chances of slipping or lead to changes in gait in order to adapt the way weight is distributed over the hooves (Barker et al., 2009). The use of rubber flooring in the alley to the milking parlours reduce lameness as rubber provides better traction than concrete and may aid with the recovery from lameness (Chapinal et al., 2013).

The use of uncomfortable bedding can also increase the risk of lameness; Barker et al. (2009) observed a positive association between SU and the use of sparse bedding on concrete, mats or mattresses. Cows increased their standing times when forced to use concrete bedding in comparison to animals using soft mats on their beds (Haley et al., 2000). Furthermore, when cows are given the choice, they preferred to use deeply bedded stalls with sawdust or sand than mattresses (Tucker et al., 2003). Chapinal et al. (2013) observed that lameness reduced in farms that used deep bedding and offered access to pasture. Cows at pasture are less likely to be diagnosed with clinical lameness than cows in a cubicle house (Olmos et al., 2009). Pasture systems can provide longer lying periods due to a comfortable lying surface and less competition for feeding or lying spaces, resulting in more resting periods (Hernandez-Mendo et al., 2007).

A cow is more likely to become lame according to the length of exposure to different risk factors (e.g. hard flooring) (Barker et al., 2009). Cows in parity equal to or higher than 4 had a greater risk of suffering with SU than cows in their first parity, and cows equal or higher than 2 had a greater risk of suffering with WLD than cows in their first parity (Barker et al., 2009). Te constant exposure to wet conditions that affects the quality of the hoof (Borderas et al., 2004), in conjunction with damaged concrete yards and badly maintain cow tracks may increase the risk of lameness (Barker et al., 2009; Barker et al., 2010). Burow et al. (2014) found that the risk of lameness reduced as cow tracks were better maintained (e.g. covered with asphalt).

1.1.7 Economic impact of lameness

Lameness has a significant economic impact on the profitability of the dairy farm. The cost of lameness can be categorised as direct and indirect costs. Lameness treatment is seen into a direct cost which in 1997 was estimated at £93.00 for a case of claw horn lesions (Kossaibati and Esslemont, 1997). When indirect costs such as reduced fertility and milk production and increased culling rate were included, a single case of lameness was calculated to cost between £74.78 and £524.43 depending on the cause (Willshire and Bell, 2009).

Lameness affects fertility in different areas of cow reproduction. Lame cows express oestrus behaviour with a lower intensity, present low ovarian activity, ovulate a low estrogenic follicle and were 3.5 times more likely to have a delayed ovulatory cycle when compare to a non-lame cow (Garbarino et al., 2004; Walker et al., 2010; Morris et al., 2011). It is not surprising therefore that lame cows express a greater calving-conception interval observed in lame cows (Hernandez et al., 2005b).

High yielding cows are more likely to suffer with lameness and a negative relationship between milk production and the degree of lameness has been observed (Hernandez et al., 2005a; Bach et al., 2007; Archer et al., 2010b; Green et al., 2010; Green et al., 2014). Clinically lame cows reduced milk production up to 4 months prior to diagnosis which continued for up to 5 months post treatment; this was associated with a loss of approximately 360 kg in a 305 day lactation period (Green et al., 2002). Amory et al. (2008) found that a case of white line disease can cause a milk loss of 370 kg while a case of sole ulcer can cause a milk loss of 570 kg. Importantly this reduction in milk production started 3 months prior to sole ulcer diagnoses and one month prior in white line disease. Lameness also causes an increase in early culling rates and carcasses tend to be of a low conformation therefore reaching a lower value (Sogstad et al., 2007a).

Farmers main reason to change from conventional parlours to AMS was the reduction of costs in farm staff (Meskens et al., 2001). The efficiency of an AMS and recovery of costs rely on frequent and consistent voluntary milkings that can benefit udder health (Svennersten-Sjaunja and Pettersson, 2008). Lameness reduces the frequency of visits (Bach et al., 2007). As in the previous paragraph, lameness may not only affect milk production in this milking system but may also increase the labour costs as farm staff are needed to fetch these animals.

1.1.8 Diagnosis of lameness

Diagnosis of lameness is commonly performed using a mobility or gait scoring system. Observers look at different postures and changes in the gait of the cows and then assign the animal a specific score. Scores range from 0 to 6, depending on the system used (Manson and Leaver, 1988; Sprecher et al., 1997; Whay et al., 1997; Flower and Weary, 2006). In the United Kingdom, the dairy industry uses a scoring system that ranges from 0 to 3; 0 being a cow with normal or sound gait and score 3 is for cows that are severely lame (DairyCo, 2009) (Table 1.2). This system is based on that described by Whay et al. (1997). Further detail of the systems commonly used can be found in Table 1.2 and Table 1.3.

Table 1.2 Description of the Dairy Co mobility score, the industry standard in the UK.

Score	Class	Behavioural observations
0	Good mobility	Walks with even weight-bearing and rhythm on all four feet, with a flat back. Long, fluid strides possible
1	Imperfect mobility	Steps unevenly (rhythm or weight bearing) or strides shortened. Affected limb or limbs not immediately identifiable
2	Impaired mobility	Uneven weight-bearing on a limb that is immediately identifiable and/or obviously shortened strides (Usually with an arch to the centre of the back)
3	Severely impaired mobility	Unable to walk as fast as a brisk human pace (cannot keep up with the healthy herd) and signs of score 2

Table 1.3 Descriptions of the most common mobility score systems used in research and on commercial dairy farms.

Reference	Score	Class	Behavioural observations
Whay et al. (1997)	1	Sound	
	2	Imperfect locomotion	
	3	Mild lameness	
	4	Moderate lameness	
	5	Severe lameness	
	6	As lame as possible while upright	
Sprecher et al. (1997)	1	Normal	Cow stands with a level back posture. Gait is normal
	2	Mildly lame	Cow stands with a level back posture but develops an arched back while waking. Gait remains normal.
	3	Moderately lame	Arched back posture always evident while standing and walking. Gait is affected and described as short-striding on one or more limbs.
	4	Lame	Arched back posture always evident and gait described as one deliberate step at a time. The cow favours one or more limbs/feet.
	5	Severe lame	Cow demonstrates an inability or extreme reluctance to bear weight on one or more limbs/feet
Flower and Weary (2006)	1	Smooth and fluid movement	Flat back, steady head carriage, hind hooves land on or in front of fore-hooves (track up), joints flex freely, symmetrical gait and all legs bear weight equally
	2	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 and all legs bear weight equally
	3	Capable of locomotion but ability to move freely is compromised	Arched back, steady head carriage, hind hooves do not track up perfectly, joints show signs of stiffness, asymmetric gait and slight limp can be discerned
	4	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 and reluctant to bear weight on at least one limb but still uses that limb in locomotion
	5	Ability to move is severely restricted and must be vigorously encouraged to move	Extremely arched back, obvious head bob, poor tracking-up with short strides, obvious joint stiffness characterized by lack of joint flexion with very hesitant and deliberate strides, asymmetric gait and inability to bear weight on one or more limbs

Validity and reliability must be measured in any scoring system. In the case of mobility score, validity can be established ensuring that the system measures a response to the source of pain and that the painful condition responds to analgesics (Weary et al., 2006). From the different mobility scores used, there are only two systems that have measured pain and used a painkiller in order to observe absence of pain after the clinical lameness has been established (Whay et al., 1997; Flower and Weary, 2006). The system described by Whay et al. (1997) was used to measure hyperalgesia in lame cows (Whay et al., 1998) and their response to analgesics (Whay et al., 2005). In the first study, cows identified as lame by the scoring system presented a lower nociceptive threshold in comparison to the non-lame cows (Whay et al., 1998). Whay et al. (2005) studied lame cow's nociceptive thresholds and locomotion score in response to the application of either a non-steroidal anti-inflammatory drug (Ketoprofen) or sterile saline. All cows improved their locomotion score but cows treated with Ketoprofen presented a modulated response to the nociceptive threshold test.

Validity of the scoring system described by Flower and Weary (2006) has been carried out by comparing the gait of cows with and without sole ulcers, observing a higher mobility score in cows with sole ulcers. Validation with analgesics was done by assessing a cows' mobility score prior to and after the administration of a local anaesthetic to the lame limb (Rushen et al., 2007). The authors observed a very small but significant difference in the mobility score between prior to and after the injection.

Reliability and repeatability of a scoring system is only achieved with proper training and experience of the observer or assessor (Whay, 2002). Only one of the studies described previously (Table 1.2 and 1.3) has measured intra and inter-observer reliability. Flower and Weary (2006) measured the intra and inter-observer reliability of the mobility scoring system and of 6 specific gait attributes (back arch, head bob, tracking up, joint flexion, asymmetric gait and reluctance to bear weight), this latter was measured in order to understand where the variation lie. The intra and inter observer reliability for the mobility scoring system was within acceptable ranges (intra-observer 0.76 and 0.85; inter-observer 0.69) (Martin and Bateson, 2010). Though, the intra and inter-observer reliability for the 6 specific gait attributes ranged from 0.35-0.9 and from 0.38-0.83 respectively. Joint flexion and asymmetric gait were the attributes with more inconsistency when scored; possibly due to lack of clear definitions or because mild gait defects could be more difficult to score (Flower and Weary, 2006).

More objective methods for lameness diagnosis are described in the literature but these are only just becoming commercially available (Kujala et al., 2008; Chapinal et al., 2011; Maertens et al., 2011). Among these, force plates are the most promising method as they can be attached to automatic milking robots (Pastell et al., 2006). Force plates were used to measure the difference in weight distribution in lame cows (Neveux et al., 2006), and found that lame cows transferred their weight to the non-lame limbs. Pastell et al. (2010) found a positive relationship between mobility score and the ratio of weight applied to the pair of legs and also observed that there was a relationship between this ratio and the type of lesion, particularly sole ulcers.

Kinematics is another quantitative method used to explore gait changes. Flower et al. (2005) were the first to use it to compare the gait profiles of cows with no visible injuries and cows with sole ulcers. They observed that healthy cows walked faster than cows with lesions, due to longer stride durations and longer strides, and that put weight on three legs when walking instead of only using two. Blackie et al. (2013) evaluated gait changes in cows with different lameness status. Researchers observed that tracking distance decreased as mobility score increased and that cows with SU shortened their back when walking in comparison to cows with SH; but they did not observed a significant difference between lame and non-lame animals in their spine posture when standing. Although, research in this area has detected good markers for SU detection (e.g. stride length), it is still unknown when cows start to modify their gait in order to compensate for the pain caused by lameness (Blackie et al., 2013).

Lying behaviour measurement has also been described as a possible diagnostic method for lameness (Ito et al., 2010). Lame cows increased their lying time in order to reduce pain in the lame limb (Ledgerwood et al., 2010) Accelerometers or pedometers attached to the cow can measure this behaviour automatically (Rushen et al., 2012). A mathematical model has been built to read and process the information from activity sensors to provide alerts when behavioural changes occur. Promising results show a sensitivity of 85.5% and a specificity of 88%, the authors suggest further research is necessary to improve the model in a similar milking system and in different types of housing and feeding systems (De Mol et al., 2013).

1.2 Pain and welfare of lame cows

1.2.1 Pain definition

The sensorial, cognitive and affective components of pain make it difficult to define in animals (Vinuela-Fernandez et al., 2007). In addition, it is accepted that pain responses change according to individual characteristics such as gender, age, disease and chronicity (Molony and Kent, 1997). Molony and Kent (1997) defined pain as *“an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal’s physiology and behaviour to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery”*.

1.2.2 Pain in lame cows

Prey animals, such as cattle, conceal pain in order to reduce attacks from predators. Lame cows may be able to mask pain as a survival strategy. This makes the visual assessment of pain in lame dairy cows more difficult, with only well trained observers detecting lame cows before further damage to the hoof occurs (O'Callaghan, 2002). The assessment of pain in cattle has focused on their responses to painful stimuli and their response to the use of analgesics.

The locomotion of the lame cows is altered due to biomechanical restriction as well as the presence of sensory pain. Whay et al. (1997) observed that lame cows were in a state of hyperalgesia. Hyperalgesia is defined as an intensified sensitivity to a painful stimulus (Vinuela-Fernandez et al., 2007). The researchers applied a mechanical nociceptive pressure with a blunt pin over the lateral side of the metatarsus until the animal reacted to the stimulus. It was observed that lame cows tolerated lower levels of pressure than non-lame cows, such that pain threshold reduced as the grade of lameness increased. Similarly, Dyer et al. (2007) applied direct mechanical pressure, using a hoof-tester connected to a pressure gauge, to affected claws. The pressure applied was positively correlated to the grade of lameness; cows with better mobility scores tolerated more pressure than those with mobility deficiencies (Dyer et al., 2007).

Further, lame cows also continued under this hyperalgesic state for up to 28 days after a lameness treatment (e.g. foot trimming) was applied (Whay et al., 1998). This was particularly evident in those with sole ulcers and white line disease. Chapinal et al. (2009b) observed different behavioural and gait changes according to the type of claw horn lesion; cows with sole ulcers when compared to cows with other type of lesions (sole haemorrhages and digital dermatitis) showed higher mobility score, arched back, asymmetric steps and increased their lying time.

The response to analgesic treatment has been used to study the relationship between pain and lameness. Whay et al. (2005) observed an increase in the nociceptive thresholds of cows that received Ketoprofen at 3.0 mg/kg of BW, particularly in those with chronic claw horn lesions. Flower et al. (2008) found that following a higher dose of Ketoprofen (3 mg/kg), lame cows distributed their weight more evenly between legs. In a similar study, Chapinal et al. (2010b) compared weight distribution, gait score, walking speed and activity between two groups of lame cows which received either 3 mg/kg of Ketoprofen for two days or saline solution; lame cows treated with Ketoprofen improved the weight distribution. Similarly, the use of local anaesthesia on the claws of the affected limb of lame cows improved mobility score as weight was distributed more evenly after the anaesthesia injection (Rushen et al., 2007).

1.2.3 Perceptions about the pain associated with lameness

Lameness was considered by DEFRA (2007) as a painful condition for the animal and a serious welfare concern. The effects of lameness on reduced fertility rate and milk production are well accepted. The perception of pain caused by lameness and recognition of its welfare importance however appears to be less well appreciated among professionals involved in the dairy industry. A survey questionnaire among cattle vet practitioners (270) and cattle foot trimmers (135) found that more than 95 and 85% respectively considered lameness as a very painful experience for the cow (O'Callaghan, 2002). When farmers were asked about their opinions towards lameness, Leach et al. (2010) found that 94% of farmers considered that “pain and suffering for the cow” was a very or extremely important consequence of lameness. Among the specific factors that encourage farmers to control lameness, pride in a healthy herd (83% of farmers) and feeling sorry for the lame cows (81%) were considered very or extremely important.

Studies about attitudes of farmers towards lameness have provided new information that can be applied in strategies to prevent and control lameness. Bruijnis et al. (2013) found (n=145 questionnaires) that 70% of farmers in the Netherlands believed that cows could suffer pain; 89% considered that clinical foot disorders affected dairy cow welfare and a similar proportion believed that subclinical foot disorders did not affect welfare. Farmers with lower prevalence of skin lesions in their herd were more likely to consider that animals feel pain as humans do (Kielland et al., 2010). In in-depth interview carried out with 12 farmers about their perception of lameness, Horseman et al. (2014) found that lameness is a term used only for severely lame cows; even when farmers recognised that lameness was a painful problem for cattle, cows with impaired mobility (score 2 of the Dairy Co mobility score – Table 1) were not considered lame and were not a priority for treatment.

In Switzerland, Becker et al. (2013) interviewed cattle practitioners (n=137), farmers (n=77) and claw trimmers (n=32), finding that 50.7% of farmers considered of absolute importance “the reduction of pain to the lowest possible level” in comparison to 25.6% of cattle practitioners. Regarding pain perception according to different treatments (e.g. excision of a sole ulcer), farmers scored the lowest in comparison to cattle practitioners and claw-trimmers. But, 74.3% of farmers were prepared to pay for analgesic treatment whilst 60.9% of cattle practitioners considered that farmers were willing to pay for analgesic treatments. In addition, 46.7% of cattle practitioners considered that price was a barrier for farmers to administer analgesic treatments, on the other hand only 10.8% of farmers agreed that price was a major concern for them to pursue analgesic treatment for their cattle.

Cattle practitioners’ attitudes towards pain in cattle can be influenced by gender and years of practice. Huxley and Whay (2006) found that cattle vet practitioners gave a rating of 6 (on a pain scale of 10 points) to lameness; females and recent graduates gave the highest scores to most disease in the survey. Only 33% of 252 participants used NSAIDs in more than half of the lameness cases they treated. In addition, respondents that gave a lower pain score to the conditions were those who did not use analgesic treatments. Becker et al. (2014) found that a veterinarian's year of graduation was positively associated with the likelihood of using analgesia when treating sole ulcers. This attitude was influenced by their knowledge about the obligation to provide analgesia (Switzerland's legislation about painful interventions in animals) and by their sensitivity to pain of dairy cows. A recent Brazilian study, found similar findings observed in Europe about veterinarians attitudes towards pain in cattle, with recent graduates using analgesia more frequently than older veterinarians (>10yrs of graduation) and female practitioners more likely to give a higher pain score to comparable conditions (Lorena et al., 2013).

1.2.4 Welfare of lame cows

FAWC (2009) promoted the concept that animal welfare be considered through the fulfilment of the 5 freedoms which include physiological needs (*freedom from thirst and hunger*), health (*freedom from pain, injury and disease*) and behavioural needs (*freedom from discomfort, freedom to express normal behaviour and freedom from fear and distress*). Milk production per cow has increased dramatically in the last 40 years, a cow now produces on average 28 L/day and high-yielding cows produce 50 L/day (Huxley and Green, 2010). In order to produce this amount of milk, a cows' metabolic rate works up to 5 times its energy maintenance requirement (Huxley and Green, 2010). High-yielding cows are more likely to become lame (Archer et al., 2010b). Animals under high metabolic pressure can be under disease risks and this can have detrimental consequences to their welfare (Broom, 2006; Huxley and Green, 2010). Lameness reduces the quality of life in dairy cows, decreasing their ability for normal functioning, causes suffering and affects their ability to perform their normal behaviours (Bruijnis et al., 2012).

Bruijnis et al. (2012) assessed the impact of lameness caused by foot disorders (e.g. SH, DD, interdigital and heel erosion, etc.) on the welfare of cows. The authors used information on pain impact, duration and incidence of the lesion to model the effects of lameness on the welfare score of cows (maximum score was 60: a cow with severe pain for a year). In addition, the model included a subclinical or clinical presentation of lameness. Findings showed that lame cows at the herd level had severe pain for 3 months. This was caused by DD, which was modelled as having the highest incidence and clinical recurrence. Subclinical disorders, like SH, also showed a high impact on cow's welfare due to a high incidence and long duration. Clinical and subclinical lameness presented a different impact at herd (54% and 46%) and at cow level (27% and 73%). Individual cow welfare is affected differently depending on their pain experience and the duration of the problem (Bruijnis et al., 2012).

Pain is an important component of lameness, though the recommended treatments do not highlight the use and importance of analgesics (O'Callaghan, 2002). Dairy cattle can remain in a hyperalgesic state for up to 28 days after foot trimming has been carried out, the use of painkillers reduces this effect (Whay et al., 2005). In addition, delayed recognition and treatment not only increases the time a cow is exposed to the painful condition, it also reduces the chances of recovery (Leach et al., 2012). Alawneh et al. (2012) showed that severely lame cows were more likely to get pick up for treatment than moderately lame cows. Meanwhile, Leach et al. (2012) observed that from 101 cows enrolled for conventional treatment at the farmer's discretion only 13 received treatment and this occurred on the 65th day after being identified as lame. Improvement in lameness treatment protocols and husbandry can dramatically improve the welfare of lame cows and can provide long-term benefits to the cow and the farmer (O'Callaghan, 2002).

Almeida et al. (2008) observed that lame cows increased their lying time, reduced time spent feeding and showed higher incidence of self-grooming in conjunction with physiological changes (e.g. reduced dehydroepiandrosterone (DHEA)). Galindo and Broom (2002) observed that lame cows received more licking from their cohorts than non-lame cows. These authors concluded that lame cows may have tried to maintain a stable relationship within the herd and may have also looked for comfort. In addition, lame animals may be under a negative emotional state due to pain caused by lameness and the lack of control over their environment (e.g. reduced mobility and feed intake). Neave et al. (2013) observed a negative judgement bias after hot-iron disbudding in dairy calves suggesting that pain in these animals can cause negative emotional states. Dairy calves were more likely to approached near-negative colours after disbudding in comparison to before, showing that these animals were expecting negative events.

1.3 Behavioural changes caused by lameness and lameness treatment

1.3.1 Behavioural changes caused by lameness

Animals modify their behaviour in order to cope with a disease, reduce further damage and aid in the recovery (Molony and Kent, 1997; Broom, 2006). Lame cows modify their gait in order to reduce pressure on the affected limb, distributing the weight evenly in the other limbs (Chapinal et al., 2009a) and reduce their activity; as a consequence a lame cow’s activity budget is altered (e.g. reduction in feeding behaviour time) (Gomez and Cook, 2010).

1.3.1.1 Feeding behaviour

Dairy cattle not only require adequate nutrition, but also the possibility to display normal feeding behaviour, in order to obtain the minimum feed intake to maintain healthy growth, reproduction and milk production, particularly for high yielding cows (≥50 L/day). Cattle manipulate their feed with the lips, teeth and tongue and they may browse the feed for palatable particles (Phillips, 2002). Dairy cattle show a lot of variability in feeding behaviour patterns between cows (DeVries et al., 2003b), though it can be affected by where, when and how they eat whatever is provided to them (Grant and Albright, 1995). When at pasture, a cows' feeding behaviour pattern can be affected by the time of milking and cows tend to spend more time eating when grazing than when indoors (O'Connell et al., 1989), as cattle are more selective when grazing (Phillips, 2002). When indoors, cows prefer to eat during the day (Albright, 1993), with two evident peaks at times when fresh feed is distributed (DeVries et al., 2003a).

Feeding behaviour changes through lactation with an increase in cows' feeding behaviour (total meal time, meal duration (minutes/meal) and meal frequency) between days 35 and 94 of lactation (DeVries et al., 2003b). Parity has an effect on feeding behaviour; multiparous cows reduced feeding frequency but had a larger intake than primiparous cows (Azizi et al., 2009). High yielding cows consumed more daily DMI (primiparous: 34.3 kg/day and multiparous: 44.5 kg/day) and had lower feeding time than low yielding cows (primiparous: 28.4 kg/day and multiparous: 38.7 kg/day) (Azizi et al., 2009).

Feeding behaviour is directly affected by disease in the dairy cow (Siivonen et al., 2011; Fogsgaard et al., 2012) and calf (Borderas et al., 2008a). In the case of lameness, results vary with some studies finding no significant difference between lame and non-lame cows for grazing (Walker et al., 2008) or total feeding time (Singh et al., 1993b; Galindo and Broom, 2002). Meanwhile other studies observed significant differences between lame and non-lame cows (Hassall et al., 1993). These discrepancies may be explained by the experimental design, as DeVries et al. (2003b) observed that meal or feeding frequency and duration may not be a reliable method to assess difference between treatment groups, due to the high variability between cows.

Cows diagnosed with acute lameness showed reduction in their feed intake and feeding time, but showed an increase in their feeding rate in comparison with non-lame cows (Gonzalez et al., 2008). These observed changes were more evident in cows diagnosed with sole ulcers. Chronically lame cows presented similar feed intake that non-lame cows but increased their feeding rate 30 days before foot trimming (Gonzalez et al., 2008). Cows with hock lesions and foot lesions were observed to eat less (cumulative losses on feed intake: 46.1 kg and 27.8 kg respectively) between the diagnosis day until recovery (70 and 56 days respectively) (Bareille et al., 2003). Almeida et al. (2008) found that clinically lame cows (foot lesions and deteriorated mobility score) had decreased feeding time, serum DHEA (<23%), and increased cortisol levels (>65%) compared to sound cows.

Findings from Palmer et al. (2012) showed that cows with higher mobility score had lower meal frequency and duration at 60 and 120 days of lactation; this reduction was due to an increase in mean meal duration, though dry matter intake was significantly lower only at 60 days of lactation. Similar findings were observed in cows housed in automatic milking systems. Bach et al. (2007) observed that total feeding time, feeding frequency and feed intake reduced as mobility score increased. First parity cows showed a higher decrease in total feeding time and feed intake than multiparous cows; whereas multiparous cows showed a larger decrease in feeding frequency than first parity cows. Overall, this suggests that primiparous and early lactating cows are most affected on their feeding behaviour when lame (Norring et al., 2014).

1.3.1.2 Ruminating behaviour

Rumination is part of the digestive process in the cow that includes regurgitation, remastication and reswallowing (Beauchemin, 1991). This cyclical process facilitates the digestion of coarse grasses. During the remastication process the bolus is mixed with saliva, which works as a chemical buffer and aids the pre-digestion process. Rumination allows the breakdown of the plant cell walls which, once the cell solutes are released, are digested by the microflora in the rumen (Beauchemin, 1991).

Rumination presents a circadian variation as cows ruminate more at night than during the day particularly after feeding (Adin et al., 2009; Schirmann et al., 2012). Cattle ruminate an average of 6.5 hours per day in 8 bouts approximately, with a mean duration of 48 minutes per bout (Phillips and Hecheimi, 1989). Most of the rumination happens when cows are lying (Schirmann et al., 2012). Rumination is affected directly by the type of feed provided, particularly by the amount of fibre available in the diet (Beauchemin, 1991). Remastication presents a regular pattern with 60 to 70 bites per minute in older cattle, though high yielding cows masticate faster in order to utilise food more efficiently (Phillips and Hecheimi, 1989).

Rumination is very susceptible to stressful events or factors including: type of housing (Singh et al., 1993a), calving and oestrous (Soriani et al., 2012) or sickness such as mastitis (Siivonen et al., 2011; Fogsgaard et al., 2012). Rumination can also be affected in calves; when young (22.83 ±3.6 days) or older (153.92 ±8.1 days) calves were experimentally injected with low doses of bacterial lipopolysaccharide, rumination was reduced during the period of peak fever (Borderas et al., 2008a). Bristow and Holmes (2007) considered rumination as an anxiety-related behaviour due to its high negative correlation to cortisol levels; they found that cows that presented with increased levels of cortisol had lower levels of rumination at pasture.

In the case of lameness there are contradictory results in comparison with changes in rumination. Differences observed between studies may lie in the type of housing, behavioural data collection and sample size (Table 1.4). For example, Walker et al. (2008) observed 59 cows (39 sound and 20 lame) for 5 days after a prostaglandin injection (PGF2α), in order to study changes in behaviour during oestrus. The study did not find any significant difference in total rumination per day between groups (lame and sound); although researchers found that, when lying, lame cows ruminated more than sound cows. Almeida et al. (2008), however, found that lame cows ruminated less than non-lame cows and that lame cows had lower DHEA and higher cortisol blood levels in comparison to sound cows.

Schirmann et al. (2009) validated the Hi-Tag rumination monitoring system (SCR Engineers Ltd., Netanya, Israel) for its use in cattle. The tag has a built-in microphone that records the distinctive sounds produce by the cow when regurgitating and ruminating. The system records rumination time, interval between boluses and chewing rate. Van Hertem et al. (2013) used this monitoring system to study changes to rumination caused by lameness. This study used rumination data and combined this with hoof health data. Including data only for lame cows, they found that lame cows ruminated 25 min less on average at night (20:01 till 04:00) in comparison with non-lame cows. The observed reduction continued for up to 2 days after treatment. In addition, researchers have studied the effect of routine foot trimming on rumination time (Van Hertem et al., 2014). In this study they observed that increase in locomotion score was negatively associated with rumination time after foot trimming (Van Hertem et al., 2014). This study did not describe the causes of lameness or whether any specific treatments were applied to the lame cows.

Table 1.4 Description of studies that looked at differences in ruminating behaviour between lame and non-lame cows using direct behavioural observations.

Reference	Housing	N	Behavioural observations	Behavioural recording time	Rumination measurement		Results	Differences
Singh et al. (1993b)	Concrete floored cubicles	10 lame cows	Direct visual observation	Every 15 minutes for 24 hours (96 observations)	Total rumination time		539 ± 17	NS
		10 non-lame cows					542 ± 16	
Hassall et al. (1993)	Pasture	10 lame cows	Direct visual observation	Every 15 minutes for 24 hours for 3 days	Mean time spent ruminating in hours	Lying down	3.3	NS
						Standing	0.95	
		10 non-lame cows				Lying down	2.55	
						Standing	0.9	
Pavlenko et al. (2011)	Cubicles with two layered rubber-foam mattresses	10 cows with DD	Direct visual observation	60 minutes observation per cow per day-2 days in total per cow	Absence or presence of the behaviour	Ruminating while standing	3.831 (Odds ratio)	p<0.001
		10 sound cows (no lesions)						
		10 cows with SU				Ruminating while standing	2.079 (Odds ratio)	P=0.08
		10 sound cows (no lesions)						
Almeida et al. (2008)	Free-stall housing	8 lame cows with visible lesions	Focal sampling and continuous recording	3 consecutive days: 2-min observations in 15 min-interval for a total of 4hours per day	Total minutes observed		18.41 ± 3.34	p=0.01
		8 sound cows no visible lesions					28.57 ± 3.06	

1.3.1.3 Lying behaviour

Lying behaviour duration changes according to age. Dairy calves at 21 days of age spent 16hrs lying (Bonk et al., 2013). Three days after calving, cows lie down 14hrs/day (Jensen, 2012); lying time increased as milk production increased. In the 1980s, lactating dairy cows were reported to spend approximately 7 to 10 hours per day lying down (Arave and Walters, 1980). By 2010, Ledgerwood et al. (2010) found that cows milked in conventional systems lay down for between 10 and 12 hours per day and in automatic milking systems Deming et al. (2013b) observed that cows spent up to 14 hours per day lying down. In the wild, it was reported that cattle lay down for approximately 8 hours per day (Hall, 1989).

The preference for lying down is higher than for other behaviours. Dairy cows deprived of lying, feeding and social behaviour, spent more time lying down than eating or in social contact once restrictions were removed (Munksgaard et al., 2005). Dairy cattle that were deprived of lying showed signs of distress and physical exhaustion (Munksgaard et al., 1999). Individual dairy cows have a preference for which side they lie on. Forsberg et al. (2008) did not find a significant difference in the preference for lying on the left or right in lactating cows, and they did not observed any relationship between lying side with parity or lactation stage. Though, cows at the end of gestation have a preference for lying down on their left side (Arave and Walters, 1980; Forsberg et al., 2008). It is possible that lying over the left side provides more comfort to the cow as the foetus predominantly occupies the right side of the abdomen.

Lying behaviour components (total duration, frequency of bouts and mean bout duration) can be affected differently by different milk production parameters (parity, days in milk and milk production). Total lying duration increases with parity and age (Norring et al., 2008; Steensels et al., 2012). First parity cows showed higher number of lying bouts but shorter mean lying bout duration in comparison to older cows (Vasseur et al., 2012). Total lying time and lying bout duration were positively correlated with stage of lactation (Vasseur et al., 2012; Deming et al., 2013a), and lying bout frequency decreased with stage of lactation (Deming et al., 2013a). Total lying duration in early lactation cows was negatively correlated with milk production (Norring et al., 2012). High yielding cows housed in farms with automatic milking systems spent less time lying down and had shorter lying bouts (Deming et al., 2013b).

Regarding the effects of milking frequency on lying behaviour; cows milked once versus twice daily did not show significant differences in total lying time (Tucker et al., 2007). A similar finding was observed when comparing cows that were milked twice versus those milked three times daily (Hart et al., 2013). Though, Osterman and Redbo (2001) observed that cows milked three times daily had higher lying times during the 4 hours before milking in comparison to those milked twice or once daily. Cows housed in automatic milking systems (with a mean milking frequency of 2.8 times/day), spent an average of 11 hours per day lying down, with a frequency of lying bouts of 10 per day and an average duration of 80 minutes per bout, similar to those observed in conventional milking parlours (DeVries et al., 2011; Deming et al., 2013b). Neither of these two studies explored the association between milking frequency and lying behaviour.

Management and housing's characteristics can affect lying behaviour. Cows provided with wet bedding showed lower lying times compared to when they had access to dry bedding (Fregonesi et al., 2007b). If given the choice between these two types of bedding, cows spent most of the time lying down on the dry bedded cubicles than on the wet ones (Fregonesi et al., 2007b). Cows were observed to lie down for longer on straw cubicles versus sawdust or sand, with mattresses being the least preferred (Tucker et al., 2003; Norring et al., 2008). Wagner-Storch et al. (2003) compared six types of bedding surfaces, their findings agree with those previously described here; in addition concrete had the least occupancy followed by rubber mats and then waterbeds. Cows may have a preference for softer surfaces (Tucker et al., 2003) and deeper bedding (Ito et al., 2014). Ito et al. (2014) observed that deep sand bedding not only increased lying time but also reduced the frequency of lying bouts and increased the lying bout duration. Cows had increased lying time and longer bout duration when housed in wider stalls (Tucker et al., 2004). Stocking density increased the variation of lying time between cows (Ito et al., 2014) and decreased lying time (Fregonesi et al., 2007a). Cows at pasture had less undisrupted and longer lying times than cows housed indoors (Olmos et al., 2009).

Lame cows showed increased lying times (Ito et al., 2009). Thomsen et al. (2012) studied 1340 cows from 42 herds in Denmark and observed an increase in total lying time and in mean bout duration as mobility score increased. Similar findings were observed by Ito et al. (2010), in addition the researchers observed a significant increase in mean bout duration when severely lame cows were compared with moderately lame and sound animals. Increases in total lying time in lame cows have been observed to occur particularly in the evening (16:01-23:00) (Blackie et al., 2011). Yunta et al. (2012) observed that moderately lame cows had longer bouts than non-lame cows, although the authors did not observe significant differences for total lying time, number of lying bouts between groups or laterality of lying behaviour. Increases in lying time were observed around feeding time, lame cows in comparison to non-lame cows took longer to stand up when feed was delivered and went back to lie down sooner (Yunta et al., 2012). In automatic milking systems, the degree of lameness was positively associated with lying time and the frequency of lying bouts (Deming et al., 2013a).

Type of lesion also affects lying behaviour patterns. Chapinal et al. (2009b) observed that cows with sole ulcers had higher lying times compared to cows with other types of lesions (DD or SH) or no lesions. This increase was due to an increase in the mean bout duration. Thomsen et al. (2012), however, found that skin lesions on the hoof (e.g. digital dermatitis) were more likely to be found on those cows with increased mean duration of lying bouts but not on those cows with horn lesions (SU, SH and WLD).

1.3.1.4 Social behaviour

Cattle live in herds as this reduces the risk of predation and increases learning through social facilitation (Dumont and Boissy, 1999). Wild cattle showed a more linear hierarchy structured than domestic cattle (Hall, 1989). Cows in enclosed housing environments may developed stable or dynamic relationships depending on management practices (Wierenga, 1990). Agonistic behaviours such as bunting, avoidance, pushing, contact head to head and threatening are included in the repertoire of dominance or displacement behaviours (Dickson et al., 1967; Reinhardt and Reinhardt, 1975). Dominance values or index of displacements are measured based on the number of times a subject displaced another and by the number of times the subject was displaced (Dickson et al., 1967; Galindo and Broom, 2000).

Social hierarchy is dynamic, dairy cows can switch rank depending on weight (Dickson et al., 1967) and age, reaching a peak in dominance at the age of 9 years (Reinhardt and Reinhardt, 1975). Younger cows showed aggressive behaviour towards older cows or towards cows of their same age group; meanwhile older cows lost their position in the rank as they lost weight (Reinhardt and Reinhardt, 1975). Arave and Albright (1976) found positive correlations between dominance rank, body weight and withers height. However a recent study carried out in a beef herd for a period of 10 years showed that social hierarchy is led by age rather than weight (Sarova et al., 2013).

Cows show their dominance status particularly in situations where space is limited; at the feeding fence, cows from the same rank status fed closer to each other compared to cows of lower rank (Manson and Appleby, 1990). Val-Laillet et al. (2008) observed that displacement interactions increased depending on a cow's motivation to gain access to the resource as well as social pressure. Researchers suggested that social dominance varies according to the resource, with high ranking cows spending more time at the feeding fence than the middle or low ranking cows. In automatic milking systems, Lexer et al. (2009) observed that low ranking cows spent more time waiting to enter the milking robot. Dominant cows produced more milk when offered forage supplement in comparison to cows low in the dominance order (Phillips and Rind).

A survival analysis on lameness during the housing period found that middle and high ranking cows had better survival rates (67% and 82% respectively) compared with low ranking cows (<40%) (Galindo and Broom, 2000). Middle and high ranking cows spent less time standing still in the passage ways than low ranking cows (Galindo et al., 2000); low ranking cows also showed less lying time and spent more time standing half in the cubicles than the other two ranking groups (Galindo and Broom, 2000). Lame cows showed less aggressive interactions in comparison to non-lame cows and solicited more licking, showing a possible way to cope with the environment and to maintain stable relationships (Galindo and Broom, 2002).

1.3.1.5 Visits to milking robots – milking behaviour

Since their introduction in the 1990's, automatic milking systems (AMS) have become more popular worldwide (De Koning, 2010). By 2008, there were 5,500 commercial farms using AMS (Svennersten-Sjaunja and Pettersson, 2008) with the latest figures from 2012 (16,000 farms worldwide) indicating that their popularity continues to grow (De Koning, 2013). It is believed that most of these farms are located in North West Europe (80%; De Koning, 2010) and their use may not just be limited to Holstein-Friesian cattle as research into their use with dairy buffalo has been reported (Caria et al., 2014).

AMS or robotic milking is a less labour intensive farming system; cows can be milked up to 5 times a day with minimum human supervision. Sensors in the machine monitor body weight, udder health and milk production (Spörndly and Wredle, 2002; De Koning, 2013). This can provide more freedom to farmers compared to conventional parlours and the opportunity to increase milking frequency resulting in an increase in milk production (Uetake et al., 1997; Meskens et al., 2001). Though the overall success of the system depends mostly on the management skills of the farmer, understanding of cow behaviour and farm design are also important (De Koning, 2013).

Milking frequency in AMS depends on cow traffic, milk yield and individual cows (Svennersten-Sjaunja and Pettersson, 2008). There are three types of cow traffic: i) free – cows have ad-libitum access to the milking robot, ii) semi forced – cows have access to feed but they can be guided through the milking robot by gates and iii) forced – cows are not allowed to feed until they have been through the milking robot (Wiktorsson and Sørensen, 2004). Cows have a higher motivation for food than for milking (Prescott et al., 1998); AMS have a built in feed bunker that provides concentrate feed to supplement the base ration (Svennersten-Sjaunja and Pettersson, 2008). Cows in free-traffic systems were fetched more frequently to the AMS, particularly during the learning or reintroduction period (first 14 days of lactation; Jacobs and Siegford, 2012b). Fetched cows showed a larger avoidance distance to a familiar person than cows that were not fetched for milking (Rousing et al., 2006). Cows managed in a forced traffic AMS had an increase in the time they spent in the feeding area compared to a free traffic AMS (Ketelaar-de Lauwere et al., 1998).

AMS provides animals with the freedom to control their daily activity, with the possibility of longer periods of lying and reduced stress at the time of milking because they are not rushed or pushed by the herdsman as they are in conventional parlours (Hopster et al., 2002); more frequent milking reduces udder pressure and at the same time reduces stress on the udder ligaments (Meskens et al., 2001; Osterman and Redbo, 2001).

Cows transferred from conventional milking parlours to AMS showed higher vocalizations and elimination behaviour and lower milk production during the first 24 hours of being transferred (Jacobs and Siegford, 2012b). Weiss et al. (2005) found that inexperienced cows (90%, n=17) learnt to go voluntarily through the AMS by day 9 of training. Inexperienced cows showed a significantly increased basal heart rate during their first two visits to the AMS in comparison to experienced cows and to their previous reading in the conventional milking parlour (Weiss et al., 2005).

High ranking cows spent less time standing in the waiting area of a semi forced traffic AMS (Wiktorsson and Sørensen, 2004) and visited the AMS more frequently between 12:00 and 18:00 hours (Ketelaar-de Lauwere et al., 1996). On the contrary low ranking cows visited the unit more frequently between 00:00 and 06:00; low ranking cows may avoid agonistic encounters with high ranking cows adapting their visits accordingly to less busy times at the AMS (Ketelaar-de Lauwere et al., 1996). Similar findings were observed in cows housed in a grazing system with AMS, low ranking cows waited longer to gain access to the milking robot (Jago et al., 2003). Using data from an AMS with free traffic, Halachmi (2009) reported that low ranking and high ranking cows waited on average 68.9 minutes and 3.5 minutes respectively to gain access to the milking robot. Helmreich et al. (2014) observed that cows with a high milking frequency spent more time in the waiting area at night.

