

**Epidemiological investigations into factors
associated with hock lesions, lameness and
fertility in dairy cattle**

by

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ABSTRACT

An investigation of 5186 hock maps, from 3691 cows with hair loss on the hocks from 76 UK dairy farms, identified six risk factors associated with area of hair loss: higher locomotion score; poor cleanliness; higher mean milk yield; low body condition score, prolonged winter housing and certain combinations of stall base and bedding materials. Another finding was significantly poor correlation between a categorical scale and the continuous measure of hair loss.

Hair loss on the hocks of 70 heifers (three herds) was observed monthly from Sep 2008-Mar 2010. The results imply that lameness precedes hair loss on the hock and not vice versa, i.e. lame animals develop hair loss rather than hock hair loss leads to future lameness. Poor cleanliness score and higher milk yield were associated positively with the risk of having hair loss on the hock.

Based on the total 6889 observations from 731 cows in four herds, cows with a greater decrease in BCS (compared to BCS at calving) had higher probability of becoming lame and lower probability of recovering. Also, increase in BCS from calving was associated with lower probability of cows moving from non-lame to lame state and higher probability from lame to non-lame. Days of lactation, months of calving and parity impacted upon both non-lame to lame and lame to non-lame transitions.

Analysis of 678 cows from four herds found that cows with chronic lameness had a lower probability of conception and a longer calving to conception period compared with healthy cows. Further, cows with lower average BCS had a lower probability of conception and were more likely to be lame. However, these factors didn't influence the likelihood of an individual AI leading to pregnancy.

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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.

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LIST OF ABBREVIATIONS

AI	Artificial Insemination service
BCS	Body Condition Score
CI	Confidence Interval/Credible Interval
Ca-Con	Days from Calving to Conception
DD	Digital Dermatitis
DIM	Days in Milk
DMI	Dry Matter Intake
Freq	Frequency
HL	Hair Loss
HR	Hazard Ratios
IQR	Inter Quartile Range
kg	Kilogram
l	Litre
m	Metre
Max	Maximum
MCMC	Markov chain Monte Carlo
Min	Minimum
Mm	Millimetre
MS	Mobility Score
NEB	Negative Energy Balance
OR	Odd Ratios
P-value	Significant value
SW	Swelling
UK	United Kingdom
UL	Ulceration
USA	United States of America

CHAPTER 1: GENERAL INTRODUCTION

1.1 Lameness

Lameness in cattle has been defined as “*lameness includes any abnormality which causes a cow to change the way that she walks, and can be caused by a range of foot and leg conditions, themselves caused by disease, management or environmental factors*” (DairyCo., 2014a). Lameness is a debilitating condition and is manifested in an abnormal walking style. It is often associated with tissue damage, pain and feeling of discomfort (O'Callaghan, 2002).

1.2 Incidence and prevalence

Lameness has been widely recognized as a major economic and welfare problem in dairy herds (Enting et al., 1997; Kossaibati and Esslemont, 1997; Kossaibati et al., 1999; Cha et al., 2010) around the world. Reported incidence rate and prevalence of lameness in dairy herds is variable; this may be due to the different types of measurement used and/or different management /risk factors. Incidence of lameness is calculated based on the number of new cases which occur in a group of animals over a specific period (Archer et al., 2010a). In the UK, the average reported lameness incidence is 50 limb cases per 100 cow-years, a range between 5-170 limb cases per 100 cow-years has been reported in previous studies (Eddy and Scott, 1980; Russell et al., 1982; Whitaker et al., 1983; Collick et al., 1989; Clarkson et al., 1996; Esslemont and Kossaibati, 1996; Whitaker et al., 2000; Hedges et al., 2001; Whitaker et

al., 2004; Archer et al., 2010a). Table 1.1 demonstrates the reported incidence of lameness in published studies across the world including the United Kingdom, Netherland, USA, Kenya and New Zealand. Overall, it seems that the lameness incidence rate has dramatically increased over recent decades.

Prevalence of lameness is calculated based on the total number of lameness cases over the total number of animals at risk at a specific time point (Archer et al., 2010a). There are numerous reports of lameness prevalence ranging from 0-69%, from many regions, including the United Kingdom, USA, Chile, Kenya, Czech, Sweden, China, Austria and Germany. Data are summarized in Table 1.2. In the UK, one study stated that farmers tend to underestimate the prevalence of lameness in their dairy herds, with 5.5% being their estimated value compared with 22.1% mean prevalence observed by an external recorder (Whay, 2002). This finding is similar to a study from the USA which reported that some herds underestimated their prevalence of lameness by up to 25% (Richert et al., 2013).

Table 1.1 Reported incidence rate of lameness in dairy cows in published studies

Country	Incidence of lameness (per 100 cows-year) Mean(range)	Measurement of lameness	Number	Remarks	Reference
UK	7.3 (0-32)	Treatment records	150 herds	Recorded by 6 veterinary practices.	(Eddy and Scott, 1980)
	5.5	Treatment records	1821 herds	Recorded by 8 veterinary practitioners.	(Russell et al., 1982)
	25 (2-55)	Treatment records	185 herds	Recorded by veterinary surgeons.	(Whitaker et al., 1983)
	24	Treatment records	90 herds	Recorded by 10 veterinary practitioners. Average herd size was 125. Lameness case recurring in the same limb within 28 days of initial treatment was excluded.	(Esslemont and Kossaibati, 1996)
	54.6(10.7-170.1) limb cases per 100 cows	Treatment records or received preventive foot-trimming	37 herds	Recorded by different people- veterinary surgeon, stockman, contractor and student. Average of size was 35-184; a new case was defined as the lameness case happen on the same cows in the different limb, or same limb more than 28days after a previous record.	(Clarkson et al., 1996)
	23.7	Treatment records	340 herds	Recorded by farmers. Average of herd size was 133 (33-432). Lameness case in the same claw within one month was excluded.	(Whitaker et al., 2000)

continued

Table 1.1 (continued) Reported incidence rate of lameness in dairy cows in published studies

Country	Incidence of lameness (per 100 cows-year) Mean(range)	Measurement of lameness	Number	Remarks	Reference
	68.9 (31.6-111.5) New cases per day	Treatment records	5 herds	Recorded by 6 veterinary practitioners. Herd size greater than 90 cows. This was an intervention study (effect of biotin supplementation).	(Hedges et al., 2001)
	20.7-23.3	Treatment records	434 herds	Recorded by farmers. Lameness case in the same claw within one month was excluded.	(Whitaker et al., 2004)
Netherland	9.3-49.2	Claw lesions diagnosed by vet	13 herds	Recorded by farmers and veterinarian. Most severe lesion was recorded. First lameness case was used in the analysis.	(Barkema et al., 1994)
Kenya	1.46 (per total cows per month)	Gait abnormality	78 herds	Recorded by researchers and farmers. Small scale herds were recruited- ten or less cows and less than 5ha of farmland. Only two visits in the study.	(Gitau et al., 1996)

continued

Table 1.1 (continued) Reported incidence rate of lameness in dairy cows in published studies

Country	Incidence of lameness (per 100 cows-year) Mean(range)	Measurement of lameness	Number	Remarks	Reference
USA	0.06; 0.15 (per total cows per month)	Treatment records	2 herds	Herd 1-Recorded by herd personnel; Herd 2-Recorded by hoof trimmer. Incidence rate calculated based on the number of lameness treatment and dividing by the number of cows during the month. Repeated cases of the same cows on different date were considered as separate events.	(Warnick et al., 2001)
	23.3	Disease treated with orthopedic blocks only	1 herd	Recorded by 3 farm employees. Only disease treated with orthopedic blocks were included (excluded all infectious disease)	(Bicalho et al., 2008)
	49.1 (per total cows per year) 1.41 (per 1000 cows per day)	Claw lesions verified by claw trimmers	4951 cows	Recorded by 2 claw trimmers. Every occurrence recorded was counted as a separate event; Incidence rate was calculated base on the new cases of claw lesion in a defined period given the number at risk.	(Sanders et al., 2009)
New Zealand	15 (12-19) (per 100 cows- days at risk)	Gait abnormality	1 herd	Recorded by farm staff. A new lameness case must 14 days after latest recorded date.	(Alawneh et al., 2011)

Table 1.2 Reported prevalence of lameness in dairy cows in published studies

Country	Prevalence of lameness Mean(range)/Mean $\pm$ SD	Measurement of lameness LS: locomotion score	Reference
UK	20.6 (2.0-53.9)	LS (scale 1-5) scale 3-5: lameness	(Clarkson et al., 1996)
	22.1 (0-50.0)	LS (scale 0-3) scale 2-3: lameness	(Whay et al., 2003)
	Organic farms: 24.2 (6.8-55.6)	LS (scale 0-3) scale 2-3: lameness	(Huxley et al., 2004)
	Zero grazing farms: 39 $\pm$ 0.02	LS (scale 1-5)	(Haskell et al., 2006)
	Grazing farms: 15 $\pm$ 0.01	scale 3-5: lameness	
	68 (48-72)	LS (scale 0-3) scale 2-3: lameness	(Archer et al., 2010b)
	36 (0-79)	LS (scale 0-3) scale 2-3: lameness	(Leach et al., 2010)
USA	Clinical lameness: 65.2	LS (scale 1-5)	(Sprecher et al., 1997)
	Severe lameness: 36.4	scale 3-5: clinical lameness	
	Summer: 21.1 $\pm$ 10.5	LS (scale 1-4)	(Cook, 2003)
	Winter: 23.9 $\pm$ 10.7	scale 3-4: lameness	
	24.6 (3.3-57.3)	LS (scale 1-5) scale 3-5: lameness	(Espejo et al., 2006)
	British Columbia: 27.9 $\pm$ 14.1	LS (scale 1-5)	(von Keyserlingk et al., 2012)
	California: 30.8 $\pm$ 15.5	scale 3-5: clinically lame	
	British Columbia: 7.1 $\pm$ 5.4	LS (scale 1-5)	
	California: 3.6 $\pm$ 4.2	scale 4-5: severely lame	
	8 (0-54)	LS (scale 1-5) scale 3-5: lameness	(Richert et al., 2013)
Chile	Large herd: 33.2	LS (scale 1-5)	(Tadich et al., 2010)
	Small herd: 28.7	scale 2-5: lameness	
Austria and Germany	34 (0-81)	LS (scale 1-5) scale 3-5: lameness	(Dippel et al., 2009)

continued

Table 1.2 (continued) Reported prevalence of lameness in dairy cows in published studies

Country	Prevalence of lameness Mean(range)/Mean $\pm$ SD	Measurement of lameness LS: locomotion score	Reference
Kenya	11.7	LS (scale 0-4) scale 2-4: clinically lame	(Gitau et al., 1996)
Czech	22 (6-42)	Lameness: any degree of limping on one or more leg.	(Dembele et al., 2006)
Sweden	4	LS (scale 0-3) scale 1-3: lameness	(Manske et al., 2002)
China	Clinical lameness: 31 $\pm$ 12 Severe lameness: 10 $\pm$ 6	LS (scale 1-5) scale 3-5: clinical lameness scale 4-5: severe lameness	(Chapinal et al., 2014)

1.3 The impact of lameness

Lameness is a multi-factorial disease that has negative impacts on the welfare and health of dairy cattle, with associated economic losses to dairy producers (Enting et al., 1997; Kossaibati and Esslemont, 1997; Kossaibati et al., 1999; Cha et al., 2010).

1.3.1 Production and economic losses

Lameness is the third most serious economic disease problem, behind the losses from mastitis and infertility (Enting et al., 1997; Kossaibati and Esslemont, 1997; Kossaibati et al., 1999; Cha et al., 2010). The cost of lameness has been categorized into direct and indirect costs, the average estimated direct and indirect costs of each lameness case were reported as £50

and £85 respectively (Kossaibati et al., 1999). The direct costs included treatment costs, cost of milk discarded, losses of shorter lactation period and waste of farmer's efforts, (e.g., labour time) while the indirect costs included loss due to increase in number of dairy cattle to be culled and reduction in fertility (Kossaibati et al., 1999). Different causes of lameness have different impacts on economic loss; sole ulcer (per case): £518.73, white line disease: £300.05, digital dermatitis: £75.57, interdigital lameness: £154.31 (Willshire and Bell, 2009).

Numerous studies have reported the impact of lameness on milk production. Lame cows had lower milk yield up to 4 months before and 5 months after lameness was diagnosed and treated, resulting in a 360kg reduction in milk yield per cow per 305 days of lactation (Green et al., 2002). Bicalho et al. (2008) also reported that total reduction in milk yield was 314-424kg per lame cow per 305 days of lactation. Archer et al. (2010b) reported that the reduction in milk yield was 350kg per cow per 305 days of lactation for cows which were severely lame in the first month of lactation. These findings demonstrate that lameness has impacts on milk yield, however, previous studies have also suggested that high milk yield is a risk factor for lameness (Green et al., 2002; Archer et al., 2010b). One possible explanation is cows with higher milk yield at higher risk of insufficient nutrition intake and longer standing time to eat and these may increase the risk to be lame. On the contrary, lame cows have lower dry matter intake and higher risk of other health diseases resulting lower milk yield (Green et al., 2002; Archer et al., 2010b).

Lameness has been associated with poor fertility, including lower conception rate, longer days from calving to conception and a higher number of artificial services per conception compared with non-lame cows (Sprecher et al., 1997; Melendez et al., 2003; Hultgren et al., 2004; Hernandez et al., 2005; Bicalho et al., 2007; Kilic et al., 2007; Machado et al., 2010). One study from Spain reported that increased calving interval and number of inseminations were associated with economic losses, with one unit change associated with the loss of \$4.90 (US dollar) and \$67.32 (US dollar) year per cow respectively (Gonzalez-Recio et al., 2004). (Further description of the relationships between lameness and fertility are described in 1.10.1.)

The impact of lameness on risk of culling/death has been published previously (Sprecher et al., 1997; Rajala-Schultz and Grohn, 1999; Booth et al., 2004; Bicalho et al., 2007; Machado et al., 2010). Lameness had up to twice the risk of being culled/dying compared with non-lame cows (Booth et al., 2004; Bicalho et al., 2007; Machado et al., 2010). Whitaker et al. (2000) reported that lameness is one of the main factors that forces farmers to involuntarily cull animals.

1.3.2 Welfare

Lameness is associated with pain and discomfort, where lame cows have been reported to have lower nociceptive threshold, indicating lame cows are more sensitive to pain (hyperalgesia) (Whay et al., 1998; O'Callaghan, 2002). The pain associated with lameness influences cows to have behaviour changes,

such as lame cows have longer lying times, spend less time eating, lower dry matter intakes, lower daily activity levels and higher frequency of abnormal lying and rising behaviours (Hedges et al., 2001; Galindo and Broom, 2002; O'Callaghan et al., 2003; Bach et al., 2007; Kilic et al., 2007; Ito et al., 2010; Häggman et al., 2012). Pain and feelings of discomfort also influences cows to change their normal gaits as demonstrated by change in mobility.

The duration and extent of pain resulting from lameness could be attributed to different causes and severity of lameness. Cows with claw horn lesions (sole ulcer and white line diseases) have longer duration of pain (lower nociceptive threshold was detected at day 28) (Whay et al., 1998) and more severe pain (higher posture scores) (O'Callaghan et al., 2003), whereas cows with acute digital tissue infection ('foul-in the foot' or digital dermatitis) have shorter duration of pain and less severe pain (lower posture scores). Generally cows with severe lameness also have severe pain (higher posture score) compared with cows with mild lameness (O'Callaghan et al., 2003).

1.4 Monitoring lameness

Numerous locomotion scoring systems have been designed to help farmers to detect lameness and for research purposes for monitoring the impact of lameness. Early detection could allow for prompt treatment and thus decrease the negative impact of lameness. Table 1.3 shows the locomotion scoring systems that have been reported in published studies (more detailed descriptions are provided in Appendix 1).