Lame cows reduce their voluntary visits to the AMS: Borderas et al. (2008b) observed that high visiting cows had lower mobility scores; only 4% of these animals had a mobility score described as slightly lame, meanwhile 32% of low-visiting cows were scored as slightly or severely lame. Visits to an AMS reduced as mobility score increased (Bach et al., 2007); this reduction was more significant in 1st calving cows. Cows with higher mobility scores were more likely to be fetched than those with low mobility scores (Bach et al., 2007). Severely lame cows were less likely to visit the AMS between 24:01-5:59 but more likely to visit the unit between 06:00-12:00 possibly when fresh feed was provided (Bach et al., 2007). No published research was identified that reported the effects of type of lameness or lameness treatment on the number of visits to an AMS.

1.3.2 Effects of lameness treatment on the behaviour of dairy cattle

Early treatment of foot lesions has been showed to improved milk production and mobility score (Leach et al., 2012), though little is known of the effects of lameness treatment on cow behaviour. As previously stated, recommended treatments for lame cows include the use of NSAIDs, foot blocks and foot trimming (Whay et al., 2005; Shearer et al., 2013). Foot blocks are recommended to reduce the weight load on the affected claw; they aim to promote healing (Shearer et al., 2013), and to improve mobility score (Wehrle et al., 2000). Foot trimming helps to clean the lesion and reduce the pressure in this location and can improve mobility (Van der Tol et al., 2004). Lameness causes behavioural changes due to the discomfort. Lameness treatment should aim to reduce the discomfort and control the pain (O'Callaghan, 2002), if this is achieved behavioural changes should be minimal.

Cutler (2012) applied foot blocks to 10 healthy cows (5 on the right hind leg and 5 on the left hind leg) and observed the lying behaviour, activity, mobility score and milk production, and compared these to the measurements of 10 healthy cows with no foot blocks. Only 6 cows retained their block until the end of the trial (28d). Block wear was higher on the cranial end than at the block's caudal end. Cows were more active before the block was applied but no behavioural changes (lying bouts and lying bout duration) were observed between groups. Blocks did affect mobility score; blocked cows had higher scores for up to three days after application. Milk production was not affected by application of foot blocks.

O'Callaghan (2003) studied the effect of lameness treatment on cow activity and mobility. The researcher treated 285 lame cows once and 35 cows twice. All cows received foot trimming according to the method described by Toussaint-Raven et al. (1985) and further treatments were applied depending on lesion type. Cows with acute lesions were assigned randomly to no further treatment or 3 days Ketoprofen treatment (3 mg/kg IM SID); cows with DD received additional antibiotics. Cows with chronic lesions were assigned randomly to no further treatment, foot block application or 3 days Ketoprofen treatment. Treatments did not improve mobility score even 30 days after treatment. Level of activity (steps/hour) increased immediately after treatment and remained higher than before treatment. Treatment results varied according to the severity of the lesions. Administration of NSAID in cows with mild acute and moderate chronic lesions increased their activity; meanwhile activity reduced for cows with mild chronic lesions and with severe acute or chronic lesions. Cows that received foot blocks reduced their activity if they were diagnosed with mild or moderate chronic lesions.

Routine foot trimming may cause changes in feeding and lying behaviour. Gonzalez et al. (2008) observed the most significant changes in non-lame cows, which increased feed intake, feeding time and feeding rate after foot trimming. On the other hand, lame cows did not show significant changes in their feeding behaviour but increased their visits to the feeding area (Gonzalez et al., 2008). Routine hoof trimming affected lying behaviour, with both lame and non-lame cows increasing their lying time for up to 5 weeks after hoof trimming (Chapinal et al., 2010a).

1.4 Statistical and epidemiological tools to study behaviour

1.4.1 Reliability and validity studies

Reliability is used particularly to measure the agreement between observers or within-observer. It is important to know if the measurements that the observer uses are repeatable and reproducible. Good or reliable measures must have good consistency (the measurement can be repeated and still measures the same thing) (Martin and Bateson, 2010). Within-observer reliability measures the consistency of the observer to measure the same thing at different times; this reliability checks should be carried out at regular times throughout the study and it can be done using videos that allow the observer to be measured at different times. The degree of agreement may be affected by sample size (Martin and Bateson, 2010).

Validity of automated tools used to measure behaviour can be carried out measuring its sensitivity (ability to detect a behaviour) and its specificity (ability to make distinction between a true behaviour) (Rushen et al., 2012). In order to carry out validity testing, the assessment needs a gold standard against which the tool can be compared. In behavioural studies, direct visual observations can be considered the gold standard against which the automated tool is compared (Rushen et al., 2012). In addition, predictive values can add information about the usefulness of a test or automated tool. These can tell us the probability of the tool to measure the behaviour that we want it to measure (Mattachini et al., 2011).

1.4.2 Multilevel analysis

Data can have a hierarchical pattern particularly when data is collected from groups of animals living on the same farm (e.g. cows within pens), or when collecting data from the same animal but in different time periods (e.g. days within cows). In the study of dairy cattle behaviour, it is important to consider this hierarchical structure as behaviour can vary both within cow and between cows (Ito et al., 2009; Weary et al., 2009). Multilevel modelling allows this structure to be included within the statistical analysis taking into consideration the random variability at and within each level (Rasbash et al., 2009). According to Stamps et al. (2012), traditional statistical analysis used previously (e.g. GLM) can reduce the chances of finding significant associations between mean scores of a behaviour and the variables of interest, as these analysis calculate the estimates of the mean scores of the behaviour without taking into consideration the within animal variation. In multilevel analysis, once these effects have been controlled for, what is left is the unpredictable component of behaviour or residuals (Briffa et al., 2013).

In addition, these models allow for a multivariable analysis approach which allows the inclusion of many independent variables that can impact on a particular outcome behaviour, for example lying behaviour can be dependant on the amount of spaces available and the bedding material (Martin and Bateson, 2010). These variables are analysed within the model at each level, thus providing an advantage over traditional statistical methods as the predictive variables are accounted for at lower levels in the hierarchy (Stamps and Blozis, 2006)

1.4.3 Case-control studies

Case control studies are epidemiological observational studies which study retrospectively the factors that elevate or reduce the risk to disease by comparing the cases (e.g. diseased animals) to controls (e.g. healthy animals) (Dohoo et al., 2003). Controls are selected from the same type of population (e.g. dairy farms with AMS) or from within the same population (e.g. cows from one farm), these animals or controls must be healthy or not suffering from the problem that is under investigation (e.g. lameness) when the study starts. The ideal ratio for case-control is 1:2 (Dohoo et al., 2003). Matching criteria are usually applied in order to select case and controls that are similar to each other reducing the confounding effects of those variables used for matching. In addition, exclusion criteria can be applied (e.g. only animals from 1st parity) as this helps to reduce the effects of unknown factors that can negatively influence the final results (Dohoo et al., 2003).

These studies are easy, quick and cheap to carry out as the data can be collected retrospectively and follow-ups can be done with no loss (e.g. time). The main disadvantage is that causation cannot be deduced, particularly if animals were infected or sick before they were exposed to the risk factor (Petrie and Sabin, 2009).

1.4.4 Longitudinal studies

The use of longitudinal studies in animal behaviour research, allows the investigation of changes in behaviour over time and are mainly use in behavioural development (Forstmeier, 2002; Martin and Bateson, 2010). In veterinary medicine, longitudinal studies are used to study the effects of treatment on a disease pattern (Petrie and Watson, 2006). In recent years, automated tools to measure behaviour have allowed researchers to include this epidemiological tool to look at the effects of husbandry or diseases on behaviour (Rushen et al., 2012).

Longitudinal studies, in comparison to cross-sectional studies (studies carried out at a specific time point or over a short period), are more powerful in detecting causal relationships, though this strength only relies on one single criterion for causality: development over time (Twisk, 2007). Among other disadvantages of this type of study are the costs involved in carrying out longitudinal studies, the time needed to collect the data and the difficulty in analysing the data as complex techniques are required (e.g. multilevel analysis) (Twisk, 2007). In animal behaviour, the drawback of longitudinal studies is that animals can get used to repetitive measurements, this can be more disadvantageous for studies of behaviour development (Martin and Bateson, 2010). Nevertheless, the main advantage of longitudinal studies are that they allow the study of the relationship between the individual development of the outcome and the individual development of the dependant variables (Twisk, 2007).

1.4.5 Randomised clinical trials

Randomised clinical trials or experimental longitudinal studies investigate the effect of an intervention (e.g. clinical treatment) over an outcome or measure of interest (Dohoo et al., 2003). Subjects are randomly assigned to one of the treatments under study, in this way the study is free from bias: assessment of the outcome is comparative (more than one treatment group or control group) and carried out free of preconceived ideas (e.g. blind assessment unaware of which treatment was applied) (Petrie and Watson, 2006). As a longitudinal study, the disadvantages are the cost involved, the time needed to collect the data and the need for complex data analysis. Additionally this study type is more likely to miss data, as subjects can be withdrawn during the study period (e.g. culled) (Twisk, 2007).

1.5 Conclusions

Lameness is a major problem to the dairy industry, particularly affecting high yielding cows. Lameness is associated with poor fertility, reduction in milk production and an increased culling rate. Claw horn lesions have been recognised as the main cause of lameness but there is limited information on how these may affect cow behaviour and later recovery rates. In addition, the increase in popularity of AMS has raised many questions regarding the welfare of cows housed in this system, as well as how lame cows cope with this environment.

Welfare of lame cows is not only affected by the pain caused by the disease, lack of prompt diagnosis and delay in the application of an appropriate treatment cause prolonged pain and directly affects cow behaviour. These have detrimental consequences on an animal's ability to cope with its environment. As documented, the lack of farmers' perception of the existence of moderately lame cows in their herds increases the chances of a cow of not being detected and properly treated. Further, when lameness is detected there is a lack of knowledge on the effectiveness of treatments and the effect of these treatments on dairy cattle behaviour.

1.6 Thesis objectives

This thesis aims to examine the effects of lameness and lameness treatment on dairy cow behaviour housed in automatic milking systems. The objectives were:

1. To assess the effect of lameness on milking behaviour in cows housed in an automatic milking system.
2. To assess the effect of lameness on rumination behaviour in cows housed in an automatic milking system.
3. To determine the effects of lameness treatment on lying behaviour in cows housed in automatic milking systems.
4. To determine the effects of lameness treatment on milking and rumination behaviour in cows housed in automatic milking systems.
5. To investigate the effect of claw horn lesions on the likelihood of recovery from lameness.

Chapter 2

Study of the impact of lameness on dairy cows visits to an automatic milking system

2.1 Introduction

Automatic milking systems (AMS) were introduced to the dairy industry approximately 20 years ago and there are approximately 9000 units being used worldwide (Halachmi et al., 2011). The most attractive farm benefits for the use of AMS are the freedom they provide farmers compared to conventional parlours as well as the opportunity to increase milking frequency resulting in an increase in milk production (Uetake et al., 1997; Meskens et al., 2001). Of equal importance, the cows may benefit from the freedom to control their daily activity, with the possibility of longer periods of lying down and reduced stress at the time of milking because they are not gathered and crowded as they are in conventional parlours (Hopster et al., 2002; Jacobs and Siegford, 2012a). Additionally, more frequent milking reduces both udder pressure and stress on the udder ligaments (Rossing et al., 1997; Meskens et al., 2001; Osterman and Redbo, 2001).

Lameness has become one of the most detrimental problems in modern dairy herds, not just for the welfare of the cow but also for the economics of the farmer (Archer et al., 2010a). Lameness is a sign of pain and discomfort at the level of the leg but more commonly at the level of the claw (Archer et al., 2010a), which leads to cows modifying their gait in order to have access to their needs (e.g. feed or social contact) (Galindo and Broom, 2002). Acute or chronic pain and stress reduce the cows' ability to control their environment (Whay et al., 1997; Galindo and Broom, 2002; O'Callaghan, 2002). The severity of lameness is positively correlated with the amount of time spent feeding (Gomez and Cook, 2010) and lying (Ito et al., 2010), particularly during oestrus (lame cows lie for longer than non-lame cows) (Walker et al., 2008). Consequently, lameness is associated with an average loss of 350kg of milk per year (Green et al., 2002; Archer et al., 2010b; Reader et al., 2011) and a reduction in reproduction success (Huxley, 2009).

Even though there are studies investigating the effect of lameness on milking behaviour (Klaas et al., 2003; Bach et al., 2007; Borderas et al., 2008b), these were carried out in farms with an average milk production of 30 L/day. The aim of the present study was to identify the impact of lameness on milking behaviour (frequency and time of visit) under a UK AMS management system in a high yielding herd. The null hypothesis was that lameness had no impact on cows' milking behaviour. The alternative hypothesis of the study was that lameness had an impact on milking behaviour.

2.2 Materials and Methods

2.2.1 Study population

The study was conducted between October and November 2011 on a 200 cows AMS unit in the midlands region of the United Kingdom, with an average milk yield per cow of approximately 11500L per 305 days. The study protocol was approved by the University of Nottingham's School of Veterinary Medicine and Science Ethical Review Committee.

The unit consisted of four pens, housing approximately 45 Holstein cows with access to one AMS (Figure 2.1) (Lely Astronaut A3, Lely UK Ltd, St Neots, UK). Each pen had three rows of free-stalls bedded with a thin layer of sawdust on a mattress base. Three of the four pens had 59 stalls and the remaining yard contained 66 stalls. All walking and standing areas were covered with rubber matting (Kraiburg, Kitt Ltd. UK); passageways were cleaned every hour by automatic scrapers. Cows had free access to the AMS at any time; a maximum of 5 milking visits per cow per day was permitted. The maximum interval allowed between milking visits was set at 12 hours. Milking attendance was monitored twice a day (at 07:00 and 15:00 hours approximately) and cows were selected if their visit frequency was considered inadequate based on their days in milk, parity and yield. Selected individuals were identified and walked into the AMS by farm staff, cow ID number was recorded.

Fresh feed was provided as a total mixed ration once per day at approximately 8.30; ration was pushed up at 10:00, 12:00, 14:00, 17:00, 20:00 and 6:00. Feed was provided along one side of each pen (approximately 36 meters) and each pen contained two large water troughs. All milking cows in the study had ad libitum access to the total mixed ration and in addition, cows were provided with an individual concentrate ration (1.5Kg/day) adjusted to the frequency of milking visits that was dispensed in a feed bunker in the AMS, each time they were milked. If the cow produced more than 23 L/day, an additional 0.16 kg per each extra litre of milk was provided each day.

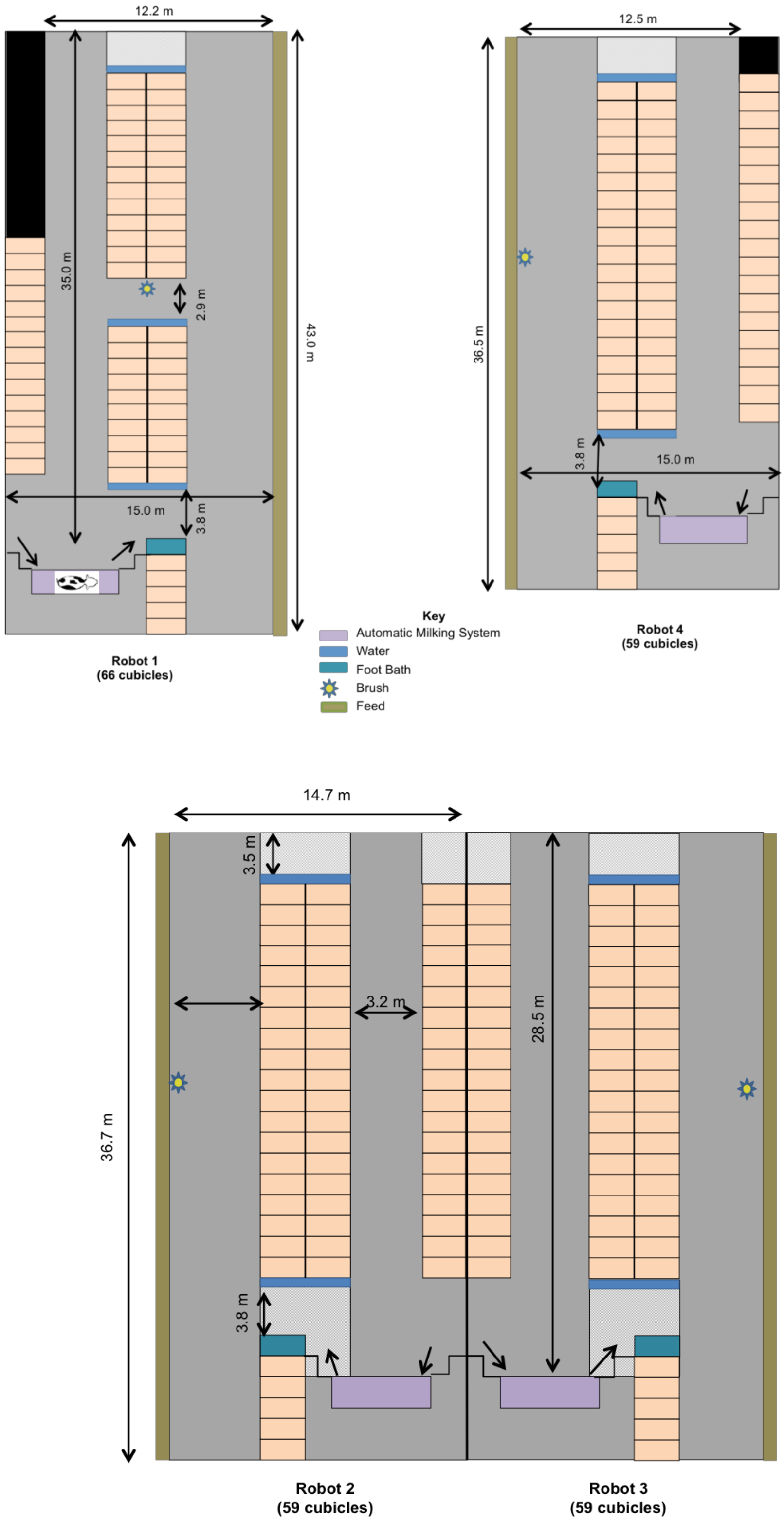

Figure 2.1 Pen layout in the automatic milking system farm**.**

2.2.2 Study design

The study was designed as a case-control observational study. Milking visit frequency and time of the milking visits to the AMS were compared between lame (case) and non-lame cows (control). A sample of the whole herd data was analysed prior to the start of the study to establish the parameters to be taken into consideration for the matching criteria and to estimate the sample size required (Stata/SE 12.0 - Stata Corp 2011, USA). Assuming an estimated reduction of 0.5 milking visits per day between case and control animals, a standard deviation in the number of visits per day of 0.86 (based on herd data) with 80% power and a confidence level of 95%, the required sample size was calculated as a minimum of 24 case and control pairs.

2.2.3 Mobility scoring

Locomotion score was carried out following the UK industry standard four point system (Appendix 1: DairyCo, 2009): score 0 a cow with good mobility, score 1 with imperfect mobility, score 2 with impaired mobility with a limb that is immediately identifiable and score 3 with severely impaired mobility. Mobility score was carried out by a single trained observer (GMP), who was trained by an experienced researcher (JH) for a period of 4 weeks.

In order to record the mobility score of each cow, each pen was locked at one end and the animals were pushed towards the other side. The mobility score assessor (GMP) stood on one side of the passageway meanwhile the animals were pushed slowly from one end to the other of the passageway crossing in front of the observer. This was repeated in each pen once per week for 7 weeks, starting at 10am and ending before 12pm.

2.2.4 Case-control selection

After each observation, case-control pairs were selected using the matching criteria outlined in Table 2.1 and blocked by pen, aiming to achieve a case-control ratio of 2:1 (Dohoo et al., 2003). Lame cows could only be enrolled onto the study once; cows classified as controls could be used more than once, if they met the matching criteria for more than one lame animal. The farm veterinarian examined and treated lame cows within 48 hours after they were enrolled onto the study. Cows were diagnosed after receiving a standard foot trim; lesions were identified following Archer et al. (2010a).

Table 2.1 Criteria for the matching selection of case-control pairs.

Matching Criteria	Case	Control
Mobility Score	2 or 3 (lame or severely lame)	0 or 1 (non-lame)
Parity	1	1
	2	2
	>2	>2
Daily Milk Production (L)	a	a ± 5
DIM	<20d	<20d
	20-180d	± 20 (min 20d)
	>180d	± 50

DIM = days in milk; a = daily milk production from the previous 24hrs (12:01 – 12:00); d= days.

2.2.5 Data collection

Following enrolment, data for each case-control pair were downloaded for a 24-hour period beginning at 12:01. Data collected included number of milking visits in the last 24 hours, time of each visit, number of refusals (the milking robot computer refused to milk the cow because the minimum-milking interval had not been reached (4 hours) and number of failures (the milking robot computer failed to attach the teat cups to the cow).

2.2.6 Statistical Analysis

Data were managed in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA). The dataset of case-control pairs included AMS pen ID (1 to 4), cow ID, case (1) or control (0), locomotion score, parity, daily milk production (last 24 hours) and days in milk (DIM). Each visit to the AMS was allocated to one of four time periods (12:01 - 18:00; 18:01 - 24:00; 24:01 - 06:00 and 06:01 - 12:00), the variable was called Visit ID model; then, each six hour period was given an identification (ID) number depending on whether it pertained to case (1, 2, 3 and 4) or control animals (5, 6, 7 and 8).

Statistical analysis was conducted using Stata/SE 12.0. (Stata Corp 2011, USA) and MLwiN (version 2.10; Centre for Multilevel Modeling, University of Bristol, UK). Parity, daily milk production and DIM were normally distributed; the Mean Paired test (Petrie and Watson, 2006) was used to compare data between groups. The total number of milking visits was not normally distributed and could not be successfully transformed; therefore Wilcoxon Signed-rank test (Petrie and Watson, 2006) was used to compare data between groups. A P value of ≤0.05 was specified to indicate significant differences. Refusals data were analyzed using Two Sample Proportion Test (Petrie and Watson, 2006) to compare data between groups.

A multilevel binomial logistic regression model was built to compare the odds of the milking visits to the AMS at specific time periods between case and control groups. The model was set with 3 levels (AMS pen=*k*, cow ID=*j* and visit ID model=*i*) and the outcome was defined as whether cows visited the milking robot during a particular time period (visit Y/N). Visit ID for cases (1-4) and controls (5-8) were the explanatory variables and were added as fixed effects. AMS pen (1-4) was also added as a fixed effect. The model was as follows:

$$\text{Logit}(\pi_{ijk}) = \beta_0 x_{0ijk} + \beta_1 x_{1ijk} + \beta_2 x_{2ijk} + e_{ijk} + u_{ijk}$$

Where π was the probability of visit/no visit to the AMS, β_0 was the intercept fixed at each level, β represented the regression coefficient of each explanatory variables and the predictor variables were represented by x. x_1 represented milking robot (4 variables) and x_2 represented visit ID (8 variables). The random error is represented by e and u at cow and pen level respectively. The model fit was checked by graphical analysis of normal distribution of residuals at level 2 (cow) and 3 (visit ID).

2.2.7 Results

A total of 38 case-control pairs were enrolled in the observation period. Two cows were used twice as controls in the pair matching. As expected due to matching there were no significant differences in parity, DIM and daily milk production between the case (lame) and the control (non-lame) cows (Table 2.2).

Table 2.2 Mean and ±SE for the matching variables for lame and non-lame cows.

Variable	Lame		Non-Lame		*P*
	Mean	±SE	Mean	±SE	
DIM	164.4	12.7	166.5	13.7	0.49
Parity	2.6	0.3	2.7	0.3	0.68
Milk production (24hrs)	40.8	1.2	41.6	1.4	0.12

Twenty-six cows were diagnosed successfully, diagnosis data was missing for 12 cases. Sixteen cows were diagnosed with claw horn lesions (sole ulcer, white line disease and/or sole haemorrhage), two cows were diagnosed with interdigital necrobacillosis, three cows were diagnosed with claw horn lesions and interdigital necrobacillosis and 5 cows were diagnosed with claw horn lesions and digital dermatitis.

The total number of visits to the AMS for cows in the case group was 164 and for the control group was 140 (Table 2.3), from which refusals represented 26%

for the former and 23% for the latter. No significant difference between groups was found for refusals from the AMS (P>0.05). A closer look at the data, in the 24 hours of observation, showed that 5 case cows were fetched in comparison to 4 control cows.

Table 2.3 Total visits and percentages for milking visits and refusals to the AMS by case and control cows.

		Milking Visits	Refusals	Total Attendance
Case	Total Visits	122	42	164
	%	74	26	100
Control	Total Visits	108	32	140
	%	77	23	100

Case cows visited a maximum of 4 times and a minimum of once during the 24-hour observation period, with a mean of 2.8 milking visits per 24 hours. Control cows had a maximum of 5 and a minimum of 2 milking visits, with a mean of 3.2 milking visits per 24 hours. When the number of milking visits to the AMS from case and control cows were compared a significant difference was observed ($z = -2.71$, $P<0.001$).

As shown in Figure 2.2, the results of the logistic regression model demonstrated that after controlling for the effect of robot, case cows were significantly less likely to visit the AMS between 24:01 and 06:00 when compared to control animals (Table 2.4). There was no significant effect of robot on the likelihood of milking visits (Table 2.4).

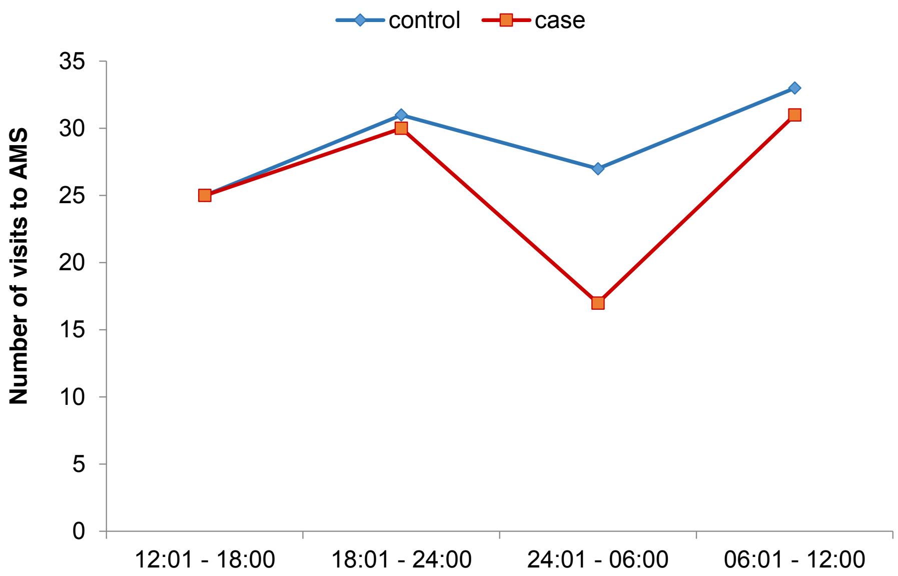

Figure 2.2 Frequency of milking visits during four time periods by case and control cows.

Table 2.4 Logistic regression model results investigating the effect of robot ID and visit times among case and control cows on the likelihood of a robot visit.

Fixed Part	Freq	Coef	SE	Odds Ratio	z	Confidence Interval 2.50%	Confidence Interval 97.50%	P
Intercept	304	0.80	0.40					
Milking robot								
Robot 1	104	Reference						
Robot 2	64	0.36	0.38	1.43	0.87	0.68	3.00	0.35
Robot 3	64	-0.07	0.36	0.93	0.04	0.46	1.88	0.84
Robot 4	72	0.18	0.36	1.20	0.27	0.60	2.42	0.60
Visit time								
24:01-06:00 (Cl)	38	Reference						
12:01-18:00 (Cs)	38	-0.25	0.50	0.78	0.24	0.30	2.07	0.62
18:01-24:00 (Cs)	38	0.43	0.54	1.53	0.63	0.54	4.38	0.43
24:01-06:00 (Cs)	38	-1.12	0.49	0.33	5.27	0.13	0.85	0.02
06:01-12:00 (Cs)	38	0.59	0.55	1.81	1.15	0.61	5.34	0.28
12:01-18:00 (Cl)	38	-0.25	0.50	0.78	0.24	0.30	2.07	0.62
18:01-24:00 (Cl)	38	0.59	0.55	1.81	1.15	0.61	5.34	0.28
06:01-12:00 (Cl)	38	0.99	0.60	2.70	2.74	0.83	8.75	0.10

Cs= Case; Cl= Control; Freq = Frequency of observations; Coef=Coefficient

2.3 Discussion

Lame cows visited the AMS less frequently than non-lame cows. Pain and discomfort caused by lameness may have reduced the cow's willingness to attend the AMS as frequently as non-lame animals. In conventional parlours lame cows are often the last to enter the milking robot (Hassall et al., 1993) and tend to walk more slowly (Chapinal et al., 2010b). Lame cows lie down for longer than their sound herd mates(Singh et al., 1993b; Juarez et al., 2003; Ito et al., 2010), even during oestrus (Walker et al., 2008), and particularly if the stalls have a comfortable bedding substrate (e.g. sand) (Gomez and Cook, 2010). Daily lying time in cows housed in an AMS was positively associated with the degree of lameness (Deming et al., 2013a). It can be postulated that the lame cows visited the AMS less because of the discomfort associated with standing and walking to the unit. If lame cows do not visit the AMS as many times as their non-lame counterparts, they have a higher chance of developing udder firmness due to milk accumulation that can cause pain (Meskens et al., 2001; Osterman and Redbo, 2001; Gleeson et al., 2007), and may increase their chances of acquiring an intra-mammary infection (DeVries et al., 2011).

Lame cows were less likely to attend the milking robot at night (24:01-06:01 hours) compared to their matching pairs (non-lame cows). Bach et al. (2007) observed a similar trend in much lower yielding cows; cows with high mobility score did not visit the AMS at night and were more likely to visit the milking robot during the morning. Previous work demonstrated that non-lame cows had a higher attendance to the milking robot between 08:00 and 19:00 hours (Wagner-Storch and Palmer, 2003). This has been associated with the distribution of fresh feed early in the morning and feed being pushed up through the day (e.g. in the morning) (DeVries et al., 2011). In addition, cows show a higher motivation for feed than for being milked (Prescott et al., 1998) and during oestrus lame cows do not change their diurnal feeding patterns (Walker et al., 2008). These findings suggest that lame cows also prefer to be active when feed is distributed, even though at this time there could be increased competition to gain access to limited resources.

Deming et al. (2013a) observed that lame cows had presented increased lying times and reduced milking frequency in AMS in comparison with non-lame cows. Similarly to conventional parlours, lame cows increased the duration of lying time at night even during oestrus when compared with non-lame cows (Walker et al., 2008; Blackie et al., 2011). There is limited information on the nocturnal activity of cows (lame or non-lame) in AMS. Helmreich et al. (2014) observed that non-lame cows in AMS lay down less at night (22:00-05:00) in comparison with day time (05:00-22:00). In the present study, lame cows visited the milking unit less at night; as previous research shows, it is possible that they may have increased their lying time during this period of the day.

One of the aims of the present study was to investigate the impact of lameness on the visits of high yielding cows. The average milk production in the present study was 41.2 L/d and the average number of visits per day was 3.2 visits. These results are much higher than those previously reported: the average milk yield ranged between 29.7 L/d and 34.7 L/d; with an average attendance raging between 2.1 and 2.6 visits per day (Klaas et al., 2003; Bach et al., 2007; Borderas et al., 2008b; Deming et al., 2013a). In AMS, milking frequency is positively associated with milk yield (DeVries et al., 2011), and negative associated with mastitis and lying times (DeVries et al., 2011; Hovinen and Pyorala, 2011); on the other hand lameness caused increase lying times (Deming et al., 2013a) and reduction in milking frequency, as seen in this study. These changes in their behaviour may increase the likelihood of mastitis in AMS. Further research is needed to investigate this association in AMS.

Bach et al. (2007) observed that lame cows needed to be fetched more frequently to be milked compared to sound cows. The work reported here did not identify a similar trend, in the present study five lame cows were fetched in comparison to four non-lame cows. Previous studies reported that milking frequency reduced as mobility score increased, suggesting that severely lame cows were more likely to have greater reduction in the milking frequency (Bach et al., 2007; Deming et al., 2013a), and consequently were more likely to be pushed. It is possible that cows in the present study may have suffered with mild or moderate lameness meaning that changes in their frequencies may have not been identify by the farmer. Also, the observation period used in this study may have not been able to capture the differences observed by Bach et al. (2007). So far, Bach et al. (2007) is the only study that mentioned this difference in fetching events, further research is needed to understand the association between lameness and fetching events as this can have additional negative effects on cow welfare and human-animal relationship (Rousing et al., 2006) and add cost to routine management activities.

This study was carried out on a commercial farm, so animals that did not attend on time were pushed through the milking machine by the farm staff. The maximum period allowed before a fetching event occurred in the study farm was 12 hours. The lack of significant difference between lame and non-lame cows on the fetched events might have been caused by factors in the environment that prevented control cows from entering the milking robot (e.g. other cow queueing) or cow factors (e.g. individual behavioural traits). Alternatively the higher yields of cows used in this study may have increased their motivation to attend the AMS, regardless of their lameness state. Future studies should investigate within cow differences on milking visits to control for individual variations. In addition, future studies on lameness in AMS should consider social interactions to understand the general impact of lameness on the welfare of cows.

Most of the lame cows in the present study were diagnosed with claw horn lesions. It has been observed that major behavioural changes occurred in cows diagnosed with DD and SU (Bareille et al., 2003; Thomsen et al., 2012). Diagnosis of lameness was only carried out in 26 of 38 cases, this was due to time constraints and lack of qualified staff to carried out the diagnosis at the time of enrolment. Further studies that include lameness diagnoses would help to determine the changes that type of lesion have over milking frequency.

2.4 Conclusions and future work

The present study demonstrated the impact of lameness on the frequency of milking visits on a high yielding AMS farm. Lame cows visited the milking robot less than non-lame cows and this reduction was most evident at night. These results have a direct impact on the welfare of lame cows as they might be more likely to have a reduction in milk yield and to increase their chances of mastitis; either of these latter can have direct impact on farm profits. In addition, little is known about lameness effects on social hierarchy in dairy cattle, and in particular in AMS where it was observed that social rank has an effect on how these milking robots are used: high ranking cows use the unit more during the day than low ranking cows. Further research is needed to investigate the effects of individual variation on milking frequency and the effects of lameness on the social hierarchy of cows housed in AMS and to investigate the real costs of lameness in these systems.

Chapter 3

Study of the impact of lameness on the rumination behaviour of dairy cows

3.1 Introduction

Cattle ruminate an average of 6.5 hours per day in 8 bouts approximately, with a mean duration of 48 minutes per bout (Phillips and Hecheimi, 1989). Cows reduce their rumination when under stress or pain: type of housing-concrete bedding (Singh et al., 1993a), grazing restrictions (Gregorini et al., 2012) and at calving or oestrous (Soriani et al., 2012). Mastitis can also reduce rumination in dairy cattle, independently of the amount of feed they consume (Siivonen et al., 2011; Fogsgaard et al., 2012). Rumination is also affected when calves are challenged with LPS endotoxin (Borderas et al., 2008a). Reduction in rumination has been associated with lower DHEA and higher blood cortisol levels (Almeida et al., 2008).

Animals under stress or suffering ill-health change their behaviour in order to reduce pain or cope with their environment to increase the chances of recovery (Broom, 2006). In conventional parlours lame cows reduce their feeding time (Gonzalez et al., 2008; Gomez and Cook, 2010), increase their lying time (Ito et al., 2010) and modify their gait in order to access their needs (e.g. feed or social contact: Galindo and Broom, 2002). In previous studies investigating the association between rumination and lameness, no definitive differences between lame and sound animals were identified. This is possibly because rumination measurement was carried out using visual observations of behaviour over relatively short periods of time and / or across relatively small numbers of animals (Hassall et al., 1993; Singh et al., 1993b; Almeida et al., 2008; Pavlenko et al., 2011).

Rumination collars have been validated and introduced to commercial farms in the last 5 years (Schirmann et al., 2009) with the data collected mainly being used to detect heat in cows (Bar and Solomon, 2010). The collar contains a tag that collects the sounds produced when a bolus is regurgitated and when a cow is ruminating. The tag processes and stores the data which are downloaded to the farm computer when the cow approaches or passes through the computer antenna (Lindgren, 2009). Data are collected in 2-hour intervals. Once in the computer, the data are presented to the farmer as total minutes per day. The collars also provide data for average interval between boluses and between chewing actions in a 24-hour period. This collar has started to be used to investigate the effect of lameness on rumination with the aim of developing an automatic tool to detect lameness. Van Hertem et al. (2013) observed that lame cows ruminated less at night (20:01-04:00) than non-lame cows before and after the diagnosis day.

The aim of the present study was to perform a detailed examination of the impact of lameness on the total daily rumination time. The null hypothesis was that total daily rumination time in cows was not affected by their lameness status. The alternative hypothesis was that total daily rumination time in cows was affected by their lameness status.

3.2 Materials and methods

All study protocols were reviewed and approved by the University of Nottingham's School of Veterinary Medicine and Science Ethical Review Committee before data collection began.

3.2.1 Study Population

The study population was described in Chapter 2 – section 2.2, for further details on the farm description and management practices please refer to that Chapter. All milking cows in the study were fed a total mixed ration (TMR) of 31.6% grass silage, 29.2% maize silage, 24.3% whole crop silage, 8.5% rapeseed meal, 3.6% nutty blend, 1.2% molasses, 0.6% fat supplement and 1.0% minerals (based on DM: 36.4% DM, 10.8% CP, 24.4% ADF, 43.0% NDF and 11.4 MJ/Kg of metabolizable energy). In addition, cows were provided with an individual concentrate ration (1.5 kg/day) adjusted to the frequency of milking visits, in the AMS. If the cow produced more than 23 L/day, an additional 0.16 kg per each extra litre of milk was provided.

The farm had a lameness prevention and control plan in operation; a fully qualified foot trimmer trimmed all feet of all animals every five months. Additionally any animals that became lame were identified and treated as soon as possible by farm staff. Lactating cows walked through a footbath containing 5% copper sulphate placed at the AMS exit for at least one day per week. Finally, the diet was fortified with 20mg of Biotin per cow per day to aid in the prevention of claw horn lesions.

3.2.2 Study Design

The study was designed as an observational longitudinal study. Cows were observed for 9 weeks; in each week they were assigned a mobility score to identify them as lame or non-lame. Total rumination time per day (averaged over 2 days) was used as the outcome of a multilevel (with three levels: pen, cow and observation week) linear regression model to investigate any association between rumination time and lameness status. Sample size was calculated using the difference between lame and non-lame cows observed in a previous published study (Almeida et al., 2008), estimated rumination time difference was 10 min, the standard deviation used was 9 min, and the calculation was assigned with 80% power and a confidence level of 95%, the required sample size was calculated as a minimum of 13 cows per group (Stata/SE 12.0 - Stata Corp 2011, USA).

3.2.3 Data collection

3.2.3.1 Mobility scoring

Locomotion scoring was carried out following the UK industry standard four point system (Appendix 1: DairyCo, 2009): score 0 a cow with good mobility, score 1 with imperfect mobility, score 2 with impaired mobility with a limb that is immediately identifiable and score 3 with severely impaired mobility. Mobility score was carried out once every 7 (±1) days by a single trained observer (GMP). Each pen was locked at one end and the animals were pushed towards the other side before the mobility score started. Then, the assessor (GMP) stood on one side of the passageway and a technician pushed the animals slowly in front of her. Cows were classified as non-lame when scored 0 or 1, and as lame when scored 2 or 3, each observation week.

3.2.3.2 Rumination data

Each cow had a rumination collar attached as a standard management procedure on the farm. The tag (Qwes-HR, Lely WestNV, The Netherlands) registered total rumination time, chews per bolus and time between boluses. The rumination tag was attached to the cow through a collar that kept the tag in position (upper left side) by a counter lead weight (Figure 3.1). Rumination data were collected continuously through a microphone within the tag and stored in the memory of the tag in blocks of 2 hours. These data were downloaded to the farm computer every time cows were milked or passed by the infrared sensors located in each robot. The full datasets could not be accessed through the farm computer; these were obtained through the manufacturer (Lely Ltd). The data were downloaded as CSV files, and then were transferred to Microsoft Excel® and plotted for analysis.