Table 1.3 A brief description of published locomotion scoring systems for cattle

Reference	Scoring system
(Manson and Leaver, 1988)	1.0-5.0 with 0.5 point intervals Based on the cow's gait on a concrete floor
(Tranter and Morris, 1991)	0-4 with 1 point intervals Based on head movement, degree of sinking of sound hindquarter, limb abduction/adduction and length of stride
(Wells et al., 1993)	0-4 with 1 point intervals Simplified from the system of Manson and Leaver (1988)
(Whay et al., 1997)	1-6 with 1 point intervals Devised from the system of Kestin et al. (1992)
(Sprecher et al., 1997)	1-5 with 1 point intervals Based on standing and walking with focus on the cow's back posture
(Winckler and Willen, 2001)	1-5 with 1 point intervals Reliability of this system has been evaluated in the study of Brenninkmeyer et al. (2007)
(Cook, 2003)	1-4 with 1 point intervals Developed from the system of Sprecher et al. (1997), Wells et al. (1993) and Manson and Leaver (1988)
(Whay et al., 2003)	0-3 with 1 point intervals Adopted by the UK dairy industry
(Flower and Weary, 2006)	1-5 with 1 point intervals A detailed description of each gait score was provided

Even though there are numerous scoring systems available, not many scoring systems have been evaluated for their reliability (intra-observer agreement and inter-observer agreement) (Thomsen et al., 2008). A modified five point scoring system (score 1= normal, score 2= uneven gait, score 3= mild lameness, score 4= lameness, score 5= severe lameness) based on Sprecher et al. (1997), Flower and Weary (2006), Haskell et al. (2006) and Rajkondawar et al. (2006) has been evaluated for reliability. The authors reported a good agreement between and within observers using this scoring system. However, authors also demonstrated lameness categories of this scoring system were not

equidistant, and therefore suggested further validation of this system (Thomsen et al., 2008).

In the UK, a 4 point scoring system described by Whay et al. (2003) is widely accepted and commonly used on farm even though evaluation of the reliability of this system has not been formally carried out (Reader et al., 2011). However, this system is simple and easily used by farmers without professional input (DairyCo., 2014b).

1.5 Causes of lameness

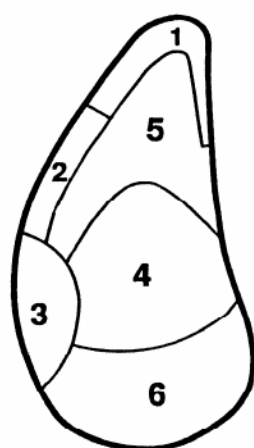
The common causes of lameness are claw horn diseases (e.g, sole ulcers, and white line disease), infectious diseases (e.g., interdigital necrobacillosis, digital dermatitis and heel erosions) and leg injuries, of which sole ulcers, white line disease and digital dermatitis are the most common (Clarkson et al., 1996; Hedges et al., 2001; Blowey, 2005; Potterton et al., 2012). Among leg injuries, hock lesions are one of the most common injuries found on dairy farms and have been thought to be one of the causes of lameness (Huxley and Whay, 2006). Further detail on claw horn lesions (sole ulcers, white line disease), digital dermatitis and hock lesions are stated below.

1.5.1 Claw horn lesions

Figure 1.1 demonstrates a commonly used claw zone diagram which can be used for recording the location of lesions. Sole ulcer, toe ulcer and heel ulcers

occur at zone 4, zone 5 and zone 6 respectively; white line disease occurs in either zone 1, 2 or 3 (Sanders et al., 2009; Shearer et al., 2012).

As mentioned above, of the claw horn lesions, sole ulcers and white line diseases are the most common (Clarkson et al., 1996; Bicalho et al., 2009). The aetiology of these conditions is not fully understood (Bicalho et al., 2009). A recent study reported that the prevalence of sole ulcers and white line disease were associated positively with the thickness of the digital cushion, possibly mediated through changes in body condition (Bicalho et al., 2009). The digital cushion is a layer under the third phalanx which acts as part of the suspension apparatus in dairy cattle. A further description of the relationships between the digital cushion and body condition score is outlined in section 1.9.1.



- Zone 1- White line at the toe
- Zone 2-Abaxial white line
- Zone 3-Abaxial wall-bulb junction
- Zone 4-Sole-bulb junction
- Zone 5-Apex of the sole
- Zone 6-Bulb

Figure 1.1 Claw zone diagram used for recording the location of lesions

(Adopted from Greenough and Vermunt, (1991))

1.5.1.1 Sole ulcers

Sole ulcer, also known as *Pododermatitis circumscripta* and Rusterholz ulcer, is a non-infectious lesion where the sole horn is damaged, leading to exposure of the corium. Primary disruption of the corium will then lead to trauma around pedal bone follow producing inflammation. It occurs at the sole bulb junction (zone 4 in Figure 1.1). Generally, sole ulcers occur in both hind legs, usually on the lateral claws, and more rarely in the front legs, usually on the medial claw. These lesions are painful and can lead to a severe mobility problem. Affected dairy cattle may change their gait in order to put more weight on the healthy claw and to avoid the affected claw touching the floor. Sole ulcers have been reported to be associated with a lower first service conception rate, a longer calving interval, a lower conception rate, a higher culling rate and lower milk yields (Hultgren et al., 2004; Amory et al., 2008; Machado et al., 2010). Pain resulted from sole ulcers is generally long lasting, therefore prevention, e.g, foot trimming, key to control (Andrews, 1992).

1.5.1.2 White line diseases

The white line, is the junction between the hoof wall and sole horn. White line disease is a non-infectious disease where foreign objects penetrate into the white line or separation occurs between the wall and the sole horn. The white line is composed of horn which is softer than horn of the wall or sole. Infection will most likely occur after foreign objects penetrate through to the corium potentially leading to abscessation or hemorrhage. White line disease,

like sole ulcer, is also associated with economic loss and has negative impacts on fertility (Amory et al., 2008; Machado et al., 2010). Generally, foot trimming is the most common treatment applied to white line disease. However, complete recovery from the disease is difficult where previous published studies have found that lower nociceptive threshold on day 28 compared to non-lame cows (Whay et al., 1998; O'Callaghan, 2002), therefore, prevention such as reducing time standing and providing better walking surface to cows is the key to reduce levels of disease (Archer et al., 2010a).

1.5.2 Digital dermatitis

Digital dermatitis (DD), also known as papillomatous digital dermatitis or Mortellaro's disease, is a very painful condition and easily spreads amongst the whole herd. Infection occurs in the soft tissue between the claws and the heel. The early lesions are dry, hyperkeratosis and painless while the severe lesions are seen as moist, fluid, matted hairs, interdigital cleft and painful (Andrews, 1992; Dopfer, 2009). DD is thought to spread to other cows through wet and moist surroundings, such as mud, manure etc. Cows with DD often change their gait and spend more time lying down in order to avoid pressure on the infected feet. Infected dairy cattle have a lower fertility rate, lower milk yield and bring economic loss to the herds (Argaez-Rodriguez et al., 1997; Cha et al., 2010). In the UK, footbathing, systemic antibiotic and individual topical treatments are common treatments used for controlling DD, of which footbathing is most frequently used by farmers (Laven and Logue,

2006). However, keeping the environment clean and dry is the key to controlling the spread of DD and economic loss due to infection.

1.6 Hock lesions

Leg injuries have been reported as causes of lameness in dairy cattle, amongst these, lesions around the hock are most common (Wechsler et al., 2000; Kielland et al., 2009), accounting for 85% of reported leg injuries. In comparison, the other leg injuries reported include: lesions at the carpus (9%), knee (3%) and other joints injuries (3%) (Busato et al., 2000; Weary and Tazskun, 2000). Lesions on the hock are thought to be caused by abrasion on hard lying surface or contact with cubicle fittings and it is logical to think that these lesions can bring discomfort and pain to dairy cattle (Huxley and Whay, 2006).

The prevalence of hock lesions has been recorded across the world including, the United Kingdom (Whay et al., 2003; Rutherford et al., 2008; Potterton et al., 2011b), Europe (Brenninkmeyer et al., 2012), Canada (Zurbrigg et al., 2005b), the United States (Barberg et al., 2007; Fulwider et al., 2007; Lombard et al., 2010; Lobeck et al., 2011), China (Chapinal et al., 2014) and Norway (Kielland et al., 2009). The reported prevalence of hock lesions varies between farms (in Table 1.4); similar to lameness, these differences could be attributed to the different housing/management system used on these farms and the different scoring systems that were employed in the studies.

Table 1.4 Reported published prevalence of hock lesions in dairy cows according to lesion types and combinations: hair loss, ulceration and swelling

Country	Prevalence of hock lesions Mean(range)	Reference
Hair loss on the hock		
UK	30.0 (14.3-65.5)	(Whay et al., 2003)
US	Compost bedding: 24.1 (2.0-43.9)	(Barberg et al., 2007)
Europe		
Germany and Austria	50 (0-100)	(Brenninkmeyer et al., 2012)
Ulceration on the hock		
UK	8.3 (0-22.5)	(Whay et al., 2003)
Europe		
Germany and Austria	36 (0-97)	(Brenninkmeyer et al., 2012)
Swelling on the hock		
UK	35.0 (22.2-66.6)	(Whay et al., 2003)
	Organic farms: 4.6	(Rutherford et al., 2008)
	Nonorganic farms: 8.3	
US	Compost bedding: 1 (0-3.3)	(Barberg et al., 2007)
Europe		
Germany and Austria	36 (0-100)	(Brenninkmeyer et al., 2012)
Combination of two or three lesions on the hock (hair loss, ulceration or swelling)		
UK	Organic farms: 37.2	(Rutherford et al., 2008)
	Nonorganic farms: 49.1	
	(Based on hair loss and ulceration)	

continued

Table 1.4 (continued) Reported published prevalence of hock lesions in dairy cows according to lesion types and combinations: hair loss, ulceration and swelling

Country	Prevalence of hock lesions Mean(range)	Reference
US	Compost barn: 0	(Fulwider et al., 2007)
	Rubber-filled mattress: 71.6	
	Sand: 25	
	Waterbed: 35.2	(Lombard et al., 2010)
	(score 1-3)	
	(Based on hair loss, ulceration and swelling)	
	23.5 (score 2-3)	(Lobeck et al., 2011)
	(Based on hair loss and swelling)	
	Cross-ventilated freestall barns:	
	3.8 (0-29.1)	
Norway	Compost bedded pack barns:	(Richert et al., 2013)
	41.2 (2.2-72.4)	
	Conventional ventilated freestall barns:	
	23.9 (0-67.9)	(Kielland et al., 2009)
	(score 2-3)	
	(Based on hair loss, and swelling)	
	19 (0-95)	(Weary and Taszkun, 2000)
	(Based on hair loss, ulceration and swelling)	
	60.5 (score1-3)	
	(Based on hair loss, and ulceration condition)	
Canada	Sawdust: 69.7 (51.9-93.5)	(Zurbrigg et al., 2005b)
	Mattress: 91.7 (84.9-97.6)	
	Sand: 23.8 (5.3-59.5)	
	(Based on hair loss and ulceration)	(Chapinal et al., 2014)
	44 (score 2- 3)	
China	(Based on hair loss, swelling and ulceration)	
	40 (6-95) (score 2-3)	
	5 (0-50) (score 3)	
	(Based on hair loss and swelling)	

1.6.1 Description of hock lesions

The hock can be divided into five areas, which are the lateral tuber calcis, lateral tarsal joint, medial tuber calcis, medial tarsal joint and dorsal tuber calcis (Figure 1.2). Hock lesions are the most commonly observed on the lateral tarsal joint and lateral tuber calcis, the medial tarsal joint is the least commonly affected site (Weary and Taszkun, 2000; Fulwider et al., 2007; Potterton et al., 2011b). The type of bedding material is suggested to be the predominant risk factor for the severity of lesions occurring at different locations of the hock. Several studies have reported that cows housed in a cubicle with mattresses often have lesions on more than one location on the hock (e.g., dorsal tuber calcis, lateral tuber calcis and lateral tarsal joint) whereas cows which are housed in a cubicle with sand, sawdust or waterbeds are likely to have lesions on just one location (dorsal tuber calcis) (Weary and Taszkun, 2000; Mowbray et al., 2003; Fulwider et al., 2007). The further explanation of the development of hock lesions on different type of bedding is presented in 1.7.2.

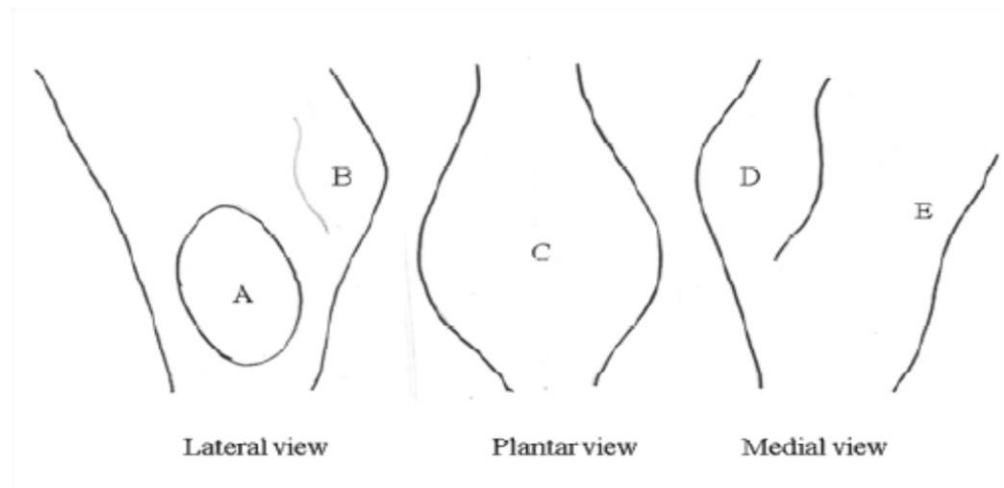


Figure 1.2 The five locations on the hock from lateral, plantar and medial view respectively

A: lateral tarsal joint, B: lateral tuber calcis, C: dorsal tuber calcis, D: medial tuber calcis, E: medial tarsal joint (Adopted from Potterton et al. (2011b))

1.6.2 Measuring hock lesions

A common standard scoring system for hock lesions does not exist, although various types of system to assess the severity of hock lesions have been described. The three main presentations of lesions over the hock are hair loss, ulceration and swelling. Different scoring system used different classification of lesions, for instance, some scoring systems merged the three presentations of lesions into one scoring system (Chaplin et al., 2000; Livesey et al., 2002; Zurbrigg et al., 2005b; Fulwider et al., 2007), two presentations of lesions either hair loss and swelling (Barberg et al., 2007; Lombard et al., 2010; Husfeldt and Endres, 2012) or hair loss and ulceration (Gustafson, 1993; Weary and Taszkun, 2000; Wechsler et al., 2000; Regula et al., 2004; Keil et al., 2006; Norring et al., 2008; Rutherford et al., 2008) and others have used a separate scoring system for each lesion (Vokey et al., 2001; Huxley and

Whay, 2006). Table 1.5 summarizes scoring systems which have been used in published studies (more detailed descriptions are provided in Appendix 2). Researchers have tended to use their own scoring systems to evaluate the severity of lesions and therefore results are difficult to compare.

The progression and development of hock lesions remains unclear. From the scoring systems which have been developed, some researchers have indicated a progression of lesion severity starting from hair loss and ending with swelling (Barberg et al., 2007; Lombard et al., 2010; Husfeldt and Endres, 2012) or terminating with ulceration (Gustafson, 1993; Weary and Tazskun, 2000; Regula et al., 2004; Norring et al., 2008; Rutherford et al., 2008); or starting from hair loss, followed by wounds and ending with swelling (Chaplin et al., 2000; Fulwider et al., 2007). There is one study from the UK which reported that the risk factors for hair loss, ulceration and swelling are unique and there is no common risk factor across all these three lesion presentations (Potterton et al., 2011a). Only one risk factor for swelling was shared with hair loss (bedding material) and none with ulceration. This finding suggests that the aetiology of these lesions may be different. Furthermore, Potterton et al. (2011a) also reported that there were five similar risk factors between hair loss (of 14 risk factors identified) and ulceration (of 10 risk factors identified) (herd size, mean milk yield, mobility score, number of days of winter housing, base material) but the fact that a large proportion of identified risk factors were unique suggests that ulceration may not be a progression of hair loss. However in the longitudinal study by Livesey et al. (2002) factors (e.g. use of mats) increased risk of both severe (e.g ulceration) and total (including hair

loss) hock lesions suggesting prevention of hair loss would help in prevention of severe hock lesions despite there could be other unique factors that could cause hair loss. Therefore, it suggests that the severity of hair loss, ulceration and swelling over the hock should be measured using the separate scoring system instead of using scoring systems which merge two or three presentations of lesions, to understand the development of these lesions.

Table 1.5 A brief description of scoring systems used to assess hock lesions in dairy cattle from published studies

Reference	Scoring system	Brief description
(Gustafson, 1993)	Categorical (5 categories) Score 0-4	Based on hair loss and ulceration
(Wechsler et al., 2000)	Categorical (4 categories) 2 categories of hairless, 2 categories of scabs or wounds	Based on hair loss and ulceration
(Weary and Taszkun, 2000)	Binary Score 1 or score 2	Based on hair loss and ulceration
(Chaplin et al., 2000)	Categorical (5 categories) Score 0-5	Based on hair loss, swelling and ulceration * This system was extended from the Gustafson (1993) scoring system.
(Vokey et al., 2001)	Categorical (3 categories) Score 0-2	Based on hair loss
	Categorical (3 categories) Score 0-2	Based on swelling
(Livesey et al., 2002)	Categorical (3 categories) Score 0-2	Based on hair loss, swelling and ulceration
(Regula et al., 2004)	Categorical (4 categories) Score 0-3	Based on hair loss and ulceration
(Zurbrigg et al., 2005b)	Categorical (4 categories) Score 0-3	Based on hair loss, ulceration and swelling

continued

Table 1.5 (continued) A brief description of scoring systems used to assess hock lesions in dairy cattle from published studies

Reference	Scoring system	Brief description
(Huxley and Whay, 2006)	Categorical (4 categories)	Based on hair loss
	Score 0-3	
	Categorical (4 categories)	Based on ulceration
(Keil et al., 2006)	Score 0-3	
	Categorical (4 categories)	Based on swelling
	Score 0-3	
(Fulwider et al., 2007)	Categorical (3 categories)	Based on hair loss and ulceration
	Hairless patches, scabs, open wounds	
	Categorical (4 categories)	Based on hair loss, swelling and ulceration
(Barberg et al., 2007)	Score 0-3	
	Categorical (3 categories)	
	No lesion, mild and severe lesions	
(Norrington et al., 2008)	Categorical (6 categories)	Based on hair loss and ulceration
	Score 0-5	
	Categorical (3 categories)	Based on hair loss and swelling
(Rutherford et al., 2008)	Score 1-3	
	Binary	Based on hair loss and ulceration
	Sound or damaged	
(Lombard et al., 2010)	Categorical (3 categories)	Based on hair loss and swelling
	Score 1-3	
	Categorical (3 categories)	Based on hair loss and swelling
(Husfeldt and Endres, 2012)	Score 1-3	

* This system was extended from the Weary and Taskun (2000) scoring system

All of these systems are inherently subjective measuring methods using categorical descriptors, consequently variations within and between observers could lead to study bias. An observer may have different interpretations for the terms used to describe each category and therefore increase the measurement variations between observers, this phenomenon is called “observer drift”(Smith, 1986). Observers may also struggle to give a score when the lesion falls between the descriptors for two scores, in this situation they will

select either of the scores depending on their preferences, resulting in unaccountable study bias (Engel et al., 2003). Yet, unlike lameness scoring there is no published study to evaluate the reliability of the categorical scoring system used to score hocks, i.e. whether there is consistency of measurement results for the same severity of lesions. The reliability of scoring system has to be investigated in order to understand whether the current scoring systems are useful as tools for scoring hock lesions in dairy cattle.

In response to the subjectivity of the scoring system, Potterton et al. (2011b) proposed a measuring method that is relatively more objective based on a hock map. The details of the hock map used are presented at Chapter 3. An observer after training could record the location, area and shape of lesions using the hock map and then use a computer program to calculate the area of the lesion. Thus the hock map provides a relatively more objective measure and continuous data on the lesion severity. No comparisons have been made to understand how this hock map measurement equates to scores on a categorical scoring scale. Furthermore, there are no studies which have investigated the risk factors associated with the severity of hock lesions, based on continuous measurements of these lesions.

1.7 Risk factors for lameness and hock lesions

A large number of risk factors for the severity of lameness and hock lesions, related to aspects of the cow's environment, management practices and

individual animal characteristics have been demonstrated in previous studies. A summary of these is discussed in the sections below.

1.7.1 Risk factors associated with lameness

Despite different causes of lameness, herd level and cow level risk factors for lameness have been published in a wide range of studies, and are outlined below.

Herd level risk factors

Farms with housed cows in cubicles or free-stalls had a higher prevalence of lameness compared with those who housed cows in a straw yard or tie stall (Sogstad et al., 2005; Haskell et al., 2006; Rutherford et al., 2009). Compared with organic farms, conventional (non-organic) farms have been reported to have a higher prevalence of lameness, possibly because organic farms have less emphasis on milk production and more on constructing a comfortable environment for cattle (Rutherford et al., 2009).

Reduction of lying comfort was associated positively with risk of lameness (Dippel et al., 2009). Dippel et al. (2009) reported that cows housed on mats or mattresses had a higher risk of lameness compared with cows on deep-bedded stalls. Cows housed on sand stalls had a lower prevalence of lameness compared to those housed on mattresses stalls (Espejo et al., 2006). Lying comfort affects the behaviour of dairy cattle (lying time, standing time) and

consequently increases the risk of lameness (Cook et al., 2008; Ito et al., 2010).

Suitable cubicle design in the housing of cattle should provide a comfortable space for the cows in order to reduce the risk of lameness. Longer cubicle lying areas have been associated with lower herd prevalence of lameness (Sogstad et al., 2005; Haskell et al., 2006; Dippel et al., 2009; Rutherford et al., 2009). Shorter lunging space and standing area in milking parlour have also been reported to increase the risk of lameness (Haskell et al., 2006; Rutherford et al., 2009). It has been proposed that inadequate cubicle design might increase the probability of abnormal lying and rising behaviour, thereby resulting in the occurrence of lameness.

Aspects of farm management practices used on farms have been investigated in various studies. The reported high prevalence of lameness in larger herds, could be due to less time being allocated to each cow and therefore a lower chance of rapid identification and treatment (Alban, 1995; Whitaker et al., 2000; Chapinal et al., 2014). Grazing practice has also been reported to influence the risk of lameness, where zero grazed cows were more likely to be lame (Haskell et al., 2006; Rutherford et al., 2009). This was possibly due to the pasture being a more comfortable surface for lying and standing therefore leading to cows having a better gait score (Hernandez-Mendo et al., 2007). Cows that receive foot trimming have a lower risk of lameness compared to cows that do not receive foot trimming (Manske et al., 2002), this is also supported by DairyCo guidelines (2014c).

Cow level risk factors

Higher parity number, older age, larger hip width and heavier cows are at a generally higher risk of lameness (Wells et al., 1993; Sogstad et al., 2005; Haskell et al., 2006; Dippel et al., 2009; Rutherford et al., 2009). An increase in milk yield has also been associated with a higher risk of lameness (Rutherford et al., 2009). Cows that were ‘ever lame’ had higher milk yield (approximately 330-342kg per cow per 305 days of lactation) compared with cows which were ‘never lame’ (Green et al., 2002; Archer et al., 2010b). Poor body condition score has been reported to be positively associated with the risk of lameness (Espejo et al., 2006; Dippel et al., 2009; Hoedemaker et al., 2009), however a full understanding of this association is still lacking. Further description of the relationship between BCS and lameness is described in section 1.9.1.

1.7.2 Risk factors associated with hock lesions

Similar to lameness, many risk factors have been described in previous studies to be associated with the presence of hock lesions. These include, housing system, lying comfort, cubicle design, management practices and factors related to the characteristics of the cow. Of these, lying comfort is considered as the predominant risk factor (Huxley and Whay, 2006; Lombard et al., 2010).

Herd level risk factors

The prevalence of hock lesions in cows housed in free-stalls has been reported to be higher than those housed in a straw yard (Haskell et al., 2006; Rutherford et al., 2008), but it was lower compared to cows housed in tie stalls (Busato et al., 2000; Regula et al., 2004). Organic farms and compost-bedded pack barns have a lower prevalence of hock lesions compared with conventional farms (Rutherford et al., 2008; Lobeck et al., 2011). In organic farms, cows are more likely to have lower cleanliness score (i.e. cleaner), lower somatic cell count and longer grazing duration compared to non-organic farms, all these factors reducing the risk of hock lesions (Ellis et al., 2007; Rutherford et al., 2008).

Shorter length of cubicle, shorter tie-chains, lower stall gradient, presence of an electric trainer, less feed space and less standing area per cow are further reported risk factors for hock lesions (Weary and Taszkun, 2000; Zurbrigg et al., 2005a; Haskell et al., 2006; Fulwider et al., 2007; Rutherford et al., 2008; Potterton et al., 2011a). Improper cubicle design and less space per cow may increase the difficulty of rising and lying (Regula et al., 2004; Tucker et al., 2004). Hocks that are more often in contact with edges and cubicle fittings, result in more lesions (Potterton et al., 2011a).

Farms with a longer duration of grazing and shorter duration of winter housing have been reported to have fewer hock lesions (Rutherford et al., 2008; Potterton et al., 2011a). A larger herd size and higher stocking rate per cubicle, where the cows have higher risk of colliding with each other in crowded

environment are associated positively with hock lesions (Rutherford et al., 2008; Potterton et al., 2011a; Chapinal et al., 2014).

Lying comfort has been suggested as the predominant risk factor for hock lesions (Huxley and Whay, 2006; Lombard et al., 2010). Harder lying surfaces are more abrasive and less protective of the hocks and therefore more lesions on the hocks were found. Farms that used mattresses in free-stalls have a higher prevalence of hock lesions compared with those using sand or waterbeds (Weary and Taszkun, 2000; Fulwider et al., 2007), but a lower prevalence compared with those using rubber mats and concrete flooring (Livesey et al., 2002; Rushen et al., 2007; Kielland et al., 2009).

Very few studies have looked into the development of hock lesions for different types of bedding materials over time. Livesey et al. (2002) investigated the development of hock lesions in cows housed in straw yards, cubicles with mats or cubicles with mattresses. After 26 weeks housing, the cows housed in cubicles with mats had the most severe (higher score) and total hock lesions (total number of hocks with maximum score), while the cows housed in straw yards had the least severe (lower score) and total hock lesions (Livesey et al., 2002). This finding is supported by cross-sectional studies which reported that straw yards are associated with fewer hock lesions (Haskell et al., 2006; Rutherford et al., 2008). Among these three types of bedding materials, straw yards were suggested to be a more comfortable lying surface for the cows, resulting in reduction in skin friction on the hocks and an increased probability of lesion recovery.

A study from US compared the effects of two alley surfaces (grooved concrete and rubber mats) and three bedding materials in the cubicle (deep sand, rubber mattresses with sawdust or concrete with sawdust) on the development of hock lesions over 15 weeks (Vokey et al., 2001). The cows housed in deep sand stalls showed a significant improvement compared with the cows which were housed on a concrete base with sawdust (Vokey et al., 2001). This is consistent with a previous cross-sectional study which found that cows housed in deep sand stalls had a lower risk of hock lesions (Weary and Tazskun, 2000).

Comparison of development of hock lesions between cows housed on mattress and deep bedded sand stalls was investigated over six weeks (Mowbray et al., 2003). The cows housed in mattress stalls had greater area of hair loss on the hock joint, but smaller area of hair loss or skin breakage on the point of the hock (Mowbray et al., 2003).

From the results outlined above, it appears that lying comfort is essential for the prevention of hock lesions in cows. However, measuring degree of lying comfort is complex. There are three main features of lying comfort, including types of base, materials used as a bedding substrate and the depth of bedding material used. Including these three features in the analysis could give us more understanding about how lying comfort affects hock lesions. Researchers tend to investigate either one or two of these three features in their studies (Weary and Tazskun, 2000; Livesey et al., 2002; Fulwider et al., 2007; Rushen et al., 2007; Kielland et al., 2009) and there is no study to investigate these three

features together in one study probably because of the numerous possible combinations of base, depth of bedding and type of bedding.

Cow level risk factors

Within the same housing system, each cow has a different risk of hock lesions. Greater age, larger hip widths, higher parity number, lower BCS, dirtier hind limb and udder, higher milk yield and lameness have been significantly associated with hock lesions (Haskell et al., 2006; Rutherford et al., 2008; Potterton et al., 2011a). Lameness is more likely to have hock lesions (Whay et al., 2003; Regula et al., 2004; Kielland et al., 2009; Brenninkmeyer et al., 2012), possibly because lame cows have a longer total lying time and an increased frequency of lying bouts (Chapinal et al., 2009; Ito et al., 2010); however this hypothesis has not been investigated in any published studies.

Comparing risk factors between studies is difficult because of the different scoring systems used. Furthermore, researchers have tended to investigate risk factors for the presence or absence of lesions and there is a lack of published work on risk factors specifically associated with the severity of a hock lesion (including risk factors associated with the severity of hock hair loss).

Hair loss is the most prevalent hock lesion on dairy farms (Huxley et al., 2004; Potterton et al., 2011a; Potterton et al., 2011b) and the direct impact of hair loss is unknown, but hair loss alone is suggested as an indicator of abrasion from the lying surface (Livesey et al., 2002; Mowbray et al., 2003; Huxley and

Whay, 2006). Understanding risk factors associated with the severity of hair loss on the hock is essential for decreasing the impact of hock lesions on farm.

1.8 Association between lameness and hock lesions

There is little evidence of the direct impact of hock lesion on the welfare of dairy cattle (Huxley and Whay, 2006; Rutherford et al., 2008; Laven and Livesey, 2011), but it is logical to think that these lesions bring discomfort and pain to dairy cattle (Huxley and Whay, 2006) and therefore they have been suggested as a welfare indicator (Whay et al., 2003; Regula et al., 2004).

A positive association between lameness and hock lesions has been reported in multiple studies (Whay et al., 2003; Regula et al., 2004; Kielland et al., 2009; Brenninkmeyer et al., 2012; Richert et al., 2013), however, the directionality of this association remains unknown. Hock lesions may predispose cows to becoming lame in the future as lesions over the hocks might influence walking posture, rising and lying behaviours and eventually increase the risk of lameness. Alternatively, lameness may increase the chance of cows having hock lesions in the future as lame cows spend a longer time lying down and show changes their rising and lying behaviour (Chapinal et al., 2009; Ito et al., 2010); this may increase the chance of having hock lesions when the hocks collide with the bedding surface or cubicle fittings more often. Understanding the directionality of the relationship between lameness and hock lesion is important to improve the health and welfare effects associated with these two conditions.

1.9 Body condition score

Body condition score (BCS) has been widely used as a management tool in the dairy industry and is well recognized by researchers, scientists, farmers and veterinarians (Gillund et al., 2001; Roche et al., 2004; Berry et al., 2007a; Berry et al., 2007b; Roche et al., 2009; Kellogg, 2010). Body condition score is used to monitor energy balance and body fat in individual cattle (Edmonson et al., 1989; Bewley and Schutz, 2008; Hoedemaker et al., 2009; Kellogg, 2010).