3.2.3.3 Milk production data

Days in milk, parity and daily milk production data were recorded and stored by the farm computer. At the end of the observational study, all data were collected using T4C software (Lely, Netherlands). A report was created using the template "Daily Production History" and data were downloaded as a CSV file, and then transferred to Microsoft Excel® for analysis.

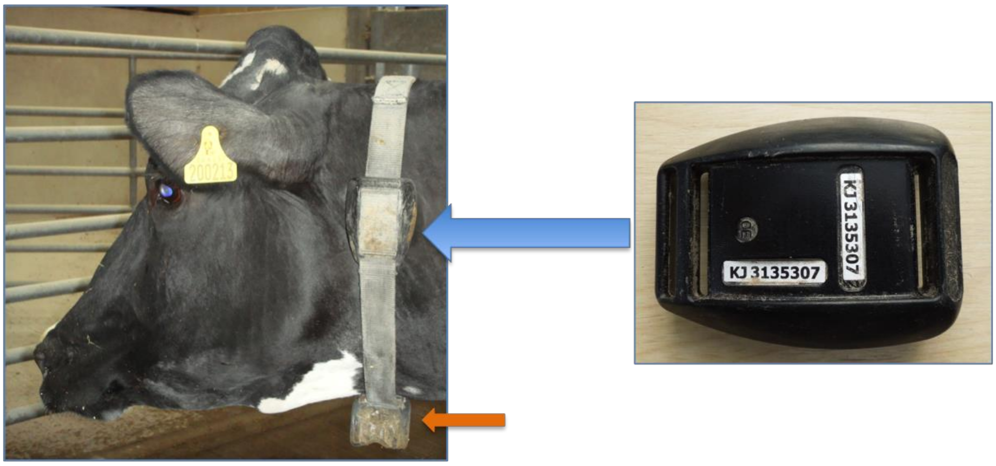

Figure 3.1 The rumination tag (picture on the right) was attached to a collar; this was maintained on the upper left side of the neck by a lead weight (orange arrow).

3.2.3.4 Body weight data

The AMS had a built in weigh scale platform that recorded body weight every time a cow was milked. Once the observational study was concluded, data were collected using T4C software (Lely, Netherlands). Data were transferred to an Excel file (Microsoft) for analysis.

3.2.3.5 Lameness treatment

Lame cows were identified and treated according to normal farm practices throughout the study period i.e. no specific treatment interventions were undertaken as part of the research.

3.2.4 Statistical Analysis

Downloaded data were managed in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA). Descriptive analysis and statistical analysis was carried out using Stata/SE 12.0 (Stata Corp 2011, USA). A multilevel regression model was built using MLwiN version 2.25 (Rasbash et al., 2009). Level of significance was set as $P \leq 0.05$ for all the experiments. Results from descriptive analysis are presented as median (IQR) and results from the multilevel model are presented as follows (Coefficient (SE)).

A multilevel linear regression model was built in order to study the association between rumination and lameness status. The model had the following form:

$$y_{ijk} = \beta_{0ijk} + \beta_1 x_{1ijk} + \beta_2 x_{2ijk} + \beta_3 x_{3ijk} + \beta_4 x_{4ijk} + \beta_5 x_{5ijk} + v_{ijk} + u_{ijk} + e_{ijk}$$

The outcome variable (y) was total rumination time averaged across the two days following the locomotion score in each observation week. The three levels of the model were AMS pen (k), cow (j) and observation week (i). β_0 was the intercept fixed at each level. β represents the regression coefficient and the predictor variables are represented by x. x_1 represents lameness status (0 = no lame, 1 = lame and 2 = lame and treated), x_2 stands for milk production (2 categories: >44 L/day or ≤44 L/day), x_3 days in milk (3 categories: 0-130 DIM, 131-260 DIM or ≥261 dim), x_4 for parity (4 categories: 1^{st}, 2^{nd} 3^{rd} or $\geq 4^{th}$ parity), x_5 for weight (3 categories: ≤550, 551-700 or ≥701 kg of BW) and *v*, *u* and *e* stands for the random error of each level. The model fit was checked by graphical analysis of normal distribution of residuals at level 2 (cow) and level 3 (observation week).

The outcome variable had a high level of outliers and it was not normally distributed. A detailed inspection of the data suggested that some of this variation could be due to missing or spurious data recording. The Fourth Spread test (Devore, 2000) was used and extreme outliers were deleted. After this procedure normality of the variable was achieved. The dependant and the independent variables were fixed to the first day of mobility score per cow to carry out the description of the herd.

3.3 Results

A total of 174 cows were observed during the study. Thirteen animals were excluded because they did not have at least 2 consecutive mobility scores and a further 11 because they had either missing data or they suffered other disease conditions (e.g. mastitis) during the observation period. Missing data were related to farm management procedures where data from cows that were sold during the observational study were deleted. Therefore statistical analysis was performed on the remaining 150 cows with a total of 1057 locomotion scores.

3.3.1 Herd description

Cows in the present study had a parity median of 2 (1-3), 50 cows were primiparous and 100 multiparous (34 cows were in 2^{nd} parity, 31 cows were in their 3^{rd} parity and 35 were in parity 4^{th}, 5^{th}, 6^{th} or 7^{th}). The median for DIM was 128 (61-219) days; 76 cows were between 0 to 130 DIM, 47 cows between 131 to 260 DIM and 27 cows were equal or more than 261 DIM. The mean milk production was 38.1 (SD 9.6). The mean body weight was 652.1 (75.4) kg.

3.3.2 Lameness prevalence

From the total of 150 cows observed during the study, 43 cows were observed ≤ 6 times and 107 cows were observed ≥7 times. Table 3.1 presents the number of cows observed per week under each category of the mobility score (0-3). Cows scored as 0 and 1 were considered as non-lame and scores of 2 and 3 as lame. Lameness presented an average prevalence of 28% during the observation period with the exception of the last week of observation (week 9: 46%) (Table 3.1). In total 110 cows were observed lame and 40 were never lame during the observation period. From these 110 cows: 2 cows were observed lame throughout the 9 weeks, 17 cows were observed lame between 5 to 8 times, 9 cows were observed lame 4 times, 42 cows were observed lame 2 or 3 times and 40 cows were observed lame once. Eleven cows were treated by the farm during the study period. Five cows were diagnosed with interdigital necrobacilosis and six cows were diagnosed with claw horn lesions (SU, WLD and SH). One of the cows was identified as lame twice and was treated again.

Table 3.1 Number of cows observed per week in each category for mobility score and percentage of lame and non-lame cows per week.

Observation Week	Mobility Score (0-3)						Total of Cows observed per week
	0	1	Non-Lame %	2	3	Lame %	
1	9	28	73	12	2	27	51
2	12	70	69	32	5	31	119
3	11	83	76	23	6	24	123
4	13	57	75	18	5	25	93
5	2	103	78	25	4	22	134
6	1	99	71	37	3	29	140
7	1	100	75	31	3	25	135
8	0	101	74	30	5	26	136
9	0	68	54	51	7	46	126

3.3.3 Total rumination time and lameness

The average rumination time for non-lame cows was 496.1 minutes/day (SD 99.3 minutes/day), for lame cows that were not treated was 499.4 minutes/day (SD

89.0 minutes/day) and for lame cows that were treated was 508.1 minutes/day (SD 71.8 minutes/day). The results of the multilevel linear regression model showed that the average rumination time over the 2 days after mobility scoring was affected by lameness status and production variables (Table 3.2). Lameness had a small but significant ($P<0.05$) negative association with rumination; rumination was reduced by 7.9 minutes per day in the two days following a lame locomotion score.

Parity and days in milk affected rumination. Cows in third or higher parity ruminated more than primiparous cows ($P<0.05$) and cows greater than 130 days in milk ruminated less than those less than 130 days in milk ($P<0.05$). Weight did not affect the amount of rumination on the observed days ($P>0.05$).

There was random variability between cows (5081.41 (619.45)) and observation week (1997.87 (93.90)). Figure 3.2 shows the residuals plots at both levels suggesting that the model was a reasonable fit to the data.

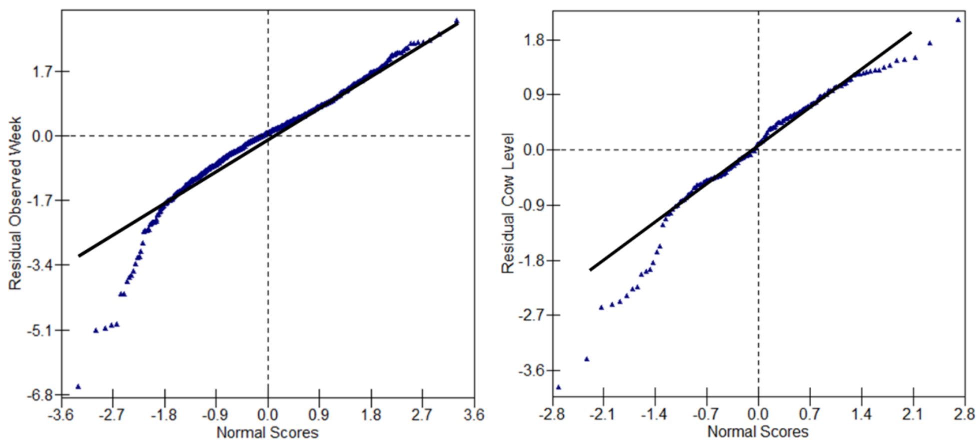

Figure 3.2 Residuals (SD 1.96) at observation week (1057 observations) and at cow level (150 cows).

Table 3.2 Results of the linear regression multilevel model: Average rumination of day 1 and day 2 after mobility score.

Model term	Freq.	Coefficient	SE	Confidence Interval		z	P
				2.50%	97.50%		
Intercept	1057	522.25	20.21				
Lameness status							
Non-Lame	758	Reference					
Lame	287	-7.88	3.93	-15.58	-0.19	-2.01	0.04
Lame and treated[1]	12	-11.98	14.59	-40.56	16.61	-0.82	0.41
Parity							
1	350	Reference					
2	250	13.20	17.03	-20.18	46.59	0.78	0.44
3	216	36.56	17.54	2.17	70.95	2.08	0.04
≥4	241	65.67	17.47	31.44	99.90	3.76	<0.001
Milk Production							
> 44 L/day	302	Reference					
≤ 44 L/day	755	-9.46	5.34	-19.93	1.01	-1.77	0.08
Days in Milk							
0 - 130 days	446	Reference					
131 - 260 days	394	-26.90	6.11	-38.89	-14.92	-4.40	<0.001
≥ 261 days	217	-35.17	10.18	-55.13	-15.22	-3.45	<0.001
Body Weight							
≤ 550 kg	64	Reference					
551 - 700 kg	660	-14.02	13.44	-40.36	12.33	-1.04	0.30
≥ 701 kg	333	-17.52	15.05	-47.00	11.97	-1.16	0.24
AMS Pen							
AMS pen 1	259	Reference					
AMS pen 2	256	-30.05	17.33	-64.01	3.91	-1.73	0.08
AMS pen 3	254	-12.76	17.86	-47.76	22.24	-0.71	0.47
AMS pen 4	288	-21.61	17.47	-55.85	12.64	-1.24	0.22

Freq.= Frequency of observations
[1]Lame and treated = cows were treated within the 48 hours after being observed lame

3.4 Discussion

The present study demonstrated that lame animals ruminated for a significantly shorter period of time each day compared to their sound herd mates, although the difference was small (~8 minutes / day). The reason for the small but significant reduction in rumination time observed in lame animals was not identified in this study. The observed reduction in total rumination time could be associated with a reduction in total dry matter intake (associated with a reduction in total feeding time) and therefore lower fibre content in the rumen. However, a previous study demonstrated that lame cows may compensate for the reduction in total feeding time by increasing their feed intake rate (Gonzalez et al., 2008). It could be postulated that consuming the total daily dry matter intake over fewer meals at an increased rate may decrease rumination, as the rumen becomes more efficient with larger amounts of feed (Beauchemin, 1991). Alternatively, the discomfort / stress associated with lameness may directly affect rumen function via central depression of the centres controlling rumination. Previous work demonstrated that rumination is negatively associated with higher levels of cortisol (Bristow and Holmes, 2007; Almeida et al., 2008).

A recent study using the same rumination data collection system, published after this study was carried out, observed a reduction in rumination time at night (20:01 – 04:00) for lame cows (208 ±6 minutes) when compared to non-lame cows (221 ±3 minutes) during the 7 days before diagnosis and treatment (Van Hertem et al., 2013). In the present study, the difference observed was 8 minutes less for lame cows compared with non-lame cows. Rumination was averaged for 2 days (48 hours) after gait assessment was carried out (by a single observer). Van Hertem et al. (2013) did not specify if they observed any difference over a 24-hour period. One of the main differences between these two studies is the way cows were detected as lame. Van Hertem et al. (2013) did not used a specific mobility score assessment, the detection of lame cows was carried out at every milking by the cow pusher who did not received any specific training in locomotion scoring. Horseman et al. (2013) found that farmers are more likely to call cows 'lame' when an animal is considered severely lame to an external observer. So, it is possible that the difference observed between studies might be due to the severity of lameness as in the present study most of the cows were classified as mildly/moderately lame.

Van Hertem et al. (2013) observed that the rumination time difference between lame and non-lame cows continued up to 24 hours after treatment. The study did not specify for how long cows were lame, at least 7 causes of lameness were identified and the type of treatment was not specified. In the present study, 12 lame cows that were treated within 2 days did not show significant differences for total rumination time when compared to non-lame cows. In previous studies where a significant reduction in total rumination time was observed, lame cows were diagnosed with DD (Pavlenko et al., 2011), or were clinically diagnosed with lameness (e.g SU or DD) (Almeida et al., 2008). The present study did not consider lameness diagnosis as part of the analysis due to the low number of cows and different lesions observed (e.g. DD, WHD, SU). It is possible that different causes of lameness and prompt treatment could have an effect on rumination time changes.

In the present study cows ruminated on average 522 minutes/day (Table 3.3). This is similarly to times reported by studies carried out using the same rumination tag (Schirmann et al., 2009; Soriani et al., 2012). In addition it was observed that cows showed significant variability in total rumination time. Similar variation has been observed in feeding behaviour (DeVries et al., 2003b). This variation may be explained by the variation in feed consumption between days (Beauchemin, 1991), which may have had an effect on the present and previous results.

During the study period, lameness prevalence on this AMS was on average 28%. This is higher than the prevalence observed in AMS farms in Canada (20%: Borderas et al., 2008b) but similar to the prevalence observed in Spain (29%: Bach et al., 2007) and in the UK (32%: Reader et al., 2011). There was an increase in lameness prevalence in the last week of the observation period; nothing could be identified to explain this increase.

In agreement with previous studies, primiparous cows ruminated less than multiparous cows (Soriani et al., 2012). The effects of parity and days in milk were large compared to the impact of lameness (Table 3.2). Environmental and social factors can increase the stress levels in primiparous cows as they are introduced for the first time to the milking herd; regrouping cows showed a decrease in rumination time, animals introduced to a new pen showed also a reduction in feeding time (Schirmann et al., 2011). Though the reduction in rumination time based on these stressors may be short lived, social structures within the herd (primiparous cows lower in rank than multiparous (Sarova et al., 2013) can impact on the feeding time and rumination time as low ranking cows avoid higher ranking cows at the time of feeding (Manson and Appleby, 1990). It was not possible to locate any current research that looked at the association between rumination time and DIM. Though the present study observed that rumination time decreased as DIM increased, this can be link to the reduction of feed intake through lactation (Norring et al., 2012).

In addition, it would be helpful to understand the impact that rumination reduction has for dairy cow welfare. It is accepted that rumination reduction is linked to lower DHEA and higher cortisol blood levels (Almeida et al., 2008); but it is uncertain how this may affect cow welfare. Further studies into changes in rumination and pain or stress markers should be pursued in order to understand the importance of this behaviour for cow welfare.

Outliers were identified during data analysis and deleted, reducing slightly the number of observations available. This did not cause any effect on the results. None of the published studies that had used this technology reported the presence of outliers (Soriani et al., 2012; Van Hertem et al., 2013). From personal communication with the manufacturer, the only technological reasons that this may happen could be either a low battery charge within the equipment or incorrect positioning of the rumination tag. In the present study both aspects were carefully checked before, during and after the data collection. Therefore, it remains possible that this data may have represented normal between cow variation within the herd. Schirmann et al. (2009) validated the system against visual observation; the authors observed that a loose or incorrectly positioned collar may have contributed to the 6.1% variation observed between direct visual observation and the rumination collar data. Even though there are published papers validating this system (Schirmann et al., 2009; Burfeind et al., 2011), it is important that future studies not only check that tags are fully charged and correctly positioned, but also run a small validation of their tags. If outliers are observed, they should be fully reported including how they were handled.

3.5 Conclusions and future work

The use of a rumination collar facilitated the data collection for the present study and allowed for a longer monitoring period in comparison to previous studies that carried out data collection by direct visual observation. By using a 24-hour automatic measurement, the present study confirmed that rumination was affected by lameness, but the reduction was of limited biological or practical significance. It is possible that rumination time reductions observed in previous studies may be dependent on the cause and chronicity of lameness; the present results highlight the need for further studies on the effects of lameness on rumination according to the type of lesion and their chronicity.

Chapter 4

Effects of lameness treatment on lying behaviour

4.1 Introduction

Prompt recognition and early treatment of lameness may increase the chances of a faster recovery, bringing less economic losses to farmers and improving the welfare of lame cows (O'Callaghan, 2002). Some of the main causes of lameness are claw horn lesions, particularly sole ulcers (Murray et al., 1996; Bicalho et al., 2007). Recommended treatments for these vary according to the type of lesions. Overall hoof trimming is applied as a herd management tool for lameness prevention and treatment, this helps with gait smoothness and rhythmicity (Blowey, 1992a; Van der Tol et al., 2004; Ouweltjes et al., 2009; Tanida et al., 2011). In the case of horn disruption, the application of a wooden block to the healthy claw is recommended. This allows some rest to the affected claw giving time for the lesion to heal and promoting the comfort of the cow (Shearer et al., 2013). Studies carried out on the use of foot blocks were focused on the type of block and locomotion recovery (Pyman, 1997; Wehrle et al., 2000).

Lameness in dairy cattle is a very painful disease that can cause hyperalgesia and changes in their behaviour (Whay et al., 1997; Almeida et al., 2008). The use of NSAIDs is recommended as part of lameness treatment; the use of analgesics can help with the healing process, reduce pain and inflammation, and improve locomotion sooner (Whay et al., 1998; Flower et al., 2008). However only 17% of interviewed farmers in a UK study (n=84) used injectable analgesics when treating claw horn lesions (Horseman et al., 2013).

Lying behaviour is affected by the degree of lameness (Thomsen et al., 2012). Lame cows increase their lying time due to an increase in bout duration; these changes were observed particularly in severely lame cows (Thomsen et al., 2012) and at evening (16:01-23:00) (Blackie et al., 2011). Lameness does not affect the laterality of lying behaviour (Yunta et al., 2012). Lesions that cause the greatest increase in lying behaviour are DD followed by SU (Chapinal et al., 2009b; Thomsen et al., 2012). In the area of lameness treatment and its effect on lying behaviour, there is very limited published research. Cutler (2012) investigated the effects of wooden blocks on healthy cow’s behaviour (n=10) and milk production; she observed that animals reduced their activity in comparison to the period before block application in comparison to control cows (no block applied), although blocks did not affect lying time or milk production. On the contrary, O'Callaghan (2003) observed that lameness treatment (NSAID, blocks or antibiotics) caused changes in the activity (steps) of lame cows depending on the type of claw horn lesion identified.

In order to recommend lameness treatments it is important to understand their impact on cow behaviour and welfare. The aims of the present study were to determine the effect of lameness treatment on the lying behaviour patterns of newly lame cows. The null hypothesis was that lameness treatment did not affect lying behaviour. The alternative hypothesis was that different lameness treatment affected lying behaviour.

4.2 Materials and Methods

4.2.1 Description of herds and farm management

Two automatic milking farms from the east midlands area of the United Kingdom were enrolled in the study. Both farms had approximately 200 Holstein dairy cows and had free access to automatic milking systems (Lely Astronaut A3 and A4, Lely UK Ltd, St Neots, UK). Farm 1 had an average milk yield per cow of 11500 L per 305 days and farm 2 had an average milk yield per cow of 11531 L per 305 days. Both farms had a year round calving pattern. Further description of the units can be found in Table 4.1.

4.2.2 Study design

The experiment was designed as an observational longitudinal study to investigate the impact of lameness treatment on the lying behaviour of lame dairy cows. Lameness treatment was applied as part of a partially blinded, randomised clinical trial looking into the individual treatment of lame cows with claw horn lesions. Lying behaviour of lame treated cows was compared to the behaviour of non-lame cows. Sample size for this study was calculated using data collected previously about the lying behaviour of 16 cows in an automatic milking system. The calculation assumed that lame cows lying behaviour increases up to 90 minutes with a power of 80% and standard deviation of 134.4 minutes, according to the results 44 cows per treatment group were required (Stata/SE 12.0 - Stata Corp 2011, USA). Prior to commencing the study, study protocols were reviewed and approved by the University of Nottingham's School of Veterinary Medicine and Science Ethical Review Committee.

Table 4.1 Management and production characteristics of the farms in the study.

General Characteristics	Farm	
	1	**2**
Number of pens	4	2
Number of automatic milking system units	4	4
Number of cows per robot	~45	~50
Maximum number of times a cow was allowed to go into the milking robot each day	5	5
Frequency of monitoring milking attendance	2	3
AMS provides a concentrate-feeding ration?	Yes	Yes
How much concentrate is provided?	Up to 9 kg/day	up to 10 kg/day
Feeding type outside of the AMS	Mixed ration	Mixed ration
Frequency of provision of fresh ration	Once a day	Once a day
Frequency of ration push up	5-6 times/day	4-5 times/day
Body weight scale built in the AMS	Yes	No
Alley floor surface	Rubber mat	Parallel grooved concrete floor
Automatic scrapers present	Yes	Yes
Frequency of automatic scrapper operation	Every hour	Every hour
Type of housing system	Cubicles	Cubicles
Type of bedding in cubicle	Mattress base covered with a thin layer of saw dust	Water beds covered with a thin layer of ash lime and Envirobed paper
Number of cubicles per cow	~1.3	~1.2
Trimmed frequency by qualified foot trimmer	Every 5 months	Every 4 months
Foot bath (5% copper sulphate): Lactation cows	Once a week	No
Foot bath (5% formalin): Dry cows and heifers	Yes	Yes

4.2.3 General experimental procedures

The present study was part of a randomised clinical trial (RCT) on lameness treatment at the cow level that ran from December 2011 until February 2013.

4.2.3.1 Mobility score

Mobility score was carried out every 2 weeks on both farms using a modified DairyCo mobility score system, which was subdivided into 6 categories as shown in Table 4.2. Cows with mobility scores of 0 or 1 were considered as non-lame and any cow with a score equal or higher than 2 were considered as lame. Cows were mobility scored prior to the examination, at 7 days after treatment and at 35 ±2days after treatment. Cows were mobility score on each farm, by a different observer per farm, which remained the same throughout the study. In both farms, cows were moved to one end of the pen and then walked quietly in front of the observer. An observer blind to the treatment administered carried out the final score at study outcome (35±2days).

Table 4.2 Randomized clinical trial mobility scoring system (Based on the DairyCo Mobility Score (DairyCo, 2009))

<table>
<tr><th>DairyCo Mobility Score</th><th>RCT Mobility Score</th><th>Descriptor</th></tr>
<tr><td>0</td><td>0</td><td>As DairyCo descriptor: Even weight bearing and rhythm, flat back, long fluid strides</td></tr>
<tr><td>1</td><td>1</td><td>As DairyCo descriptor: Uneven rhythm or weight bearing but affected limb not immediately identifiable, back may be raised. Walking velocity normal.</td></tr>
<tr><td rowspan="2">2</td><td>2a</td><td>Mild asymmetry in hind-limb movement. Decreased stride length on affected limb and slightly decreased stance duration with a corresponding increase in limb flight velocity on the non-affected side. Walking velocity remains normal. Back may be raised.</td></tr>
<tr><td>2b</td><td>Moderate asymmetry in hind-limb movement. Decreased stride length on affected limb and a distinct decrease in stance duration. Limb flight on the non-affected limb is correspondingly faster and the overall walking velocity is reduced. Back usually raised.</td></tr>
<tr><td rowspan="2">3</td><td>3a</td><td>Severe asymmetry in hind-limb movement. Marked decrease in stride length on affected limb and very short stance duration. Limb flight on non-affected limb rapid and walking velocity reduced such that cannot keep up with healthy herd. Back raised.</td></tr>
<tr><td>3b</td><td>Minimal or non-weight bearing on affected limb (hops). Back raised. Reluctant to walk without encouragement</td></tr>
</table>

4.2.3.2 Mobility score reliability

To test mobility score reliability cows with different mobility scores were selected and encouraged to walk slowly in front of a camera over a firm, level surface. The camera (Nikon Coolpix AW110-Nikon) was position on one side of the track using a tripod, so one lateral view of each cows was recorded. Videos of cows walking slowly and for at least 3 strides were selected. A total of 40 video clips were used to create a series of videos to test the reliability of the DairyCo mobility scoring system (scores 0-3); this was conducted with 2 observers, with an expected inter-observer reliability (ρ_1) of 0.85, acceptable (ρ_0) at 0.6 or higher, with α=0.05 and β=0.2 (Walter et al., 1998). The final film was edited using Movie Maker (Microsoft Corp., Redmond, WA). Each clip was 4 to 5 seconds long and was repeated twice in succession. There was a 3s gap in between videos. Clips were randomly distributed in the movie.

The two observers were given the mobility score chart to review for 5 minutes, immediately prior to commencing the reliability test. After this, each observer was given a score sheet that contained the number of the clips sequentially in a column and the scores 0-3 in a row to tick. Once the video was finished the sheets were collected. To measure intra-observer reliability, observers scored the film for a second time after 4 hrs.

4.2.3.3 Cow enrolment

4.2.3.3.1 *Selection of cows to be treated*

Cows were selected for treatment if they had two consecutive non-lame mobility scores followed by a lame mobility score, and only presented with one of the hind limbs lame. Once a cow was selected, a qualified veterinarian carried out a standard foot trim: toe length cut evenly on both claws, at least 7.5cm in length; followed by creation of a dish at the axial sole. After this, diagnosis was carried out following the descriptions in Table 4.3. Only cows diagnosed with claw horn lesions

(Sole Haemorrhage or Sole Ulcer, White Line Disease or 'Other') on a single claw were selected for treatment. Treatments started in January 2012 and continued until January 2013.

Table 4.3 Descriptors used to diagnose the lesions for cows enrolled in a randomised clinical trial (Archer et al., 2010a). Blue circles indicate the lesions.

Picture	Name	Abbreviation	Description
	Sole Ulcer	SU	Broken sole surface with or without granulation tissue.
	Sole Haemorrhage	SH	From diffuse to severe bruise on the horn of the sole. The colour ranges from light pink to very dark red or purple.
	White line disease	WLD	A lesion of any severity at any location on the white line. Lesions can vary from diffuse red/pink marks to complete separation of the wall.
	Other		Two or more of the previously described claw horn lesions on a single claw (pictures shows WLD and SH).

Cows were not considered for the study for the following reasons: if claw horn lesions were present on both claws (Figure 4.1), if the foot had an infectious problem (e.g. Digital Dermatitis or Interdigital Necrobacillosis) or an interdigital growth, or if the farmer had treated the cow on the same leg in the previous 120 days or the animal had received parenteral antibiotics within the previous 14 days.

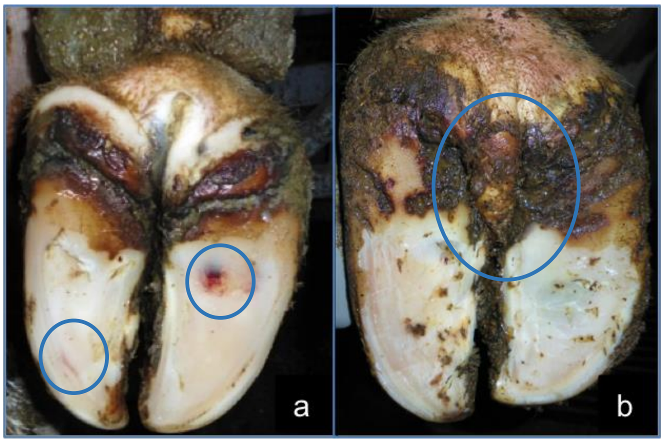

Figure 4.1 (a) Hind feet affected with claw horn lesions on both claws (Lateral claw (right) has a sole ulcer and medial claw (left) has sole haemorrhage) and (b) hind feet affected with interdigital growth. Blue circles highlight the lesions.

4.2.3.3.2 *Selection of control cows*

A cow was selected as a control if she had 3 mobility scores as non-lame (0 or 1) prior to the treatment day of her matched enrolled animal and had no disease events in the last month (e.g. no mastitis). Control cows were matched by pen (housed in the same pen), parity (in the same parity) and DIM (± 20 days) to the enrolled animals. Cows that were enrolled for treatment were not considered as controls. Cows that were selected as controls and became lame at least 35 days after they were selected as controls were considered to be enrolled for treatment.

4.2.3.4 Treatments

Lame cows that were accepted for the study (after therapeutic foot trimming and diagnosis) were allocated to one of 4 treatments. Random allocation to a treatment was blocked by farm and diagnosis (Table 4.3). The same qualified veterinarian that carried out the diagnosis applied the treatments to maintain consistency throughout the study.

The treatments were as follows:

- Treatment 1: Therapeutic trim only (Standard Dutch foot trim followed by trim and investigation of lesions then removal of diseased horn (Toussaint-Raven et al., 1985)).
- Treatment 2: Therapeutic trim and foot block (applied to the healthy claw) (Blowey, 1992a).
- Treatment 3: Therapeutic trim and NSAID (3 mg/kg bodyweight of Ketoprofen IM once per day for 3 days) (Whay et al., 2005).
- Treatment 4: Therapeutic trim, foot block and NSAID (as described above).

Treatments were applied with the cow restrain in a trimming chute (Figure 4.2.a). Cows that were part of the study were re-examined 7±2days after treatment, and foot blocks were re-applied if necessary (e.g. if the block was no longer present). At 28±2 days after treatment animals within treatment groups 2 and 4 were visually re-assessed and the foot block was removed if still present.

Figure 4.2 Application of treatments: (a) cows were put in a trimming chute, then (b) foot trimming was conducted (c) and a block, NSAID or both were applied.

4.2.4 Production and health status data

Daily milk production, days in milk and parity data were collected using the on farm management system T4C software (Lely, Netherlands). Health status data were collected for the duration of the study.

4.2.5 Specific Experimental Procedures

4.2.5.1 Lying behaviour measurement

Lying behaviour was defined as cows lying in a resting position in the cubicle, with four legs resting parallel to the floor. It could be in any of the four positions described by Anderson (2008) (Long, short, wide or narrow) over the left or right side. Data collected included total lying time, number of lying bouts per day, average time of each lying bout and total minutes per day spent lying on each side.

4.2.5.2 Lying behaviour observation period

Cows were observed between July 2012 and March 2013. Each animal (control and treated cows) were fitted with accelerometers (Figure 5.3) (Onset Pendant G data loggers, Onset Computer Corporation Pocasset, MA) following a standard operating procedure for hobo loggers (Appendix 2). Accelerometers were set to record y (lying behaviour) and z (laterality of lying behaviour) axis at 1-minute intervals (as per Appendix 3).

Accelerometers were attached immediately after treatment and started recording at 23:59 hours on the day they were attached (~10:00am), following an habituation period of approximately 14 hours (Gibbons et al., 2012). Accelerometers were attached to the non-lame leg of enrolled cows (Table 4.3); for control cows the accelerometer was attached to a leg chosen at random by tossing a coin. They were left attached for a total of approximately 7 days. As all the cows had at least 5 days of data collected and 3 days of data are the minimum to obtained a representative measure of lying behaviour (Ito et al., 2009), this number of days was used for analysis. Observation periods were from 00:01 to 24:00 hours of each observed day.

Figure 4.3 Left: A Hobo® accelerometer and Right: Accelerometer attached with vetwrap.

4.2.5.3 Accelerometer validation and reliability

Validation and reliability of the accelerometer was carried out as explained in Appendix 3. In brief, accelerometers were attached to eight South Devon beef cows and their calves for 3 days and video recorded during the same period. Data from accelerometers and video recordings were compared. For both groups of animals total lying times recorded by the HOBO highly correlated with the data from the video recordings (R^s =0.99; P<0.001); similar findings were observed for frequency of lying.

4.2.6 Data analysis

Data were downloaded to Microsoft Excel 2010 (Microsoft Corp., Redmond, WA). Descriptive and statistical analyses were carried out using Stata/SE 12.0 (Stata Corp 2011, USA). Multilevel regression models were built using MLwiN version 2.27 (Rasbash et al., 2009). Level of significance was set as $P \leq 0.05$ for all experiments. Results from multilevel models are presented as follows (Coefficient

(SE)). The weighted kappa (k_w) was used to calculate the intra and inter-observer reliability for mobility score. The interpretation of the k_w was conducted using Landis and Koch (1977) where ≤0=poor, 0.01-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial and 0.81-1.00 = almost perfect.

Lying behavior (total lying time, number of bouts per day and average lying bout duration) and laterality of lying behaviour data from 78 cows (44 enrolled and 34 controls) was downloaded from the accelerometers using Hoboware®Lite Software Version 3 (Onset Computer Corporation, Pocasset, MA) and then transformed and modified accordingly using Software Macro Hobo 3D Microsoft Excel® (Gibbons et al., 2012). Total lying time was defined as the total number of minutes spent lying down per day. Number of bouts per day was defined as the number of times a cow was lying down and average bout duration was calculated by dividing the total lying time by the number of bouts per day. Values for daily milk production, parity and days in milk were fixed to the first day of observation. As target sample size was not reached, post-hoc calculations were conducted using *simpower* analysis from Stata/SE 12.0 (Stata Corp 2011, USA).

4.2.6.1 Lying behaviour

Total lying time (min/day) followed a normal distribution but average bout duration (min/bout) was right skewed and was transformed (square root) to achieve a normal distribution. Both variables were analyzed independently using multilevel linear regression models. Number of bouts per day followed a Poisson distribution and therefore was analyzed with a multilevel Poisson regression model. The outcome variable (y) was one of the three variables (Total lying time, number of bouts and average bout duration per day). All the three models had 2 levels: cow (j) and observed day (i). β_0 was the intercept fixed at each level. β_s represented the regression coefficient, the predictor variables are represented by x (Table 4.4) and e and u stand for the random error of cow and observed day level respectively.

The multilevel linear regression models for total lying time and for average lying bout duration outcomes had the following form:

$$y_{ij} = \beta_{0ij} + \beta_1 x_{1ij} + \beta_2 x_{2ij} + \beta_3 x_{3ij} + \beta_4 x_{4ij} + \beta_5 x_{5ij} + u_{ij} + e_{ij}$$

The models' fit was checked by graphical analysis of normal distribution of residuals at level 2 (cow) and level 1 (observed day).

Table 4.4 Description of variable *x* used in the multilevel models to investigate lying behaviour in a lameness treatment study.

Variable	Name	Categories and descriptor
x_1	Treatment	0 = control (non-lame and not treated) 1 = trim only 2 = trim and block 3= trim and NSAID 4=trim, block and NSAID
x_2	Daily milk production	Continuous variable total litres per day
x_3	Days in Milk (DIM)	1 = ≤100 days 2 = 101-200 days 3 = ≥201 days
x_4	Parity	1= 1st parity 2= 2nd parity 3= 3rd or higher parity
x_5	Farm and pen	1 - 4 = farm 1 (4 pens) 5 - 6 = farm 2 (2 pens)
x_6	Left hind limb treated	0= not treated 1= treated
x_7	Position of accelerometer: Left hind limb	0= accelerometer not on left hind limb 1= accelerometer on left hind limb

A multilevel Poisson model was built for number of bouts per day outcome and had the following form:

$$\text{Log}(\pi_{ij}) = \beta_{0ij} + \beta_1 x_{1ij} + \beta_2 x_{2ij} + \beta_3 x_{3ij} + \beta_4 x_{4ij} + \beta_5 x_{5ij} + u_{ij} + e_{ij}$$

In this latter model, Log (π_{ij}) is the log of the expected value of the number of bouts per day. The model was fitted with Monte Carlo Markov Chain (MCMC) methods for 500,000 iterations with a burn in of 500. Visual analysis was performed on the chain mixing and stability of the model (Appendix 4).

Treatment days were divided in to four time periods (12:01 - 18:00; 18:01 - 24:00; 24:01 - 06:00 and 06:01 - 12:00) to investigate any difference between treatment groups on lying behaviour throughout the day (total lying time per time period). One way ANOVA was used to investigate this difference, and Bonferroni test was used to identify where the difference lay (Petrie and Watson, 2006).

4.2.6.2 Laterality of lying behaviour

Laterality of lying behaviour was analysed as a percentage of time that a cow spent on each side per day. Only proportion of time spent on the left side was used for further analysis. A multilevel logit binomial model was built to analyse the association between treatment and percentage of time spent on the left side. The denominator was set up as 100. The model had 2 levels: cow (*j*) and observed day (*i*). β_0 was the intercept fixed at each level. β_s represent the regression coefficient, the predictor variables are represented by *x* (Table 4.4) and *u* and *e* represented the random error at cow and observed day level respectively. The model presented the following form:

$$\text{Logit}(\pi_{ij}) = \beta_{0ij} + \beta_1 x_{1ij} + \beta_2 x_{2ij} + \beta_3 x_{3ij} + \beta_4 x_{4ij} + \beta_5 x_{5ij} + \beta_6 x_{6ij} + \beta_7 x_{7ij} + u_{ij} + e_{ij}$$

The model's fit was checked by graphical analysis of normal distribution of residuals at level 2 (cow) and level 1 (observed day).

4.3 Results

4.3.1 Mobility score inter- and intra-observer reliability

The intra-observer reliability for the mobility score (0-3) was K_w= 0.69 (95% CI: 0.60-0.78) for observer 1 and K_w= 0.73 (95% CI: 0.66-0.76) for observer 2; and for the lameness status (non-lame (0-1 scores) or lame (2-3)) was K_w= 0.89 (95% CI: 0.76-1.00) for observer 1 and K_w= 0.80 (95% CI: 0.61-0.98) for observer 2. Inter-observer reliability results for the mobility score (0-3) was K_w= 0.49 (95% CI: 0.43-0.62), and for the lameness status (non-lame (0-1 scores) or lame (2-3)) was K_w= 0.36 (95% CI: 0.08-0.64).

4.3.2 General description

A total of 44 newly lame dairy cows were enrolled onto the study and 34 matched control (non-lame) dairy cows. Data collection was conducted from July 2012 until January 2013. The estimated required sample size for the study was not achieved as fewer cows than expected fulfilled the enrolment criteria for the study from which this subset of animals was drawn (from 100 lame cows examined only 21 and 23 lame cows were enrolled from farm 1 and farm 2 respectively). Thirteen cows on farm 1 and 21 cows on farm 2 were matched to lame cows on each farm. Due to the matching criteria it was not possible to find a control cow for every treated cow. Only one treated cow had 2 control cows (farm 2). The distribution of cows on each farm according to the treatment applied is shown in Table 4.5.

Table 4.5 Number of non-lame cows and treated lame cows according to the treatment applied by farm and pen.

Farm	Pen	Non-lame cows	Treatment groups				Number of treated newly lame cows
			Trim	Trim + Block	Trim + NSAID	Trim + Block + NSAID	
1	1	3	2	1	2	1	6
	2	2	1	1	1	1	4
	3	5	3	2	0	3	8
	4	3	1	1	1	0	3
2	5	10	1	3	3	2	9
	6	11	5	3	4	2	14
Number of cows in each group		34	13	11	11	9	44

Twenty-three treated cows had the accelerometer attached to the right leg and 21 to the left leg. For the control cows accelerometers were attached randomly, 17 had the accelerometer attached to the left leg and 17 had the accelerometer on the right leg. Twenty-two cows were lame on the left leg and 22 on the right leg.

Nine of the non-lame control cows had mobility score 0 and 25 cows had score 1 when observations began. Twenty one treated lame cows were diagnosed with SH / SU, from these 16 were mobility scored 2a, 4 cows were mobility scored 2b and 1 was mobility scored 3a. Ten of the cows diagnosed with WLD (n=11) were mobility scored 2a and 1 was mobility scored 3a. Twelve cows were classified as diagnosis “Other”, from these 10 cows had mobility score 2a, 1 cow had mobility score 2b and 1 cow had mobility score 3a.