Similar to locomotion scoring systems, there are various types of scoring systems described for assessing body condition of cows, a five point scoring systems with 0.25 or 0.5 intervals is commonly used in the UK (systems developed by Wildman et al. (1982) and later modified by Edmonson et al. (1989)). All systems start with the lowest value indicating the thinnest animals and the highest value indicating the fattest animals (Bewley and Schutz, 2008). There are several studies which have reported the body condition scoring system developed by Edmonson et al. (1989) to be a valid tool when compared to the ultrasound measurement of subcutaneous fat (Domecq et al., 1995; Hussein et al., 2013). Compared to ultrasound, measuring BCS is simple, less costly and easier to perform on farm.

Cows have different BCS throughout lactation. DairyCo (2014d) have suggested target values for BCS at every stage of lactation (Table 1.6). In early lactation, cows usually have lower BCS and the BCS will then increase

until the end of lactation (Figure 1.3). Cows in low BCS are experiencing negative energy balance (NEB) (Coffey et al., 2002; Ahmed et al., 2010; Kellogg, 2010) where intake is insufficient compared to energy used, therefore cows will mobilize body tissues and fats to support the energy deficiency. A wide range of studies have shown that cows experiencing NEB (low BCS) have a higher risk of metabolic disorders, higher risk of lameness, higher risk of endometritis, elevated somatic cell counts, lower milk yield, poorer reproductive performance, poorer fertility rate and a higher risk of culling (Suriyasathaporn et al., 1998; Heuer et al., 1999; Espejo et al., 2006; Berry et al., 2007b; Dippel et al., 2009; Hoedemaker et al., 2009; Kellogg, 2010; Machado et al., 2010; Bicalho, 2012).

Table 1.6 Target BCS of dairy cattle at every stage of lactation

Stage of lactation	Target BCS
At calving	2.5-3.0
60 days post-calving	2.0-2.5
100 days before drying off	2.5-3.0
At drying off	2.5-3.0

(Adopted from DairyCo., (2014d))

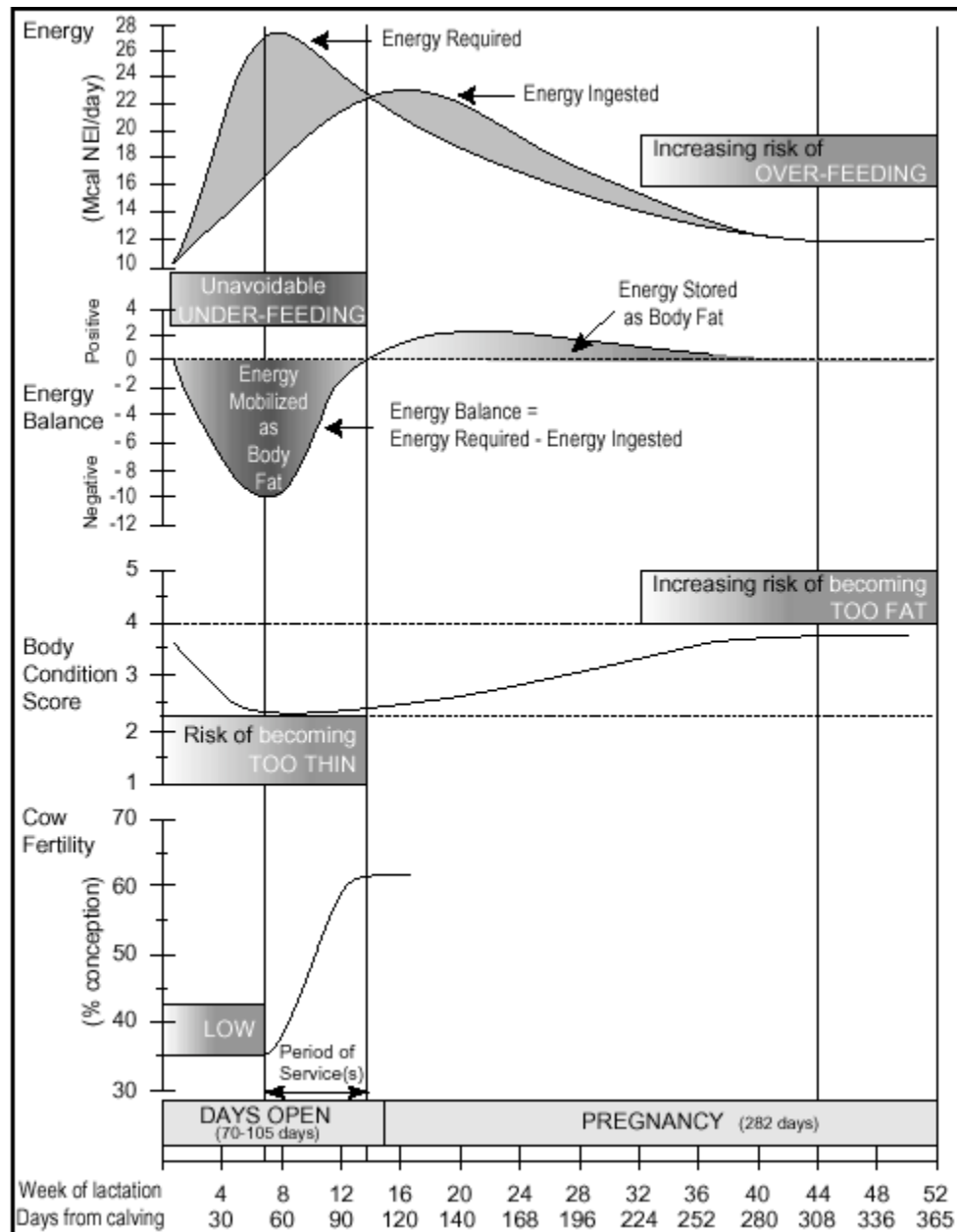


Figure 1.3 Changes of body condition score, milk yield and feed intake throughout lactation (Adopted from Howard et al., (1994))

1.9.1 Association between lameness and body condition score

Cross-sectional studies have reported a negative association between BCS and the risk of lameness (Espejo et al., 2006; Dippel et al., 2009; Hoedemaker et al., 2009), however there is limited evidence on the directionality of the

relationship. A study conducted in the UK reported that cows with prior low BCS ($BCS < 2.5$) had a higher risk of treatment for lameness in a 30 day risk period (Green et al., 2013). The findings of Green et al. (2013) suggested that low BCS is causing cows to have a higher risk of claw horn disease. Bicalho et al. (2009) in a cross-sectional study reported that lame cows are more likely to have thinner digital cushion compared with non-lame cows and reported a significant positive association between BCS and thickness of the digital cushion, where the cows with low BCS had thinner digital cushion compared with cows with higher BCS. Cows in low BCS (experiencing NEB) will mobilise fat from their whole body possibly including the digital cushion to support the energy deficiency, therefore it is plausible that this will result in cows having a higher risk of lameness. However, larger longitudinal studies are needed to explore this relationship further. The study by Green et al. (2013) was conducted on one farm and there are no further investigations of the dynamics of changes of BCS on the risk of lameness published. Furthermore, there is no published work to investigate the changes of BCS on the risk of recovery from lameness. Understanding the complex relationship between lameness and body condition score could decrease their impacts on the health of dairy cattle and minimize the associated economic losses.

1.10 Fertility

Poor reproductive performance has been considered as one of the largest economic losses and welfare concerns in dairy herds (Kossaibati et al., 1999; Gonzalez-Recio et al., 2004). Genetic selection for high milk yield is thought

to be one of the reasons for poor reproductive performance (along with lameness) (Oltenacu and Broom, 2010). A decline in reproductive performance has been reported worldwide including the United Kingdom (declined from 55.6% to 39.7%) (Royal et al., 2000), Spain (declined from 42.3% to 33.1%) (Lopez-Gatius, 2003; Gonzalez-Recio et al., 2004) and the United States (declined from 65% to 40%) (Lucy, 2001).

There are several reproductive performance indicators widely used by researchers. These are: probability of conception, days from calving to conception, calving interval, days to first service, first service conception rate and number of services per conception. Over the past decades, a decline in first service conception rate, prolonged days from calving to conception and lower conception rate have been reported (Hillers et al., 1984; Domecq et al., 1997; Royal et al., 2000; Lopez-Gatius, 2003; Gonzalez-Recio et al., 2004). Increased number of services and days from calving to conception are associated positively with economic losses (Gonzalez-Recio et al., 2004; Inchaisri et al., 2010), and poor reproductive performance also influenced the culling rate (Grohn and Rajala-Schultz, 2000). Whitaker et al. (2000) reported that fertility was the main reason cows were culled or sold.

The impacts of lameness and poor body condition on fertility have been receiving increasing concern from around the world. Understanding the complex relationships among lameness, poor body condition and fertility is essential to maintain optimal fertility.

1.10.1 Association among lameness, body condition score and fertility

The link between lameness and fertility is unequivocal, numerous studies have reported that lame cows are more likely to have a lower successful rate of first service (Hultgren et al., 2004; Kilic et al., 2007), lower conception rate (Hernandez et al., 2001; Melendez et al., 2003; Bicalho et al., 2007; Kilic et al., 2007; Machado et al., 2010), longer days from calving to conception (Collick et al., 1989; Sprecher et al., 1997; Hernandez et al., 2001; Melendez et al., 2003; Bicalho et al., 2007; Kilic et al., 2007), longer days of calving to first service (Collick et al., 1989; Sprecher et al., 1997; Kilic et al., 2007) and a longer calving interval (Hultgren et al., 2004), however other studies have reported no differences between lame cows and non-lame cows in selected reproductive performance indicators (e.g. days from calving to conception, days from calving to first service, successful rate of first service and calving interval) (Cobo-Abreu et al., 1979; Dohoo and Wayne Martin, 1984; Barkema et al., 1994; Melendez et al., 2003; Sogstad et al., 2006; Peake et al., 2011), possibly due to the different measurement of lameness used and the timing of lameness recorded.

The severity of the impacts of lameness on fertility could be attributed to different causes of lameness, for example, previous studies have reported that lame cows with claw lesions and multiple lesions were less likely to conceive compared with healthy cows (Collick et al., 1989; Hernandez et al., 2001; Machado et al., 2010), whereas lame cows with interdigital necrobacillosis or

papillomatous digital dermatitis (PDD) were reported to have no significant differences compared with healthy cows (Hernandez et al., 2001).

Even though there is strong evidence showing negative association between lameness and fertility, the reasons behind this relationship remain unclear. Lameness is a debilitating condition, associated with tissue damage, pain and a feeling of discomfort (O'Callaghan, 2002). Pain and feelings of discomfort could lead dairy cattle to have a longer duration of lying, less feeding time and lower dry matter intake, resulting in weight loss and negative energy balance influencing follicular development and ovulation performance (Whay et al., 1998; Galindo and Broom, 2002; O'Callaghan, 2002; Bach et al., 2007; Ito et al., 2010).

Pain from lameness can be stressful, and this stress might reduce fertility by interfering with normal follicular activity, such as reduction in the amounts of gonadotrophin-releasing hormone (GnRH) and luteinising hormone (LH) leading to lower oestradiol production by slowing follicular growth and reducing the expression of oestrus behaviour (Dobson and Smith, 2000; Dobson et al., 2008). Increased levels of stress also influences the immune system of dairy cattle (greater ratio of neutrophil and lymphocyte and CD14 expression) which has its impact on fertility (O'Driscoll et al., 2009; Tomlinson et al., 2012).

Lame cows spent more time lying down during the oestrus period leading to lower expression of oestrous behaviour and lower frequency of mounting

(Sood and Nanda, 2006; Walker et al., 2008; Crowe and Williams, 2012), this could be because of the pain and stress from lameness.

The impacts of lameness on fertility maybe mediated through the changes of BCS. Lamé cows are more likely to have low BCS (Espejo et al., 2006; Dippel et al., 2009; Hoedemaker et al., 2009) and multiple studies have shown that low BCS has impacts on fertility, however inconsistent results of the effects of BCS on the fertility have been published; for example, cows with low BCS had a lower probability of conception (Suriyasathaporn et al., 1998; Machado et al., 2010) and a longer calving to conception interval (Ruegg and Milton, 1995; Markusfeld et al., 1997; Lopez-Gatius et al., 2003; Jilek et al., 2008; AL-Hilfee, 2009; Hoedemaker et al., 2009; Machado et al., 2010), higher numbers of services per conception (Amer, 2008; Jilek et al., 2008), longer days from calving to first service (Pryce et al., 2001; Jilek et al., 2008; Hoedemaker et al., 2009), lower first service conception rates (Suriyasathaporn et al., 1998; Loeffler et al., 1999; Pryce et al., 2001), a shorter calving interval (Pryce et al., 2001) and more days from first service to conception (Jilek et al., 2008). However, other published studies have found no effect on the calving to conception interval (Ruegg and Milton, 1995; Heuer et al., 1999; Jilek et al., 2008), probability of conception (Suriyasathaporn et al., 1998), days from calving to first service (Jilek et al., 2008), first service conception rate (Domecq et al., 1997; Markusfeld et al., 1997; Heuer et al., 1999; Pryce et al., 2001; Tillard et al., 2008), days from calving to first service (Ruegg et al., 1992; Ruegg and Milton, 1995; Pryce et

al., 2001) and number of services per conception (Ruegg et al., 1992; Ruegg and Milton, 1995; Heuer et al., 1999).

Similarly to lameness, the physiological mechanisms behind the impacts of low BCS on fertility remain unclear. One possible explanation was cows with low BCS experiencing negative energy balance; this condition influences ovulation, quality of oocytes and regulation of gene expression, thereby affecting the risk of pregnancy (Lopez-Gatius et al., 2003; Kadokawa and Martin, 2006; Roche et al., 2009). Alternatively, cows with low BCS were more likely to be lame (Espejo et al., 2006; Dippel et al., 2009; Hoedemaker et al., 2009), therefore the pain and stress resulting from lameness may reduce the risk of pregnancy.

The extent of the effect of lameness on reproductive performance might be attributed by the duration of cows being lame as cows lame for a long period are logically having longer duration of pain and lower feed intake and this longer duration of conditions could affect the reproductive performance. Furthermore, exploring the impacts of lameness and BCS on the likelihood of each AI leading to pregnancy could provide a better understanding of the impacts of lameness and BCS on the overall reproductive performance of dairy cattle. Regular recording of mobility and body condition score of dairy cattle is suggested as this could provide more information about the condition of cows in order to devise a strategy to maintain optimum reproductive performance in dairy herds.

1.11 Conclusion

The high prevalence of lameness and hock lesions in dairy cattle is of utmost health and welfare concern. A list of identified risk factors associated with the absence or presence of hock lesions (hair loss, swelling and ulceration) has been described in previous studies, however, whether these risk factors also contribute to the severity of different types of hock lesions (in animals which have lesions) remains unknown.

Hock lesions have been suggested to be a cause of lameness in previous studies, however the directionality of the relationship between lameness and hock lesions remains unknown, i.e., whether lameness predisposes to hock lesions or vice versa. Given the directionality of development of hock lesions is yet unclear, further understanding of the directionality of the relationship between lameness and different types of hock lesions is crucial to help identify ways to reduce the prevalence of hock lesions and lameness in dairy cattle.

Further investigation of the directionality of BCS and risk of lameness has been conducted by Green et al. (2013) on one farm. Yet, there was no exploration of the dynamics of change of BCS on the occurrence of lameness. There is also no previous work exploring whether change in BCS has an impact on recovery from lameness.

Cows with chronic lameness (long period of lameness/repeated lameness cases) may have poorer reproductive performance as a longer duration of ill

health may have serious negative effects on hormone levels and metabolism affecting their reproductive performance. However, there is no study to support this hypothesis. Generally, researchers tend to study single lameness cases, mostly the first lameness case to occur or a single lameness event in a specific time period.

The objectives of this PhD were to use a range of advance statistical modelling techniques to:

- i) Investigate the risk factors associated with area of hair loss measured by lesion area on hock maps and the correlation between measured area on the hock maps and scores given on a categorical scale (Chapter 3).
- ii) Investigate the association between lameness and the presence of hair loss on the hock and other related factors in dairy cattle in a longitudinal study (Chapter 4).
- iii) Investigate the effect of change in BCS and other risk factors on the transition from non-lame to lame and lame to non-lame and the correlation between both transitions (Chapter 5).
- iv) Investigate the effect of lameness and BCS on the likelihood of an artificial insemination (AI) leading to a pregnancy, probability of conception and days to conception (Chapter 6).

CHAPTER 2: STATISTICAL BACKGROUND AND METHODOLOGY

2.1 Analytical epidemiological studies

The definition of epidemiology as stated in the Dictionary of Epidemiology is “*The study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems*” (Last, 1983). In other words, epidemiology is the study of investigating the patterns, causes and effects of health related problem in defined populations (Ressing et al., 2010). Epidemiological studies can be categorized as either experimental or observational (Hoffmann and Lim, 2007). The main types of observational study are descriptive studies and analytic studies (Hajat, 2011). Descriptive studies aim to give a description of disease occurrence, such as which animals are affected, where they are located and when the disease occurred (Grimes and Schulz, 2002; Hajat, 2011). Analytical studies aim to investigate and identify the factors associated with the presence of disease within a population, or to compare a group of animals with disease to a group of healthy animals (Hajat, 2011). The three most common types of analytic observational studies are cross-sectional studies, cohort studies (one form of longitudinal studies) and case-control studies (Ressing et al., 2010); further descriptions of cross-sectional studies and longitudinal studies are provided in the next section as these studies were conducted as part of this thesis.

2.1.1 Cross-sectional studies

Cross-sectional studies, also known as prevalence studies, are a type of observational study which are carried out at one specific time point or over a short period or time (Levin, 2006; Ressing et al., 2010). These studies are descriptive studies and are generally used for providing a snapshot of a population at a particular time point, such as the frequency of a disease and its characteristics in a population. In these studies, data are collected with various characteristics, where a set of potential risk factors are associated with an outcome. Cross-sectional studies are useful for investigating associations between risk factors and the outcome, but not for investigating cause and effect relationships. These studies are low cost and are quick and easy to conduct for generating hypotheses compared to longitudinal studies, making inferences about a population of interest and providing the prevalence of an outcome in order to support further research (Levin, 2006).

2.1.2 Longitudinal studies

In a longitudinal study, the outcome of interest is measured repeatedly over a period of time. Longitudinal studies are often used to observe changes in a disease over time (Holmbeck et al., 2006; Ployhart and Vandenberg, 2010). In comparison to cross-sectional studies, these studies have more power to detect causal relationships, but less power compared with experimental studies (Holmbeck et al., 2006). The drawbacks of these studies are the amount of time needed to conduct them and the cost of data collection can be relatively

high (Louis et al., 1986; Newman, 2010). Furthermore, subjects may drop out of a study, perhaps die or move out of the population, affecting the power or sample size at the end of the study (Louis et al., 1986). Since a similar group of subjects are observed repeatedly over time, there is a correlation within subjects and thus complex statistical techniques are required to analyse the data (Diggle, 2002; Ployhart and Vandenberg, 2010).

2.2 Statistical modelling

“Successful modelling of complex data is part science, part statistics, part experience, but a major part is logic or common sense” (Ughade, 2013). The two most common analytical tools are statistical modelling and stratification (Ughade, 2013). In the past, stratification was commonly used by researchers, however, it has several limitations, such as there might be a small number of objects/animals in the individual strata resulting in biased estimation; have difficulty in the interpretation of results from several levels of strata; and this method is based on a non-parametric approach (Ughade, 2013). Due to these complexities, this method is hard to apply in the epidemiological research. Statistical modelling has the ability to handle these limitations, thus it has become the most commonly used method for various types of research.

In the field of veterinary epidemiology, statistical modelling can be used to understand the factors that influenced the occurrence of disease which could be used to devise control strategies (Dubé et al., 2008; Ughade, 2013). Evaluation of the impacts of control strategies can also be evaluated using

statistical modelling (Dubé et al., 2008). This method is needed for decision makers involved in the planning of control strategies for specific diseases. For example, data collected from dairy farms could be analysed using statistical modelling in order to provide an understanding of which factors influenced a particular disease. This information is useful for farmers to uptake best practices to improve the health and welfare of their animals.

2.2.1 Multilevel modelling

Data with variables at different levels of hierarchy are common in veterinary sciences, and are referred to as a hierarchical data structure. For instance, data collected on dairy farms may have a number of levels; dairy cattle may be the level 1 unit clustered or nested within level 2, where level 2 is the dairy farm. Generally, there are more variations between dairy cattle from different dairy farms than dairy cattle within the same farm. Dairy cattle in the same farm are more likely to be similar e.g. in terms of exposure or a certain type of management. Ignorance of this variability may lead to wrong conclusions being drawn (Goldstein, 2010; Khan and Shaw, 2011), for instance, the probability of hock lesions of dairy cattle from same farm may have lower variability compared to the dairy cattle from other farms as they share the same common exposures, such as types of bedding, management practices and environmental factors. Multilevel modelling has become an important tool to analyse this type of data. The variability associated with each level of nesting is accounted for in the analysis (Atkins, 2005). Other advantages of using multilevel modelling include its flexibility in handling time predictors and

missing data compared to other statistical tools (Kwok et al., 2008). There are a wide range of multilevel models, the type of dependent variable is used to decide which kind of multilevel model is used for analysis.

2.2.1.1 Multilevel multivariable linear regression model

Regression analysis is a technique used to measure the relationship between a response variable and explanatory variables. Multivariable regression analysis is a technique used when more than one explanatory variable is present and is widely used in the epidemiology of animal science (Rasbash et al., 2012).

A multilevel multivariable linear model is generally employed to analyse a continuous dependent variable in cases of a clustered dataset (Rasbash et al., 2012). Model specifications are

$$y_{ij} = \beta_0 + \beta_1 X_j + \beta_2 X_{ij} + u_j + e_{ij}$$

$$u_j \sim N(0, \sigma_u^2), \quad e_{ij} \sim N(0, \sigma_e^2)$$

where y_{ij} is the dependent variable, β_0 is the intercept and the subscripts i and j represent the first and second levels respectively. β_1 and β_2 are the coefficients of the explanatory variables of X_j and X_{ij} respectively and u_j and e_{ij} are the random effects of the residual variation between the second and the first level respectively, which are assumed to follow a normal distribution with mean zero and variance σ^2 . Models can be fitted using iterative generalized least squares estimation (IGLS) (2.4.1). The model fit is checked by residual plots at each level (Goldstein, 2010). Residual plots could be used for checking the

model assumptions for each level, i.e. normality of random effects and the presence of any outliers (Cox and Snell, 1968; Singer, 2011; Rasbash et al., 2012). A normal distribution curve is presented when the normality assumption is obtained (Rasbash et al., 2012). If there are any outliers in the residual plots, omitting the outliers is used to check whether the differences will influence the parameter estimate (Goldstein, 2010; Rasbash et al., 2012). This method has been applied in Chapter 3 for analysis of a cross-sectional dataset.

2.3 Statistical analysis of longitudinal datasets

Longitudinal studies are becoming increasingly common in the field of veterinary epidemiology. The outcomes are repeatedly collected over time (Edwards, 2000), for example, mobility score and body condition score of dairy cattle are observed monthly throughout the study period (Chapter 5). These data are correlated within subjects and thus require complex statistical techniques for analysis (Edwards, 2000). Multilevel modelling is used to analyse longitudinal studies as there are a minimum of two levels of data; measurement at level 1 nested within individuals at level 2 (Steele, 2008).

Timing of disease occurrence is another outcome that researchers are interested in investigating in longitudinal studies (Steele, 2005, 2008). These data are referred to as ‘event history data’ and the related analyses, known as survival analysis.

2.3.1. Event history data

Event history data is longitudinal data of the timing of the occurrence of the disease(s) or interest (Steele, 2005). For example, lameness histories of dairy cattle include the timing from non-lame to lame; or conception histories include the duration from calving until conception. These data are generally complex, and may have features such as repeated events (repeated occurrence of lameness throughout the study), multiple origin states (non-lame or lame at the start of lactation), multiple transitions of diseases (non-lame to lame and lame to non-lame) (Steele et al., 2004). Thus, event history analysis, also known as survival analysis, duration analysis or hazard modelling is recommended for analysing these event history data (Steele, 2005).

2.3.1.1 Issues in the analysis of event history data

In event history data, there are two features which are worthy of particular attention. Firstly, some objects are lost to follow up due to death, leaving the population or early exit from the study. This is called data censoring (Steele, 2005; Liu, 2012). There are a number of types of censoring, including: a) Right censoring where no event occurs during the study, an object is lost to follow up or leaves the study before study period ends; b) Left censoring refers to an object which has an event occurrence before the study period starts; c) Interval censoring describes an event which occurred within study period, but at an unknown time; d) Left truncation describes when participants

are exposed to the risk of experiencing the event before the study period begins (Fox, 2002; Steele, 2005; Liu, 2012).

Left censoring, left truncation and interval censoring are difficult to include in the analysis, therefore the most common approach is to exclude these objects from the study. Right censored data cannot be simply excluded as it can lead to study bias. The best way to handle right censored data is to include them in the analysis and define them as non-informative censoring (Steele, 2005).

In addition to censoring, another feature that must be considered in the event history data is the handling of time varying covariates (Steele, 2005). Variables with fixed effects refer to the value of variables which are constant throughout the study, such as breed and farm ID; variables which change over time are called time varying covariates, e.g., BCS of cows measured weekly/biweekly/monthly. Generally, researchers will select the value of this variable at a specific time point and ignore the following information (Steele, 2005), for instance, BCS at the start of the study period. Time varying covariates include useful information and this can be exploited to explain dynamic complex relationships between variables, hence a discrete time event model could be utilized to investigate time varying covariates (2.3.3.2).

2.3.2 Descriptive definitions used for time to event data

Survival analysis, also known as event history analysis is used to analyse the duration of time until the occurrence of the event (Smith et al., 2003; Steele,

2005). Before the occurrence of the event, every individual is at risk of having the event.

Hazard function ($h(t)$) is the probability of an event occurring at time t given that no event has occurred before time t . It can also be interpreted as the risk of experiencing event at time t .

The hazard function is defined as:

$$h(t) = \lim_{dt \rightarrow 0} \left(\frac{\Pr(t \leq T < t + dt | T \geq t)}{dt} \right)$$

$\Pr(t \leq T < t + dt | T \geq t)$ is the probability of the event occurring at time $t + dt$ given that no event occurs before time t .

Survival function $S(t)$ is the probability of the event that has not occurred before time t (Steele, 2005). Survival function is:

$$\begin{aligned} S(t) &= \Pr(T > t) \\ &= 1 - F(t) \end{aligned}$$

Survival function must decrease from the start of study, with $S(0)=1$ and $S(t) \rightarrow 0$ when $t \rightarrow \infty$.

2.3.3 Modelling event history data

Event times are measured in either continuous or discrete time, this aspect is important in deciding which type of event history modelling is used. The most flexible continuous time model is the Cox proportional hazard model (Cox

model) whereas the most common discrete time model is the discrete time event model (Steele, 2005, 2008).

2.3.3.1 Continuous time event history model- Cox proportional hazard model (Cox model)

The Cox model is the most common event history model used in survival analyses (Steele, 2005). Cox models have a number of attractive features, including: a) flexibility when the baseline hazard function is unspecified; b) simple and easy to interpret results, e.g., relative risks, hazard ratios or odds ratios. The Cox model is based on the non-parametric approach which does not require any particular distribution of data, however, the assumption that the effects of covariates must be constant over the survival time must be met (Smith et al., 2003).

In Cox models, the hazard at time t for each individual i is :

$$h_i(t) = h_0(t) \exp(\beta_i X_i)$$

where β_i is a vector of regression coefficients.

The Cox model with natural log is written as:

$$\text{Log}[h_i(t)] = \text{Log}[h_0(t)] + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_i X_i$$

Where $\beta_1, \beta_2, \dots, \beta_i$ are the coefficients of the explanatory variables of $X_1, X_2, \dots, X_i$ respectively. Exponents of the β can be interpreted as hazard ratio or relative risk. If there is a categorical variable, $\beta=0$ ($e^\beta=1$) indicates there is no

effect on the hazard; $\beta > 0$ ($e^\beta > 1$) indicates a higher hazard compared with the reference group; $\beta < 0$ ($e^\beta < 1$) indicates a lower hazard compared with the reference group. For continuous variables, e^β indicates the changes of hazard for each 1 unit change in x (Steele, 2005).

To use Cox models, the data must fit a proportional hazard assumption where the effect of covariates must be constant over time. This assumption could be checked either graphically (e.g, Kaplan Meier estimated survival curves) or using a statistical method (e.g., log rank test) (Walter, 2009; Liu, 2012). If the covariates meet the proportional hazards assumption, Kaplan Meier estimated survival curves should show the parallel lines (Abeysekera and Sooriyarachchi, 2009; Walter, 2009). If the effect of a covariate is time dependent (non proportional hazard over time), this covariate has to be excluded from the analysis, the stratified Cox model or a discrete time event model (2.3.3.2) could be used for further investigation (Steele, 2005).

Schoenfeld's global tests are suggested for checking the fitted Cox model and each of the covariates in the fitted model to ensure the proportional hazard assumptions are met (Abeysekera and Sooriyarachchi, 2009). A Cox-Snell residual plot can be used to test the model fit and detect outlying values (Abeysekera and Sooriyarachchi, 2009; Liu, 2012). A straight line plot indicates the data fit the model well (Abeysekera and Sooriyarachchi, 2009; Ravangard et al., 2011).

2.3.3.2 Discrete time event history model

Discrete time event history models have several advantages compared with continuous time models; they can be used for fitting data with non proportional hazards and time varying covariates (Steele, 2005).

One disadvantage of this model is that data has to be restructured into the person period format prior to analysis (Steele, 2005). The binary response $y_{i(t)}$ is coded as below:

$$y_{i(t)} = \begin{cases} 0 & t < y_i \\ 0 & t = y_i, \delta_i = 1 \\ 1 & t = y_i, \delta_i = 0 \end{cases}$$

Where y_i indicates the observed event at censoring time t either coded zero if no event occurred or coded one if the event occurred, δ_i represents censoring. For instance, if an individual experiences an event at the fifth visit, then, the $y_{i(t)}$ will be $\{y_{i(1)}, y_{i(2)}, y_{i(3)}, y_{i(4)}, y_{i(5)}\} = \{0, 0, 0, 0, 1\}$. If an individual is censored at $t=5$, it will be $\{0, 0, 0, 0, 0\}$.

The discrete time hazard at modelled time t is the probability of an event during time t , given that no event occurred in time $t-1$ (Steele, 2005, 2008), described below (for a binary response):

$$h_{i(t)} = \Pr\{y_{i(t)}=1 | y_{i(t-1)}=0\}$$

The logit link function of this model can be written as:

$$\text{Logit}[h_{i(t)}] = \text{Logit}\left[\frac{h_{i(t)}}{1-h_{i(t)}}\right] = \alpha(t) + \beta'X_{i(t)} + u_i$$

$$u_i \sim N(0, \sigma_u^2)$$

where $\alpha_i(t)$ is an effect of duration t , β' represents the coefficients of the vector of covariates, covariates $X_{i(t)}$ may be fixed or time varying effects and u_i represents the random effect between individual levels which were assumed to follow a normal distribution with a mean of zero and variance σ_u^2 . $\alpha_i(t)$ is a categorical variable with a piecewise constant function. Models can be fitted using quasi-likelihood methods and followed by Markov chain Monte Carlo (MCMC) methods with 500,000 iterations and a burn in of 5,000. (The chain mixing and stability should be checked visually (Browne, 2009).)