During the first five days of observation 4 cows lost their blocks and consequently had a new block attached on the 7th (±1) day following initial treatment. Four of these cows were diagnosed with SH/SU, of which one cow received trim and foot block as treatment (Treatment 2) and 3 animals received trim, foot block and NSAID as treatment (Treatment 4). Table 4.6 shows the number of cows in each treatment group according to diagnosis that were used in the final analysis.

Table 4.6 Number of cows in each treatment group categorised by diagnosis.

Treatment	Diagnosis			Total
	SU/H	WLD	Other	
Trim	5	4	4	13
Trim + Block	4	3	3	10
Trim + NSAID	6	2	3	11
Trim + Block + NSAID	2	2	2	6
Total	17	11	12	40

Table 4.7 presents the description of the treated and control dairy cows for milk production variables. As expected, due to matching criteria, there were no significant differences between treated and control groups for these variables. There

were 3 cows that had missing daily milk production data. For the other variables (Parity and DIM) there were no missing data.

Table 4.7 Descriptive analysis of production variables (Mean - SD and Median - IQR) fixed to the first day after treatment. (N) refers to the number of cows with data for daily milk yield.

Treatment	N	Daily Milk Yield*a		Parity1a		DIM*a	
		Mean	SD	Median	IQR	Mean	SD
Control	34 (33)	36.9	10.7	2	1-3	211.2	118.0
Trim	13 (14)	37.7	9.0	3	2-4	198.1	135.6
Trim + Block	10	32.4	10.7	2	1-4	266.5	123.9
Trim + NSAID	11 (10)	42.5	9.8	3	2-4	152.1	77.9
Trim + Block + NSAID	6	39.6	6.8	2.5	2-3	199.7	133.6
All cows	71 (68)	37.5	10.1	2	1-3	206.6	119.3

*One way ANOVA; [1] Kruskal-Wallis, [a]No significant differences

Results from the post-hoc analysis showed that the simulated power with the final sample size was 0.56 with a level of significance of 0.05.

4.3.3 Lying behaviour on first 5 days of observation

Table 4.8 describes the distribution of the lying behaviour (total lying time, number of bouts and average bout duration) on the first observation day after treatment.

Table 4.8 Mean (SD) and median (IQR) of lying behaviour variables according to treatment group.

Treatment	N	Total Lying (min/day)		Bouts/day		Average bout duration (min/bout)	
		Mean	SD	Median	IQR	Median	IQR
Control	34	685.8	153.7	10	8 - 13	61.0	53.5-82.1
Trim	13	727.1	181.4	9	7 - 14	72.8	68.5-78.8
Trim + Block	10	840.6	151.1	12	12 - 13	67.7	54-83.3
Trim + NSAID	11	732.6	197.4	13	8 - 14	71.9	56.1-88.3
Trim + Block + NSAID	6	676.8	175.8	10	7 - 12	76.2	60.7-85.0

4.3.3.1 Total lying time in the first 5 days after treatment

Figure 4.4 shows the distribution (Mean and SE) of total lying time per day by treatment. In the multilevel linear regression model there was a significant association between cows treated with trim and block and total lying time per day ($p<0.05$). Cows in this treatment group were lying longer than matched-control cows (Table 4.9). Pen by farm variable also had significant association with total lying time per day. Cows in pen 3 in farm 1 and cows in pen 1 in farm 2 showed less lying time per day when compared with cows in farm 1 in pen 1. No other variable had a significant association with total lying time. A significant ($P<0.01$) random variability was observed for cow level (17060.83 (3062.39)) and for observed day level (5861.35 (492.74)). Analysis of the residuals for the cow and observed day levels are presented in Figure 4.5 and suggest model was a good fit for the data.

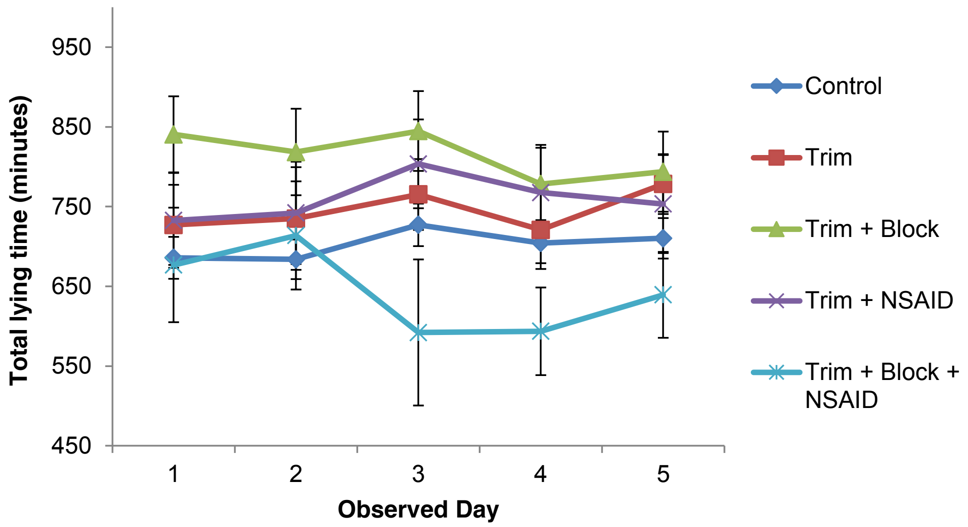

Figure 4.4 Mean (SE) of total lying time (min) per day in each treatment group

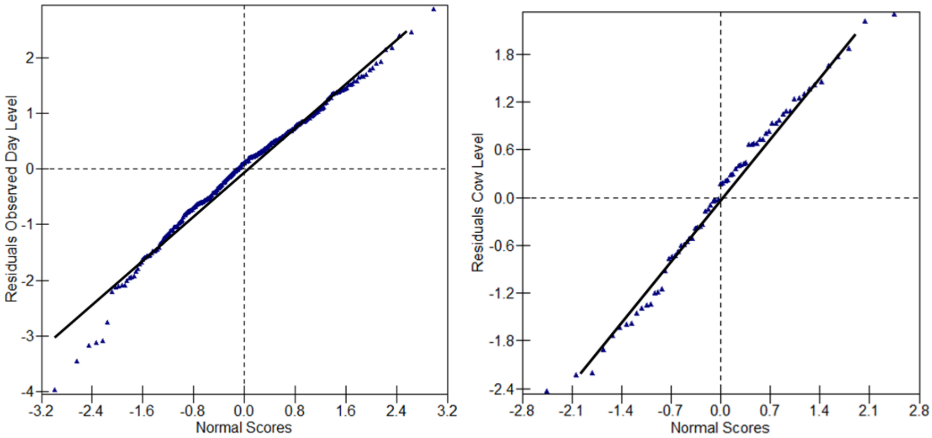

Figure 4.5 Q-Q plots of residuals distribution (SD 1.96) at observed day (354 observations) and cow (71 cows) levels for the total lying time model.

Table 4.9 Results of the multilevel linear regression model on total duration of lying behaviour per observed day after treatment.

Model term	Freq.	Coefficient	SE	Confidence Interval		z	P
				2.50%	97.50%		
Intercept		792.97	84.37				
Farm and Pen							
Farm1 Pen 1	45	Reference					
Farm1 Pen 2	30	-116.43	74.87	-263.18	30.31	-1.56	0.12
Farm1 Pen 3	60	-131.97	62.70	-254.86	-9.09	-2.11	0.04
Farm1 Pen 4	30	-140.76	76.52	-290.73	9.22	-1.84	0.07
Farm2 Pen 1	85	-166.17	60.61	-284.97	-47.36	-2.74	0.01
Farm2 Pen 2	120	-50.35	58.00	-164.04	63.33	-0.87	0.39
DIM							
DIM ≤ 100	85	Reference					
DIM 101-199days	104	11.56	48.19	-82.90	106.01	0.24	0.81
DIM ≥200	176	44.59	45.96	-45.49	134.67	0.97	0.33
Milk Production							
Daily Milk Production L		-0.11	1.35	-2.75	2.53	-0.08	0.94
Parity							
Parity 1	105	Reference					
Parity 2	80	-65.54	55.52	-174.35	43.28	-1.18	0.24
Parity ≥3	180	-11.15	45.05	-99.45	77.14	-0.25	0.80
Treatment							
Control	170	Reference					
Trim	65	31.07	49.05	-65.07	127.21	0.63	0.53
Trim+Block	50	120.27	50.44	21.41	219.12	2.38	0.02
Trim+NSAID	55	53.88	53.14	-50.27	158.02	1.01	0.31
Trim+NSAID+Block	30	-31.91	66.63	-162.50	98.67	-0.48	0.63

Freq. = Frequency of observations

4.3.3.2 Total number of lying bouts in the first 5 days after treatment

Figure 4.6 shows the median of the number of lying bouts per day by treatment. The results of the multilevel Poisson regression model showed no significant association between number of lying bouts per day and the model variables (Table 4.10). Cow level presented a significant (P<0.01) random variability (0.07 (0.02)) in the model. The model presented a visually stable chain mixing (Appendix 4).

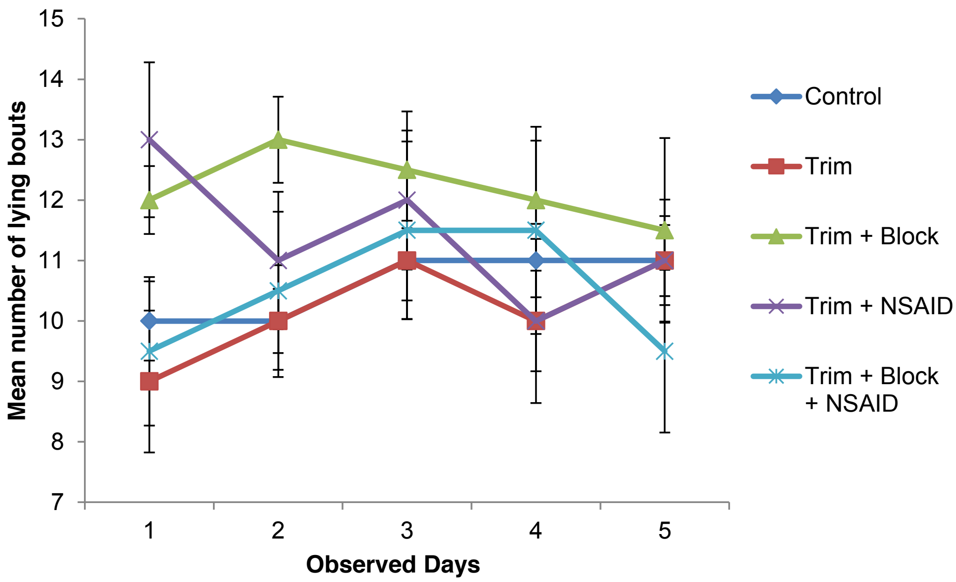

Figure 4.6 Median of number of lying bouts (SE) in each treatment group per day.

Table 4.10 Results of the multilevel Poisson regression model on the total number of lying bouts per observed day after treatment.

Model term	Freq.	Coefficient	SE	Confidence Interval		z	P
				2.50%	97.50%		
Intercept		2.43	0.22				
Farm and Pen							
Farm1 Pen 1	45	Reference					
Farm1 Pen 2	30	-0.06	0.16	-0.38	0.27	-0.34	0.74
Farm1 Pen 3	60	-0.07	0.14	-0.33	0.20	-0.50	0.62
Farm1 Pen 4	30	-0.17	0.17	-0.50	0.15	-1.04	0.30
Farm2 Pen 1	85	-0.10	0.13	-0.36	0.16	-0.78	0.44
Farm2 Pen 2	120	-0.16	0.13	-0.41	0.09	-1.27	0.20
DIM							
DIM ≤ 100	85	Reference					
DIM 101-199days	104	0.00	0.11	-0.21	0.21	0.02	0.99
DIM ≥200	176	0.08	0.11	-0.13	0.29	0.75	0.46
Milk Production							
Daily Milk Production L		-0.001	0.004	-0.01	0.01	-0.25	0.80
Parity							
Parity 1	105	Reference					
Parity 2	80	0.06	0.12	-0.18	0.30	0.50	0.61
Parity ≥3	180	0.08	0.10	-0.12	0.27	0.77	0.44
Treatment							
Control	170	Reference					
Trim	65	-0.09	0.11	-0.30	0.12	-0.85	0.40
Trim+Block	50	0.13	0.11	-0.08	0.34	1.19	0.24
Trim+NSAID	55	-0.01	0.12	-0.24	0.21	-0.12	0.90
Trim+NSAID+Block	30	-0.15	0.15	-0.43	0.14	-1.00	0.32

Freq. = Frequency of observations

4.3.3.3 Average lying bout duration in the first 5 days after treatment

Figure 4.7 shows the mean (SE) of the average lying bout duration per day by treatment. The results of the multilevel linear regression model showed no significant association for any of the variables with average lying bout duration per day (Table 4.11). A significant ($P<0.01$) random variability was observed for cow level (0.47 (0.09)) and for observed day level (0.59 (0.05)). Analysis of the residuals for the cow and observed day levels are presented in Figure 4.8 and suggested a good fit.

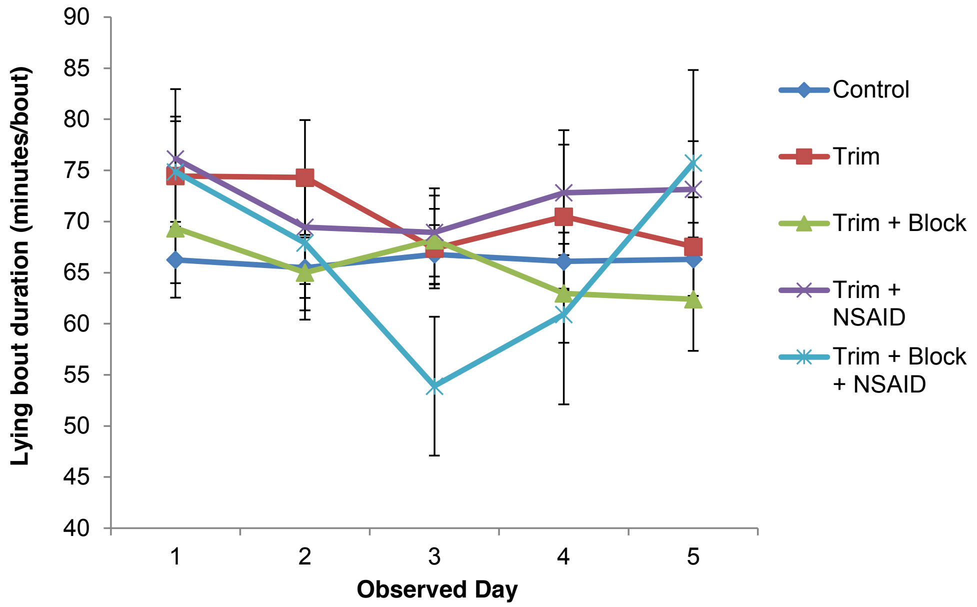

Figure 4.7 Mean (SE) of lying bout duration in each treatment per day.

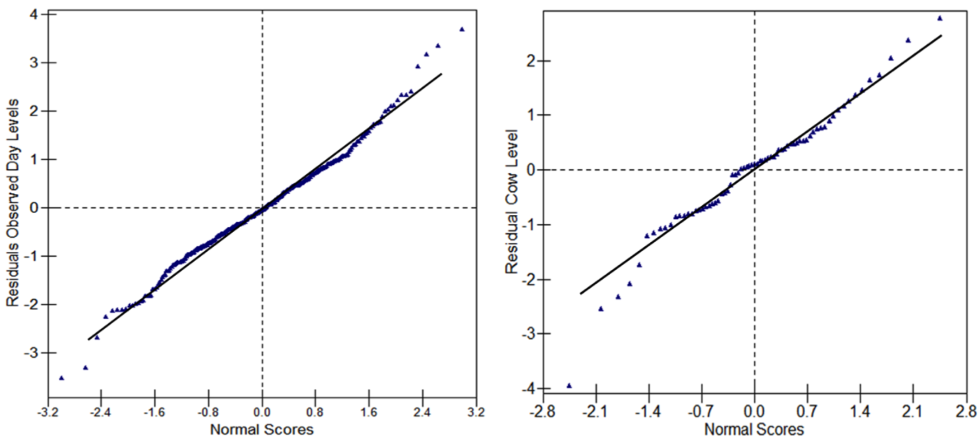

Figure 4.8 Q-Q plots of residuals distribution (SD 1.96) at observed day (354 observations) and cow (71 cows) levels for the mean bout duration model.

Table 4.11 Results of the multilevel linear regression model of average lying bout duration (square root) per observed day after treatment.

Model term	Freq.	Coefficient	SE	Confidence Interval		z	P
				2.50%	97.50%		
Intercept		75.86	0.55				
Farm and Pen							
Farm1 Pen 1	45	Reference					
Farm1 Pen 2	30	-0.52	0.43	-1.36	0.31	-1.23	0.22
Farm1 Pen 3	60	-0.51	0.36	-1.21	0.18	-1.44	0.15
Farm1 Pen 4	30	-0.08	0.43	-0.93	0.77	-0.18	0.86
Farm2 Pen 1	85	-0.50	0.34	-1.18	0.17	-1.46	0.14
Farm2 Pen 2	120	0.41	0.33	-0.23	1.06	1.25	0.21
DIM							
DIM ≤ 100	85	Reference					
DIM 101-199days	104	0.07	0.28	-0.48	0.61	0.24	0.81
DIM ≥200	176	-0.17	0.27	-0.71	0.36	-0.63	0.53
Milk Production							
Daily Milk Production L		-0.01	0.01	-0.03	0.01	-0.70	0.48
Parity							
Parity 1	105	Reference					
Parity 2	80	-0.53	0.31	-1.15	0.08	-1.69	0.09
Parity ≥3	180	-0.25	0.26	-0.75	0.25	-0.98	0.33
Treatment							
Control	170	Reference					
Trim	65	0.47	0.28	-0.08	1.01	1.68	0.09
Trim+Block	50	0.08	0.29	-0.48	0.64	0.28	0.78
Trim+NSAID	55	0.32	0.30	-0.27	0.92	1.07	0.28
Trim+NSAID+Block	30	0.41	0.38	-0.33	1.15	1.08	0.28

Freq. = Frequency

4.3.3.4 Total lying time by time of day

When total lying time was distributed by time periods throughout the day, it was observed that cows in the trim and block group lay down significantly longer than control cows in all the periods (Period 1: Mean difference (Md) = 27.6min, P= 0.04; Period 2: Md= 31.1min, P= 0.01; Period 3: Md= 27.7min, P= 0.01; Period 4: Md= 26.5min, P=0.04) (Figure 4.9).

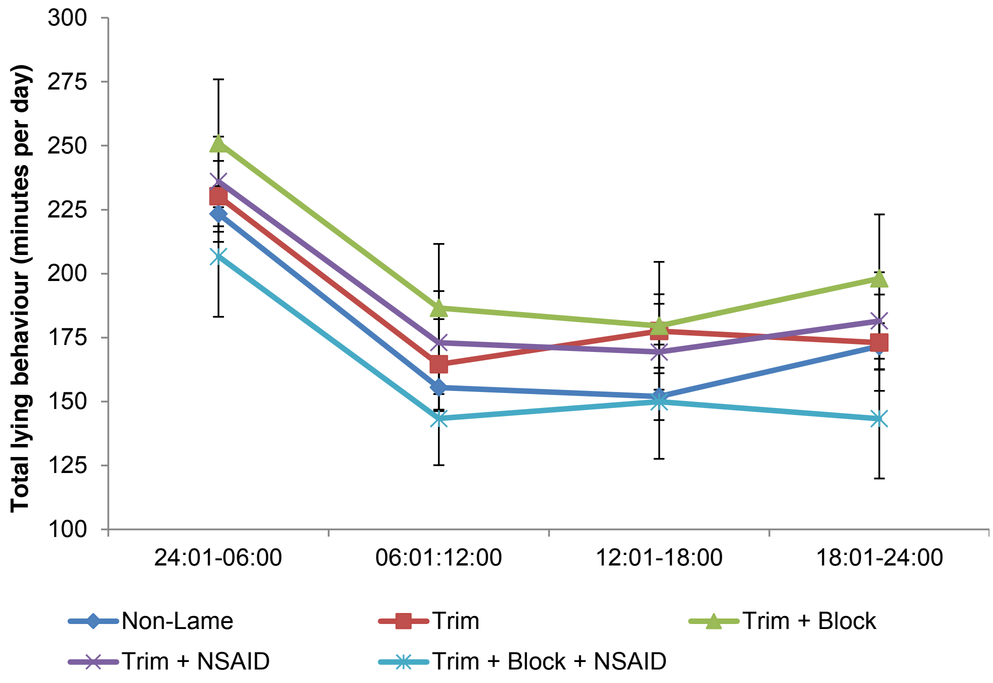

Figure 4.9 Mean (SE) of total lying time of each treatment group per time period.

4.3.4 Laterality of lying behaviour in the first 5 days of observation

Individual non-lame cows showed a preferred side to lie on during the observed period, with 70.6% of cows lying most of the time on their left side and the remaining 29.4% of cows preferred to lie down on their right side (Figure 4.10). On average non-lame cows spent 57% of their time lying on their left side.

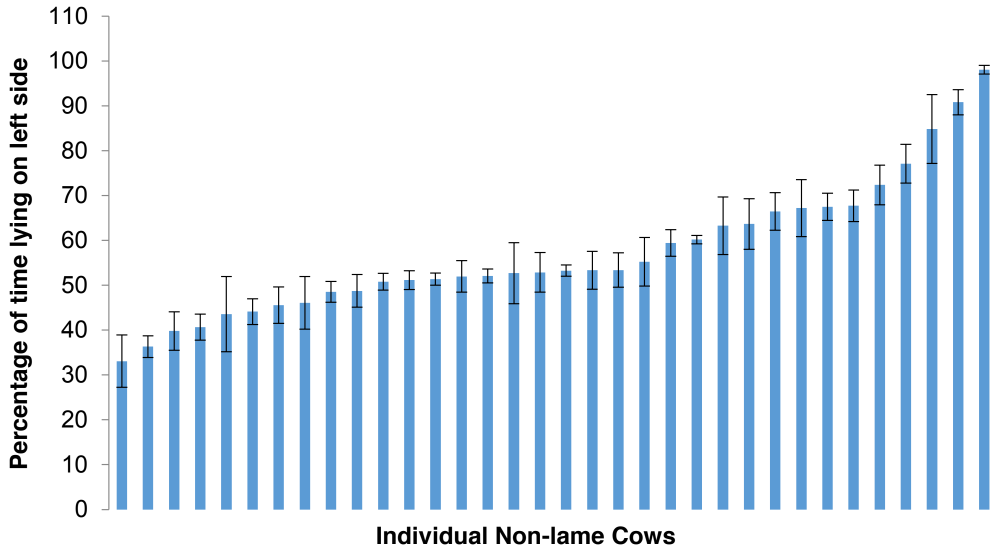

Figure 4.10 Mean (SE) percentage of time that individual non-lame cows spent lying on their left side during the observed days.

Results from the multilevel binomial logit model are presented in Table 4.12. Cows were more likely to lie down on the left side if they were housed in pen 1 on farm 2 in comparison to cows housed on Farm 1 pen 1 (OR: 2.36; CI: 1.40-3.98). Cows with higher milk production were more likely to lie down on their left side than cows with lower milk production (1.01; 1.01 – 1.02). No treatment effect was observed on the laterality of lying behaviour. Neither side where the accelerometer was attached or treated leg had an influence on the likelihood of cows lying on their left side. A significant (P<0.01) random variability was observed for cow level (Coefficient: 0.32 (SE: 0.06)). Analysis of the residuals for the cow and observed day levels are presented in Figure 4.11.

Table 4.12 Multilevel binomial logit regression model results for the outcome: Proportion of time spent lying on the left side.

Model term	Freq.	Coefficient	SE	Odds ratio	z	Confidence Interval		P
						2.50%	97.50%	
Intercept		-1.08	0.33					
Farm and Pen								
Farm1 Pen 1	45	Reference						
Farm1 Pen 2	30	0.31	0.33	1.37	0.91	0.72	2.60	0.340
Farm1 Pen 3	60	0.51	0.28	1.66	3.43	0.97	2.85	0.064
Farm1 Pen 4	30	0.31	0.33	1.37	0.92	0.72	2.60	0.338
Farm2 Pen 1	85	0.86	0.27	2.36	10.43	1.40	3.98	0.001
Farm2 Pen 2	120	0.46	0.25	1.59	3.44	0.97	2.60	0.063
DIM								
DIM ≤ 100	85	Reference						
DIM 101-199days	104	-0.19	0.20	0.82	0.93	0.56	1.22	0.335
DIM ≥200	176	0.33	0.19	1.39	3.06	0.96	2.01	0.080
Milk Production								
Daily Milk Production L		0.013	0.004	1.01	10.56	1.01	1.02	0.001
Parity								
Parity 1	105	Reference						
Parity 2	80	0.19	0.24	1.20	0.61	0.76	1.91	0.435
Parity ≥3	180	0.36	0.19	1.43	3.52	0.98	2.09	0.061
Treatment								
Control	170	Reference						
Trim	65	-0.29	0.24	0.75	1.54	0.47	1.19	0.215
Trim+Block	50	-0.04	0.24	0.96	0.02	0.60	1.54	0.875
Trim+NSAID	55	0.27	0.25	1.32	1.20	0.81	2.15	0.273
Trim+NSAID+Block	30	0.59	0.30	1.81	3.82	0.99	3.27	0.051
Left hind limb treated								
No treated	270	Reference						
Treated	100	-0.09	0.23	0.92	0.14	0.59	1.43	0.704
Accelerometer attached on left hind limb								
No attached	188	Reference						
Attached	182	0.18	0.13	1.19	1.83	0.92	1.54	0.177

Freq. = Frequency of observations

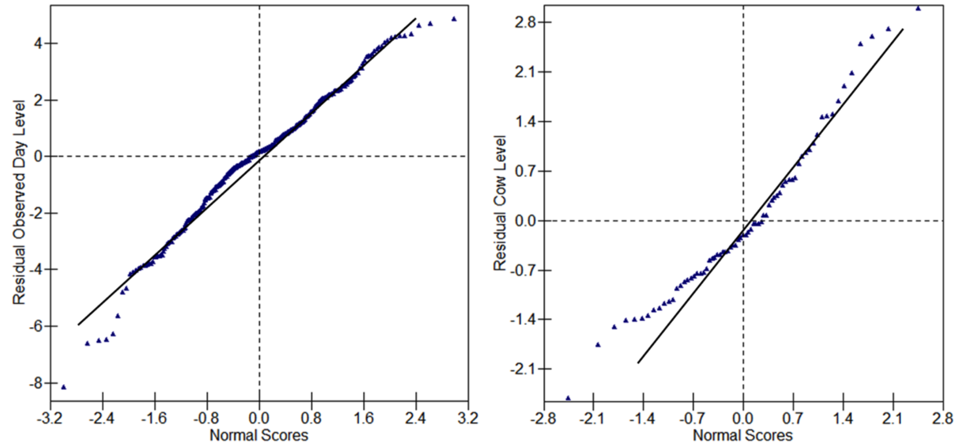

Figure 4.11 Q-Q plots of residuals distribution (SD 1.96) at observed day (354 observations) and cow (71 cows) levels for laterality of lying behaviour model.

4.4 Discussion

Total lying time was affected by lameness treatment. Newly lame cows treated with a therapeutic trim and foot block lay down longer than non-lame control cows. Treatment did not have any other significant effect on the other variables of lying behaviour (number of bouts/day and average bout duration). This is the first study to assess the effect of lameness treatment on lying behaviour under a randomized controlled clinical trial. Previously, two studies assessed the effects of hoof trimming (Chapinal et al., 2010a) and NSAID (Chapinal et al., 2010c) on the behaviour of lame cows which included lying behaviour.

In the present study, lame cows treated with trim and foot block increased their lying time, possibly due to the pain or discomfort caused by the block and their lameness state. Cows that received a three day course of NSAID in addition to the block did not show any increase in their lying time in comparison to non-lame cows. The use of NSAIDs improved the locomotion score of lame cows (Flower et al., 2008), cows distributed more evenly their weight on the hind legs (Chapinal et al., 2010b) and modulated the hyperalgesia present in lame cows (Whay et al., 2005). In addition, the application of foot blocks to non-lame cows only increased their mobility score but lying time was not affected (Cutler, 2012). On the contrary, a foot trim followed by the application of a foot block caused a reduction in activity in cows with mild or moderate chronic foot lesions (SU and WLD) and no improvements in cows with severe chronic foot lesions in comparison to before treatment (O'Callaghan, 2003). Therefore, it is possible that the combination of foot blocks and NSAID may prevent the discomfort caused by the block and reduce the pain caused by claw horn lesions.

The other lameness treatments (trim only, trim and NSAID, and trim, block and NSAID) did not show any effect on lying behaviour. The present findings disagree with Chapinal et al. (2010c), who observed increased lying times in the 2 days immediately following treatment in both of their experimental groups (hoof trimming and saline or NSAID (2.2 mg/kg of BW of Flunixin meglumine) injection). Even though this effect was not significant on day 3 and 4 after treatment in the group treated with the NSAID, the authors concluded that hoof trimming may have caused some discomfort to lame cows therefore causing the increase in lying times (Chapinal et al., 2010c). In the present study, professionally trained veterinarians conducted hoof trimming using the 5 step Dutch trimming method, and treatments were randomly applied according to lesion. There is a lack of standardised methods for hoof trimming in the literature, few authors discuss the technique applied and as in the case of Chapinal et al. (2010c), the authors only stated that the technique was applied by a trained hoof trimmer and that the hoof capsule was reshaped. It could be possible that a different technique or the instruments used to apply the hoof trimming might be the cause of differences observed between studies or that cows may have been severely lame. On the other hand, according to the post-hoc power calculation, it is possible that a type II error may have occurred which meant that the non-significant effect for the other treatments observed may be the results of a small sample size.

Lameness increases the total lying time and bout duration (Ito et al., 2010; Thomsen et al., 2012), or lying time and bout frequency as observed in AMS (Deming et al., 2013a). Ito et al. (2010) observed that bout duration and total lying time were higher in severely lame cows than in moderately lame cows. In the present study neither of these variables were significantly increased in lame cows treated with trim and foot blocks in comparison to non-lame cows. The previous studies did not specify chronicity of lameness or type of lesions; the present study looked at the behaviour of newly lame treated cows diagnosed with claw horn lesions. Then, it is possible that chronicity and type of lesions, as well as treatment, may have different effects on lying bout duration and lying frequency.

It is important to consider that cows enrolled in the present study were new cases of lameness; cows must have become lame at some point in the 2 weeks prior to treatment. Therefore their lying behaviour may not have changed dramatically as observed in previous studies investigating lying times between lame and non-lame cows (Ito et al., 2010; Thomsen et al., 2012). Also, most of the cows were diagnosed as mildly lame, Yunta et al. (2012) did not observe significant lying time differences between moderately lame (Mobility score 1-5: cows with score 3 and 4) and sound cows (score 1). Due to the nature of the study we were unable to record the “normal” lying behaviour of the lame cows when they were sound or before treatment, but we used sound cows that did not receive treatment. These cows showed an average lying time of 702 minutes/day (11.7 hrs/day) similar to those observed in healthy cows housed in automatic milking systems (Deming et al., 2013b). Even though this study does not provide any clear evidence, it can be postulated that prompt intervention may prevent the significant changes in lying behaviour observed in previous studies (e.g.Ito et al., 2010).

Lame cows that received hoof trimming, NSAID and foot block were more likely to have a better mobility score 35 days after treatment (Thomas et al., 2015), and did not show any significant differences in lying behaviour when compared to non-lame cows. This combination of treatment has not been studied before. It has been observed that the 5 step Dutch hoof trimming improves gait and smoothes rhythmicity in non-lame cows (Van der Tol et al., 2004), in the case of lame cows it may have helped to release pressure from the lesions (e.g. sole ulcers) as part of the technique includes removing any damaged hoof and clearing the lesion. The function of the foot block is to raise the healthy claw so it removes the weight bearing from the affected claw; this provides adequate time for the new horn to grow avoiding further damage (Shearer et al., 2013). NSAIDs' function is to reduce pain and control further inflammation. The present findings in combination with previous research may suggest that this treatment protocol may be not only beneficial for a prompt recovery but also to improve the welfare of lame cows as it does not affect their behaviour.

Farm and pen had an effect on total lying time, cows housed in pen 3 in farm 1 and cows housed in pen 1 in farm 2 lay down less than cows housed in pen 1 farm 1. Lying behaviour can be affected by the type of bedding (Tucker et al., 2003), and stocking density (Fregonesi et al., 2007a). No clear explanation could be drawn from the statistical and descriptive analysis in the present study, though it is possible that other factors like individual pen management e.g. introduction of new cows into those pens, may have caused this association. Finally, there is always the possibility that this is part of normal individual group differences.

Cows treated with trim and foot block showed increased lying time throughout the whole day in comparison to non-lame cows i.e. the increase in lying time was distributed evenly. There is limited information on the lying behaviour of lame cows housed in AMS. To the author's knowledge there is only 1 peer-reviewed study (Deming et al., 2013a) published in this area. The authors found that lameness and delivering feed twice a day (in comparison with once a day) was associated with increased lying time. Cows with feed available throughout the day might be more efficient when eating (reducing unnecessary standing time) (Deming et al., 2013b). This may explain why lame cows in the present study did not show reduced lying times in a particular time period as in both farms feed was pushed up at least twice a day, providing them with readily available food at all times.

Overall, cows had a tendency to lie down on their left side, although there was a high variability between individuals (Figure 4.10). Individual variability has been observed previously (Tucker et al., 2009; Gibbons et al., 2012). In the present study control cows spent on average 57% of their time lying over their left side, slightly higher than previous studies that used the same data logger. Medrano-Medrano-Galarza et al. (2012) and Ledgerwood et al. (2010) observed that healthy cows spent on average 53% lying over the left side. Preference for the left side might be associated with the stage of pregnancy which becomes more evident when closer to calving (Forsberg et al., 2008; Tucker et al., 2009). On the other hand, it has been suggested that rumen fill may influence the preference for lying on the left side, though Tucker et al. (2009) did not find any association between the time since eating and time spent eating with lying side preference. In the present study it was not possible to capture information about the stage of pregnancy or feed intake. Future studies considering lying side preference must include feed intake and stage of pregnancy to understand more clearly these possible associations.

Moderate lameness does not have an effect on lying side (Yunta et al., 2012). The present study did not observe a significant effect of treatment on the laterality of lying behaviour of mildly lame cows. This was surprising as the application of a foot block, in particular, may have been expected to have an impact on the side that cows preferred to lie on. When a cow wants to lie down, first she kneels down on her fore limbs (one at a time), and as she lowers down her back she places one of her hind limbs under her belly and the other rests upwards. When a cow wants to stand up, they use the outer hind leg to thrust themselves upwards. So it was assumed that cows may prefer to lie down over the side that had the foot block to make a stable and more comfortable rising platform, as the opposite foot will have a flatter and more comfortable surface to drive upwards through. Consequently, it was interesting to observe no effects of lameness treatment (type of treatment and side where it was applied) on the laterality of lying behaviour. It could be assumed that cows may have adapted quickly to the discomfort or the unstable surface provided by the foot block when rising and lying down.

No significant effect was observed on lying side preference either caused by where the treatment was applied (left or right leg) or for the side to which the accelerometer was attached. This latter finding agrees with a previous study that used a bigger data logger, where researchers did not observe changes in laterality preference when data loggers were attached to either (right or left) or both hind legs (Gibbons et al., 2012).

Cows housed in pen 1 in farm 1 were more likely to lie down on their left side than cows on farm 1 pen 1. It has been proposed that housing (free-stall) may increase the individual preferences (Phillips et al., 2003) and cows may be more likely to switch sides when housed on a comfortable surface (e.g. well bedded mattress) (Tucker et al., 2009). No clear causation was drawn from the present study regarding housing conditions in that particular pen. In addition, it was observed that cows that produce more milk were more likely to lie down on their left in comparison to cows that were producing less. Milk yield has been associated with hair loss, ulceration and swelling at the hock (Potterton et al., 2011; Lim et al., 2015). There are no published studies that have looked at preferences of lying side and milk production. It is possible that udder size may play an important role in lying preferences which may have an impact on hock lesion development. This area warrants further investigation in future studies.

Measures of activity (e.g. lying behaviour) have been suggested as a good method to assess the effects of lameness treatment (Chapinal et al., 2010c). The present study demonstrated that lying behaviour is a useful tool to study changes caused by lameness treatment. Lying behaviour in cows is very variable even when studied at the farm level (Ito et al., 2009). Ideally, lying behaviour should be measured before and after any treatment is applied so comparisons can be made at the cow level. The present experimental design did not allow measurement of lying behaviour before treatment was applied. The use of repetitive measurements and the selection of matched control cows were used to account for the lack of within cow measurements. In addition, the target sample size was not reached; this may have reduced the power of the study to detect significant differences among variables.

Analyses of the present study were carried out only considering cows as lame or non-lame. The intra-observer reliability in this case (lame and no-lame) was almost perfect (Observer 1: K_w=0.89; Observer 2: K_w=0.80), each observer was in charge of one farm; then individual observer error was contained within farm. On the other hand the inter-observer reliability was fair (K_w=0.36, range: 0.08-0.64), which means that there is the possibility that cows between farms were not similarly selected. Though, these results are relevant for the selection of cows, this may have not affected the measurement of lying behaviour which was not directly dependent on mobility score.

4.5 Conclusions and future work

This is the first study to measure the effects of lameness treatment on lying behaviour. The study found that the application of trim and a foot block caused an increase in lying behaviour and the increase in lying behaviour was distributed throughout the day. Foot blocks may cause discomfort to the lame cows as they increased their lying time in comparison to non-lame cows, suggesting that their welfare may be compromised. Cows that received trim, foot block and NSAID did not show any significant difference in lying behaviour in comparison to non-lame animals. Lameness treatment and accelerometer attachment side did not affect cows lying side preference. The findings suggest that the application of trim, foot block and NSAID may be more suitable not only from the clinical side but also for cow welfare as it does not affect their behaviour.

Further studies are required to understand lying preference and its association with milk production. Results from the present study only apply to cows with mild lameness that received prompt treatment and that were diagnosed with a claw horn lesion in one claw. Further studies are required to understand how chronicity and cause of lameness affects lying behaviour and how the treatment of these conditions affects the behaviour of lame cows.

Chapter 5

Effects of lameness treatment on milking and rumination behaviour

5.1 Introduction

Lame cows alter their behaviour to avoid further damage to the affected area and to reduce discomfort (Molony and Kent, 1997). Observed behavioural changes go from reduction in food intake to increases in resting behaviour (Deming et al., 2013a; Norring et al., 2014). As discussed previously, lameness affects voluntary visits to the milking robot (Chapter 2). AMS gives cows freedom to approach the unit/robot when convenient. In the case of the relationship between rumination behaviour and lameness there are contradictory findings (Chapter 1: Table 3). Recent technological advances allow researchers to standardised data collection, using rumination collars (Schirmann et al., 2009). A recent study using this technology observed a 25 minutes reduction in rumination at night (20:01-04:00) in lame cows in comparison with non-lame cows (Van Hertem et al., 2013). This reduction remained for two days after treatment (treatment not specified) (Van Hertem et al., 2013). From the studies presented in this thesis, it was observed that lame cows reduced their rumination time by 8 minutes per day and visited the AMS less particularly at night in comparison to non-lame cows (Chapter 2 and 3).

Prompt treatment reduced the prevalence of lameness for up to 4 weeks after treatment (Leach et al., 2012). Lameness treatment may help to reduce pain and discomfort caused by the lesions (O'Callaghan, 2002); which may be reflected on the behaviour of lame cows. However, information regarding on how lameness affect rumination and visits to the AMS is scarce. Therefore the aim of the present study was to describe the effect of lameness treatment on milking frequency and the time of milking, and total rumination time. The null hypothesis was that treatment did not cause changes in the behaviour of lame treated cows compared with control cows. The alternative hypothesis was that treatment caused changes in the behaviour of lame treated cows in comparison to control cows.

5.2 Materials and methods

5.2.1 Description of herds and farm management

The present study was carried out in the same farms described in Chapter 4. For details about the herds and farm management please refer to Chapter 4, section 4.2.1.

5.2.2 Study design

This study followed similar observational longitudinal design to that in Chapter 4. The present study was designed to investigate the impact of lameness treatment on total rumination time and milking visits. As in Chapter 4, behaviour (rumination and milking behaviour) of lame treated cows was compared to the behaviour of non-lame cows. Additional information on the study design can be found in Chapter 4, section 4.2.2.