2.3.3.3 Multilevel multistate discrete time event history model

Compared to discrete time event history models, multilevel multistate discrete time event history models can model data with multiple events (transitions). In real biological data, objects/animals often experience repeated events during the study period, e.g, a cow may experience lameness multiple times in the study period. Due to this they will have multiple states and then multiple transitions from a state. For instance, a repeatedly lame cow will have 2 transitions in this data, including transition from the non-lame state to the lame state (lameness) and from the lame state back to the non-lame state (recovery from lameness). A continuous period for an individual in a particular state is called an episode (Steele, 2005, 2008). A ‘duration clock’ is turned to ‘zero’ when a cow changes to the other state. Only one transition has occurred in each episode therefore they are mutually exclusive. In the past, the simple way to handle these data was fitting each transition into a separate model (Steele, 2005), however multistate models can handle both transitions in the same

model. The advantages of this approach are a) a measure of the covariate effects are the same in both transitions; b) they provide a measure of the residual correlation between two transitions (Steele et al., 2004; Steele, 2005).

Similar to the discrete time event history model, the data must be expanded and restructured into the person period format before analysis (a detailed description of the method for expanding and restructuring data is presented in Chapter 5). This event history data has two levels where level 1 is an episode nested within each individual (level 2) (Goldstein et al., 2002). The event indicator is coded as 1 or 0 depending on the occurrence of an event: if no event occurs, zero is coded; one is coded if event occurs (change to the other state). The hazard at time t for each episode j of cow k for each state i is

$$h_{ijk(t)} = \Pr\{y_{ijk(t)} = 1 | y_{ijk(t-1)} = 0\}$$

where i indexes the state, j indexes the episode and k indexes the individual.

A logit link function can be written as:

$$\text{Logit}[h_{ijk(t)}] = \beta_0 i + \alpha_i(t) + \beta x_{ik(t)} + u_k$$

$$u_k \sim N(0, \sigma_u^2)$$

where $\beta_0 i$ is a state specific intercept, $\alpha_i(t)$ is an effect of duration which is a categorical variable with piecewise constant step function for each state, $\beta x_{ik(t)}$ refers to the fixed or time varying effects of the vector of covariates for each state and u_k represents the random effect of the individual level for each state which are assumed to follow a multivariate normal distribution with

covariance and non-zero correlation between random effects. The correlation estimate indicates the directions of both transitions.

2.4 Estimation method

The common estimation method used for normal and binomial outcome is Iterative Generalised Least Squares (IGLS) and Markov chain Monte Carlo (MCMC) respectively.

2.4.1 Iterative Generalised Least Squares (IGLS)

The Iterative Generalised Least Squares (IGLS) algorithm, based on the frequentist approach is used to estimate the regression coefficients in a multilevel linear regression model (Goldstein, 1986; Dohoo et al., 2003; Browne, 2009). The estimation procedure is an iterative process and the procedure iterates the estimation of the value for each parameter until estimates obtained from two consecutive cycles reach convergence (Browne and Draper, 2006; Goldstein, 2011). This method produces maximum likelihood estimates of the parameters when the residuals follow a normal distribution (Dohoo et al., 2003). Likelihood (or likelihood function) refers to the probability of observing the data, based on the unknown parameters and the fixed data (Dohoo et al., 2003).

2.4.2 Markov chain Monte Carlo (MCMC)

In the logistic regression model, a model is initially fitted by using quasi-likelihood estimation methods followed by an estimation method called Markov chain Monte Carlo (MCMC) (Browne, 2009). Likelihood estimation methods can lead to estimation bias (Browne and Draper, 2006). Therefore, MCMC method in the Bayesian framework is used to avoid this issue. MCMC estimation is calculated the probability of posterior distribution for parameter based on the prior distribution with the observed data (Dohoo et al., 2003; Browne, 2009) by Hybrid Metropolis-Gibbs sampling estimation (Browne, 2009). In the MCMC analysis, the particular starting values for Markov chains are used (based on the quasi-likelihood estimation) and each new value is generated stochastically based on the previous value. Due to the poor Markov chain mixing at the initial stage of analysis, a certain number of initial iteration (burn-in length) have to be discarded and the Markov chains then converge to an equilibrium distribution (Browne, 2009). The burn-in length and chain length sufficient and long enough to reach convergence can be diagnosed in the MCMC trajectories (Browne, 2009).

After running MCMC, the chain mixing and stability of each parameter are then evaluated in the MCMC trajectories window (Browne, 2009). One of the MCMC trajectories will be used for further explanation (Figure 2.1). A trace plot (upper left graph) shows that there is a good mixing chain and a smooth histogram in the Kernel density plot (upper right graph) indicates that estimation of parameter is under a normal distribution. Autocorrelation (ACF)

and partial autocorrelation plot (PACF) shows that the line is closely to 0 at the end of analysis, meant that the chain is sufficiently close to independent and identically distributed data (Browne, 2009). Monte Carlo Standard Error (MCSE) shows that the chain is enough to get the accurate mean estimate. The length of Markov Chain used in the model is sufficient and long enough for effective convergence based on the Raftery-Lewis diagnosis and Brooks-Draper diagnosis (Brooks and Gelman, 1998, Raftery and Lewis, 1992). The Deviance Information Criterion (DIC) diagnosis is checked to ensure that the best model is obtained (smaller DIC value indicate better model) (Browne, 2009).

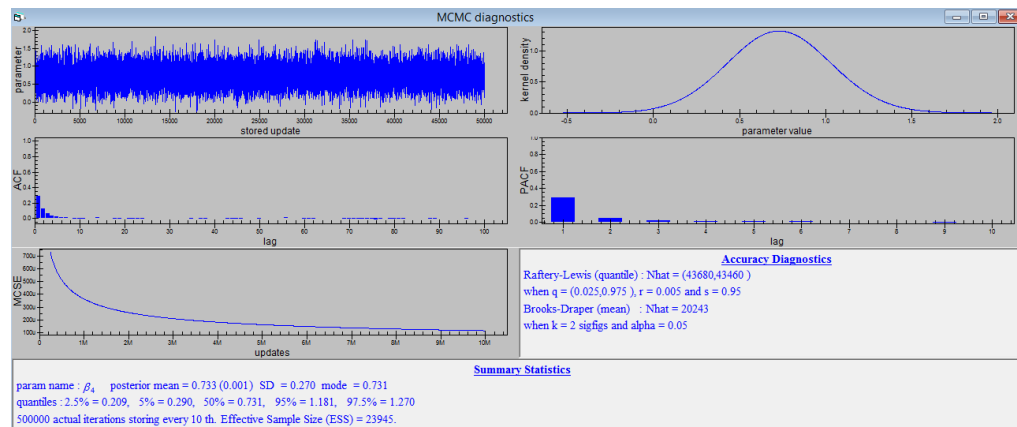


Figure 2.1 An example of MCMC trajectories window

2.5 Conclusion

Various methods of advance statistical modelling could be helpful for researchers to produce robust models to explain the complex relationships that exist in variables collected and in handling large amount of information (Ughade, 2013). With an increasing number of herds which are now routinely collecting data, (e.g locomotion score, body condition score, hygiene score,

behaviour of cows, milk yield), a much bigger and detailed dataset could be explored with advanced statistical techniques to predict diseases, evaluate risks and perform economic analyse.

CHAPTER 3: AREA OF HAIR LOSS IN DAIRY COWS WITH HOCK LESIONS: A STUDY OF ITS RISK FACTORS AND CORRELATION TO CATEGORICAL SCALE ON 76 DAIRY FARMS IN THE MIDLANDS REGION OF THE UNITED KINGDOM

3.1 Introduction

Leg injuries in cattle commonly occur on protruding areas such as the carpus, hock, fetlock, tarsus and hip (Weary and Taszkun, 2000). Amongst leg injuries, lesions around the hock are the most common (Wechsler et al., 2000; Kielland et al., 2009). Their presence has been reported across the world including the United Kingdom (Whay et al., 2003; Rutherford et al., 2008; Potterton et al., 2011b), Germany and Austria (Brenninkmeyer et al., 2012), Norway (Kielland et al., 2009), the United States (Fulwider et al., 2007; Lombard et al., 2010), Canada (Weary and Taszkun, 2000) and China (Chapinal et al., 2014). Hock lesions have been described at five locations (lateral tuber calcis, lateral tarsal joint, medial tuber calcis, medial tarsal joint and dorsal tuber calcis) with the lateral tarsal joint and tuber calcis reported as the most common and the medial tarsal joint as the least common site (Weary and Taszkun, 2000; Fulwider et al., 2007; Potterton et al., 2011b). One study in southern British Columbia reported that approximately 76% of hocks had lesions at more than one location (Weary and Taszkun, 2000).

The impact of hock injuries on the welfare of the animal is largely unknown (Huxley and Whay, 2006; Rutherford et al., 2008; Laven and Livesey, 2011).

However, it has been assumed that the severity of hock lesions reflects the degree of comfort of the lying surface (Livesey et al., 2002; Lombard et al., 2010; Lobeck et al., 2011; Brenninkmeyer et al., 2012), which may impact on welfare and health (Haskell et al., 2006; Huxley and Whay, 2006; Brenninkmeyer et al., 2012). Hock lesions are associated positively with lameness (Whay et al., 2003; Regula et al., 2004; Kielland et al., 2009; Brenninkmeyer et al., 2012); lame cows have more swelling at the hocks (Whay et al., 2003; Rutherford et al., 2008). Positive correlations between hock lesions and injuries at other locations such as teat injuries and skin injuries have also been reported (Regula et al., 2004; Sogstad et al., 2006) suggesting they may be of use as welfare indicators (Whay et al., 2003; Regula et al., 2004).

The severity of hock lesions has been described to vary from hair loss to open wounds, swelling and ulceration (Weary and Taszkun, 2000; Kielland et al., 2009). There is a lack of understanding of development and progression of these hock lesions. One study in the UK has reported that risk factors of each presentation/lesion e.g. hair loss, ulceration and swelling are unique (Potterton et al., 2011a) and based on these findings suggested that there might not be a linear progression of the hock lesions from hair loss to ulceration as suggested in the some scoring systems (Gustafson, 1993; Weary and Taszkun, 2000; Regula et al., 2004; Norring et al., 2008; Rutherford et al., 2008). Of these lesions, hair loss is the most common in dairy herds (Huxley et al., 2004; Potterton et al., 2011a). Another longitudinal study (Livesey et al., 2002) found that factors that increased the risk for total lesions (including hair loss)

also increased the risk of most severe hock lesions. The impact of hair loss to dairy cattle is unknown, however hair loss alone is suggested as an indicator of abrasion from the lying surface (Livesey et al., 2002; Mowbray et al., 2003).

Although categorical scales have been used to assess the severity of lesions, there is no commonly used, standard scoring system. Researchers have used a variety of categorical scoring systems to grade severity (Weary and Tazskun, 2000; Regula et al., 2004; Barberg et al., 2007; Rutherford et al., 2008; Kielland et al., 2009; Potterton et al., 2011b). There is no evidence of the reliability and validity of these scoring systems, nor how these scores equate to the area of skin affected with lesions.

The majority of studies on hock lesions have investigated risk factors associated with the presence or absence of lesions (Regula et al., 2004; Rutherford et al., 2008; Kielland et al., 2009; Potterton et al., 2011a); Potterton et al. (2011a) has reported that there are 14 risk factors associated with presence of hair loss (locomotion score, days of winter housing, parity, mean milk yield, breed, freestall base material, freestall bedding material, application of hygiene products, height of lowest side rail at front end of freestall, time mats and mattresses have been in freestall, distance from neck rail to rear of the freestall, height of neck rail, length of freestall and herd size). There is no published data on risk factors associated with an increase in area of hair loss on hocks. The aims of this study were to examine the area of hair loss lesions on the lateral aspect of the hock using detailed hock maps and to use these data to investigate: i) the risk factors associated with area of hair

loss measured, and ii) the correlation between measured area on the hock maps and scores given on a categorical scale.

3.2 Materials and methods

3.2.1 Data set and study methodology

A detailed description of the dataset and the study methodology used to generate it has been published (Potterton et al., 2011a; Potterton et al., 2011b), a brief description is provided below. Seventy seven farms in the Midlands region of the United Kingdom were recruited and visited during the winter housing period of 2007/2008, one farm was excluded as the animals could not be readily assessed by the observer. Approximately 50 cows were selected randomly from each herd for assessment. Each farm was visited once and all cow and farm assessments were conducted by a single observer (SLP).

Assessments of animal

Selected cows were assessed for a wide range of animal characteristics including body condition score (scale 1-5) (Wildman et al., 1982), mobility score (scale 0-3) (Whay et al., 2003), cleanliness score on tail, left and right flanks, lower and upper hind limbs on left and right side (scale 0-4) (Whay et al., 2003) (higher score indicates dirtier hocks) and rising behaviour (time taken to rise, intention movements (where an animal appears to be seeking a lunging space, but does not proceed to lunge), sideways lunge movements,

repeated attempts at rising, a pause of greater than 2s while the animal is resting on its knees with its back legs extended, slip during rising, dog-sitting and contact with the freestall fittings). Hair loss on both hocks of each study animal were scored separately using four point categorical scales (score 0-3): hair undisturbed with no loss (score 0); area of hair loss less than approximately 2cm in diameter (score 1); area of hair loss between approximately 2cm and 2.5cm in diameter (score 2); area of hair loss greater than approximately 2.5cm in diameter (score 3) (Whay et al., 2003; Huxley and Whay, 2006). Additionally, the area and shape of hair loss lesions at three locations over the hock (lateral, dorsal and the medial hocks) were recorded using hock maps (Potterton et al., 2011b), a full description of methodology used for hock maps are presented in 3.2.1.1. All lesions over both hocks for each study animal were recorded on separate hock maps; the areas affected by hair loss were measured as partial hair loss and complete hair loss. If there was any swelling on along with hair loss it was recorded too.

Following data collection, milk records and farm data were obtained to gather information on breed, age, parity, days in milk, duration of winter housing and milk yield (mean milk yield from the 3 most recent monthly milk records).

Assessments of environmental

A detailed evaluation of the farm and animal environment was conducted including assessments of the loafing area, feed area passageways and freestalls. The structure and dimension of cubicles were measured using a tape

measure or a digital laser distance meter (Disto A3, Leica DISTO). The nature of base and bedding material were recorded and the mean depth of bedding was measured at three separate points, each 40cm in from the rear of the bed; at the midpoint across the width of the freestall and then at 2 further points, one 40cm on either side of the midpoint. The condition and features of cubicles were observed. Observer interviewed the farm owners to collect the information of the farm and housing management practices.

3.2.1.1 Hock maps

Hock map, a method for measuring hock lesions was proposed by Potterton et al. (2011b) which was suggested as a more useful research tool to capture lesions over the hocks was used in the study (Laven and Livesey, 2011). The map is available in the Appendix 3. The location (lateral, dorsal and the medial hocks), area and shape of lesions were recorded on the hock maps using different outline colours: blue line- partial hair loss, green line-complete hair loss. Hock maps then were scanned and stored electronically; areas of partial hair loss and complete hair loss were calculated automatically using in Matlab® (The Mathworks Inc., Natick, USA).

The programme of analysis hock maps was developed in collaboration with Chris Brignell from the School of Mathematical Sciences and Andrew French from the School of Biosciences at the University of Nottingham. The lesion areas were initially presented in pixels. An approximate scaling factor was used to convert the areas in pixels into areas in cm².

3.2.2 Statistical analysis

3.2.2.1 Data analysis (Hock map selection)

A total of 3691 cows from 76 farms were selected for inclusion in this dataset. Out of 7382 hocks, 6896 (3447 left hocks and 3449 right hocks) had complete information on hair loss. The remaining 486 hocks were excluded because of missing data (n=56), animals which could not be assessed e.g. too flighty to approach (n=31), and dirty hocks which meant the hock condition could not be accurately recorded (n=399).

Of the 6896 hocks, 6884 had complete hock maps; 12 maps could not be used due to technical difficulties (the hock maps were too dirty or had duplicate lines drawn indicating the area of lesions). A total of 1276 hock maps were excluded from the dataset because no hair loss was documented (these hocks were also free from any other lesion). There were 5608 hocks remaining in the dataset with 5528 hocks having some area of partial or complete hair loss on the lateral side.

Out of 5352 hocks with an area of partial hair loss on the lateral side, 2296 hocks (43%) also had an area of complete hair loss. Of the 2296 hocks that had an area of both partial and complete hair loss, 2143 (93%) hocks had an area of complete hair loss which was surrounded by an area of partial hair loss, whereas only 95 hocks (4%) had an area of complete hair loss which was not surrounded by an area of partial hair loss. Fifty eight (3%) hocks had

some area of complete hair loss which was surrounded by an area of partial hair loss plus another area of complete hair loss that was not surrounded by an area of partial hair loss. Examples of these areas of hair loss are presented in Figure 3.1. The 95 hocks with an area of complete hair loss which was not surrounded by an area of partial hair loss and 58 hocks with an area of complete hair loss surrounded by an area of partial hair loss plus another area which was not surrounded by an area of partial hair loss were excluded from the dataset. Finally, 13 hocks were excluded from the dataset because the animal identity could not be confirmed. The final dataset used in the univariable and multivariable analysis contained a total of 5186 hocks.

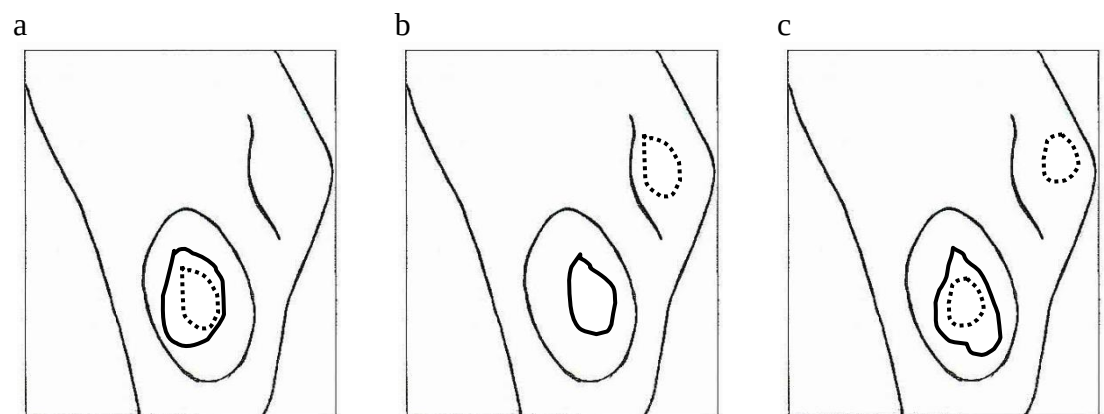


Figure 3.1 Examples of different locations of partial hair loss and complete hair loss on the lateral surface of the hock

a) Area of complete hair loss which is surrounded by an area of partial hair loss. b) Area of complete hair loss which is not surrounded by an area of partial hair loss. c) Area of complete hair loss which is surrounded by an area of partial hair loss plus another area of complete hair loss which is not surrounded by area of partial hair loss.

(Key: straight line- area of partial hair loss; dash line- area of complete hair loss)

3.2.2.2 Risk factors associated with area of hair loss on the lateral surface of the hock

As the majority of hock maps (79%, 5431/6884) had some area of partial hair loss with 99% (5352/5431) having some hair loss on the lateral area; a statistical model with 5186 observation as described was constructed to explore factors associated with area of hair loss on the lateral hock. Of 5186 hocks, 5086 (98%) had also been noted with some degree of swelling.

The dataset was screened for errors and missing values, outliers and normality of outcome variables. Frequency tables were created. Log (base e) transformation was performed to normalize area of hair loss. Descriptive analyses were done in Stata version 12 (StataCorp, USA).

A multilevel linear model was built with three levels: farm, cow and hock (Rasbash et al., 2012). The outcome was a log transformed area of hair loss on lateral surface of hock. A total of 94 potential risk factors were tested in the analysis and are presented in Table 3.1. Univariable analysis was performed and those variables with significant values, ($p \leq 0.10$), were retained and taken forward for further analysis. Variables with large numbers of missing values were excluded. A stepwise regression selection method was then used to obtain final model. The model was created using MLwiN version 2.25 (Centre for Multilevel Modelling, University of Bristol) and fitted using iterative generalized least squares estimation. Variables with a $p \leq 0.05$ were retained in the multivariable model.

The outcome variable was log transformed and therefore model specifications were

$$\text{Log}(y_{ijk}) = \beta_0 + \beta_1 X_{ijk} + f_k + u_{jk} + e_{ijk}$$

$$f_k \sim N(0, \sigma_f^2), u_{jk} \sim N(0, \sigma_u^2), e_{ijk} \sim N(0, \sigma_e^2)$$

where y_{ijk} is an area of hair loss on the lateral surface of the hock_{ijk}, β_0 is the intercept, the subscripts i, j, k represent the hock, cow and farm levels respectively. β_1 is the coefficients of explanatory variables of X_{ijk} and f_k, u_{jk}, e_{ijk} as random effects of residual variation between farm, cow and hock level respectively which assumed to follow a normal distribution with mean zero and variance σ^2 . The Chi square test/Fisher's exact test was used to test association between these categorical explanatory variables. If variables were strongly associated, only one of the variables was selected. The model fit was checked by residual plots at the levels.

Table 3.1 List of 94 potential risk factors for area of hair loss on the lateral surface of the hock

Cow level risk factors	
Categories	Descriptions
Cow characteristics	BCS, locomotion score, cleanliness score, parity, age, breed
Milk yield	Days in milk, most recently recorded milk yield, previous lactational 305 day yield, mean milk yield from the 3 most recent monthly milk records
Cow behaviours	Rising and lying behaviours
Farm level risk factors	
Environment assessments	Measurement and condition records of feed space, passageways, loafing areas, floor type, bedding materials, cubicle types, depth of bedding materials
Stall measurements	Total length, distance from the curb to the brisket positioner, length of any mat or mattress, width, curb height, width of curb left exposed when a mat or mattress was present, height of brisket positioner, distance from the neck rail to the curb (on the diagonal), height of neck rail, height of the lowest side rail at both the rear (40 cm in from rear of bed) and front of the freestall (at point of brisket positioner), and distance between lower and upper side rails at the front end of the freestall (at point of brisket positioner)
Cubicle features	Proportion of cubicles with broken side, neck rails, incorrectly positioned mats and mattresses, nonparallel side rails, side lunge space available on just one side, interrupted forward lunge or bob space and directly facing a wall
Management practices	Hygiene products related variables, herd size, stocking rate, days of winter housing, frequency of bedding materials was replenished

3.2.2.3 Correlation between hair loss measured by area on the hock maps and scores given on a categorical scale

The area of hair loss on the lateral surface of each hock measured on the hock maps was compared with the respective categorical scores on a scale of 0 to 3 assigned to that hock (Potterton et al., 2011b). Mann-Whitney tests (Petrie and Watson, 1999) were used to assess the differences in the area of lesion among

scores on the categorical scale. A p -value of less than or equal to 0.05 was specified to indicate significant differences.

3.3 Results

3.3.1 Risk factors associated with area of hair loss on the lateral surface of the hock

There were 19 significant variables with p -value less than or equal to 0.10 associated with area of hair loss on the lateral surface of the hock in the univariable analysis (Table 3.2). Of these, six risk factors remained in the final multilevel linear model (Table 3.3). The intercept as mean area of hair loss across all cows was 22.31cm². Coefficients for each parameter were presented as the ratio of the mean of selected parameters over the reference group, where coefficients over 1 indicated a greater mean area of hair loss and coefficient less than 1 was a smaller mean area of hair loss compared with the reference group respectively. Residual plot at each level indicated a good model fit (Figure 3.2).

Table 3.2 List of significant variables ($p \leq 0.10$) associated with area of hair loss on the lateral hock surface in the univariable analysis

Cow characteristic factors	Total cleanliness score
	Breed
	Parity
	Mean milk yield (kg)
	Body condition score
	Locomotion score
	Days of winter housing
Farm management factors	Base and bedding material in the freestall
	Mean depth of bedding material (m)
	Total loafing area in cubicle yards (m ²)
	Length of feed space per cow in cubicle yards (m/cow)
	Age of mats and mattresses within cubicles (years)
	Frequency of application of hygiene products to cubicles (week)
	Frequency of re-bedding (week)
	Cubicle type
	Width of cubicle (m)
	Distance from the neck rail to the rear of the cubicle on the diagonal (m)
	Height of the lowest side rail at the head of the cubicle (m)
	Distance between the side rails at the head of the cubicle (m)

Cows with locomotion score 3 (i.e. severely impaired mobility; (coefficient: 1.22 (confident interval: 1.06-1.39)) had a significantly larger area of hair loss compared with cows with good mobility (score 0). Cows with a total cleanliness score between 10-18 (1.17 (1.03-1.33)) i.e. moderately dirty, had a significantly greater area of hair loss on the hock compared with those cows which had a lower cleanliness score. An increase in mean milk yield was positively associated with area of hair loss; cows producing between 25.1-40.0kg (1.11 (1.03-1.19)) and 40.1-58.1kg (1.25 (1.09-1.43)) had significantly larger areas of hair loss compared with cows producing less than 25.0kg of milk. Cows with a BCS of 2 (0.90 (0.82-0.98)) were significantly more likely

to have a smaller area of hair loss compared with cows with a BCS between 1 and 1.5.

The number of days of winter housing was positively associated with area of hair loss; cows housed between 41-76 days (1.24 (1.04-1.48)) and more than 76 days (1.19 (1.00-1.41)) had significantly larger areas of hair loss compared with those housed less than or equal to 40 days. Cows housed in stalls with a concrete base and sand bedding (0.46 (0.30-0.70)) or a concrete base with chopped straw bedding (0.27(0.15-0.52)) had a significantly smaller area of hair loss compared with cows housed in stalls with a concrete base and unchopped straw or rape straw bedding. Cows housed in stalls with a mattress base and unchopped straw or rape straw (1.47 (1.04-2.09)) had a significantly larger area of hair loss compared with cows housed in stalls with a concrete base and unchopped straw or rape straw bedding.

Table 3.3 Risk factors associated with area of hair loss on the lateral hock surface in the multilevel linear model

Variable	Freq. of hocks	Coeff.	95% C.I.		p-value	
Intercept	5186	22.31cm ²				
Locomotion score						
Score 0	2240	Ref.				
Score 1	892	1.03	0.94	1.14	0.49	
Score 2	1521	1.01	0.93	1.10	0.78	
Score 3	354	1.22	1.06	1.39	0.004	*
¹ Total cleanliness score						
2-9	437	Ref.				
10-18	4492	1.17	1.03	1.33	0.02	*
19-27	133	1.18	0.91	1.52	0.21	
Mean milk yield (kg)						
2.4-25.0	1800	Ref.				
25.1-40.0	2093	1.11	1.03	1.19	0.01	*
40.1-58.1	435	1.25	1.09	1.43	0.002	*
Body condition score						
1-1.5	1339	Ref.				
2	2143	0.90	0.82	0.98	0.01	*
2.5-4.5	1655	0.93	0.84	1.02	0.14	
Days of winter housing						
2-40	1045	Ref.				
41-76	1073	1.24	1.04	1.48	0.02	*
>76	2082	1.19	1.00	1.41	0.04	*

continued

*p-value ≤ 0.05, Ref.: reference, Freq.: frequency, Coeff.: coefficient, C.I.: confident interval

¹The total cleanliness score was a summation of 7 separate areas including the tail, left and right flanks, left and right lower hind limbs and left and right upper hind limb.

Table 3.3 (continued) Risk factors associated with area of hair loss on the lateral hock surface in the multilevel linear model

Variable	Freq. of hocks	Coeff.	95% C.I.		<i>p</i> -value	
Base and bedding material in the freestall						
Concrete with unchopped straw or rape straw	235	Ref.				
Concrete with sawdust or wood shaving	133	1.16	0.72	1.88	0.54	
Concrete with sand	169	0.46	0.30	0.70	<0.001	*
Concrete with chopped straw	46	0.27	0.15	0.52	<0.001	*
Mattress with sawdust or wood shaving	760	1.02	0.73	1.42	0.92	
Mattress with unchopped straw or rape straw	208	1.47	1.04	2.09	0.03	*
Mattress with chopped straw	185	0.78	0.42	1.44	0.43	
Mattress with other bedding	86	0.76	0.41	1.41	0.38	
Mat with sawdust or wood shaving	485	0.78	0.56	1.09	0.14	
Mat with sand	34	0.72	0.37	1.40	0.34	
Mat with unchopped straw or rape straw	58	1.42	0.76	2.66	0.28	
Mat with chopped straw	223	1.02	0.67	1.55	0.93	
Mat with other bedding	78	0.78	0.42	1.47	0.45	
Other base with sawdust or wood shaving	163	1.22	0.76	1.97	0.41	
Other base with unchopped straw or rape straw	688	0.97	0.70	1.33	0.84	
Other base with other bedding	88	0.76	0.40	1.43	0.39	

**p*-value ≤ 0.05.

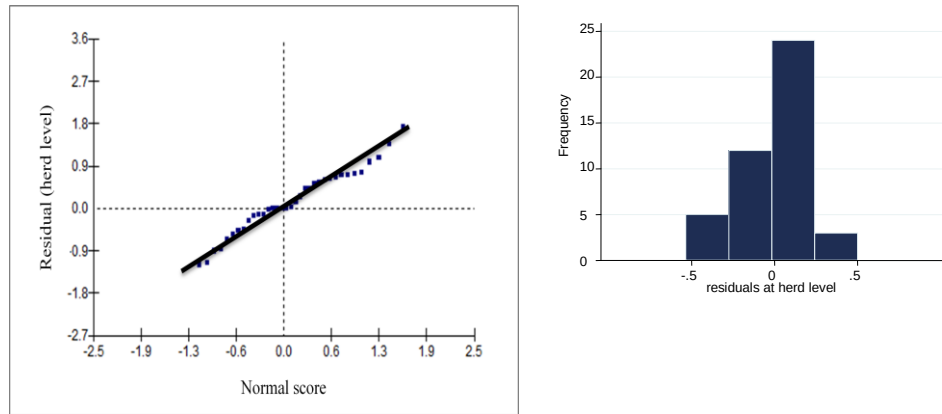
Random variability between farms: 0.072 (0.018); random variability between cows: 0.18 (0.016); random variability between hocks: 0.37 (0.15).

There were significant associations between some variables in the final multivariable model and other explanatory variables; mean milk yield was significantly positively associated with parity, hence, only mean milk yield was included in the model. There were significant associations between the type of base bedding used in the stall and the mean depth of the bedding material. Out of the 208 observations (hock level) from mattress based stalls, 164 (79%) and 44 (21%) observations had a mean depth of straw or rape straw bedding between 0-0.02m and 0.03-0.04m respectively (Table 3.4). Sixty three (27%), 84 (36%) and 88 (37%) observations from concrete base stalls had a mean depth of straw or rape straw bedding between 0-0.02m, 0.03-0.04m and 0.05-0.06m respectively. Of the 169 observation (hock level) from concrete base stalls, 41% (70) and 59% (99) of observations had a mean depth of sand bedding between 0.03-0.04m and 0.07-0.12m respectively while all 46 (100%) observations had a mean depth of chopped straw bedding between 0.07-0.12m in concrete base stalls.