5.2.3 Sample size

Sample size for rumination behaviour was calculated assuming an estimated difference of 8min per day between lame and non-lame cows, a standard deviation of 90min with 80% power and a confidence level of 95% required, the required sample size was calculated as 47 cows per treatment group (Stata/SE 12.0 - Stata Corp 2011, USA). In the case of milking behaviour, the findings from Chapter 2 were used to estimate the required sample size per treatment group. Assuming an estimated difference of 0.5 milking visits per day between lame and non-lame cows, a standard deviation in the number of visits per day of 0.70 with 80% power and a confidence level of 95%, the required sample size was calculated as a minimum of 18 cows per treatment group (Stata/SE 12.0 - Stata Corp 2011, USA).

5.2.4 General experimental procedures

Selection and treatment of lame cows followed the same experimental procedure described in Chapter 4, section 4.2.3. In brief, only cows diagnosed with claw horn lesions (Sole Haemorrhage or Sole Ulcer, White Line Disease or ‘Other’) on a single claw were selected for treatment. Selection of control cows followed the matching criteria described in Chapter 4, section 4.2.3.3.1. In brief, a cow was selected as control if she had 3 mobility scores as non-lame (0 or 1) prior to the treatment day of her matched enrolled animal.

5.2.5 Specific experimental procedures

5.2.5.1 Milking behaviour data collection

Milking behaviour was considered as the number of voluntary visits of a cow to the milking robot and the time of day when these happened (Figure 5.1). The AMS computer system recorded when the cow entered the milking robot (start of event) and ended once the cow left (end of event). Data collection followed the same procedure described in Chapter 2, section 2.2.4.

Figure 5.1 Automatic milking robot with a cow at the entrance and cow in the unit being milk while eating concentrate.

5.2.5.2 Rumination behaviour data collection

The present study used the same rumination collar described in Chapter 3, section 3.2.3.2. For the present study, Lely engineers collected data from the farms' computer matrix.

5.2.5.3 Behavioural observation period

The present study included the whole duration of the RCT (Chapter 5) in which cows were observed between December 2011 and March 2013. Data for the total rumination time and milking visits were collected continuously as standard procedure on the farms, so each cow was observed on the day prior to treatment (day 0) and on the following 5 days after treatment (treated days). For both periods, observations were made from 00:01 to 24:00 hours per observed day.

5.2.6 Data analysis

Data were downloaded to Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) with descriptive and statistical analyses carried out using Stata/SE 12.0 (Stata Corp 2011, USA). Multilevel and single level regression models were built using MLwiN version 2.27 (Rasbash et al., 2009) to investigate the association between lameness and lameness treatment, and milking and rumination behaviour. Level of significance was set as $P \leq 0.05$ for all the analysis. Results from models are presented as follows (Coefficient (SE)). A dichotomous variable (Yes or No) called Pushed was created to control for the time cows were pushed through either the robot in the morning (7-8am) or in the afternoon (2-3pm). One-way ANOVA test was used to compare milk production variables between groups. As sample size was not reached for rumination behaviour, post-hoc calculations were made using *simpower* analysis from Stata/SE 12.0 (Stata Corp 2011, USA).

5.2.6.1 Milking behaviour data analysis

A single level Poisson regression analysis was built to study the association between milking behaviour and lameness status on day 0 (day before treatment). The model took the following form:

$$Log\ (\pi_j) = \beta_{0j} + \beta_1 x_{1j} + \beta_2 x_{2j} + \beta_3 x_{3j} + \beta_4 x_{4j} + \beta_5 x_{5j} + \beta_6 x_{6j} + e_j$$

$$y_j \sim Poisson\ (\pi_j)$$

The outcome variable (y_i) was number of visits at cow level (j) on day 0. β_0 was the intercept and $\beta_{1\text{-}5}$ represented the regression coefficient. The predictor variables were represented by x_1 (lame or non-lame) and $x_{2\text{-}6}$ (Table 5.1). The first category of each variable was used as a reference. Log ($\boldsymbol{\pi}_j$) was the log of the expected value of the number of visits and e was the random error at cow level. The model fit was checked by graphical analysis of distribution of deviance residuals; AIC values were used to find the best model.

Table 5.1 Description of variable *x* used in the single and multilevel models.

Variable	Name	Descriptor
x_1	Treatment	5 categories: 0 = control (not treated), 1 = trim only, 2 = trim and block, 3= trim and NSAID, 4=trim, block and NSAID
x_2	Daily milk production	continuous variable total litres per day
x_3	Days in Milk (DIM)	3 categories: 1 = <100 days, 2 = 101-200 days, 3 = ≥201 days
x_4	Parity	3 categories: 1= 1st parity, 2= 2nd parity and 3= 3rd or higher parity
x_5	Farm and pen	6 categories: 1 to 4 belong to each pen on farm 1 and 5 and 6 to each pen on farm 2
x_6	Pushed	No (0) or Yes (1)

The number of milking visits between treatment and control groups on day 0 was compared using Kruskal-Wallis test and Wilcoxon Rank Test (Petrie and Watson, 2006). Then, a multilevel Poisson regression analysis was carried out to study the effect of lameness treatment on the total visit number per observed day (5 days). The model took the following form:

$$Log\ (\pi_{ij}) = \beta_{0ij} + \beta_1 x_{1ij} + \beta_2 x_{2ij} + \beta_3 x_{3ij} + \beta_4 x_{4ij} + \beta_5 x_{5ij} + \beta_6 x_{6ij} + \beta_7 x_{7ij} + e_{ij}$$

$$y_{ij} \sim Poisson\ (\pi_{ij})$$

The outcome variable (y_{ij}) was number of visits per observed day (i) within cow (j). β_0 was the intercept fixed at all levels. $\beta_{1\text{-}7}$ represented the regression coefficient and the predictor variables were represented by $x_{1\text{-}6}$ (Table 5.1). The first category of each variable was used as a reference. Number of milking visits on day 0 was used as a reference for the following visits and it is represented by x_7. Log ($\boldsymbol{\pi}_{ij}$) was the log of the expected value of the number of visits and e was the random error at cow level. The multilevel Poisson regression analysis model was followed by

Monte Carlo Markov Chain (MCMC) methods with 500 000 iteration and a burn in of 5000. The chain mixing and stability were evaluated visually (Browne, 2009).

Treated days were divided in four time periods (12:01 - 18:00; 18:01 - 24:00; 24:01 - 06:00 and 06:01 - 12:00) to study the effect of treatment on the time of milking visits. The number of visits was grouped into one of the 4 periods. Wilcoxon rank-sum test (Petrie and Watson, 2006) was used to compare the number of milking visits between treatment groups in each individual period.

5.2.6.2 Rumination behaviour data analysis

Total rumination time did not follow a normal distribution, thus square transformation was applied to normalise it for both of the analysed periods (day 0 and treated days). Results presented herein are as they were obtained from the model.

On day 0 (prior to treatment), a single level linear regression model was built to study the association between rumination time and lameness status. The model had the following form:

$$y_i = \beta_{0i} + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \beta_4 x_{4i} + \beta_5 x_{5i} + e_i$$

The outcome variable (y_i) was total rumination time on day 0 at cow level (i). β_0 was the intercept and $\beta_{1\text{-}5}$ represented the regression coefficient. The predictor variables were represented by x_1 (lame or non-lame) and $x_{2\text{-}5}$ (Table 6.1), and e was the random error at cow level. The first category of each variable was used as a reference. The model fit was checked by graphical analysis of normal distribution of residuals.

A multilevel linear regression model was built to study the effect of lameness treatment on the total rumination time per observed day (5 days). Before the model was built, the total rumination time between treatment and control groups was compared using Kruskal-Wallis test (Petrie and Watson, 2006). The model had the following form:

$$y_{ij} = \beta_{0ij} + \beta_1 x_{1ij} + \beta_2 x_{2ij} + \beta_3 x_{3ij} + \beta_4 x_{4ij} + \beta_5 x_{5ij} + e_{ij} + u_{ij}$$

The outcome variable (y_{ij}) was total rumination time per observed day (i) nested within cow (j). β_0 was the intercept fixed at each level and $\beta_{1\text{-}5}$ represented the regression coefficient and the predictor variables were represented by $x_{1\text{-}5}$ (Table 5.1). The random error at cow and day level were represented by e and u respectively. The first category of each variable was used as a reference. The model fit was checked by graphical analysis of normal distribution of residuals at observed day and cow level.

5.3 Results

5.3.1 Descriptive analysis

There were a total of 172 observations, of which 79 were events for non-lame cows and 93 events for treated cows. A total of 158 cows were observed during the period studied. Ten cows were observed first as controls and then observed as cases; time in between control and case observations was a minimum of a 30 days. Only 79 treated cows had a matched control. Four cows were used as control animals on two occasions; time in between control and control observations was a minimum of 30 days. Table 5.2 shows number of cows per pen according to treatment group with the total number of observed events of 172.

Three cows from treatment 2 (Trim + Block) and 6 cows from treatment 4 (Trim + Block + NSAID) lost their blocks during the first 7 days after treatment. As it was unknown when they lost their blocks, data from these cows were excluded from the analysis. Statistical analysis was performed with the remaining 84 cases and 79 controls (Table 5.3). Only for rumination, data from two cows were lost due to technical problems with the rumination collars during data collection period. One cow belonged to the non-lame group and the other cow to the trim and NSAID group (diagnosed with WLD). Rumination analysis was performed with the remaining 161 cows (82 cases and 79 controls).

Table 5.2 Number of cows in each farm and pen according to treatment group.

Farm	Pen	Non-Lame	Trim	Trim + Block	Trim + NSAID	Trim + Block + NSAID	Cows per pen
1	1	5	3	4	3	1	16
	2	9	1	1	3	3	17
	3	14	5	2	3	6	30
	4	5	2	4	2	1	14
2	5	22	5	3	8	4	42
	6	24	8	9	5	7	53
Cows per treatment		79	24	23	24	22	172

Table 5.3 Number of cows used for data analysis divided by treatment group and diagnosis (SU/SH =Sole Ulcer or Sole Haemorrhage, WLD = white line disease, Other = SU/SH and WLD were observed at the same time).

Treatments	Non-Lame	SU/SH	WLD	Other	Cows per treatment
Non-Lame	79				79
Trim		12	5	7	24
Trim + Block		10	6	4	20
Trim + NSAID		12	5	7	24
Trim + Block + NSAID		5	4	7	16
Cows per diagnosis	79	39	20	25	163

Table 5.4 shows milk production variables by treatment group. As expected, there were no significant differences (P > 0.05) between treated and controls cows for any of these variables.

Table 5.4 Mean and SD of milk production variables calculated for the first day of observation for non-lame, lame treated groups and all observed cows.

Variables		Non-Lame	Trim	Trim + Block	Trim + NSAID	Trim + Block + NSAID	All cows
Parity	Mean	2.5	2.9	2.5	2.6	2.1	2.5
	SD	1.7	1.1	1.8	1.4	0.9	1.5
DIM	Mean	190.1	207.6	248.1	168.9	173.4	195.0
	SD	104.3	120.8	176.2	78.8	100.6	115.3
Daily Milk Production	Mean	37.1	39.4	35.6	38.2	37.3	37.4
	SD	11.0	11.7	11.3	11.6	9.8	11.0

Prior to treatment day, in the control group 16 cows were scored 0 and 63 were scored 1. Seventy-four of the lame cows were scored 2a, 8 cows were scored 2b, 1 cow was scored 3a and 1 cow was scored 3b.

5.3.2 Milking behaviour

5.3.2.1 Number of milking visits on day 0

Between lame and non-lame cows

Median milking visits was 3 visits per day (IQR: 2-4) for non-lame and lame cows. No significant association was observed between lameness and milking visits on day 0 (P=0.79), even after being pushed into the milking robot was considered into the model (Table 5.5). Results from the single level model showed a significant (P<0.05) positive association between milk production and number of visits (Table 6.6), with cows visiting more often producing more milk. No other significant (P>0.05) associations were observed (Table 5.5). Graphical analysis of the model's residuals is presented in Figure 5.2 which shows a normal distribution.

Table 5.5 Single level Poisson regression model results for number of milking visits on day 0 (prior treatment).

Model term	Freq.	Coefficient	SE	Confidence Interval		z	P
				2.50%	97.50%		
Intercept		0.581	0.268				
Farm and Pen							
Farm1 Pen 1	15	Reference					
Farm1 Pen 2	17	-0.204	0.202	-0.60	0.19	-1.01	0.31
Farm1 Pen 3	29	-0.268	0.187	-0.63	0.10	-1.43	0.15
Farm1 Pen 4	14	-0.34	0.221	-0.77	0.09	-1.54	0.12
Farm2 Pen 1	39	0.012	0.178	-0.34	0.36	0.07	0.95
Farm2 Pen 2	49	-0.096	0.166	-0.42	0.23	-0.58	0.56
Parity							
Parity 1	56	Reference					
Parity 2	33	-0.074	0.136	-0.34	0.19	-0.54	0.59
Parity ≥3	74	-0.163	0.114	-0.39	0.06	-1.43	0.15
DIM							
DIM ≤ 100	36	Reference					
DIM 101-199days	47	0.112	0.132	-0.15	0.37	0.85	0.40
DIM ≥200	80	0.103	0.126	-0.14	0.35	0.82	0.41
Milk Production							
Daily Milk Production L		0.017	0.005	0.01	0.03	3.40	<0.001
Pushed							
No	112	Reference					
Yes	51	0.099	0.096	-0.09	0.29	1.03	0.30
Lameness							
Non-Lame	79	Reference					
Lame	84	-0.025	0.092	-0.21	0.16	-0.27	0.79

Freq. = Frequency of observations

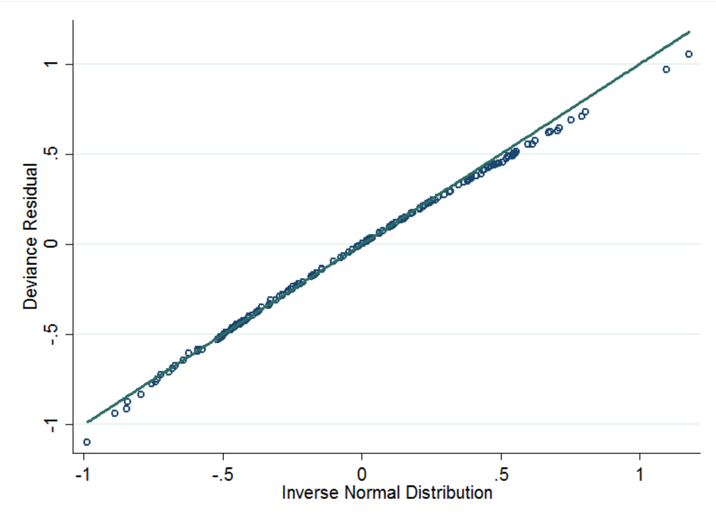

Figure 5.2 Q-Q plot of deviance residuals of the single Poisson regression model.

Between control and treated lame cows

On day 0, the number of milking visits between treatment and control groups showed a significant difference (P<0.05), cows in the trim and block treatment group had less visits than cows in the control group (z=2.83; P=0.005) (Figure 5.3).

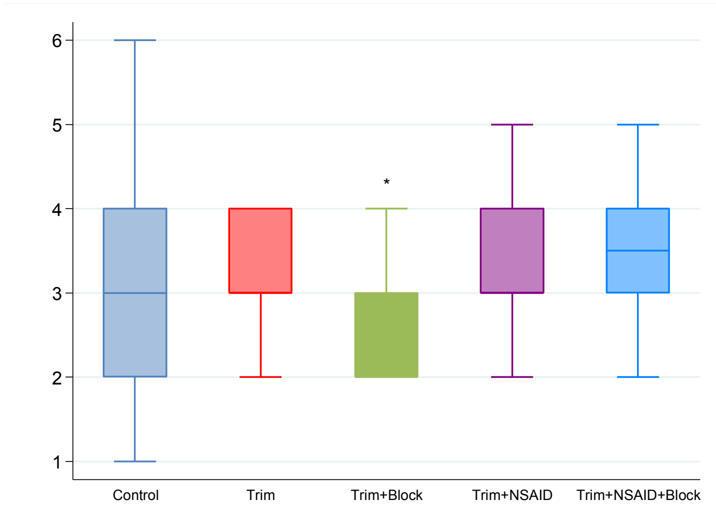

Figure 5.3 Median and IQR of the number of milking visits by treatment and control groups on day 0 (* shows significant difference).

5.3.2.2 Milking visits after treatment

Figure 5.4 shows the distribution of milking visits per day according to treatment groups. Results from the multilevel Poisson regression analysis showed that lameness treatment did not have any effect (P>0.05) on the number of milking visits (Table 5.6). The number of visits was positively associated with milk production (Table 5.6). After variables were added, there was a significant random variability at cow level (P<0.01; 0.002 (0.001)) in the model. The model presented a visually stable chain mixing (Appendix 5).

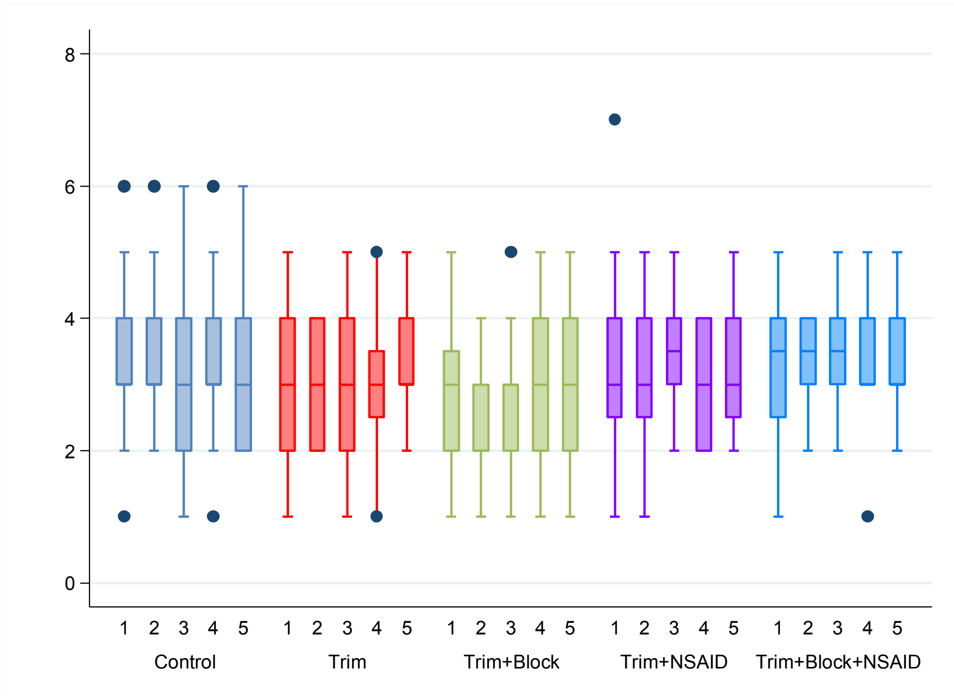

Figure 5.4 Median and IQR of number of visits to the automatic milking robots according to treatment during the observed period (treated days) (dots represent outliers).

Table 5.6 Multilevel Poisson regression analysis results for the number of milking visits during treated days (5 days).

Model term	Freq.	Coef.	SE	Confidence Interval		z	P
				2.50%	97.50%		
Intercept		0.23	0.14				
Farm and Pen							
Farm1 Pen 1	75	Reference					
Farm1 Pen 2	85	-0.01	0.10	-0.20	0.18	-0.07	0.94
Farm1 Pen 3	145	-0.07	0.09	-0.24	0.11	-0.72	0.47
Farm1 Pen 4	70	-0.16	0.11	-0.37	0.04	-1.54	0.12
Farm2 Pen 1	195	0.09	0.08	-0.07	0.25	1.11	0.27
Farm2 Pen 2	245	0.04	0.08	-0.11	0.19	0.57	0.57
Parity							
Parity 1	280	Reference					
Parity 2	165	0.01	0.06	-0.12	0.13	0.08	0.94
Parity ≥3	370	-0.06	0.05	-0.16	0.04	-1.25	0.21
DIM							
DIM ≤ 100	180	Reference					
DIM 101-199days	235	0.00	0.06	-0.12	0.12	-0.02	0.99
DIM ≥200	400	0.01	0.06	-0.11	0.12	0.10	0.92
Milk Production							
Daily Milk Production L		0.01	0.002	0.01	0.02	6.50	<0.001
Number of visits							
Day 0		0.15	0.02	0.10	0.19	6.35	<0.001
Pushed							
No	593	Reference					
Yes	222	0.03	0.05	-0.06	0.12	0.71	0.48
Treatment							
Control	395	Reference					
Trim	120	-0.07	0.06	-0.19	0.05	-1.11	0.27
Trim+Block	100	-0.001	0.07	-0.14	0.14	-0.01	0.99
Trim+NSAID	120	-0.03	0.06	-0.15	0.09	-0.50	0.62
Trim+NSAID+Block	80	-0.03	0.07	-0.17	0.11	-0.42	0.67

Freq. = Frequency of observations; Coef. = Coefficient

Figure 5.5 shows the mean (±SE) of milking visits per period of time for each of the treatment groups. In comparison to non-lame cows, lame cows treated with trim and block visited the milking robots significantly less frequently at night (24:01 – 06:00; $p > 0.001$) and during the evening (18:01-24:00; $p = 0.01$) (Figure 5.5). Cows that were trimmed only visited the milking robot less frequently than non-lame cows during the morning (06:01-12:00; p = 0.03) and afternoon (12:01-18:00; P= 0.03).

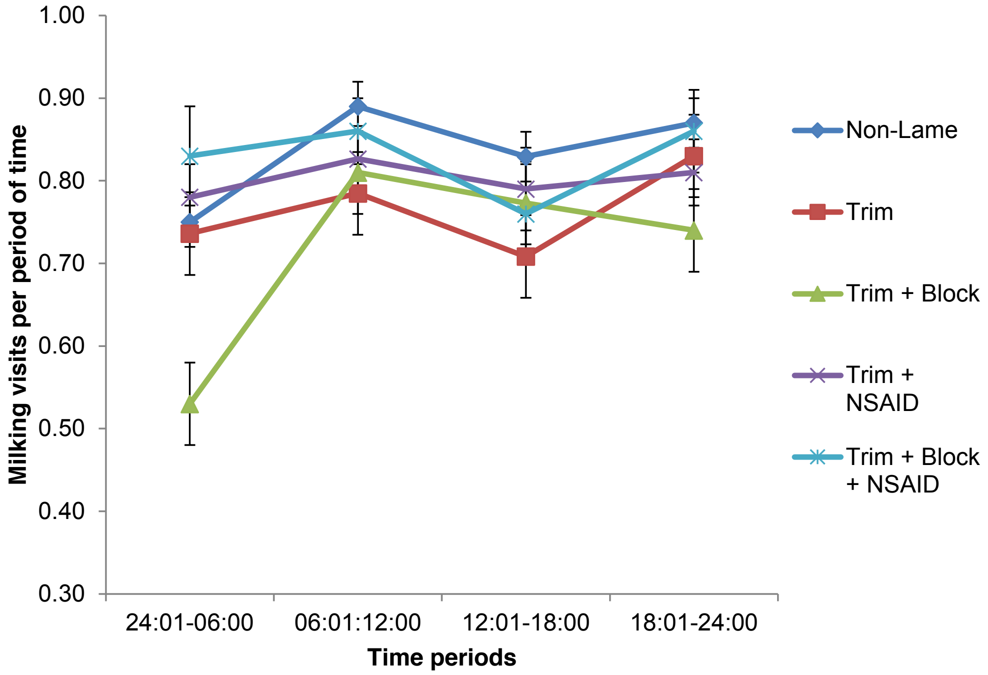

Figure 5.5 Mean (±SE) of number of visits to automatic milking robots during the observed period presented by time periods.

5.3.3 Rumination behaviour

5.3.3.1 Total rumination time on day 0

Analysis on day 0 was carried out only on 160 cows as a control cow was moved to another pen and rumination data were lost. Mean rumination time for non-lame (n=83) and lame cows (n=77) was 455.4 (SE: ±12.5) minutes and 463.3 (SE: 11.5) minutes respectively. Results from the single level model showed no significant association (P>0.05) between lameness and rumination time (Table 5.7). A significant positive association was observed between parity and rumination, with cows of 3 or more parities showing higher rumination than first parity cows. There was a significant random variability (P<0.05) among cows in the model. Graphical analysis of the residuals is presented in Figure 5.6 that shows normal distribution of the residuals.

Table 5.7 Single level linear regression model results for total rumination time (square) for day 0.

Model term	Freq.	Coefficient	SE	Confidence Interval 2.50%	Confidence Interval 97.50%	z	P
Intercept		224298.34	36607.00				
Farm and Pen							
Farm1 Pen 1	15	Reference					
Farm1 Pen 2	15	-42264.09	31494.93	-103994.15	19465.96	-1.34	0.18
Farm1 Pen 3	28	-23725.27	27865.62	-78341.88	30891.34	-0.85	0.39
Farm1 Pen 4	14	-6589.80	32293.08	-69884.24	56704.64	-0.20	0.84
Farm2 Pen 1	39	8165.54	27237.29	-45219.55	61550.63	0.30	0.76
Farm2 Pen 2	49	-13734.08	26007.45	-64708.68	37240.52	-0.53	0.60
Parity							
Parity 1	55	Reference					
Parity 2	33	27660.68	20080.82	-11697.73	67019.09	1.38	0.17
Parity ≥3	72	39701.80	16792.10	6789.28	72614.32	2.36	0.02
DIM							
DIM ≤ 100	33	Reference					
DIM 101-199	47	-14443.03	20018.49	-53679.26	24793.21	-0.72	0.47
DIM ≥200	80	-33638.29	18487.95	-69874.67	2598.10	-1.82	0.07
Milk Production							
Daily Milk Production L		171.33	716.10	-1232.21	1574.88	0.24	0.81
Lameness							
Non-lame	77	Reference					
Lame	83	146.29	13673.96	-26654.68	26947.26	0.01	0.99

Freq. = Frequency of observations

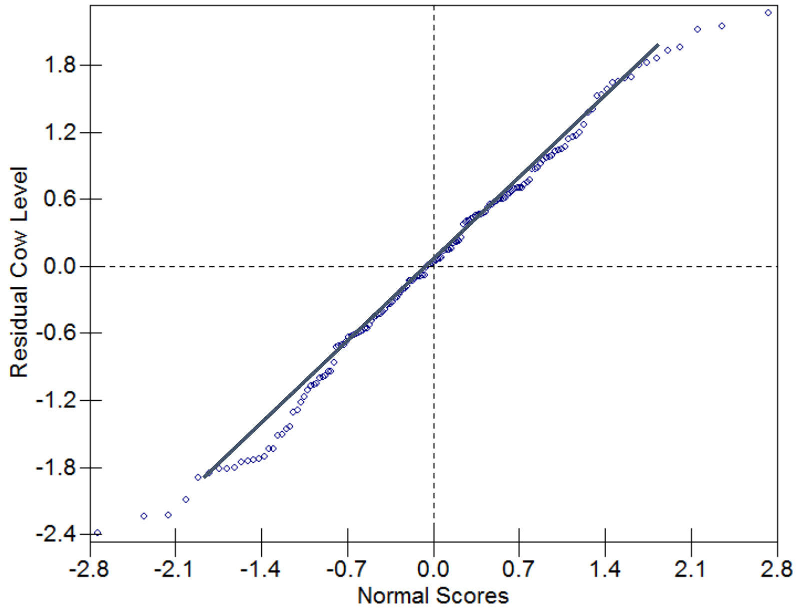

Figure 5.6 Q-Q plot of residuals of the single level linear regression model.

There was no significant difference between treatment and control groups in their total rumination time on day 0 (P=0.69) (Figure 5.7).

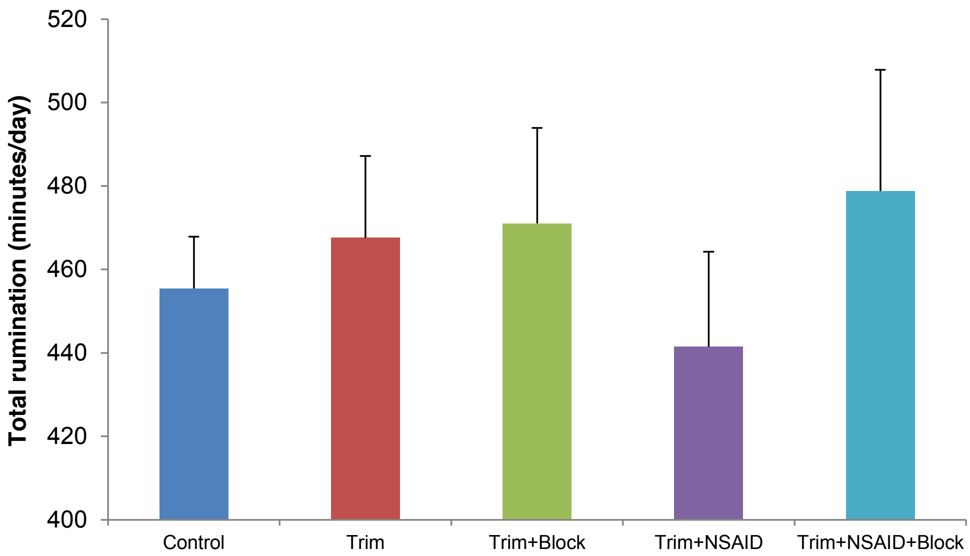

Figure 5.7 Mean (±SE) of the total rumination time by treatment and control groups on day 0.

5.3.3.2 Total rumination time on treated days

Figure 5.8 shows the distribution of total rumination per day according to treatment groups during the observed days; cows receiving the trim and NSAID treatment ruminated less than control cows (Figure 5.8, Table 5.9). Results from the multilevel linear regression model showed that control cows ruminated on average 471.3 minutes per day; lame cows treated trim and NSAID ruminated 59.1 minutes significantly ($P<0.05$) less than control cows (after controlling the effects of other variables) (Table 5.8). Parity was positively associated ($P<0.05$) with total rumination time per day (Table 5.8). Older cows (≥2 parities) ruminated more than younger cows (first parity; $P<0.05$). Days in milk were negatively associated with total rumination time (Table 5.9); cows further into lactation (≥200 days) ruminated less than cows at the beginning of the lactation period (<100 days). Cow (74825.95 (25688.50)) and day of observation (39438.64 (9365.22)) presented a significant ($P<0.05$) random variability. Residuals were close to normality; removal of the outliers (2.15% omitted) did not cause any difference to the final model. Graphical analysis of the model residuals is presented in Figure 5.9 that shows normal distribution.

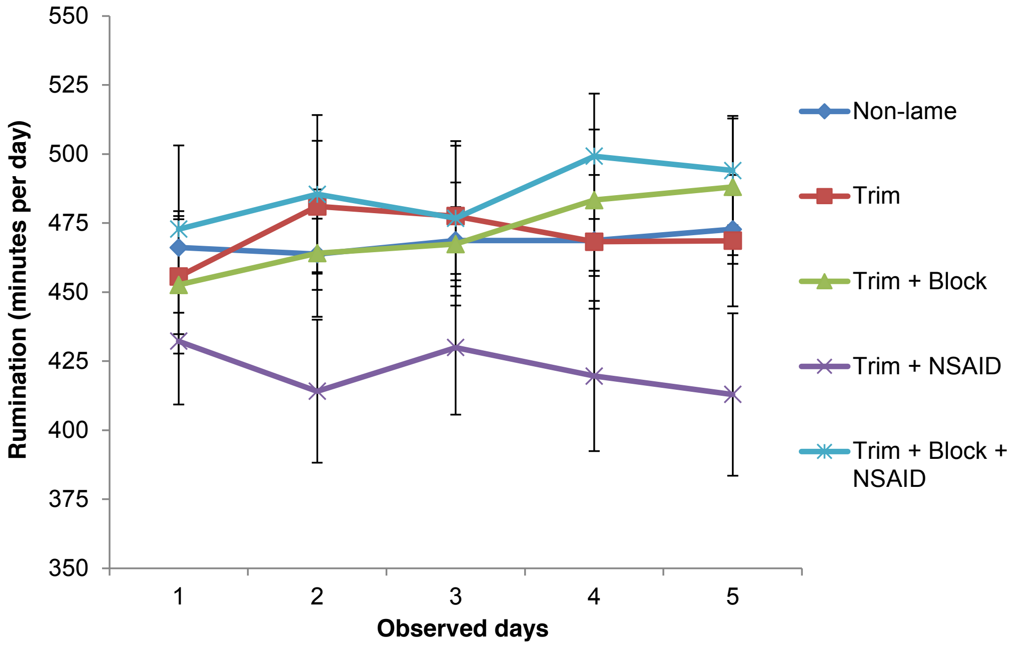

Figure 5.8 Mean (±SE) of total rumination time per day after treatment according to treatment groups.

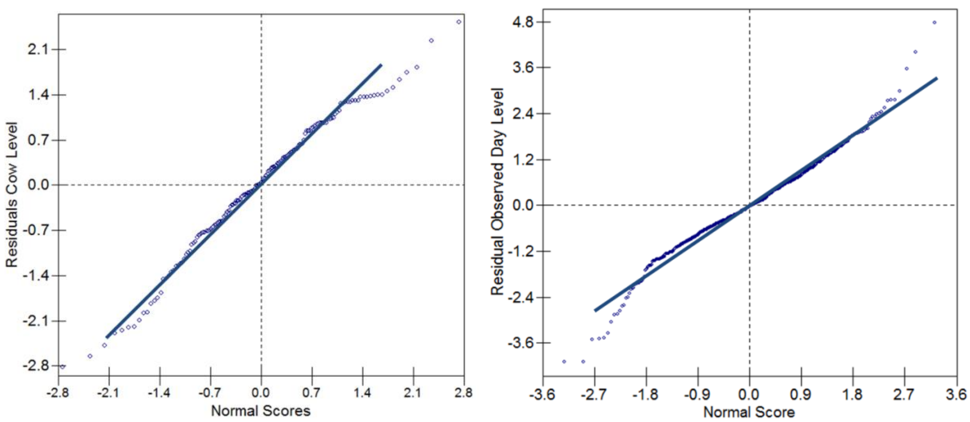

Figure 5.9 Q-Q plots of residuals distribution at cow and observed day level of the multilevel linear regression model.

Table 5.8 Multilevel linear regression model results for total rumination time (squared) per day after treatment.

Model term	Freq.	Coefficient	SE	Confidence Interval		z	P
				2.50%	97.50%		
Intercept		222111.52	26461.98				
Farm and Pen							
Farm1 Pen 1	75	Reference					
Farm1 Pen 2	75	-58156.71	29209.11	-115406.56	-906.86	-1.99	0.05
Farm1 Pen 3	145	-21562.83	25644.95	-71826.94	28701.28	-0.84	0.40
Farm1 Pen 4	70	3632.61	29668.60	-54517.85	61783.06	0.12	0.90
Farm2 Pen 1	195	15917.92	25008.30	-33098.36	64934.19	0.64	0.52
Farm2 Pen 2	245	328.97	23835.64	-46388.89	47046.83	0.01	0.99
Parity							
Parity 1	275	Reference					
Parity 2	165	51460.98	18420.45	15356.91	87565.06	2.79	0.005
Parity ≥3	365	44520.75	14710.55	15688.08	73353.43	3.03	0.002
DIM							
DIM ≤ 100	170	Reference					
DIM 101-199	235	-23356.55	18445.07	-59508.88	12795.78	-1.27	0.21
DIM ≥200	400	-34022.26	16671.94	-66699.26	-1345.25	-2.04	0.04
Milk Production							
Daily Milk Production L		302.71	239.94	-167.56	772.99	1.26	0.21
Treatment							
Control	390	Reference					
Trim	120	-13977.84	18552.80	-50341.32	22385.65	-0.75	0.45
Trim+Block	100	-360.99	20001.27	-39563.48	38841.51	-0.02	0.99
Trim+NSAID	115	-52200.70	18609.41	-88675.13	-15726.26	-2.81	<0.001
Trim+NSAID+Block	80	9015.36	21731.60	-33578.58	51609.30	0.41	0.68

Freq. = Frequency of observations

5.4 Discussion

5.4.1 Milking behaviour

There was no significant association between lameness status and number of milking visits on the day before treatment. This finding disagrees with what was observed in Chapter 2 where a significant difference in the number of milking visits was observed between lame and control cows. Other studies have previously shown that lameness severity is negatively associated with the number of milking visits (Bach et al., 2007; Deming et al., 2013a). In the present study cows must have become lame within two previous weeks prior to diagnosis, cows were mainly mild to moderately lame and the lesions studied were only claw horn lesions (39 cases with SU/SH, 20 cases with WLD and 25 cases diagnosed as other). Lameness chronicity and causes were not considered for cows in chapter 2; it is likely that cows were more chronically lame and suffering from a wider range of lesions. Neither Bach et al. (2007) or Deming et al. (2013a) considered the type of lesions in their analysis. It is probable that the difference in results between the two studies reported in the present studies was due to differences in the severity and chronicity of lameness. It could be postulated that chronicity and the severity of lesions can influence the frequency of cows visiting the milking robots.

Following treatment, there was no significant effect of lameness treatment on the frequency of visits between control and treated cows. This was after controlling for the effects of number of visits on day 0 and pushing events. Before treatment, cows that were selected for trim and block (treatment 2) were already visiting the milking robot significantly less. By chance, cows with lower visit frequency were selected for this treatment group despite the fact that the present study applied treatments randomly.

In Chapter 4 it was observed that cows treated with trim and block increased their lying time so it was expected that this could have affected milking frequency. This was not the case, cows treated with trim and foot block visited the milking unit with the same frequency as non-lame control cows. O'Callaghan (2003) observed that cows diagnosed with mild and moderate hoof lesions and treated with foot blocks decreased their activity (i.e. steps/hour) in comparison to those cows treated only with hoof trimming. In the current study, it is possible that cows in this treatment group may have not changed their visit frequency to the milking robot but may have changed the time spent in the feeding area or other preferred areas that were not measured in this study.

Previous studies observed significant differences in activity (i.e. steps) and lying behaviour following trimming with or without NSAID (O'Callaghan, 2003; Chapinal et al., 2010a; Chapinal et al., 2010c). Voluntary attendance to the milking robot is associated with cow activity, if this is impaired, the frequency of visits may be directly affected (Klaas et al., 2003). However, in the present study neither cows treated with trim nor cows treated with trim and NSAID showed significant differences on their visits to the AMS. Chapinal et al. (2010c) observed that all treated cows (trimmed with or without NSAID) increased their lying time immediately after treatment (day 1 and 2), with only those treated with NSAID increasing their activity on day 3 and 4 after treatment. In the mentioned study cows were treated the day after mobility score (Chapinal et al., 2010c). Since lame cows in the present study were treated within 2 days of diagnosis, it is possible to suggest that not only chronicity of lameness but also prompt treatment (within two days of being identified as lame by mobility score) may have contributed to the lack of any reduction in the visits of lame cows in this study.

Even though it was not possible to record which cows were pushed through the milking machine before or after treatment, a variable controlling for being pushed was added to the model. This variable was constructed with information provided by the farmers; on both farms cows highlighted by the system were pushed through the AMS at 07:00-08:00 and 14:00-15:00. This variable did not have a significant effect and the other variables were not affected by the inclusion of this variable in the model. The lack of specific data regarding which cows were pushed or not complicates the interpretation of the results; however the fact that the results remained the same after this variable was included in the model confirms the finding that newly lame cows that received prompt treatment do not change the overall number of visits they make to the AMS.

Cows treated with trim and block visited the milking robot less frequently than non-lame control cows between 18:01 and 06:00 hours. In a previous chapter (Chapter 2), it was observed that lame cows were less likely to visit the AMS between 00:01 and 06:00 hours in agreement with Bach et al. (2007). Previous studies have indicated that cows adapt their frequency of visits according to feeding time, which also impacts standing time, i.e cows stand for longer during a time closer to feeding (DeVries et al., 2011; Deming et al., 2013a). The farms used in the present study provided fresh feed at 08:00 approximately and pushed the feed up to 6 times per day. Thus, it could be suggested that cows treated with trim and block preferred to lie down at night and use the milking robot when feed was available as previous studies have observed.

Montgomery et al. (2012) observed a significant increase of activity at night in lame cows treated with hoof trimming. In the present study trim only cows visited the milking robot less frequently than non-lame control cows between 06:00 and 18:00 hours suggesting reduction in their activity during this period despite no changes in lying time for this group being observed (Chapter 5). It could be possible that social interactions may have affected the use of the milking robot by this group. Ketelaar-de Lauwere et al. (1996) reported that high ranking cows were more likely to visit the milking robot between 12:00 and 18:00 hours.

Non-lame cows in the present study showed an average milking frequency of 3.2 visits per day. This is higher than reported previously for AMS (2.4 visits/day: Wagner-Storch and Palmer, 2003; 2.2 visits/day: Bach et al., 2007; 2.6 visits/day: Deming et al., 2013a). The average milk yield in the present study was 37.1 L/day; comparable to that reported by Deming et al. (2013a) in a Canadian farm (34.7 L/day), higher than that reported by Bach et al. (2007) in a Spanish farm (30.7 L/day), but lower than that reported by Wagner-Storch and Palmer (2003) for a farm in the USA (58.1 L/day). The present study observed a positive association between milk production with number of milking visits before and after treatment. This result agrees with previous studies (Wagner-Storch and Palmer, 2003; Pettersson et al., 2011), it has been observed that increasing the milking frequency can have a direct impact on milk yield (Svennersten-Sjaunja and Pettersson, 2008). Interestingly, cows in the present study had lower milk yield but higher milk frequency than those reported by Wagner-Storch and Palmer (2003).

5.4.2 Rumination behaviour

Before treatment there was no significant association between total rumination time and lameness status. This result disagrees with what was observed in Chapter 3 where a modest difference (8 minutes less) between lame and non-lame cows was found. Previous studies found contradictory results between lame cows and non-lame cows (Hassall et al., 1993; Singh et al., 1993b; Almeida et al., 2008; Pavlenko et al., 2011). Comparison between the results reported here and previous studies is difficult as they used direct visual observation to monitor rumination. Moreover, when compared to a study that used a rumination collar similar to the one used in the present study, our findings disagree with those presented by Van Hertem et al. (2013). This latter study detected a significant reduction in the rumination time at night period (20:00 – 04:00), with the seven day average rumination time (night) being significantly lower in lame cows (208 min) compared with non-lame cows (221 min). However, the study population enrolled by Van Hertem et al. (2013) included animals diagnosed with different ailments, ranging from skin infections between the claws to double sole. On the other hand, studies using visual observation identified a significant difference in rumination time between lame and non-lame cows using animals diagnosed with DD, interdigital dermatitis and claw horn lesions (Almeida et al., 2008; Pavlenko et al., 2011). Interestingly none of these studies described lameness chronicity. Lame cows in the present study were diagnosed with mild lameness and claw horn lesions. It is possible that mild and acute lameness caused by claw horn lesions may not cause a significant reduction in rumination time.

Non-lame cows in the present study ruminated on average 455.4 min per day. This is lower than that reported from an Israeli farm and from an Italian farm using the same rumination collar (542 min: Soriani et al., 2012; 468 min: Van Hertem et al., 2014). These differences in total rumination time may be caused by differences in feeding systems or other factors that were not included in our analysis (e.g. environmental temperature).

This is the first study to investigate the use of rumination behaviour to detect the effect of lameness treatment on dairy cow behaviour. Lame cows treated with hoof trimming did not have any significant differences in rumination time. This is in disagreement with previous reports, in which cows that received a routine foot trimming showed a reduction in rumination time possibly caused by a reduction in feeding time due to long waiting times leading to a period of fasting before foot trimming (Van Hertem et al., 2014). In the present study, only selected cows had their hooves trimmed and there were no waiting times as each cow was collected individually from their home pen and treated immediately.

Cows treated with trim and NSAID ruminated 59 minutes less per day than non-lame cows. It is possible that these cows may have reduced their overall activity as observed by O'Callaghan (2003), the authors found that cows with mild chronic lameness that received NSAID and trim reduced their activity in comparison to non-lame cows. However, no significant changes were observed in lying behaviour for this treatment group (Chapter 5). Fitzpatrick et al. (2013) did not observed any significant changes on rumination time of cows with endotoxin-induced clinical mastitis that were treated with an NSAID (Meloxicam). In addition no significant changes on rumination time were observed in the group of cows that received

NSAID and a foot block in this study. Then, it is possible that NSAID was not the cause of the difference observed in the trim and NSAID group. An investigation on the differences between treatment groups prior to treatment did not find any significant difference. It is likely that cows in the trim and NSAID group reduced their rumination time due to a change in behavioural priorities that was not considered in this study (e.g. feeding behaviour) or that the difference observed between treatments may have been a chance finding in this study.

Cows treated with a foot block and trim did not have a reduced rumination time in comparison to non-lame cows. It was hypothesised that cows treated with a foot block and trim may showed a reduced rumination time as it was observed that this group had longer lying times in previous chapters (Chapter 5). It is possible that cows in this group may have changed their time budget reducing the time spent on other behaviours that were not considered in this study (e.g. feeding time), or that they increased their biting rate when feeding as observed previously in lame cows (Gonzalez et al., 2008). On the other hand, target sample size was not achieved for rumination behaviour. Results from the post-hoc analysis showed that the power obtained with the current number of cows was low. Therefore non-significant findings should be interpreted with caution.

Overall, there was a positive association between rumination time and parity. Before treatment, cows with 3 or more parities ruminated more than first parity cows; after treatment rumination was positively associated with parity. This finding agrees with what was observed in Chapter 3 and other studies (Norring et al., 2012; Soriani et al., 2012). Multiparous cows had higher rumination times than primiparous cows before and after calving. Hart et al. (2013) did not observe significant differences in rumination time between multiparous and primiparous cows housed in an AMS. It is possible that in the latter study a lack of power may have caused this result, as the study only used 12 cows.

Following treatment DIM was negatively associated with rumination, cows with greater or equal to 200 DIM ruminated less than cows with less than 100 DIM. This latter finding agrees with what was observed in Chapter 3. It has been reported that DIM was positively associated with feeding time and negatively associated with feeding rate (Norring et al., 2014). Consequently, it is possible that cows with higher DIM may spend more time feeding but feed consumption is lower and consequently their rumination time was reduced.

5.5 Conclusions and future work

Lameness did not have any significant effect on milking visits and rumination time in cows housed in automatic milking systems diagnosed with acute and mild lameness caused by claw horn lesions. However lameness treatment differentially affected cow behaviour. Lameness treatment did not affect the number of milking visits, but cows treated with trim and block showed less visits to the automatic milking system than non-lame cows between 18:00 and 06:00 hours, a period when routine farm management activities are reduced. Trim and NSAID treatment reduced the rumination time of lame cows in comparison to non-lame cows.

Further studies on the behaviour of dairy cows are needed in particular in cows housed in automatic milking systems. Results from the present study can be applied only to lame cows under similar circumstances: newly lame, on a single hind-limb and with only one claw affected with claw horn lesions, receiving similar treatments. Additional studies are required to study the effects of other type of lameness (e.g. DD or bilateral) and their treatment on cow behaviour. Future studies in AMS should include farm management practices as variables (e.g. pushed cows).

Chapter 6

Effect of claw horn lesion type at the time of treatment on lameness prognosis

6.1 Introduction

Claw horn lesions account for 27% of lameness observed in free-stall farms in Canada (Cramer et al., 2008); sole ulcers (SU) and white line disease (WLD) have been reported as the most common lesions in UK (Green et al., 2002; Leach et al., 2012; Green et al., 2014). SU and WLD caused depressed nociceptive thresholds on the day of diagnosis day in comparison with non-lame cows, and this continued for the following 28 days (Whay et al., 1998). Tadich et al. (2010) observed that SU and double sole were linked with poor locomotion; similarly Flower and Weary (2006) and Chapinal et al. (2009b) reported that SU was linked with higher mobility scores, increased back arch, joint flexion and asymmetric steps in comparison to cows with no lesions.

SU and WLD can cause milk losses of approximately 570 and 370 kg respectively (Amory et al., 2008). Sogstad et al. (2006) observed that the severity of

WLD and SU could affect reproductive performance and can be associated with some production diseases. Meanwhile, moderate and severe sole haemorrhage (SH) were positively associated with milk fever, and all the severity types of SH were positively associated with reproductive disorders such as anoestrus, cystic ovaries or silent oestrus (Sogstad et al., 2006). Additionally, SU and SH have been positively associated with a reduction in longevity and an earlier culling (Booth et al., 2004; Sogstad et al., 2007a).

There is limited information on the effect of claw horn lesion type on lameness recovery. Early diagnosis and treatment of lameness may improve prognosis and recovery rates (Leach et al., 2012). When earlier lameness interventions are applied, this are more likely to be carried out on less severe lesions therefore the likelihood of recovery may be improved (Leach et al., 2012). Leach et al. (2012) reported that early treatment of cows with a mobility score 2 (i.e. mild lameness), within 2 days of detection, reduced lameness prevalence when compared with delayed treatment. In this early intervention study, milder lesions (i.e. bruising) were observed in early treated cows, with more ulcers present when treatment was delayed.

The aim of this study was to examine whether the type and frequency of claw horn lesions in newly lame cows at the time of corrective foot trimming had an effect on the probability of recovery from lameness after treatment. In addition, the sub aim of the study was to evaluate if the categorical descriptors of the lesions compared with an objective measurement such as area or length. The null hypothesis stated that the type and frequency of claw horn lesion(s) did not affect recovery from lameness after treatment. The alternative hypothesis stated that the type and frequency of claw horn lesion(s) affected recovery from lameness after treatment.

6.2 Materials and methods

6.2.1 Study dataset

The present study used data collected during a randomised clinical trial (RCT) carried out between January 2012 and February 2013 (Chapter 4, section 4.2). The RCT was designed to compare three treatments for claw horn lesions against a positive control group, which only received a therapeutic trim. The protocol of the RCT was reviewed and approved by the University of Nottingham's School of Veterinary Medicine and Science Ethical Review Committee prior to the start of the study.

6.2.1.1 Study population and selection procedure

Five commercial farms within close proximity to the University of Nottingham were enrolled in this study. The participating farms had between 187 and 353 cows in their herds with 305d adjusted milk yields ranging from 7,394 to 11,579 L. Three of the farms housed their cows all year round and the remaining farms housed their cows over winter (October until March). Two farms milked their cows with automatic milking systems, as explained in Chapter 4 section 4.2, and the remaining used a conventional parlour milking twice a day. All the farms had free-stalls with mats, mattresses or waterbeds; passageways and standing areas were concrete except on Farm 2 which had rubber matting throughout and Farm 3 which had rubber matting only at the feed face of the high yielding cows group. For further information on the farms refer to table 4.1 (Chapter 4) and to table 6.1.

The selection of cows for the RCT study followed the requirements described in Chapter 4 (4.2.3 General experimental procedure). In brief, cows were selected for the RCT if they had two consecutive non-lame mobility scores followed by a lame mobility score and only presented lameness on one of the hind limbs. Once a cow was selected, a qualified veterinarian carried out foot trimming and diagnosed the lesions. One of four other veterinarians carried out foot trimming when holiday and other cover was required. Cows diagnosed with claw horn lesions on a single claw were selected for enrollment. Excluded cows had lesions on both claws, significant heel horn erosion (HHE), DD or another infectious disease. All excluded cows received a therapeutic trim, identical to that received by the positive control group before returned to their group.

Table 6.1 Management and production characteristics of the farms in the study (farms 3,4 and 5).

General Characteristics	Farm		
	1	3	5
Number of cows	270	141	181
Number of milking cows	239	129	153
Average milk yield/year	7900	8800	10300
Cows housed all year round	No	No	Yes
Type of grazing	Paddock	Paddock	N/A
Feeding type	MR	MR	MR
Frequency of provision of fresh ration	2	2	2
Alley floor surface	Concrete	Concrete	Concrete
Automatic scrapers present	No	Yes	Yes
Frequency of automatic scrapper operation	N/A	Twice per hour	Twice per hour
Type of housing system	Cubicles	Cubicles	Cubicles
Type of bedding in cubicle	Mattress	Mattress	Mattress
Number of cubicles per cow		172	372
Trimmed frequency by qualified foot trimmer	Every 5 months	Every 6 weeks	When needed
Foot bath (5% copper sulphate): Lactation cows	Daily (formalin)	3 times a week	Daily (formalin)
Foot bath (5% formalin): Dry cows and heifers	No	No	No

6.2.1.2 Mobility scoring

Mobility scoring was carried out every two weeks on every farm by 4 observers between December 2011 and February 2013. Three observers had one farm assigned and one observer was in charge of two farms. On AMS farms, as described in Chapter 4, cows were grouped on one end of each pen and moved slowly in front of the observer. In the conventional milking farms, mobility score was carried out with cows walking over a flat surface, after morning milking when cows left the milking parlour. Mobility score was carried out using the Dairy Co mobility score as described in Chapter 4 (section 4.2.3.1).

The inter- and intra-observer reliability for MS was measured as explained in Chapter 4 (section 4.2.3.2). Briefly, the calculation was carried out for four observers, with an expected inter-observer reliability (ρ_1) of 0.85, acceptable (ρ_0) at 0.6 or higher, with α=0.05 and β=0.2 (Walter et al., 1998). This gave a minimum of 21 clips necessary to carry out the reliability. The video showed cows walking slowly for at least 3 strides; the clips were randomly distributed in the movie. The four observers were provided with a mobility score sheet and the Dairy Co mobility score chart. The movie was observed twice with a lag of at least 4 hours between observations.

6.2.2 Description of lesion scoring study

6.2.2.1 Animals

Data from cows selected for the present study were drawn from both the enrolled and excluded groups from the RCT. Cows were selected from the enrolled group if they were treated with a therapeutic trim only (Standard Dutch foot trim followed by trim and investigation of lesions then removal of diseased horn (Toussaint-Raven et al., 1985)). From the group excluded from the RCT, selected cows were those who only had claw horn lesions. Cows with DD or other infectious condition were not considered for this study. Finally, cows were only eligible for inclusion if they were mobility scored 2 weeks after treatment and they had not received further treatment by the farmer. If selected cows were still lame at the two week outcome point, they were kept in the dataset if they were lame on the treated leg (cows that became lame on a different leg after treatment, were excluded).

6.2.2.2 Sample size

Sample size was calculated based on the rate of recovery published by Groenevelt et al. (2014). Using a one proportion score test in Stata/SE 12.0 (Stata Corp 2011, USA), with an expected 80% rate of recovery with a confidence level of 95% and a power of 80%, the calculation indicated a sample size of 86 cows was required.

6.2.2.3 Hoof photographs

As part of the study, pictures were taken from the hoof corresponding to the lame leg after a thin layer of claw horn had been removed. The claw’s surface was cleaned with water and dried with paper towels. Photographs were taken using a Sony Cybershot camera (DSC-W170 10.1 megapixels – Sony Europe Limited). A small board (101 x 228 mm) was held next to the hoof listing the cow’s number and the date to identify each image. Only images of good quality (i.e. in focus) and well identified (data on marker board) were used in this study.

6.2.2.4 Lesion identification and scoring

Lesion identification, classification and location were carried out following a standard methodology developed at the University of Nottingham (Table 6.2) based on Sogstad et al. (2007a), Greenough and Vermunt (1991) and Leach et al. (1998). A single observer identified and classified the lesions by claw. The area or length of each lesion and the identification board width were measured using the ImageJ 1.49p software (Wayne Rasband NIH, USA). This software provided information of length and area in pixels. In order to obtain the data in millimetres, the identification board width (101mm) was used as a reference to adjust the size data for camera distance from the foot. Data was transferred to the Excel® Lesion Scoring Input Form (developed by R Newsome, University of Nottingham), where the location of each lesion was added (Figure 6.1).

Table 6.2 Classification and description of claw horn and other claw lesions (University of Nottingham, 2015).

Type of lesion	Classification	Description
No Lesion		No claw horn lesion or any other lesion is identified in the foot.
Ulcer	Mild	Small penetration of sole surface, corium not expose or granulation tissue is not observed in any of the locations of figure 6.1-A.
	Severe	Penetration of sole surface with exposure of corium and/or granulation tissue present in any of the locations of figure 6.1-A.
Haemorrhage	Mild	Presence of diffuse light pink and/or yellow coloration in any of the locations of figure 6.1-A.
	Moderate	Presence of dark pink coloration in any of the locations of figure 6.1-A.
	Severe	Presence of very dark red or purple coloration in any of the locations of Figure 6.1-A.
White Line Haemorrhage	Mild	Presence of diffuse light pink and/or yellow coloration in any of the locations of figure 6.1–B.
	Moderate	Presence of dark pink coloration in any of the locations of figure 6.1-B.
	Severe	Presence of very dark red or purple coloration in any of the locations of figure 6.1-B.
White Line Separation	Mild	Dark coloured marks along white line in any location of figure 6.1-B.
	Moderate	Deep fissures and/or impacted areas along white line in any location of figure 6.1-B.
	Severe	Very deep or profound fissure, with the presence of corium involved and/or purulent exudate, necrosis, granulation tissue, separation of wall or sole in any location of figure 6.1-B.

Zones of the sole were identified following the map described by Leach et al. (1998) (Figure 6.1-A). White line zones were defined using anatomical features as follows. An ellipse was drawn on the sole area of each picture, the limits of the main long ellipse axis where the outer edge of the white line at the corner of the toe and the caudal extent of the white line at the heel. Then, the abaxial border of the ellipse was extended to meet the abaxial white line. This gave three well defined areas: abaxial 1, abaxial 2 and axial that allowed for consistency through-out the study (Figure 6.1-B). Information of the presence (Yes=1) or absence (No=0) of heel horn erosion (i.e. irregular horn surface with or without deep horn grooves that may expose the corium), under-run (i.e. horn is separated at the grooves and formed a flap at the bulb of the heel) and interdigital growth was recorded.

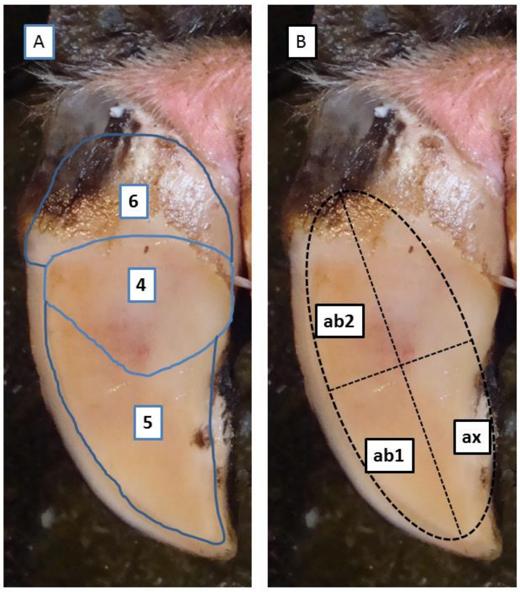

Figure 6.1 Zones of the distal surface of the claw used to describe location of the lesions observed. On the left (A) for ulcers and haemorrhage 4= sole, 5= toe, 6= heel; and on the right (B) for white line ab1= abaxial wall zone 1, ab2, abaxial wall zone 2, and ax= axial wall (Modified from Leach et al., 1998).

The ability of the observer to repeat the measurement of an area was tested by tracing the same outline in the same picture at the beginning and at the end of a picture analysis session (3 sessions). Intra-observer reliability for the lesion identification was measured prior to commencing the picture analysis, twice during the picture analysis (at picture 50 and picture 100) and at the end of the picture observation. The observer was tested using a PowerPoint presentation containing 25 pictures of different lesions; a single lesion was circled in each picture for identification (Appendix 7). The observer identified the lesion according to the categories described in Table 6.2 and also noted their location accordingly. The same set of 25 pictures was used at each of the four testing sessions; in each session pictures were re-organised to avoid observer expectations (e.g. memorising the position of a lesion within the set of pictures).

6.2.3 Statistical analysis

Lesion measurement data was transferred to an excel datasheet (Microsoft Excel 2010, Microsoft Corp., Redmond, WA, U.S.A.) and pixels were transformed into mm for the length of WLH/S lesions and in mm^2 for the area of SH or SU lesions. Cross multiplication was used to obtain the measurements (area and length) in mm. This was calculated by dividing the measurement of the board (101mm) by the length in pixels in the image to obtain the converting factor of 1mm in pixels. This factor was then used to calculate the length of each lesion in mm. In the case of area, the converting factor of 1mm in pixels was squared in order to obtain the value of $1mm^2$ in pixels. This squared factor was used to calculate the area of each lesion in mm^2.

A table was constructed containing: farm ID, cow ID, limb or foot treated (right or left), claw (lateral and medial), recover at 2 weeks (yes or no), mobility score before treatment, one claw affected (yes or no), type of lesion (no lesion, ulcer, haemorrhage, white line disease (white line separation or/and haemorrhage), others (a combination of the previous lesions with/without HHE and/or under-run)), columns for each claw horn lesion type presence (yes or no), area/length measurement and frequency, HHE (yes or no) and under-run (yes or no). An additional variable was coded to control for variation between treatment operators (1 for the main veterinarian and 2 for other veterinarians). Data for severity category for each type of lesion was consolidated to obtain a total area and frequency per claw horn lesion type to evaluate the main aim of this study. Data for area according to lesion severity was used to evaluate whether the categorical descriptors of the lesions matched objective measurements of lesions such as area or length.

Descriptive analyses and reliability analysis were carried out using Stata/SE 12.0 (Stata Corp 2011, USA). The weighted kappa (k_w) was used to calculate the intra and inter-observer reliability for mobility score. The interpretation of the k_w was conducted using Landis and Koch (1977) where ≤0=poor, 0.01-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial and 0.81-1.00 = almost perfect. Area for each lesion severity category was not normally distributed and it was not possible to reach normality after transformation; so the Kruskall Wallis test was used to compare severity categories for each lesion type (Petrie and Watson, 2006). A P-value of ≤ 0.05 was considered as a significant difference. A multilevel logistic regression model was built to examine if claw horn lesions type area and presence (yes or no) affected the likelihood of recovery. The model was built using MLwiN version 2.27 (Rasbash et al., 2009) and took the following form:

$$\text{Log}\ (\pi_{ij}) = \beta_{0ij} + \beta_1 x_{1ij} + \beta_2 x_{2ij} + \beta_3 x_{3ij} + \beta_4 x_{4ij} + \beta_5 x_{5ij} + \ldots + \beta_{14} x_{14ij} + e_{ij}$$

$$y_{ij} \sim \text{Poisson}\ (\pi_{ij})$$

The outcome variable (y_{ij}) was treatment recovery (yes or no) within claw (i) and within cow (j). β_0 was the intercept fixed at all levels. $\beta_{1\text{-}5}$ represented the regression coefficient at each explanatory variable. The predictor variables were represented as follows x_1= farm, x_2= mobility score at treament, x_3= cow with one claw affected, x_4= hoof trimming operator, x_5= ulcer area, x_6= presence of ulcer (yes/no), x_7= haemorrhage area, x_8= presence of haemorrhage (yes/no), x_9= WLH lenght, x_{10}= presence of WLH (yes/no), x_{11}= WLS length, x_{12}= presence of WLS (yes/no), x_{13}= HHE (yes/no) and x_{14}= under-run (yes/no). Log ($\boldsymbol{\pi}_{ij}$) is the log of the expected value of recovery. Results from the model are presented as odd ratio (OR) and confidence interval [CI]. Frequency and presence of claw horn lesions by type showed high collinearity, therefore only the presence of each claw horn lesion type variables was kept in the final model. The multilevel logistic regression analysis model was followed by Monte Carlo Markov Chain (MCMC) methods with 500 000 iteration and a burn in of 5000. The chain mixing and stability were evaluated visually (Browne, 2009).

6.3 Results

6.3.1 Reliability of mobility score and reliability of lesion identification

The intra-observer overall average K_w agreement for mobility score (0-3) was moderate K_w = 0.70 (95% CI: 0.61-0.73) with a range of 0.62 – 0.76; similarly for lameness status (no-lame (0-1 scores) or lame (2-3 scores)) the overall average K_w agreement was moderate K_w = 0.74 (95% CI: 0.55-0.93) with a range of 0.43 – 0.89 (Table 6.3). In the case of inter-observer analysis, the overall average agreement K_w for mobility score (0-3) was fair K_w = 0.40 (95% CI: 0.33-0.61) with a range of 0.39 – 0.55; similarly for lameness status (non-lame (0-1 scores) or lame (2-3 scores)) the overall average K_w agreement was fair K_w = 0.37 (95% CI: 0.16-0.70) with a range of 0.36 – 0.55.

Table 6.3 Kappa weighted between and within observers reliability for mobility score (1-4) and lameness status (Lame/Non-lame).

Observers	Score (0-3)			
	1	2	3	4
1	0.73 (0.60-0.76)	0.49 (0.43-0.58)	0.47 (0.24-0.65)	0.48 (0.35-0.62)
2		0.69 (0.60-0.78)	0.44 (0.20-0.57)	0.39 (0.34-0.51)
3			0.76 (0.70-0.92)	0.55 (0.42-0.70)
4				0.62 (0.54-0.72)
	Lame/Non-lame			
1	0.79 (0.61-0.98)	0.36 (0.10-0.64)	0.40 (0.12-068)	0.43 (0.15-0.72)
2		0.89 (0.76-1.00)	0.45 (0.18-0.72)	0.37 (0.10-0.64)
3			0.85 (0.69-1.00)	0.55 (0.29-0.81)
4				0.43 (0.15-0.72)

The intra-observer overall average K_w agreement for lesion classification was almost perfect K_w = 0.87 (95% CI: 0.75-0.96) with a range of 0.64 – 1.00. In the case of measuring the same claw 6 times by the same observer using the image analysis software, the standard deviation was on average 3% of the mean of each measurement assessed (e.g. area), ranging from 7.18% to 1.38%.

6.3.2 Cows description

A total of 136 cows were included in the analysis. Of these, 11 became lame in a different leg two weeks after treatment and 6 were diagnosed with DD. Data from these 17 cows were excluded from further analysis. One hundred and forteen cows were mobility score 2 and five cows were scored 3 at the time of treatment. Ninety one cows were from the group of non-enrolled cows from the RCT and 28 cows were from the enrolled group. The median lactation number was 2 (range: 2-4; n=107), median DIM was 137 days (range: 71-243; n=107) and average milk production was 37.0±1.2 L/month (mean±SE; n= 85). However, information about parity, DIM and milk production was missing for some cows.

6.3.3 Prevalence and description of claw horn lesions

Seven cows had no claw horn lesions and none of the 119 cows were diagnosed with an interdigital growth. One hundred and twelve cows were diagnosed with claw horn lesions but lesion prevalence varied according to claw. Three cows had both claws affected with haemorrhage, three cows had both claws affected with white line lesions (haemorrhage and separation), 51 cows had both claws affected by different combinations of lesions (e.g. lateral claw with haemorrrhage and WLH, medial claw with an ulcer and WLH), and the remaining 55 cows had different combinations of lesions by claw (e.g. one claw with haemorrhage and the other claw with a sole ulcer).

There were a total of 238 claws observed. Haemorrhage was the lesion most frequently observed with a total of 216 lesions, most of these were on the lateral claw (Table 6.4 and Figure 6.2). White line haemorrhage was the second most frequently observed, with a total of 133 lesions (Table 6.4 and Figure 6.2). White line separation followed with a total of 58 lesions observed. Ulcers were the least frequent claw horn lesion observed, with a total of 47 observations most of them located on the lateral claw (Figure 6.2).

The mean measured ulcer area was significantly different between severity categories mild and severe (H=4.55, 1d.f.; $P<0.05$) (Figure 6.3). The mean measured haemorrhage area was significantly different between severity categories mild, moderate and severe (H=91.02, 2d.f.; $P<0.05$) (Figure 6.3). The mean WLH and WLS lengths were significantly different between severity categories mild, moderate and severe (WLH: H=40.17, 2d.f.; $P<0.05$; WLS: H=7.61, 2d.f.; $P<0.05$) (Figure 6.4).

Table 6.4 Distribution of claw horn lesion type by severity across 112 cows.

Lesion	Classification	Lateral Claw	Medial Claw	Total
Ulcer	Mild	25	12	37
	Severe	8	2	10
Haemorrhage	Mild	87	40	127
	Moderate	36	21	57
	Severe	18	8	26
White Line Haemorrhage	Mild	47	39	86
	Moderate	22	12	34
	Severe	10	3	13
White Line Separation	Mild	18	20	38
	Moderate	9	8	17
	Severe	3	0	3
Heel horn erosion		32	37	69
Under run		10	15	25

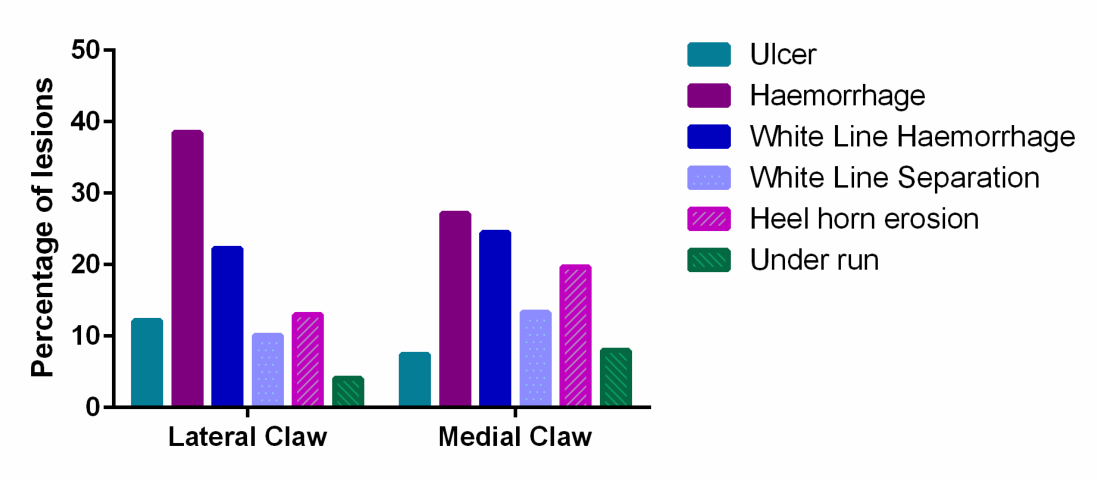

Figure 6.2 Percentage of individual lesions by claw.

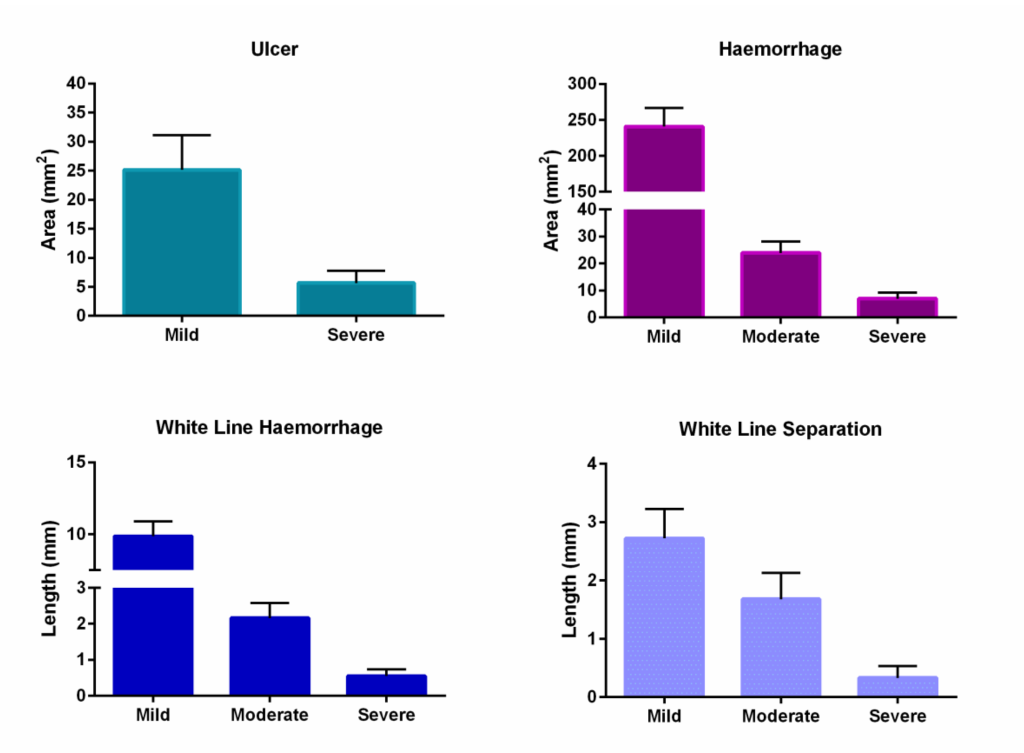

Figure 6.3 Distribution of area/length of each claw horn lesion type measured on pictures by severity scored on a categorical scale.

6.3.4 Claw horn lesions and recovery 2 weeks after treatment

There was a 79.8% (n=95 cows) recovery rate from lameness 2 weeks after therapeutic hoof trimming. Results from the final model showed that cows assigned a mobility score of 3 at the time of treatment were significantly less likely to recover from lameness compared to cows with mobility score 2 (OR: 0.15 [0.02-0.86]) (Table 6.5). Also, cows with a single claw affected were significantly less likely to recover from lameness than those with both claws affected (OR:0.37 [0.15-0.93]). Hoof trimming operator had a significant effect on the likelihood of recovery from lameness 2 weeks after therapeutical hoof trimming (Table 6.5), animals treated by the primary operator were more likely to recover. Only WLH lesions had a significant impact on the likelihood of recovery from lameness. Recovery of cows with WLH depended on the length of the lesions (OR:1.05 [1.005-1.091]) (Table 6.5). There was no significant (P>0.05) effect of other type of lesions on the likelihood to recovery from lameness 2 weeks after treatment (Table 6.5). The model presented a visually stable chain mixing with graphs presented in Appendix 6.

Table 6.5 Multilevel logistic regression analysis of the likelihood of recovery from lameness caused by claw horn lesions 2 weeks after therapeutic trimming.

Model term	Freq.	Coef	SE	OR	z	Confidence Interval		P
						2.50%	97.50%	
Intercept		2.13	0.59					
Farm								
Farm 1	48	Reference						
Farm 2	38	0.29	0.65	1.33	0.19	0.37	4.78	0.66
Farm 3	6	-0.47	1.15	0.62	0.17	0.07	5.91	0.68
Farm 4	58	0.73	0.61	2.08	1.45	0.63	6.89	0.23
Farm 5	88	0.38	0.59	1.46	0.41	0.46	4.61	0.52
Mobility score at treatment								
MS 2	228	Reference						
MS 3	10	-1.93	0.91	0.15	4.49	0.02	0.86	0.03
Cow with one claw affected								
No	182	Reference						
Yes	56	-1.00	0.47	0.37	4.50	0.15	0.93	0.03
Operator								
Operator 1	216	Reference						
Operator 2	22	-1.68	0.61	0.19	7.57	0.06	0.62	0.01
Ulcers								
Area (mm2)	238	-0.006	0.004	0.99	2.25	0.99	1.00	0.13
No	194	Reference						
Yes	44	0.96	0.85	2.61	1.28	0.50	13.70	0.26
Haemorrhage								
Area (mm2)	238	0.001	0.001	1.00	1.00	1.00	1.00	0.32
No	92	Reference						
Yes	146	-0.09	0.46	0.92	0.04	0.37	2.26	0.85
White line haemorrhage								
Length (mm)	238	0.05	0.02	1.05	4.80	1.005	1.09	0.03
No	137	Reference						
Yes	101	-2.20	0.68	0.11	10.45	0.03	0.42	0.001
White line separation								
Length (mm)	238	-0.03	0.03	0.98	0.69	0.92	1.03	0.40
No	188	Reference						
Yes	50	0.54	0.89	1.71	0.36	0.30	9.84	0.55
Heel horn erosion								
No	169	Reference						
Yes	69	-0.44	0.56	0.65	0.62	0.22	1.93	0.43
Under-run								
No	213	Reference						
Yes	25	-0.04	0.68	0.96	0.00	0.25	3.61	0.95

Freq.= Frequency of observations; Coef= Coefficient

6.4 Discussion

6.4.1 Lesion prevalence and effect on the recovery from lameness

As reported by Leach et al. (2012) and Groenevelt et al. (2014) from other recent UK studies, claw horn bruising was the lesion most prevalent in the present study and had the largest lesion areas across all the severity classifications. Sole bruising or haemorrhages have not been associated with poor locomotion score (Flower and Weary, 2006), on the other hand SU have been strongly associated with poor locomotion score even 4 weeks before diagnosis (Chapinal et al., 2009b). Only 47 claws were diagnosed with SU (plus other lesions) from a total of 286 claws. Most of the animals in this study had a combination of lesions per claw or per foot: it is difficult to make comparisons between studies as some combine all the lesions observed per foot or have only considered moderate to severe lesions (Chapinal et al., 2009b; Tadich et al., 2010).

Most of the lesions observed were classified as mild and were also the largest lesions, regardless of the type. As Groenevelt et al. (2014) suggested it is possible that these lesions may have been undereported previously. Cows in the present study became lame within the previous 2 weeks before treatment. The fact that animals had poor mobility scores suggest that there was a trauma at the level of the corium manifested through the presence of haemorrhages, which may predispose to more serious lesions if left untreated (Sogstad et al., 2007b; Groenevelt et al., 2014). Most of the claw horn lesions were observed in the lateral claw. This agrees with the literature (Ahrens et al., 2011) and it could be explained by the anatomical and overloading differences observed between digits (Van der Tol et al., 2003).

It is interesting to observe that severity of a lesion seemed not to be related to size. In every claw horn lesion type observed, mild lesions were significatively larger in area or length in comparison with moderate and severe lesions. The descriptors used in the present study were developed by the research group in the School of Veterinary Medicine and Science, based on descriptors reported previously (Leach et al., 1998; Sogstad et al., 2005). Descriptors reported previously have not included size, they have been based solely on the appearance of lesions. The work reported here suggests that lesion size may well be an important aspect of lesion pathogenicity. Future studies investigating lesion type and severity should include the area of the lesion as part of the analysis as this may be of biological importance. Further work is needed to investigate how the combination of area and severity and lesion type and severity impact on recovery (the analysis employed in this study did not allow both lesion type and lesion severity to be included in the final model).

The presence of WLH had a significant effect on the likelihood of recovery from lameness at 2 weeks after treatment, cows that had WLH were less likely to recover. White line lesions have been linked to both milk loss and lameness (Barker et al., 2007; Amory et al., 2008). Interestingly, the length of WLH was positively correlated with the likelihood of recovery i.e. cows with longer lesions were more likely to recover from lameness 2 weeks after treatment. It could be possible that the haemorrhage observed at the white line was caused several weeks before and the lesions observed were the vestiges of more severe damage (Flower and Weary, 2006). Alternatively, mild white line haemorrhage lesions were significantly larger than the other severity categories of WLH; consequently longer lesions are more likely to represent milder lesions which could be more likely to recover. White line lesions are usually classified as WL disease; and in some cases is not reported when claw horn lesions are studied (Chapinal et al., 2009b; Blackie et al., 2013). To the author's knowledge, this is the first study to report associations between lesion type at the time of treatment and the likelihood of recovery. The results can provide useful prognostic information for clinicians and foot trimmers treating lesions in the field. It was not possible to investigate the effect of both lesion type and lesion severity in this study. The present results identify the importance of further work to understand these associations and their causes.

Lame cows that were mobility score 3 before treatment were less likely to recover than those that were mobility score 2. To the best of the author's knowledge, this is the first time this association has been reported. Mobility score 3 cows did not have a particular lesion, all animals had different combinations of lesions per claw. Descriptively, the mobility score 3 animals had a parity median (median=6) higher than the cows with mobility score 2 (median=2). Older cows are more likely to suffer with SU and WLD (Barker et al., 2009), and cows above parity 3 had a lower probability of transition from the lame to the non-lame state in comparison to cows in parity 1 (Lim et al., 2015). Whay et al. (1997) observed that lameness severity was positively associated with an increase in sensitivity to mechanical noxious stimuli. This hyperalgesia persisted for at least 28 days after the lameness was treated (Whay et al., 1998). Then, it is possible that these animals may have been in a higher hyperalgesic state making them less likely to recover sooner in comparison to cows with mobility score 2. This finding must be taken into consideration by the industry as additional care should be taken when treating cows which are mobility score 3, particularly those treating lame cows should consider the administration of NSAIDs to more severely lame cows when they are treated.

Cows with one claw affected were less likely to recover. Van der Tol et al. (2002) observed that the lateral claw of the hindlimbs bears more weight than the medial claws when cows are standing. It is possible that these cows were using their lateral claw to bare the weight after treatment rather than using their medial claw. There were a total of 28 cows with a single claw affected, from these 24 cows had the claw horn lesions on the lateral claw. It could be suggested that the treatment may have realesed the pain caused by the lesions but did not allow enough support for the affected claw to rest in order to recover. On the other hand, cows with both claws affected, may have removed completely the weight from the affected limb providing a good recovery to both claws.

The recovery rate two weeks after treatment was 79% similar to that observed by Leach et al. (2012): in their study approximately 75% of the newly lame cows recovered 2 weeks after treatment. A prompt intervention is more likely to encounter mild lesions that are less complicated to treat, increasing the chances of a promt recovery and consequently less lameness in the following lactations (Groenevelt et al., 2014). It is possible that the type of lesion and its severity, measured by area or frequency, might not be as important as the early diagnosis and treatment of these lesions.

Lame cows treated by operator 1 were more likely to recover than those treated by the other operator. Animals in the present study were treated using the 5 Point Standard Dutch Foot Trim (Toussaint-Raven et al., 1985). Previous research has suggested that foot trimming may cause pain and discomfort (Chapinal et al., 2010c; Van Hertem et al., 2014). None of these previous studies specified which hoof trimming technique was used. Findings from the present study suggest that following a standard technique, a good recovery rate can be achieved. There is little research on hoof trimming techniques and their impact on recovery rates. So further work is required to understand how hoof trimming techniques may influence recovery, this will help the industry to implement a succesful and standarised technique for the treatment of lameness.

This study avoided the use of subjective assesment of lesions (Leach et al., 1998). Other studies have used the sum of adjusted severity scores in an attempt to emphasized the severity per claw or per foot in order to provide a measure of perceived biological importance to each type of lesion. As stated previously, this type of classification or scoring may be significantly under representing the importance of mild lesions. It is also necessary to consider that it is rare to observed one single lesion per claw or per foot, so any future study on claw horn lesions must take this into consideration.

6.4.2 Reliability of mobility score and lesion classification

The UK standard mobility scoring system was used in this study to enable effective communication of results to the industry. This 4 point system was design to be straight forward to implement on farm. The intra-observer overall average reliability for the mobility score was moderate (K_w=0.70) raging from 0.62 to 0.76. This result is slightly lower than that reported by Flower and Weary (2006) (r^2=0.80, 1-5 scores) but higher that the one reported by Thomsen et al. (2008) after observers received training (K_w=0.53, 1-5 scores). The observers in the present study had experience working with cattle but they did not have previous experience in mobility scoring prior to undertaking this work. It has been observed that previous experienced in the use of a mobility score can affect positively the intra-observer reliability (Main et al., 2000).

The inter-observer overall average reliability was lower (K_w=0.37, range 0.36-0.55) for lameness status than the one obtained by Barker et al. (2010) (K_w range: 0.67-0.93) who used the same mobility scoring system used in this study. For the whole 4 point scale, Barker et al. (2010) reported a percentage agreement between 61.3% and 83.3%. Even though it is not possible to obtain total agreement between observers (Flower and Weary, 2009), the current categories in the mobility score used in the present study may not be well explained and some of the gait attributes might be difficult to assess or identify (e.g. shortened strides). Flower and Weary (2006) observed that the descriptor “tracking up” resulted in a r^2=0.88 for intra-observer reliability and a r^2=0.83 for interobserver reliability, and the "asymmetric gait" descriptor had the lowest intra- and inter-observer reliability (r^2=0.42 and r^2=0.48 respectively).

A univariable model was constructed to assess the effect of scorer on the recovery and no significant effect was observed. Even though the inter-observer repeatability for mobility score was fair this did not affect the results of the present study. Even though this mobility score was developed to assist farmers and the industry to identify lame animals, it is necessary to investigate what can affect the lack of consistency to improve this methodology.

The intra-observer reliability for lesion classification was almost perfect (Kw=0.87), similar to the one observed by Leach et al. (1998). Lesion classification in the present study followed previous classifications and descriptions based on Leach et al. (1998) and Sogstad et al. (2005). A high level of reliability may have been possible due to an explicit criteria or well explained descriptors for each category. The observer did not have previous experienced in lesion classification but had veterinary training, this may have facilitated recognition and understanding of the method. Further investigation (e.g. inter-observer reliability) is necessary to understand if this lession classification methodology can be applied in other studies with multiple observers.

6.5 Conclusions and future work

Lame cows that were mobility score 3 at the time of treatment, with one claw affected and with white line haemorrhage were less likely to recover from lameness at 2 weeks after treatment. In addition, cows with longer white line haemorrhage are more likely to recover; this may be linked to the severity of the lesion (e.g. more mild lesions). Mild claw horn lesions were significantly larger than severe lesions. Further work is needed to understand the effect of lesion severity on the recovery from lameness, and on how hoof trimming techniques may influence the likelihood of recovery from lameness.

Chapter 7

General discussion and conclusion

7.1 Main results and implications

Previous research has investigated the behavioural changes caused by lameness, though there is limited research investigating the outcomes of lameness treatment and how these may affect cows behaviour. This thesis aimed to improve our understanding of how lameness and lameness treatment affected cow behaviour in AMS, and to investigate if claw horn lesion types affected the likelihood of recovery from lameness.

The studies in this thesis demonstrated that lameness treatments could modify behaviour in dairy cows. In Chapter 4 and 5, lameness treatment affected overall total daily lying time but did not affect lying frequency or lying bout duration compared to non-lame control animals (Chapter 4), and lameness affected total rumination time (Chapter 5). These findings should be considered when applying any treatment, to avoid further complications or undesirable outcomes (e.g. reduction in production). As for veterinarians and farmers, the use of NSAID in lame cows should be encouraged as this may reduce the pain caused by lameness and the discomfort associated with a foot block if applied (Chapter 4). Further research could help to understand this association observed in this thesis (cows treated with NSAID and foot block did not change their behaviour), the industry could benefit of case-control studies looking at lameness treatment of claw horn lesions with and without NSAID. Though it has been studied before, new studies can look at the application of NSAIDs to newly lame cows in comparison to more chronically lame cows, or to different types of lesions within each chronicity group. On the other hand little is known if a higher dose might bring faster recovery from more severe lesions or in cases were more than one claw or limb is affected.

Prompt recognition and treatment of animals, in this case for lameness, showed that early treatment might aid recovery. Newly lame cows (cows were mobility scored every 2 weeks and were only enrolled if they had two non-lame scores followed by a lame score) may not have changed their behaviour as the lesions that they had were more likely to be early and mild (e.g. haemorrhages: Leach et al., 2012). The industry should encouraged farmers to carried out fortnightly checks on their herds to identify promptly the animals that need treatment. Also, a good record keeping can aid in detecting which animals are at risk of becoming lame (e.g. those that have previously been lame). Overall, promoting early interventions not only improves welfare and the reduction of the behavioural changes observed in lame dairy cows, it also may reduce the production losses (e.g. milk production) caused by lameness.

Results from this study should encourage the industry to further investigate the most effective treatment protocols for lame cows. Cows welfare does not only relate to comfort and management, it should also cover the prompt diagnosis and application of effective treatments. Detrimental behavioural changes (e.g. feeding time reduction) caused by veterinary treatments should be avoided. The industry should encourage veterinarians and farmers to keep a good record of lameness treatments administered and the time taken from diagnosis to full recovery. This data could be use by the industry for longitudinal studies to investigate the success of these treatments; also this data can help assurance bodies to assess the welfare of the herd in conjunction with other welfare outcomes.

Mobility score is use widely by the industry to identify lame animals. The poor reliability between and within observer demonstrated in this thesis highlights the importance for further research on the use of this particular score; further investigation is needed to improve this tool. In the meantime, the results in this thesis should not only encourage veterinarians and farmers to keep good records of this assessment but ideally to delegate this job to a single person in each herd. A thorough training in mobility score should be provided to all people involved within the industry area before this assessment is used.

This thesis used different technology to assess animal behaviour and the impact of diseases on it, as seen in lameness. An increased use of accelerometers, rumination collars and AMS within the industry not only facilitates the assessment of dairy cow activity; the data gathered by these technologies can also be used by farmers, veterinarians and researchers to assess the impact of management and veterinarian interventions on the daily behaviour of dairy cows. As shown in this thesis, these technologies have little impact on the normal behaviour of the animals so the data generated can also be use by welfare assurance bodies to assess farm compliance with good management practices (e.g. bedding quality - lying times) and veterinary interventions (e.g. disease checks - rumination time or milking visits). Further research is needed to study the impact of lameness in AMS production due to the behavioural changes that this disease causes, this information can help to encourage AMS herd managers to implement standard operation procedures for early identification and treatment of lame cows to minimise the impacts to their milk production in this system.

In Chapter 6 severity of the claw horn lesions played a role in the likelihood of recovery. This should encourage farmers and veterinarians to treat any lame cow as early as possible. For further research in the area of claw horn lesion severity, it is necessary to develop a simple and straightforward claw horn lesion assessment in order to facilitate standardised data collection and lesion diagnosis. Finally, as seen in Chapter 6, foot trimming operator had an effect on the likelihood of recovery, this highlights the importance of clinical auditing to identify less successful operators and the reason for their poorer success, so that successful technique can be propagated through the industry.

Results from this thesis showed that early interventions when treating lameness may help to overcome the behavioural changes previously reported and achieve good recovery rates. In order to confirm the findings from this thesis, the industry would benefit from a longitudinal study looking at the effects of early interventions, applying similar treatments to those administered in this thesis, measuring not only clinical outcomes (e.g. recovery rates), but also production, behaviour and welfare outcomes (e.g. lying times); in addition, further research in newly lame and early treated cows could gather data on the likelihood of these cows becoming lame after treatment and if treatment of these repeat episodes have the same recovery rate as the first case. Any research carried out in the area of veterinary treatments should consider the collection of behaviour as a measure of welfare; little is known about the impact of many veterinarian treatments on the behaviour of domestic species and how this may impact on welfare and clinical outcomes.

Finally, it is necessary to investigate if the management of animals in their early life has any impact on their future incidence of lameness as adults. There are two principal areas in the raising of young stock that could be investigated: feeding and housing. Dairy calves are usually fed with milk replacers (i.e. alternatives to whole cows milk), little is known about the impact of this on the health and growth of these animals in the long term. A study could compare the likelihood of becoming lame between calves that were raised with whole milk or those raised with milk replacers. In the case of housing, calves and heifers are often kept in pens with concrete flooring; a study comparing the different types of housing with the likelihood of becoming lame in the first parity could add valuable information to the area of lameness risk factors and control.

7.2 Limitations and future work

Even though total daily rumination time did not seem to be a good indicator for lameness (only 8 minutes difference between lame and non-lame cows); it is important to investigate how this behaviour is affected by lameness. So far studies in this area have focused on comparing rumination time between lame and non-lame cows or comparing changes in behaviour over time. In Chapter 3, no intervention was carried out (e.g. hoof trimming, lesion identification) but further investigation about the cause of lameness may have helped to increase our knowledge on which type of lameness was the cause of the rumination changes observed both here and by other researchers. In addition, it was observed that there were some outliers in the rumination data, whilst this could have been due to technical problems with the monitoring equipment; it could also be a normal pattern within rumination behaviour linked to differences between individuals.

The studies presented in Chapter 3 and 5 used data collected with an automatic rumination collar that not only allowed the collection of more objective rumination data but also allowed for longer periods of continuous data recording.

For research purposes, it would be helpful if the system within the rumination tag collected data either per minute or per hour. This format would facilitate more informative studies of rumination circadian pattern, and allow detailed investigations of how lameness and other diseases or management, impact on rumination behaviour.

The present thesis was funded by a scholarship from the University of Nottingham International Office and had a limited budget. Data collection was necessarily carried out within this budget and with limited technical support. In addition, data collection in Chapter 4 was carried out as part of a bigger project and due to the nature of the experimental design; it was not possible to know which cows were going to be included in the study prior to their enrolment on the day of treatment. Consequently, it was not possible to collect lying behaviour data before treatment. In addition, the primary project was budgeted to run for 12 months, so it was not possible to extend the data collection period for the project in Chapter 4 beyond this point. Future studies on behavioural changes caused by lameness treatments within randomized clinical trials should take into consideration the time it takes to select suitable animals for behavioural studies and be adequately powered for expected study outcomes.

Behavioural studies should control for within cow differences, as animals tend to have individual behavioural pattern. Most of the studies in this thesis compared behavioural changes between two groups of cows (lame and non-lame). It may have been interesting to consider these individual characteristics on the data collection in Chapters 4 and 5 including a longer period of observation prior to treatment, taking baseline measurements, and including a control cow that received a sham foot trimming and a saline injection to control for stress caused by the procedures which may impact on cow behaviour. Future research in cattle behaviour could include or study these individual differences particularly in AMS where there is limited research in cow behaviour. Also, it is important to acknowledge that within a herd there may be individuals that display a behaviour with no discernable reason (e.g. cows which never go through the milking robot unless pushed by the herdsman – Personal Observation), so caution should be taken when running behavioural studies comparing changes due to disease (e.g. lameness).

During data collection, it was discovered that the farms enrolled in the studies in Chapter 4 and 5 were updating and overwriting their database as soon as a cow was sold or sent to the abattoir. These changes in the database included the loss of milk production information for study animals. Even though this problem only led to the loss of data from a small number of animals, this reduced the number of animals in the final analysis. Future studies carried out in commercial settings should take into consideration these aspects of farm management and establish good lines of communication with farmers to avoid problems with loss of data.

The experimental protocol for the studies described in Chapters 4 and 5 was designed to enrol only cows lame on one hind limb and that presented with claw horn lesions in only one claw (as foot blocks should only be applied to healthy claws). Consequently, the interpretation of findings from the present study is limited to cows that suffer with mild and acute lameness, and that are diagnosed with claw horn lesions in one claw on a single hind limb. These results are important for cow welfare and farm production, but it is necessary to conduct further research on chronic and more complex cases of lameness (e.g. both hind limbs affected). These studies could also gather information on behaviour in order to assess the impact on cow welfare. In addition, it is recognised that different types of lameness affect cow behaviour and production differently, consequently it is important that diagnosis is considered in any study looking into lameness treatment and behaviour. The industry would benefit from this information in order to provide more targeted advise on how to treat lameness depending on the type of lesion present. Ideally, a study looking at treatment should consider making comparisons of the lesions before and after treatment, this could increase our knowledge on how claw horn lesion recovery progresses over time.

Results from Chapter 4 and 5 highlighted the importance of other behaviours when assessing the effects of lameness and lameness treatment on dairy cow welfare. It is recommended that feeding behaviour and feed intake should be considered in future studies investigating the effect of lameness and lameness treatment. Reduction in feeding and rumination behaviour does not only affect the welfare of cows, it can also reduce milk production. In addition, there are many different types of foot blocks available in a range of material and shapes. Little research on the effects of foot blocks on dairy cow behaviour and how this translates into milk production have been conducted. The industry would benefit from a study comparing activity and recovery rates using different foot blocks (e.g. wooden blocks vs rubber blocks) which take into account different types of farm facilities; for example from personal observation, cows wearing a wooden block in farms with concrete flooring were more likely to slip when walking than cows housed in farms with rubber flooring.

7.3 Final conclusion

This thesis has contributed to the existing knowledge in how lameness affects cow behaviour before and after treatment, and the importance of prompt recognition and early treatment to avoid further compromises to cow welfare. It has also improved our understanding of how claw horn lesion type may affect recovery from lameness. Studies on the effects of lameness on dairy cattle behaviour found that lameness affected the number of milking visits and total rumination time; but these changes were not observed in cases of mild and acute lameness. Significant behavioural changes observed in published research studies may have been caused by the chronicity of the disease suffered by animals included in those studies.

It is important to recognize that delays in the detection and treatment of lameness, in particular in AMS, can have an impact on farm profits as they not only cause direct milk loss but also increase staff cost. Results from the present thesis suggest that the dairy industry should focus their efforts on prompt recognition of lameness, application of effective treatments as soon as lame animals are identified and standardised foot trimming practices. This approach will not only improve dairy cow welfare, but also will reflect on farm profit by limiting the hidden costs of lameness.

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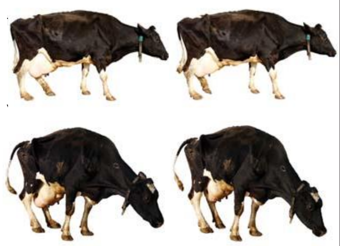
Appendix 1 - Dairy Co Mobility Score

DairyCo Mobility Score

Category of score	Score	Description of cow behaviour	Suggested action
Good mobility 	0	Walks with even weight bearing and rhythm on all four feet, with a flat back. Long, fluid strides possible.	• No action needed. • Routine (preventative) foot trimming when/if required. • Record mobility at next scoring session.
Imperfect mobility 	1	Steps uneven (rhythm or weight bearing) or strides shortened; affected limbs or limbs not immediately identifiable.	• Could benefit from routine (preventative) foot trimming when/if required. • Further observation
Impaired mobility 	2	Uneven weight bearing on a limb that is immediately identifiable and/or obviously shortened strides (usually with an arch to the centre of the back).	• Lame and likely to benefit from treatment. • Foot should be lifted to the cause of lameness before treatment. • Should be attended to as soon as practically possible.
Severely impaired mobility 	3	Unable to walk as fast as a brisk human pace (cannot keep up with the healthy herd) and signs of score 2.	• Very lame. • Cow will benefit from • Cow requires urgent attention, nursing and further professional advice. • Cow should not be made to far and kept on a straw yard or at grass. • In the most severe cases, culling may be the only possible

Appendix 2 - Standard Operating Procedure for the use of Hobo data loggers (Courtesy of Dr Anne Marie de Passillé and Dr Jeff Rushen)

To launch HOBOs

1. Click on the HOBOware icon in the desktop.
2. Connect USB station to the computer.
3. Attach the HOBO logger to the USB base station matching the ridge to the base, Fig1.
4. At the bottom right of the program window appears “1 device connected”.

Figure 1 HOBO and USB base station

5. Click on the launch “device”  icon, a window “launch logger” appears (Fig2).
   a. In this new window: check for the <u>battery</u> to be 100%
   b. **Description**: Put the name of the file to be recorded. Lying Behaviour Treatment Project:
      FarmCode_HoboNumber_dd-mm-yy (e.g. 01_UoN14_200051_12-05-12).
   c. **Channels to log**: Select 1 and 2.

   <u>If you want to measure also **laterality** you must choose 2 and 3 (Y and Z axis) instead of 1 and 2.</u>

   d. **Logging interval**: Normal at 1 min
   e. **Launch Option**: Delayed.
   Set the date and time to start logging. Set the day of the installation and the time as 10:00:00 AM if the visit will be in the morning and 02:00:00 PM if the visit will be in the afternoon.

6. Click on "**Launch**". Wait for the HOBO to be ready that is when the window disappears.

7. Before you take out the HOBO from the USB coupler, click on "Device Status"  icon, in the top left of the window. Check that all the information that holds is right (Fig3):

a. **Description**: right name.
b. **Channels to log**: Select 1 and 2.
c. **Logging interval**: Normal at 1 min
d. **Launch Option**: Delayed with the right time.

In the "Current reading" you can check if the axis is moving on screen by moving the HOBO. This will confirm that the HOBO is ready to use.

Click OK and you can release the HOBO from the USB station.

It will take no more than 2 min to launch a HOBO.

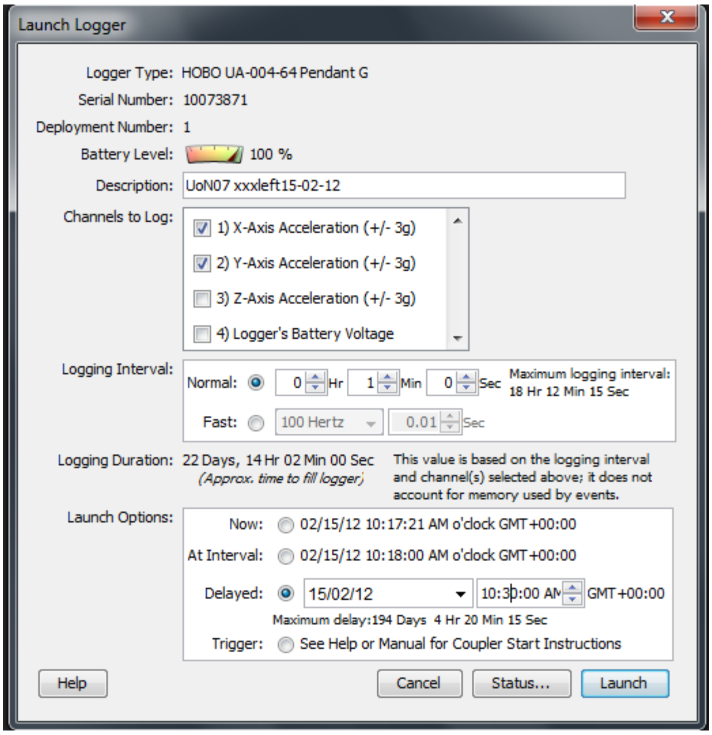


Figure 2 Launch logger screen

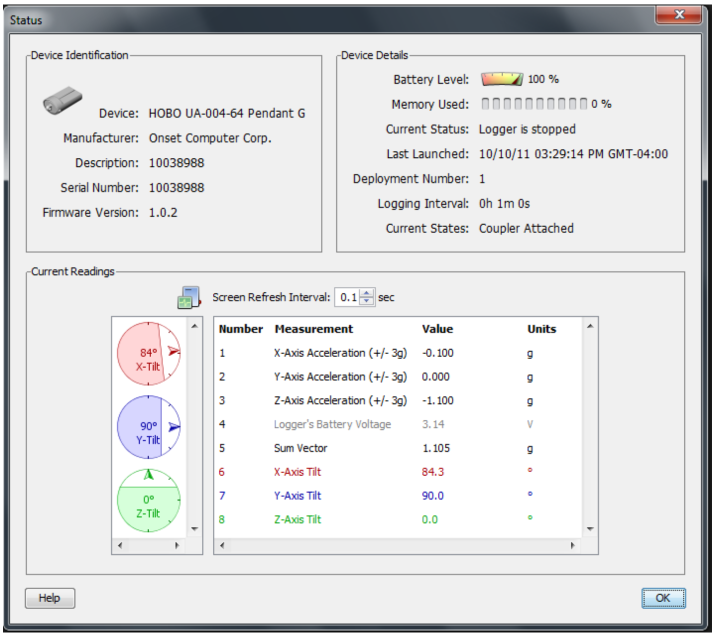


Figure 3 Device status window

To prepare HOBOs

Once you have **Launch** a HOBO, you need to prepare it before it is place in the animal:

1. Wrap the HOBO in a 10-15 cm of Vetwrap, making two layers.
2. Position the ridge of the HOBO facing down and the biggest end of it facing your left hand (Fig 4).
3. Write HOBO number (e.g. 14) over the vetwrap using a permanent marker (Fig 4). Underline number 6 or 9 to avoid confusion.
4. Put a piece of foam and wrap the foam and hobo together. Write the Hobo Number again (Fig 4).

This preparation must be done before HOBOs are taken to the farm.

Figure 4 Preparing Hobo

To attach HOBOs

Once in the farm try to keep the vetwrap dry all the time. Once wet reduces it sticky properties.

1. Wrap one layer of vet wrap on the leg of the cow above the metatarsophanlangeal joint (Fig 5).
2. Hold the roll, do not cut it.
3. Place the HOBO in the lateral side of the leg with the number written on it facing you and in the right position.
4. Continue wrapping over the HOBO until is secured, approx. 5 to 6 layers.
5. Cut the vet wrap using a safe knife or safety cutter.
6. Press well the end of the vet wrap or stuck it at one of the ends.

A third of vet wrap is needed per logger.

A finger must be easy to insert between the vet wrap and the skin. If you cannot it is too tight so you need to reapply. If you can insert more than one finger, it is too loose so you need to reapply. In this case the HOBO might rotate or get lost.

HOBO must be attached to the sound leg. Tick in the HOBO form in which leg it was attached.

Figure 5 Hobo position

To detach HOBOs from the cow's leg

1. Using a safety knife cut the vet wrap off.
2. Unwrap the HOBO but not the packing that contains the number.
3. Discard the vet wrap.

Record in the HOBO form the number of the cow and the number of the HOBO that has been taken out. Check for coincidences.

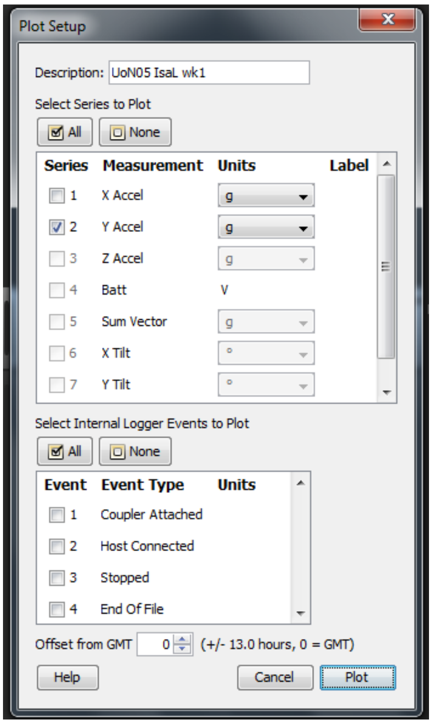
To read the device

1. Open HOBOware software.
2. Connect the USB base station to the computer.
3. Attach the HOBO logger to the USB base station matching the ridge to the base, Fig1.
4. At the bottom right of the program window appears “1 device connected”.
5. Click on the readout icon 
6. Click stop in the window that appears and wait until the data is downloaded.
7. Save the file as: xxx.hobo

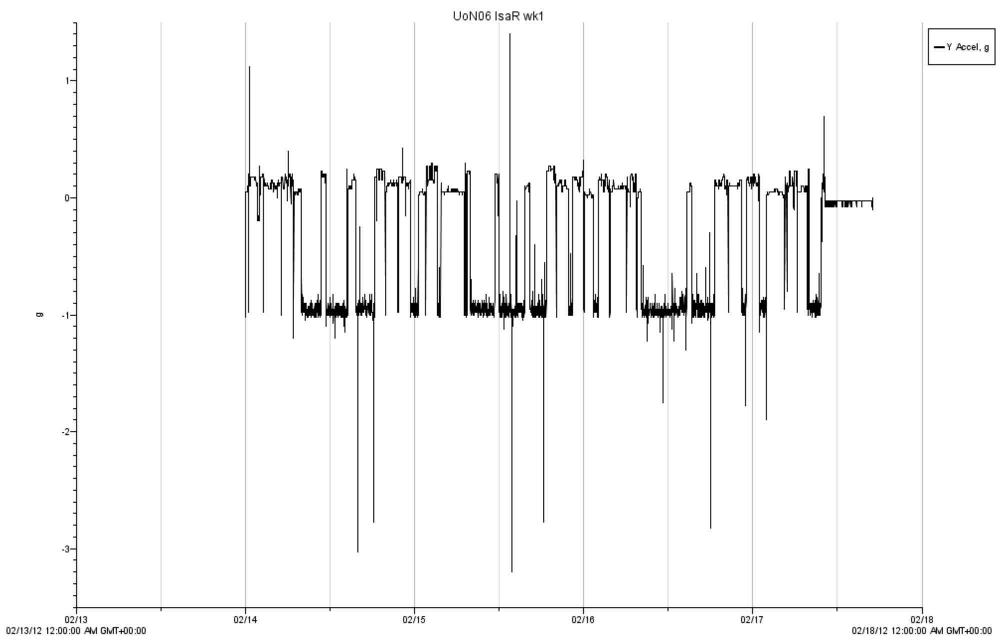
Remember to put in the name the:

FARMID = Farm ID
XXXX = cow number
first date = date ON
second date = date OFF
NB = hobo number.
For example: ON9146_5086_2010-11-22_2010-11-26_22.hobo

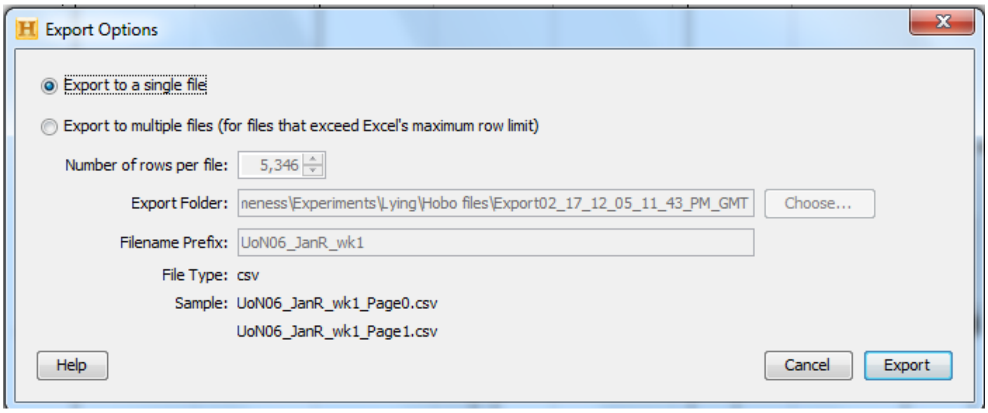
8. Once save the “plot setup” window appears, select ONLY the ‘**Y Accel**’ (i.e. uncheck every other options – you can do this by clicking on ‘none’ in the top box), and uncheck all options (‘none’ again) in the bottom box. Then click on ‘**plot**’.
9. For **LATERALITY** select ‘**Y and Z Accel.** Uncheck all options (‘none’ again) in the bottom box. Then click on ‘**plot**’ and continue with the instructions.



10. A graph will show. Check the graph to make sure that it looks 'right' (Figure 9). Make a note if: 1) there are some hours or days missing - day(s) may need to be adjusted before the macro is run (Identifiable by straight horizontal lines)



11. Click on the excel  icon with the upward arrow (**export table data** – between close (**×**) and print), then click on '**export**' when the pop up window shows. Save as a *csv* file with the same name as the graph.



12. **Close** (**×**) the graph and disconnect the logger from the base station.

To transform the data from HOBO output

1. If you are measuring **only lying behaviour** use the files in the **Macro-Lying folder**.

2. If you are measuring **also the laterality** of the behaviour use the files in the **3D Macro folder**.

3. Each folder contains an SOP on how to proceed.

Appendix 3 - Evaluation of a 3-dimensional accelerometer to measure lying behaviour in beef cattle

The present study aims to evaluate the accuracy of measuring lying behaviour (lying side, total lying time and number of lying bouts) in 1st parity beef cows and their calves. The alternative hypothesis was that there was a difference in lying behaviour between video analysis and data logger measurements. The null hypothesis was that there was no difference in lying behaviour between video analysis and data logger measurements.

Materials and methods

The present study was reviewed and approved by the University of Nottingham's School of Veterinary Medicine and Science Ethical Review Committee before data collection began.

Animals, housing and management

This study was conducted between January and February 2014 on a farm located in the north east of England. Sixteen pairs of 1st parity South Devon beef cows with their calves were housed in individual pens within 6 hours of calving for up to 3 days. Cows weighed 558 ±59 kg and had a Body Condition Score of 2 (IQ 2-2.5) on a scale of 1 to 5. Eight cows had an unassisted calving, four had a mild pull calving (calf was pulled manually) and four had a hard pull calving (use of Jack or calf puller, or up to 3 people were required). There were nine bull calves and seven heifer calves. Bull calves weighed 41 ± 4kg and heifer calves weighed 39 ± 3 kg.

Prior to calving cows were fed *ad-libitum* straw and hay with liquid molasses supplement and had free access to a water trough. After calving cows were fed *ad-libitum* straw and twice daily concentrate. Individual pens measured 4m by 4m and had a concrete floor covered with straw bedding. Fresh bedding was added every 24 hours. Each cow and calf pair were kept in these pens for up to 3 days after calving. Then they were moved to a group pen.

Behavioural recordings

Lying and standing times were recorded continuously using eight ceiling mounted Infra Red LEDs CCTV cameras (OBK20B: 1/3” Sony Colour, Gamut, Bristol, UK) connected to a digital video recording box (8DVRLAN, Gamut, Bristol, UK). Each camera was positioned 2.6m above the individual pens (Figure 1). Individual pens were identified clearly with the number of the cow written on a white board that was visible from the camera. Cameras were set to record continuously at high quality and 30 frames/second.

Lying behaviour was defined as when cow or calf was lying on the sternum or side with head rested or lifted and standing behaviour was defined as body upright supported with at least 3 legs. Total time per behaviour started at the minute the behaviour was observed and concluded when the behaviour was changed. Lying and standing events were defined as the frequency that the behaviour was observed. In addition, lying side (left or right) was recorded as total time spent and number of events per lying side.

Video analysis was carried out using The Observer® XT 11.5 (Noldus, Information Technology b.v., Wageningen, The Netherlands). A total of 12 hours of video per pair (cow-calf) was analysed. Video analyses were from 6 to 24 hours after calving. Each 24 hours was divided in blocks of 6 hours and the 3 first hours of each block were continuously analysed. Intra-observer reliability was 99.02% and 99.99% agreement for total duration and frequency respectively for all the behaviours (lying and standing behaviour). Reliability was calculated using The Observer® XT 11.5 reliability analysis.

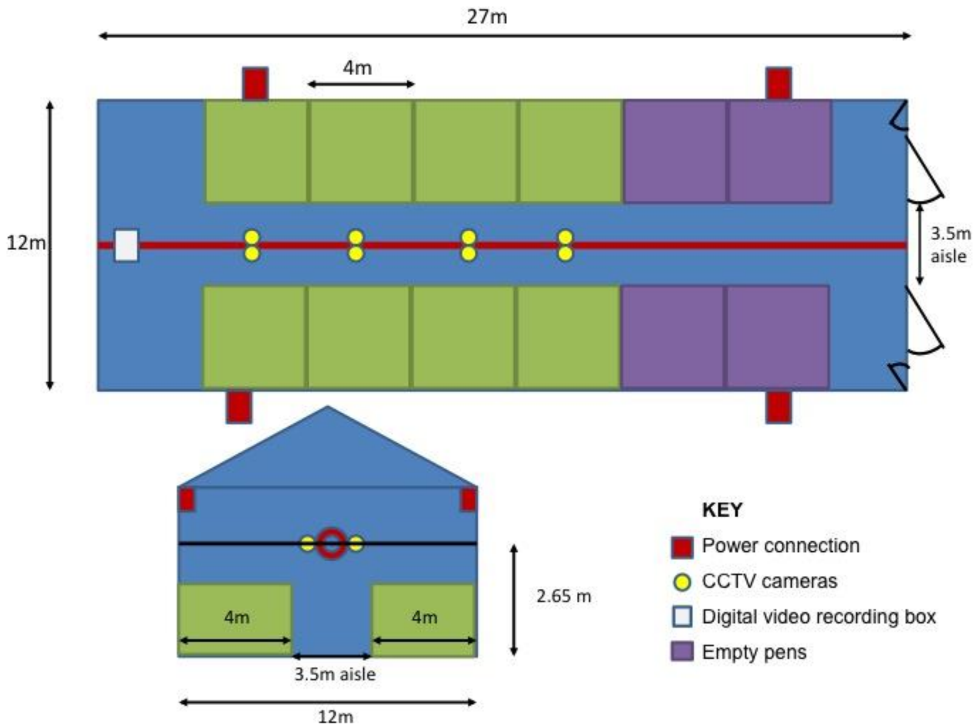


Figure 1 Layout of individual pens used for the behavioural assessment

HOBO Pendant G Data Loggers

Onset Pendant G is a tilt switch activated logger that can be attached to the leg of cattle; they measure 50x30x23 mm and weigh 17 g. It is able to measure acceleration (*g* force) or vertical tilt at x-, y- and z-axis. *G* forces goes from 0 to 6.4 and considering that leg is horizontal, values on y-axis ≥ 2.55 indicate lying and values < 2.55 indicate standing behaviour; similar values are use for x-axis and standing behaviour. Z-axis is used to measure laterality of lying behaviour, the values of *g* force will depend where the accelerometer is attached (right or left hind leg) (Ledgerwood et al., 2010). Accelerometers were set to record the y-axis (to evaluate lying behaviour) and z-axis (to evaluate laterality of lying behaviour) at a logging interval of 1 reading per minute and g forces as a unit (Figure 2) (Gibbons et al., 2012).

Animals were fitted with an accelerometer (HOBO Pendant G Acceleration Data Logger, Onset Computer Corporation, Pocasset, MA, USA) within the first 6 hours after calving. The accelerometer was attached using Vet Wrap cohesive bandage (3M Products, St. Paul, MN) to the lateral side of either hind leg above the metatarsophalangeal joint (Figure 3). Onset HOBOware® Lite Software Version 2.2.1 (Onset Computer Corporation, Pocasset, MA) was used to download data from accelerometers which then were exported to Microsoft Excel® (Microsoft Corporation). The Software Macro Hobo 3D Microsoft Excel® was used to modify and edit data.

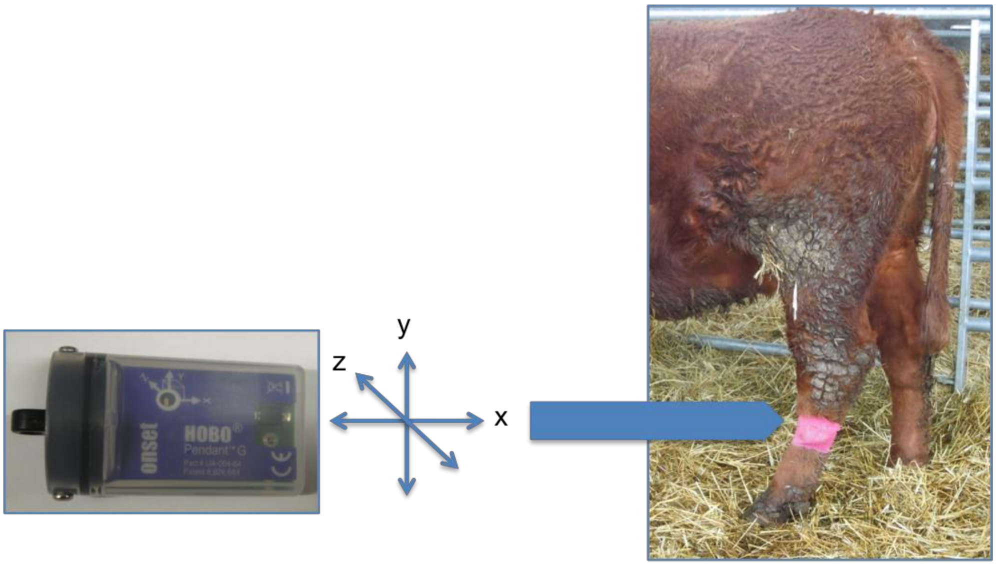


Figure 1 Position of Onset Pendant G data logger and axis (y, x and z) when cow is standing.

Figure 2 Attachment of accelerometers: calf with accelerometer on right hind leg (left picture) and researcher attaching accelerometer on the right hind leg of cow (right picture).

Statistical analysis

Statistical analysis was carried out on only 14 pairs (cow and calf) as an accelerometer was not attached to the first calf as she presented with locomotion problems, and missing hours were detected on the recordings of the second pair of cow and calf. Data for laterality of lying behaviour in calves was not considered as the accelerometers rotated around the leg in most of the calves. The lying and standing behaviour data from The Observer® XT 11.5 was exported to Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) to carry out data analysis manipulations. Statistical analysis was conducted using Stata/SE 12.0 (Stata Corp 2011, USA) and Microsoft Excel 2010 (Microsoft Corp., Redmond, WA).

Data from cows and calves were analysed independently. Correlation between visual observations and data from HOBO was analysed in a 2x2 contingency table to calculate the sensitivity (HOBO correctly identified lying events identified by the video: Yes or No) and specificity (HOBO correctly identified the standing events identified by the video: Yes or No) of the HOBO to detect the behaviours observed in the video (gold standard). Predictive values were calculated: Negative Predictive Value (NPV= Probability that the HOBO identify as a negative a lying event that was identify by the video (Yes or No)) and Positive Predictive Value

(PPV= Probability that the HOBO identified as positive a lying event that was not identified by the video (Yes or No)).

Pearson correlation test was used to study the correlation between the data obtained from the video analysis (total time lying and standing) and the data from the HOBO for cows and calves. Means and SEs for total lying and standing times were calculated for cows and calves. Only for cows' data, the percentage time spent lying on left side observed on video was compared with the data obtained from the HOBO. Level of significance was set as $P \leq 0.05$ for all the analysis.

Results

Eight calves and six cows had an accelerometer attached to their left hind leg and six calves and eight cows had it attached to the right hind leg. HOBO had a specificity and sensitivity of 1.00 to detect lying events in cows and specificity and sensitivity of 0.99 and 0.94 respectively in calves. The PPV and NPV in the case of cows was 1.0 for both and in calves 0.99 and 0.93 respectively.

Mean and ±SE of lying and standing behaviour variables for cows and calves are presented in Table 4.1. Total lying times recorded by the HOBO was highly correlated with the data obtained from the video for cows: $R^s = 0.99$ ($P < 0.001$) and for calves: $R^s = 0.99$ ($P < 0.001$). Total standing times followed a similar pattern, for cows: $R^s = 0.99$ ($P < 0.001$) and for calves: $R^s = 0.99$ ($P < 0.001$). Frequency of lying and standing behaviour detected by video analysis were highly correlated with the ones detected by the HOBO; for cows: standing and lying $R^S = 1.0$ ($P < 0.001$) and for calves: $R^S = 1.0$ ($P < 0.001$). In the case of cows, the percentage of time spent over the left side recorded by the HOBO was highly correlated ($R^s = 0.96$, $P < 0.001$) with the data obtained from the video analysis.

Table 1 Lying and standing behaviour variables (Mean and SE) for HOBO and video recordings during the observation period (12hrs).

Behaviours	Heifers				Calves			
	Video		Hobo		Video		Hobo	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Lying								
Duration (min/12hr)	244.1	25.7	244.6	25.8	575.9	21.0	580.5	21.1
Frequency (bouts/12hr)	6.80	0.80	6.80	0.90	13.86	0.74	13.86	0.74
Mean bout duration (min/bout)	39.6	3.8	39.7	3.8	42.5	2.1	42.8	2.1
Proportion Left Side	57.9	7.5	59.3	7.3				
Standing								
Duration (min/12hr)	448.0	22.9	449.6	23.2	123.4	9.9	121.3	9.8
Frequency (bouts/12hr)	8.00	0.80	8.00	0.80	11.50	0.80	11.50	0.80
Mean bout duration (min/bout)	64.0	8.3	64.3	8.3	11.4	1.2	11.2	1.1

In the case of cows, the percentage of time spent over the left side recorded by the HOBO was highly correlated (R^s =0.96, P<0.001) with the data obtained from the video analysis. Cows showed a high variation for this preference (lying over left side) with a range between 13% and 100% (Figure 6.4).

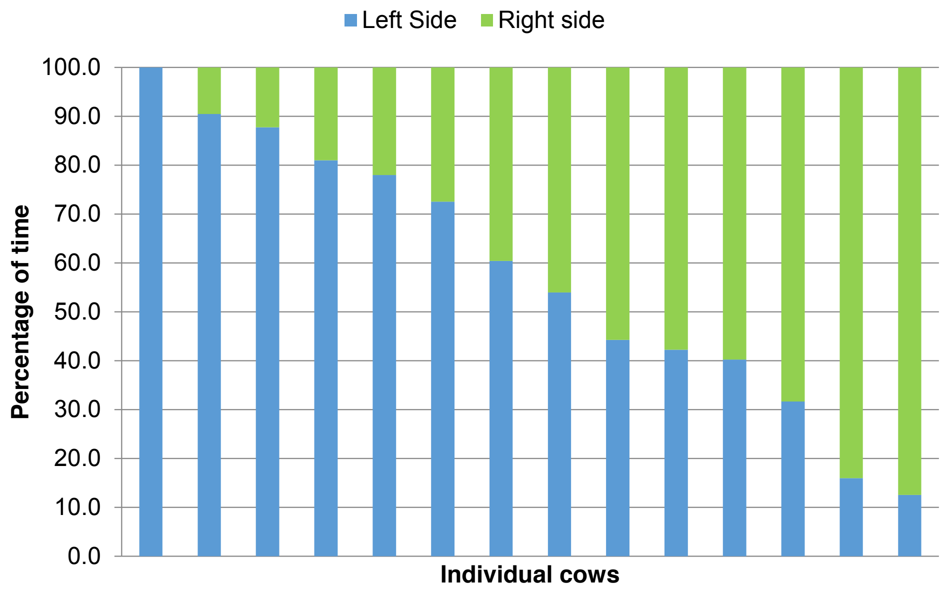


Figure 4 Percentage of time individual cows (n=14) spent lying on each side during video observation (12hrs).

Discussion

HOBO data logger had a high specificity and sensitivity for measuring lying events in beef cattle. In beef cows, the Hobo data logger showed a high correlation with total lying and standing times, bouts and the percentage of time spent on left side. These findings agree with what it has already been observed in dairy cattle (Ledgerwood et al., 2010; Mattachini et al., 2013). Similar findings were observed in beef calves, HOBO data logger presented a high correlation with total lying and standing times and bouts in calves. In the case of beef calves, it was not possible to measure laterality of lying behaviour as the accelerometer rotated around the leg. As explained by Bonk et al. (2013), calves's legs are more rounded and slimmer than adult cows. Lying times in beef calves was 575 min during the 12 hour observation period, this finding is proportional to what was observed by Jensen (2011) in dairy calves on the 3rd day after birth (1120 min/24hr).

The present study observed individual preference on the lying side, with 8 cows showing from 50 to 100% preference to lie down over the left side to the remaining cows showing less than 50% preference. Even when the data collected correspond to only 12hrs, it can show that cows have individual preference when displaying this behaviour as it was suggested previously (Tucker et al., 2009; Gibbons et al., 2012).

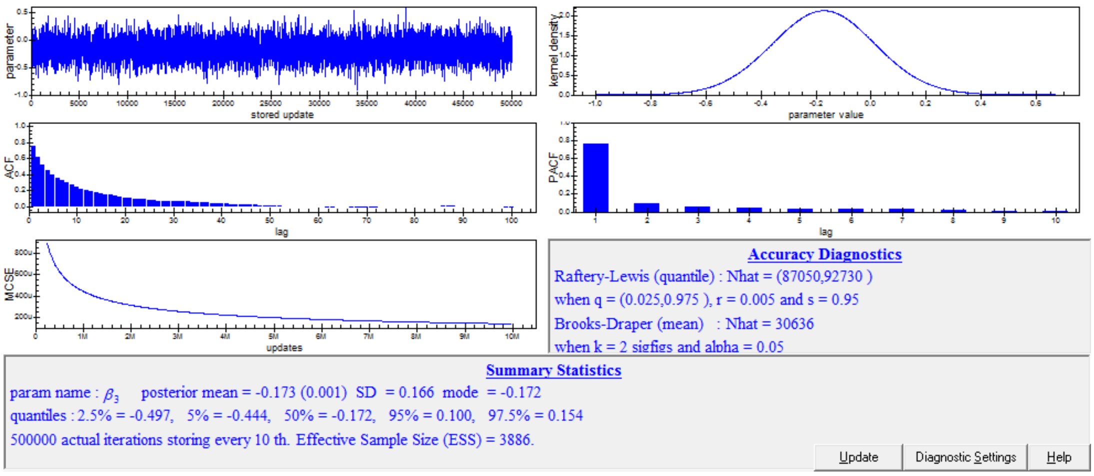
This is the first study to validate the use of HOBO data logger in beef cattle. Automatic methods for measuring behaviour are allowing researchers to reduce bias in collected data, reduced behavioural changes due to the presence of the observer and reduced the time invested in data collection. The method presented in this study is a promising tool that has been used with no problems in dairy cattle and that can be used in beef cattle with the same confidence. The understanding of natural behaviour patterns allows researchers to identify changes on cattle's behaviour that can be used as key health and welfare indicators for veterinary practice and research.

Conclusions and further work

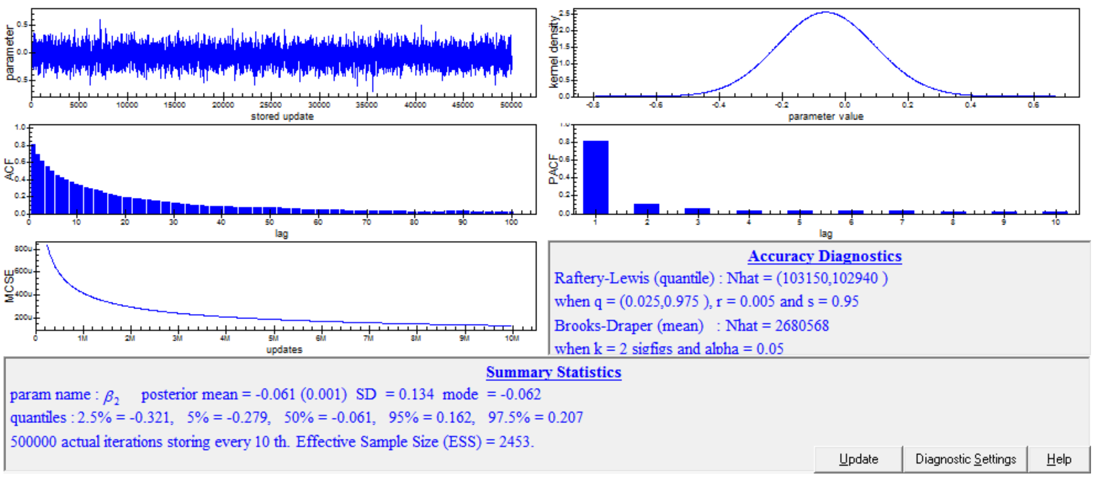
HOBO Pendant G Acceleration Data Logger presented an excellent specificity and sensitivity to measure lying behaviour in beef cows and calves. Laterality of behaviour was measured with the same accuracy that was reported previously in dairy cows. In the case of calves, it was not possible to measure laterality of lying behaviour as the accelerometer rotated around the leg as it has reported in dairy calves. Further studies are required to understand the lying behaviour of beef cattle during this crucial period and its importance.

Appendix 4 – Visual Analysis of Chain Mixing and Stability for Chapter 4 Effects of lameness treatment on lying behaviour

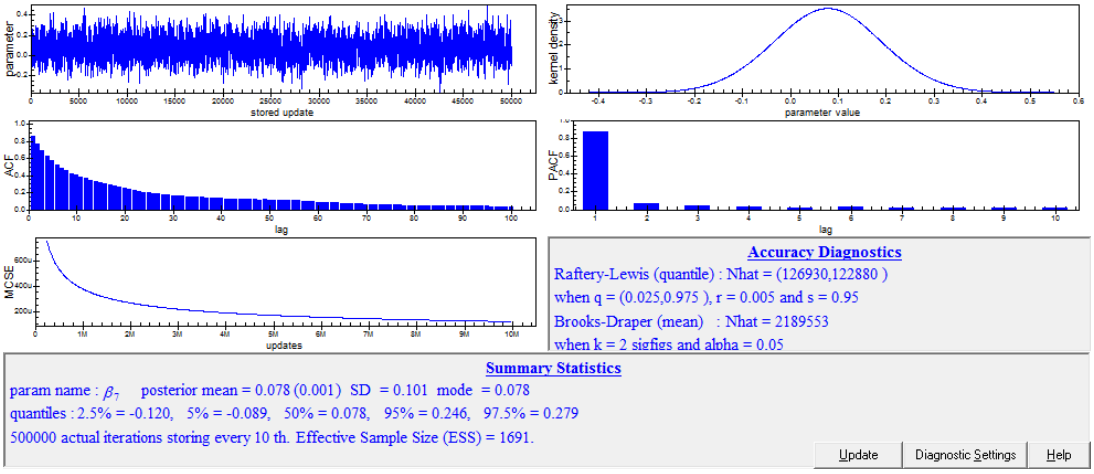
B3=-0.173(0.166)



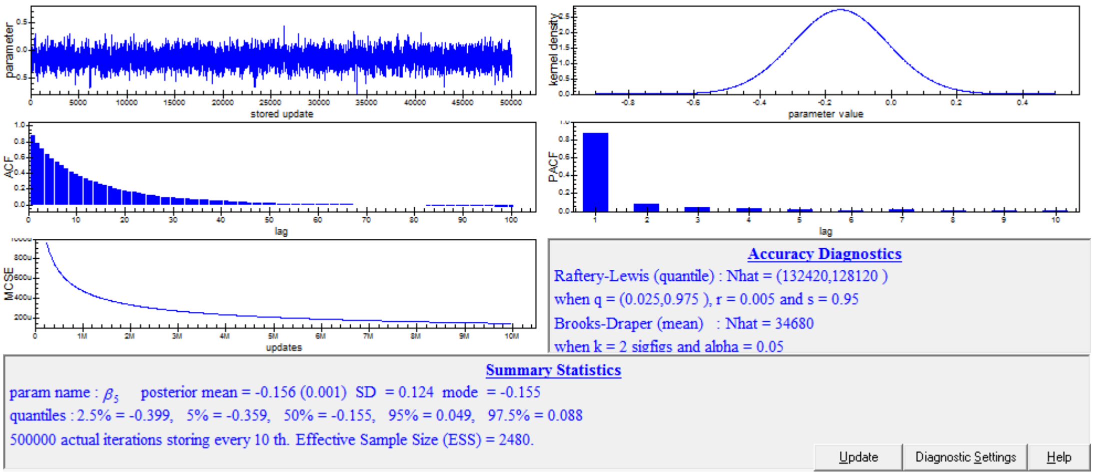
B2=-0.061(0.134)



B7=0.078(0.101)

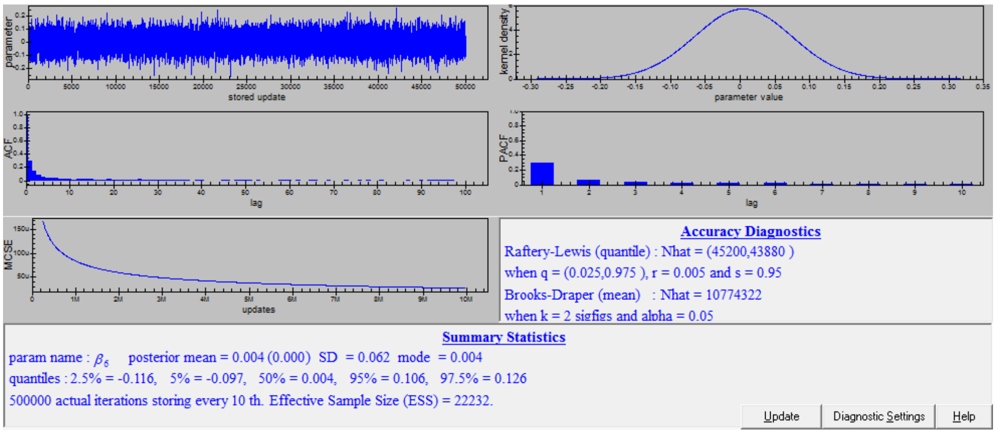


B5=-0.156(0.124)

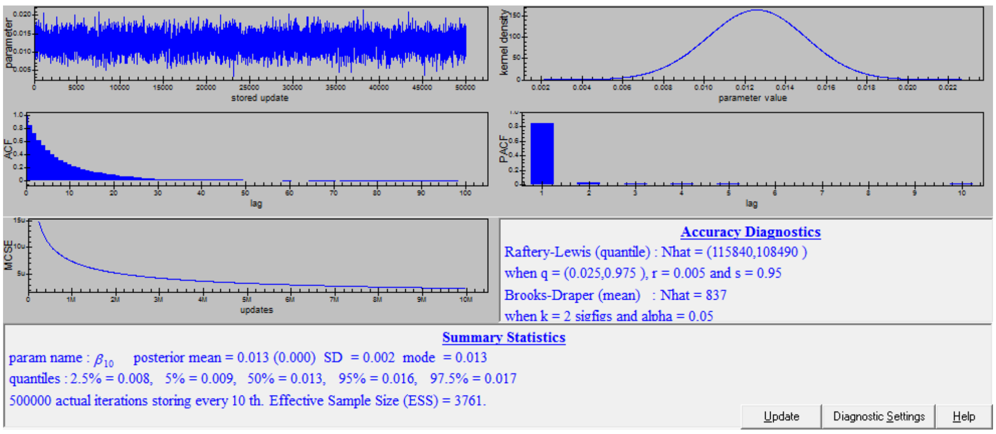


Appendix 5 – Visual Analysis of Chain Mixing and Stability for Chapter 5 Effects of lameness treatment on milking and rumination behaviour

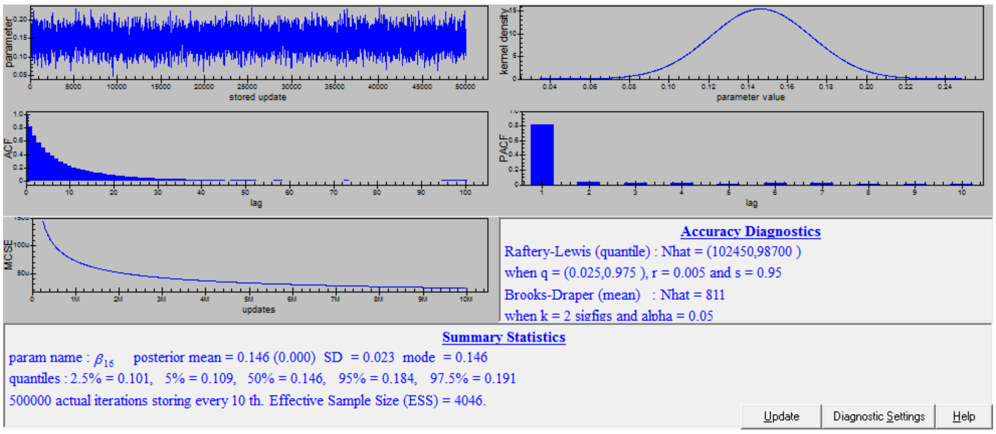
B6 = 0.01 (0.06)



B10= 0.01(0.002)

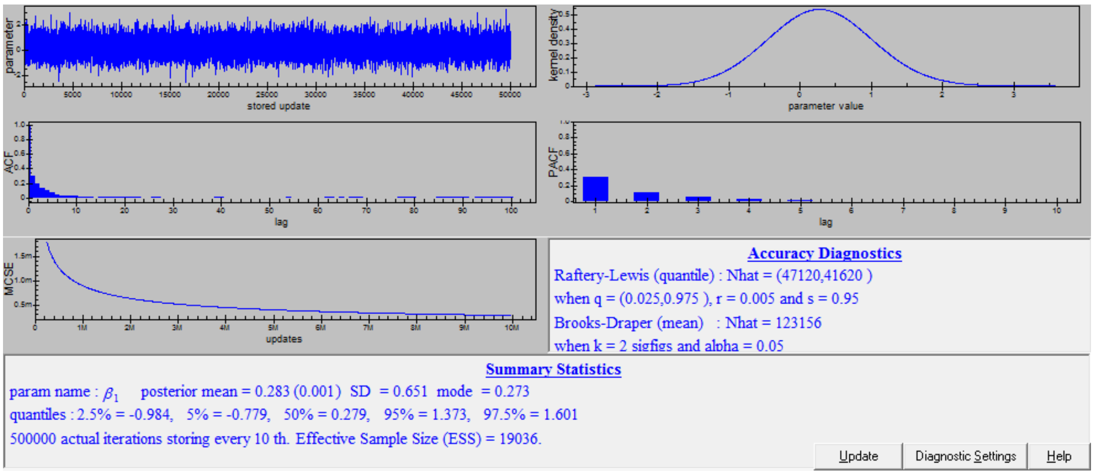


B16= -0.03(0.07)

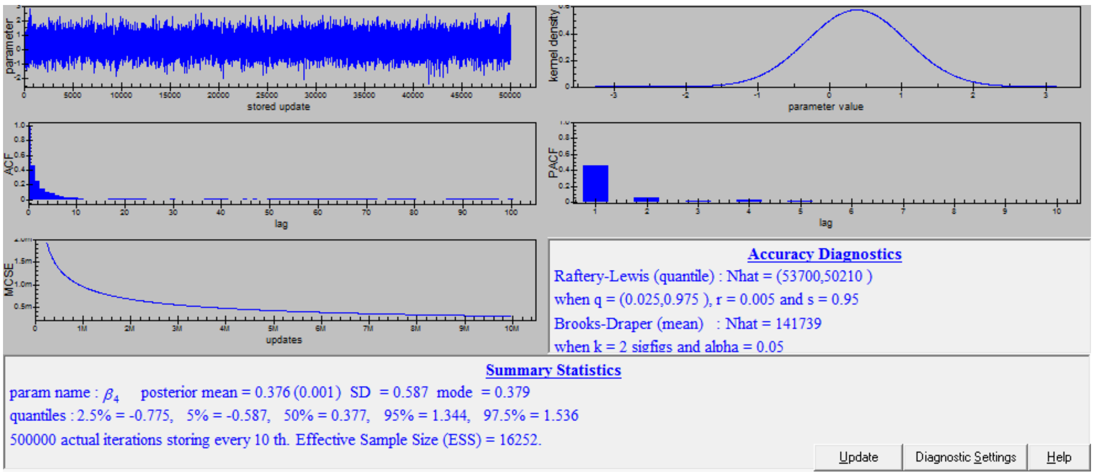

parameter
stored update
kernel density
parameter value
ACF
lag
PACF
lag
MCSE
updates
Accuracy Diagnostics
Raftery-Lewis (quantile) : Nhat = (102450,98700)
when q = (0.025,0.975), r = 0.005 and s = 0.95
Brooks-Draper (mean) : Nhat = 811
when k = 2 sigfigs and alpha = 0.05
Summary Statistics
param name : β_{16} posterior mean = 0.146 (0.000) SD = 0.023 mode = 0.146
quantiles : 2.5% = 0.101, 5% = 0.109, 50% = 0.146, 95% = 0.184, 97.5% = 0.191
500000 actual iterations storing every 10 th. Effective Sample Size (ESS) = 4046.
Update
Diagnostic Settings
Help

Appendix 6 - Visual Analysis of Chain Mixing and Stability for Chapter 6 Effect of claw horn lesion type at the time of treatment on lameness prognosis

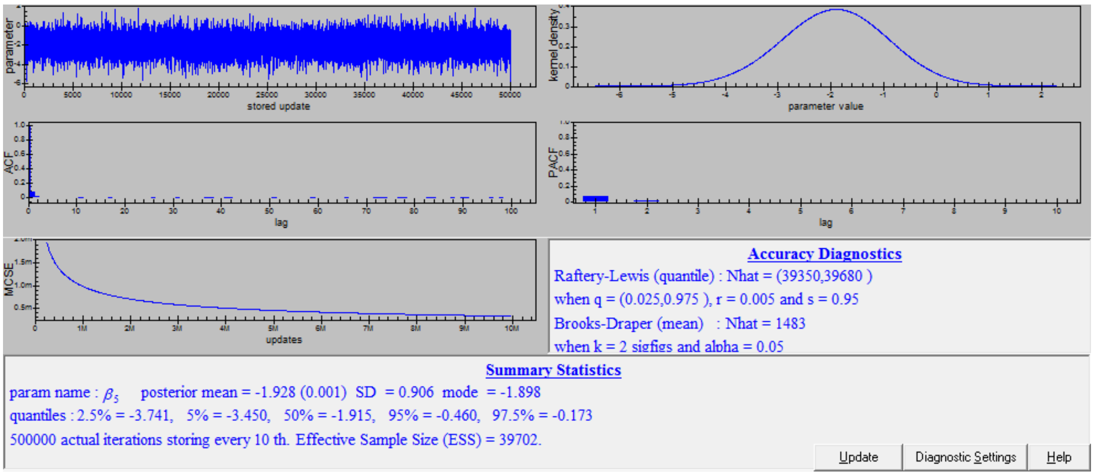
B1=0.283(0.651)



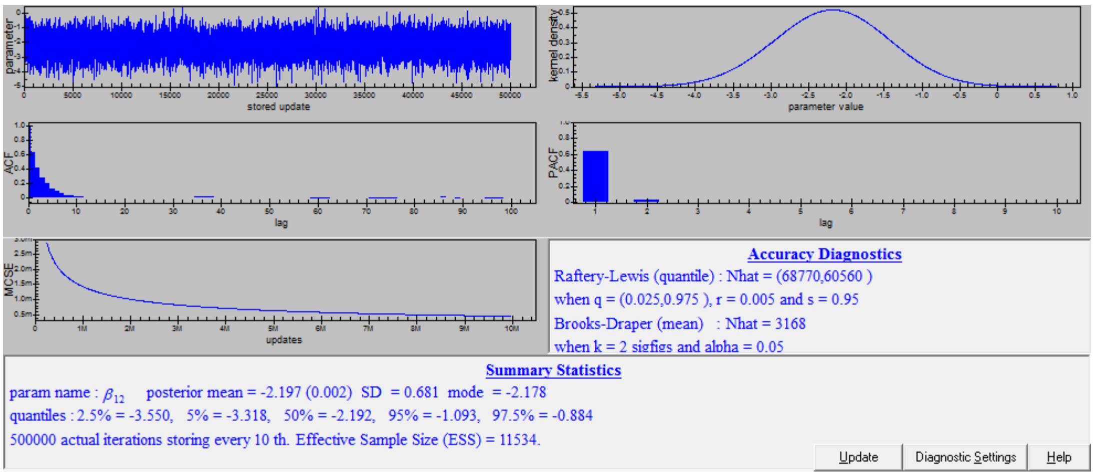
B4=-0.376(0.587)



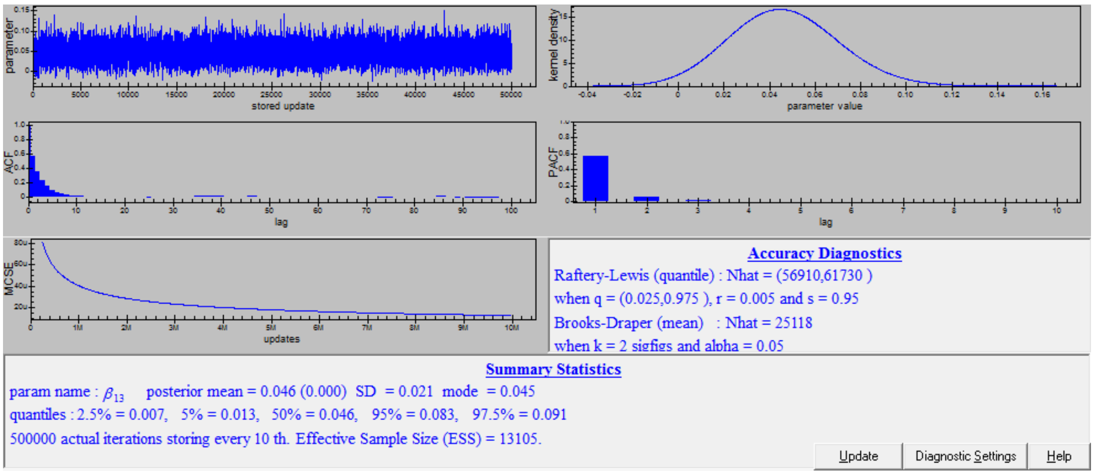
B5=-1.928(0.906)



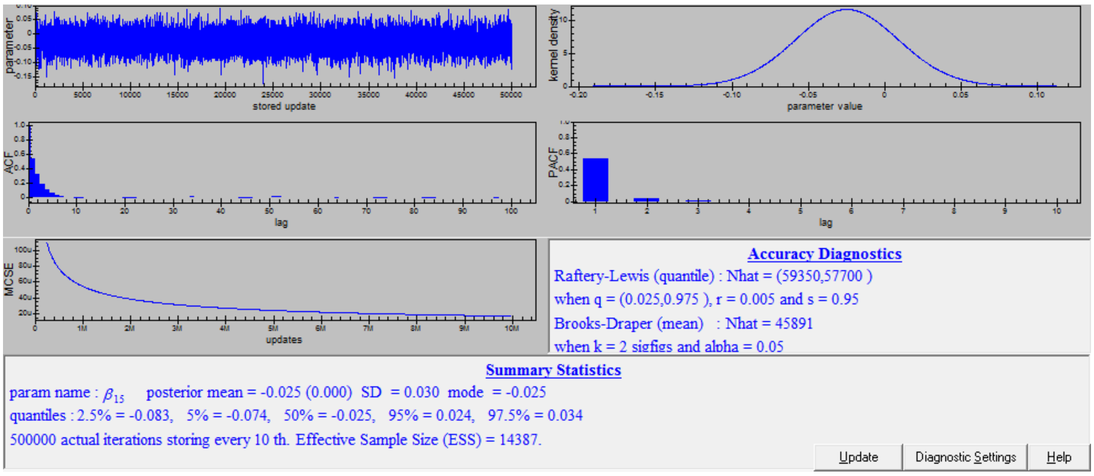
B12=-2.197(0.681)



B13=0.046(0.021)



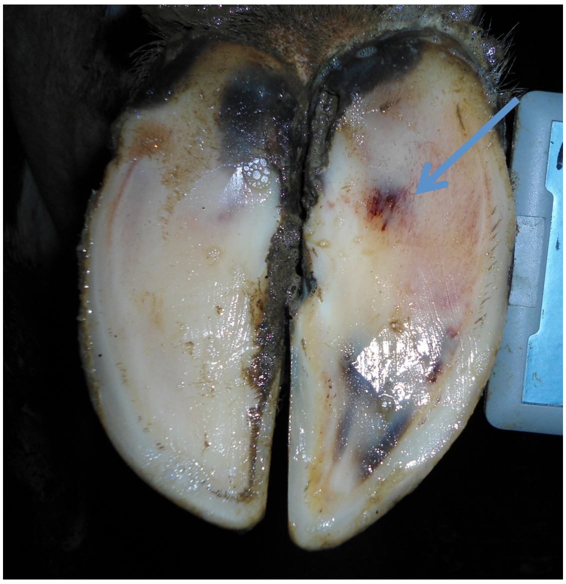
B15=-0.025(0.030)



Appendix 7 - Reliability of claw horn lesion scoring

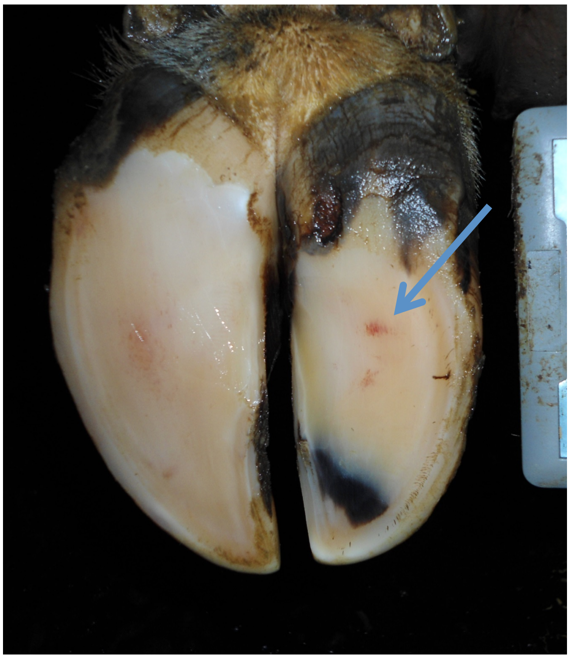
Reliability was carried out using 25 pictures, the slides were presented individually and an arrow pointing out the lesion to be identify. A label with lesion identification has been added at the bottom of each picture for the information of the reader.

Lesion Scoring Reliability



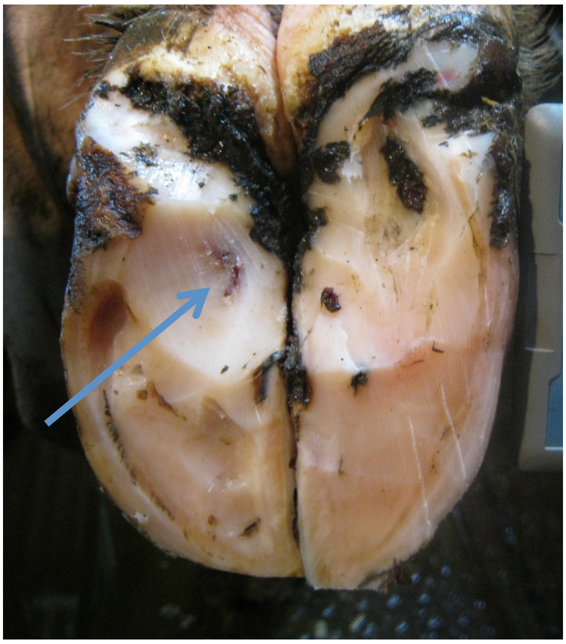
Picture 1

Identified as severe haemorrhage



Picture 2

Identified as moderate haemorrhage



Picture 3

Identified as mild ulcer

Picture 4

Identified as moderate white line haemorrhage

Picture 5

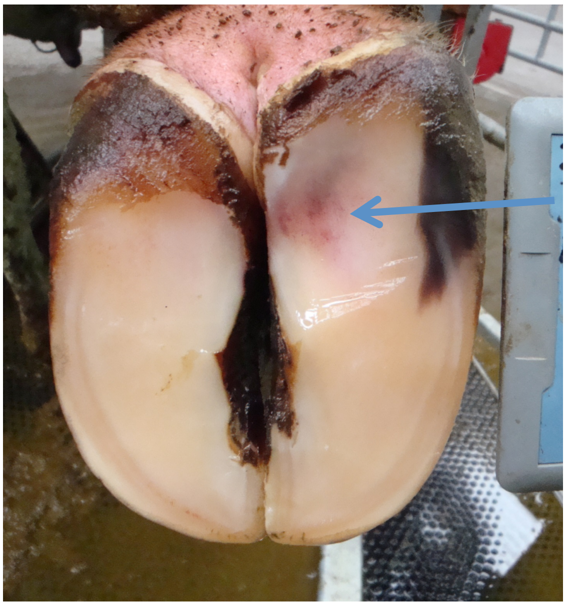
Identified as severe haemorrhage

Picture 6

Identified as severe ulcer

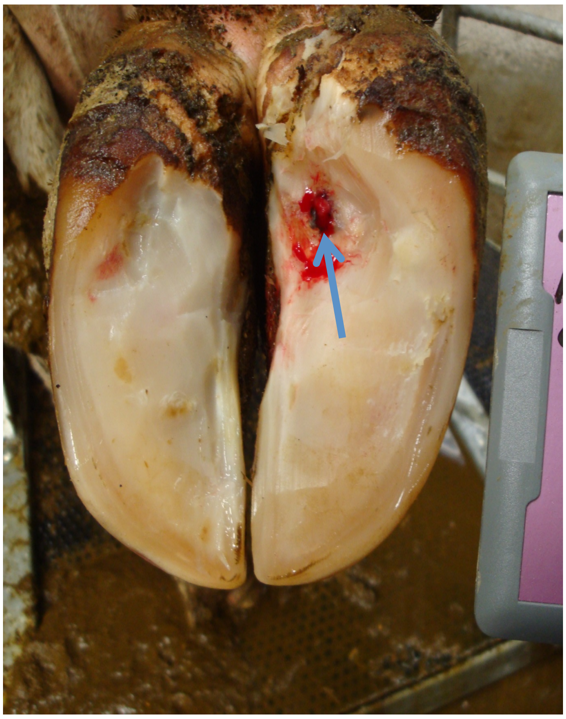
Picture 7

Identified as mild haemorrhage



Picture 8

Identified as moderate haemorrhage



Picture 9

Identified as mild haemorrhage at the WL

Picture 10

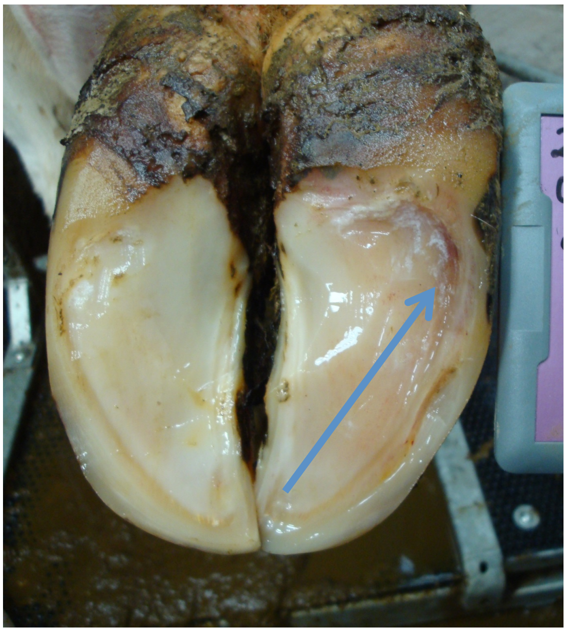
Identified as severe ulcer.

Picture 11

Identified as moderate separation

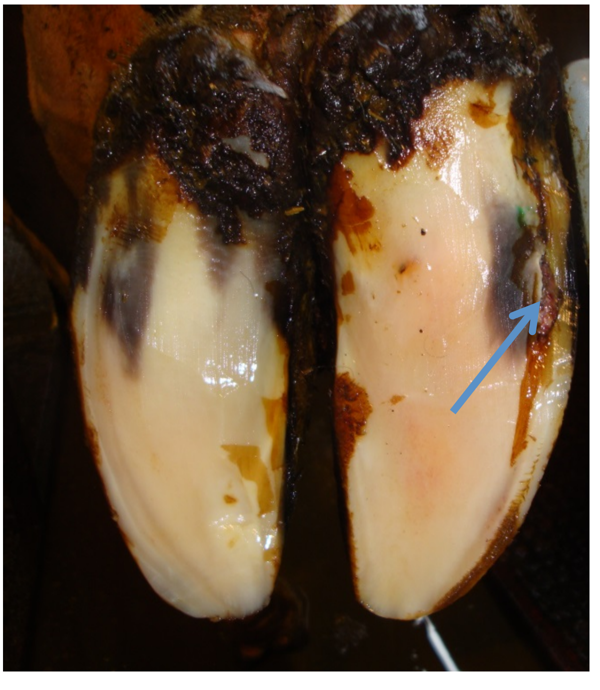
Picture 12

Identified as severe haemorrhage



Picture 13

Identified as severe separation



Picture 14

Identified as severe haemorrhage

Picture 15

Identified as mild haemorrhage

Picture 16

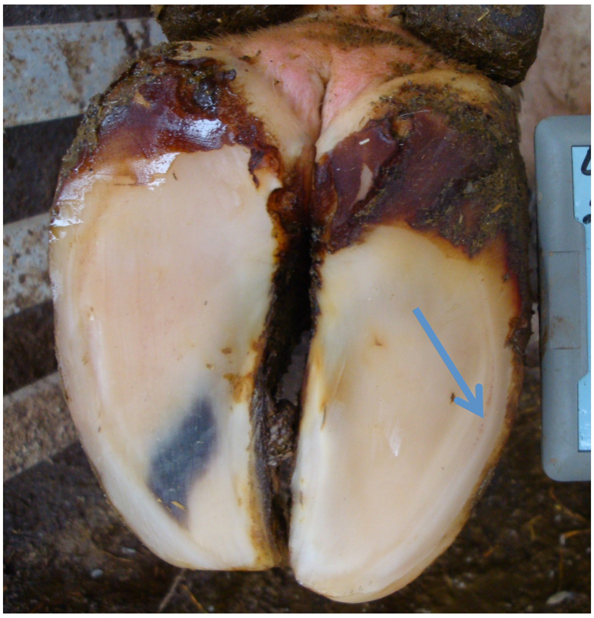
Identified as moderate haemorrhage

Picture 17

Identified as severe haemorrhage

Picture 18

Identified as moderate haemorrhage

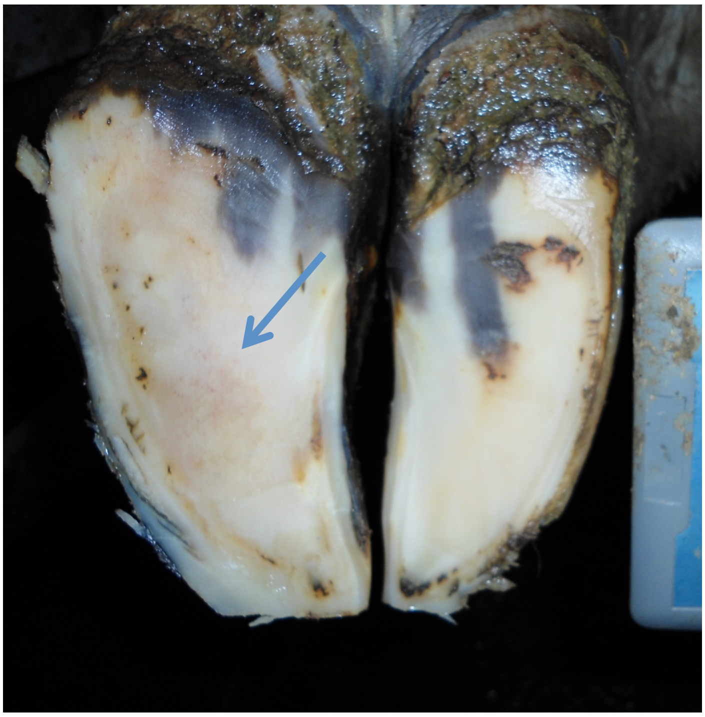


Picture 19

Identified as moderate haemorrhage

Picture 20

Identified as mild haemorrhage



Picture 21

Identified as no lesion

Picture 22

Identified as mild separation

Picture 23

Identified as no lesion

Picture 24

Identified as mild haemorrhage

Picture 25

Identified as mild ulcer