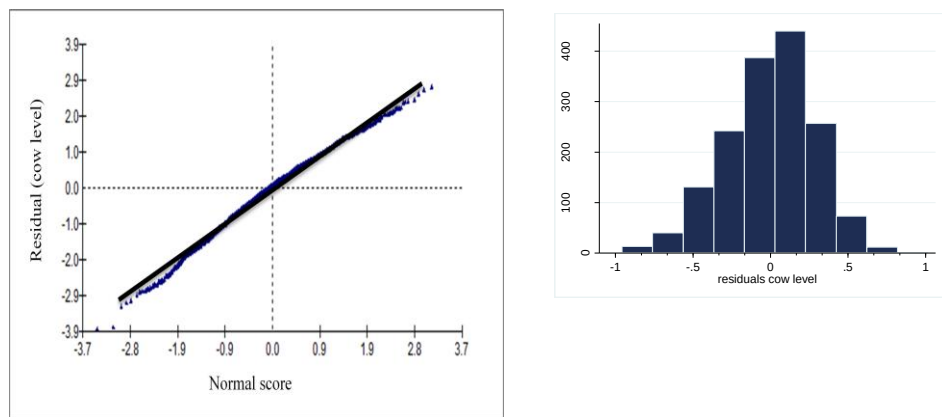
Table 3.4 Mean depth of bedding material in freestalls with different base and bedding materials

Base and bedding	Mean depth of bedding material (m)			
	0-0.02m	0.03-0.04m	0.05-0.06m	0.07-0.12m
Mattress with unchopped straw or rape straw	164 (79%)	44 (21%)		
Concrete with unchopped straw or raped straw	63 (27%)	84 (36%)	88 (37%)	
Concrete with sand		70 (41%)		99 (59%)
Concrete with chopped straw				46 (100%)

a.



b.



c.

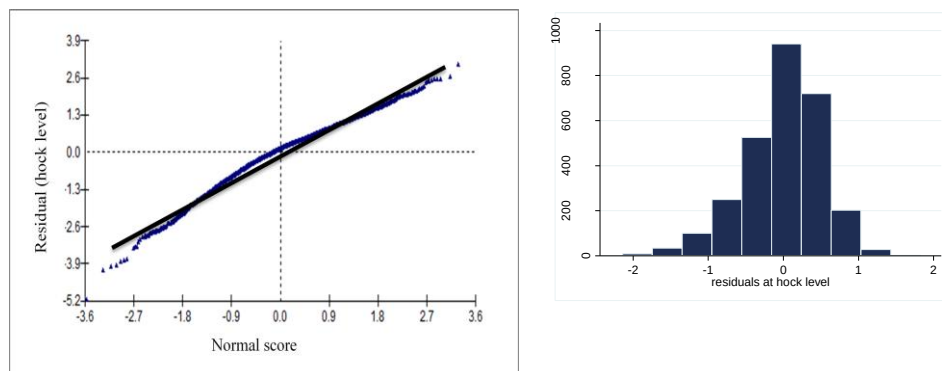


Figure 3.2 Residual plots for herd (a), cow (b) and hock (c) levels of multilevel linear model of area of hair loss on the lateral surface of the hock respectively

3.3.2 Correlation between hair loss measured by area on the hock maps and scores given on a categorical scale

There were 2072 hocks with an area of hair loss on the lateral surface only. The minimum and maximum values for the area of hair loss were 0.38cm² and 141.39cm². The distributions of area of hair loss within their allocated categorical scores are presented in Table 3.5, Figures 3.3a and 3.3b respectively. Of the 2072 hocks which had areas of hair loss recorded, 1215 (59%), 562 (27%) and 295 (14%) hocks were scored 1, 2 and 3 for hair loss on the categorical scale, respectively. The median values of area of hair loss for scores 1, 2 and 3 were 23.56cm² (Inter quartile range (IQR): 8.29 – 47.52 cm²), 20.25cm² (IQR: 9.00 – 40.51 cm²) and 42.37cm² (IQR: 28.22 – 63.46 cm²) respectively.

The median area of hair loss was no significant difference between scores 1 and score 2 ($z= 1.58$, $p=0.11$); score 3 had a significantly higher area of hair loss compared with score 2 ($z = -11.53$, $p<0.001$).

Table 3.5 Distribution of area of hair loss within their allocated categorical scores

Categorical scale	Area of hair loss (cm ²)	
Score	Freq. (%)	Median (IQR)
1	1215 (59%)	23.56 (8.29-47.52)
2	562 (27%)	20.25 (9.00-40.51)
3	295 (14%)	42.37 (28.22-63.46)
Total	2072	

Freq.: Frequency, IQR: Inter quartile range

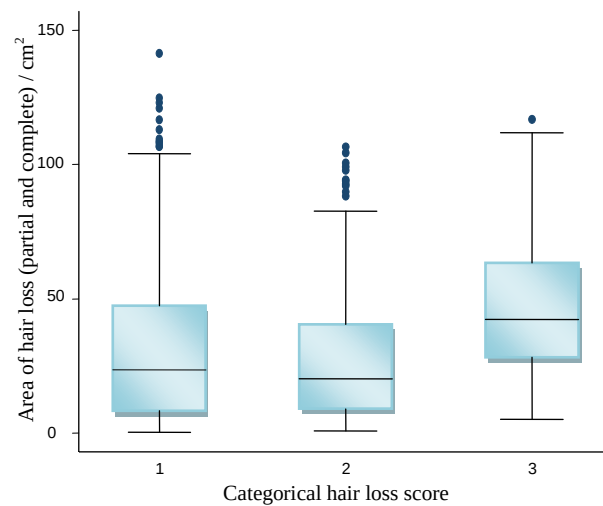


Figure 3.3 The distribution of area of hair loss on the lateral hock scored on a categorical scale

3.4 Discussion

To the author's knowledge, this is the first study to investigate risk factors associated with area of hair loss on the hocks, based on the area measured on a continuous scale. Out of 94 potential risk factors, only six risk factors were

found to have significant associations with area of hair loss. These factors were: locomotion score, body condition score, cleanliness score, mean milk yield, days of winter housing, base and bedding material in the freestall; where body condition score and cleanliness score were not associated with presence of hair loss based on the study of Potterton et al. (2011a). Of the 14 risk factors which were identified by Potterton et al. (2011a), eight risk factors were not found significant in the study, including application of hygiene products, features of freestall design (height of lowest side rail at front end of freestall, distance from neck rail to rear of the freestall, height of neck rail, length of freestall), the time mats and mattresses have been in freestall, breed and herd size. There remains a possibility that other factors which contribute to the occurrence of lesions in the first place, might not be associated with area of hair loss. Alternatively, the different results between studies is possibly due to the different measurements of hair loss on the hocks were used.

Combination of base and bedding material had significant associations with area of hair loss in this study. There are many possible different combinations of base and bedding materials used on dairy farms in the UK, which increases the difficulty of investigating lying comfort as a risk factor for hair loss. The results reported here demonstrate that on a similar base surface (concrete), sand and chopped straw were more protective than unchopped straw or rape straw. It has been suggested that sand is more likely to stay on the lying surface and compress less (Weary and Taszkun, 2000) and thus is more likely to offer protection. Furthermore, sand is an inert and

inorganic materials which is unlikely to allow pathogen growth (Kudi et al., 2009; DairyCo., 2013) whereas straw is an organic material which has higher risk of pathogen growth (Kudi et al., 2009). Compared to cows housed on sand, cows housed on straw were dirtier and had longer durations of lying, resulting in the development of lesions on the hocks (Norrington et al., 2008; Andreassen and Forkman, 2012). Chopped straw is suggested to have better absorbency compared to unchopped straw (Tuytens, 2005), and is widely used on UK dairy farms (DairyCo., 2013). This could be one possible explanation for the finding that cows housed on chopped straw had smaller areas of hair loss compared with cows housed on unchopped straw.

Lying comfort has been suggested as a predominant risk factor of hock lesions (Huxley and Whay, 2006; Lombard et al., 2010); however, lying comfort is hard to measure as it depends on types of base, materials used as a bedding substrate and the depth of bedding material used. The finding from this study showed a correlation between combinations of base and bedding material used and the depth of bedding (Table 3.4). Types of base and bedding materials are difficult to change, however depth of bedding material has more potential to be easily manipulated on farm. The results suggest that cows housed on thicker layers of bedding materials had a smaller area of hair loss even when the base was harder. For instance, concrete is harder than mattresses, however, by applying a thicker layer of straw or rape straw on top of concrete surfaces, this may increase the degree of lying comfort and decrease the area of hair loss compared with cows housed on mattresses with

thinner layers of similar bedding materials even though the concrete base is harder.

Milk yield was a risk factor associated positively with area of hair loss, agreeing with previous studies (Sogstad et al., 2006; Rutherford et al., 2008; Potterton et al., 2011a), however the causal relationship between milk yield and hock lesions is unclear. Cows with higher milk yield potentially have bigger udders and body size (Lin et al., 1987), which cause the cows to widen the distance between the limbs while walking and lying down. This may lead to body protrusion such as hocks crashing into cubicle fittings more often, and thereby increasing the chance of lesion development. Alternatively the pain and discomfort from hock lesions may decrease dry matter intake, affecting milk production directly. A decline of milk production leads to economic losses for farmers, so, it is important to understand the dynamics of the relationship between milk yield and hock lesions.

A wide range of studies found that the lameness and lower BCS associated positively with hock lesions (Whay et al., 2003; Regula et al., 2004; Kielland et al., 2009; Potterton et al., 2011a; Brenninkmeyer et al., 2012); this association was also demonstrated in the current study but Potterton et al. (2011a) found that there was no significant association between BCS and presence of hair loss. The current study design could not demonstrate the directionality of relationships among lameness, BCS and hock lesions, and these relationships have not been examined longitudinally. There are few possible explanations of the relationships between them. For example, cows

with lower BCS had higher risk of lameness, possibly due to thinning of the digital cushion which has been associated with mobility problems (Bicalho et al., 2009). Thus lame cows spent more time lying and changed their rising behaviours (Chapinal et al., 2009; Ito et al., 2010), resulting in more abrasion between hock and base material. Another possible explanation is that hock lesion may be the cause of lameness; these lesions may bring discomfort and pain to cows leading them to change their normal gait, given most hocks with hair loss had some degree of swelling although the severity/area of swelling was not investigated in this study. Furthermore, discomfort and pain feeling from hock lesions may reduce feed intake and feeding time, resulting in negative energy balance (low BCS) and mobilization of the fat in whole body of dairy cattle including digital cushion might increase the risk of lameness. Alternatively, lame cows may have more difficulty standing up or lying down, increasing the frequency with which bony protrusions such as the hock rub against the lying surface or curb, resulting in a larger area of hock hair loss. Finally cows with lower BCS may have lower feed intake and insufficient nutrition; this unhealthy condition might cause easy hair loss to the cows when hocks collide to the hard surface. The directionality of relationship between lameness vs hock lesions and between lameness vs BCS are investigated in Chapter 4 and Chapter 5 of this thesis respectively.

Moderately dirty cows had greater areas of hock hair loss compared with cleaner cows, however, very dirty cows showed no difference, possibly due to a small number of very dirty cows (133hocks) in this dataset. Other studies have found similar results (Regula et al., 2004; Zurbrigg et al.,

2005a) but Potterton et al. (2011a) showed no significant association. It is difficult to draw out possible explanations for this relationship as animal cleanliness itself is likely to be linked with other factors, such as condition of housing, environment, management factors, husbandry practices and other unidentified variables which were not measured in this or other studies. There is no published study investigating the causality of animal cleanliness and hock lesions, however previous studies have found that dirtier cows had higher risk of lameness, mastitis and lower milk quality (Mülling et al., 2006; Kelly et al., 2009; Sant'anna and Paranhos da Costa, 2011), thus farmers should always be encouraged to keep their animals as clean as possible.

The current study found that cows housed for longer periods had greater areas of hair loss on the hocks. This finding was possibly associated to the length of exposure to the lying surface (e.g. hocks are exposed to abrasive materials for longer) in comparison to those cows housed for shorter periods. Other studies have found positive associations between the duration of housing and hock lesions (Rutherford et al., 2008; Potterton et al., 2011a), however this is one of the farm management practices which is difficult to change due to climatic condition and the types of farm systems used.

The hock map proved useful for capturing large amounts of details such as severity of lesions, regions affected and lesion size. Comparison of the hair loss area on hock maps and the scores given on categorical scale indicate that the severity of hair loss at score 1 and score 2 are hard to differentiate. This

may suggest that an ordinal categorical scale for hair loss in its current form is not capturing accurately the severity of lesions. Also, categorical scales could not capture the lesions in details (e.g, size and location) compared to hock map. One of the disadvantages of hock map is this measurement is still considered as subjective measurement as the hand drawn hock maps may be influenced by the observer's judgement and introduce bias. Therefore the further investigation on the validity and reliability of this measurement is suggested. One of the drawbacks of hock maps is that skin condition such as redness, scars, thickness, bareness and swelling could not be recorded using the current form of maps. Improvements to the hock map are required to ensure it is more practical and manages to capture all pertinent aspects of the lesion. Undoubtedly hock maps are time consuming to complete, however, more precise details of lesions could be captured in comparison with the use of ordinal categorical scales. Thus hock maps are recommended for monitoring the development of hock lesions over time. Nevertheless, ordinal categorical scales remain a simple, easy and time efficient method for use on farm. However they may not accurately record the lesion, especially if categorical scales merge hair loss, ulceration and swelling into one category. Whilst they may not accurately capture all lesion detail, they are likely to remain in use for herd level assessments because of their speed and ease of use. The validity of categorical scales has not been evaluated experimentally. Future work is required to validate the scales that are currently used to identify their limitations and support their use on-farm.

Even though there are no published studies showing the impact of hair loss to the cows, it is logical to assume that cows with greater hair loss have poorer welfare (e.g, more painful feeling). Also hair loss is also present with swelling/other lesion making hard to tease out the patterns in a cross-sectional study. It's suggested a longitudinal study for development of hock lesion and their impact is needed. Furthermore, the investigation of risk factors associated with severity of swelling and ulceration are required to provide better understanding of lesion aetiology, ultimately so that control strategy to reduce the prevalence of hock lesions can be devised.

3.5 Conclusion

This study investigated the risk factors associated with area of hair loss in dairy cows which had hock lesions. Of the 94 potential risk factors which were included in the analysis, only six (milk yield, days of housing, locomotion score, cleanliness score, body condition score and lying surface) were significant in the final model. Categorical scale might not be a valid tool to differentiate the extent (based on area) of hair loss on the hock and hock maps offer a good alternative, especially hock maps could be used in longitudinal studies to monitor development and progression of hair loss over time. Further work is needed to better understand the aetiology and control of this prevalent condition which has implication for both the welfare and productivity of intensively managed dairy cows.

CHAPTER 4: INVESTIGATIONS INTO THE ASSOCIATION OF LAMENESS WITH HAIR LOSS ON THE HOCKS IN DAIRY CATTLE IN A LONGITUDINAL STUDY

4.1 Introduction

Hock lesions are one of the welfare concerns in dairy herds and have an indirect impact on the welfare and health of dairy cattle (Haskell et al., 2006; Huxley and Whay, 2006; Brenninkmeyer et al., 2012). It is assumed that these lesions may cause pain or discomfort to the cows (Huxley and Whay, 2006) and are correlated positively with other health problems such as teat injuries and skin injuries (Regula et al., 2004; Sogstad et al., 2006).

Hock lesions are associated positively with lameness, one of the most serious and costly welfare issues (Regula et al., 2004; Kielland et al., 2009) and lameness itself has been linked with many other health problems, for instance, an increase in the risk of culling (Rajala-Schultz and Grohn, 1999), decrease fertility rate (Bicalho et al., 2007) and lower milk yield (Green et al., 2002). Lame cows produce lower milk yields compared with healthy cows and consequently cause economic loss to dairy farmers (Bicalho et al., 2008; Sulayeman and Fromsa, 2012).

The association between lameness and hock lesions has been established in cross-sectional studies (Whay et al., 2003; Regula et al., 2004; Kielland et al., 2009; Brenninkmeyer et al., 2012), however the cross-sectional study design

lacks the power to establish the cause and effect in the relationship between two conditions i.e. the directionality of the association. Some longitudinal studies investigated the development of hock lesions when cows were housed on different stall surfaces (Vokey et al., 2001; Livesey et al., 2002; Mowbray et al., 2003; Norring et al., 2008) but the temporal association between lameness and hock lesions was not investigated. Lameness may increase the chance of cows having hock lesions in the future as lame cows spend longer time lying down and lameness changes their rising and lying behaviour (Chapinal et al., 2009; Ito et al., 2010); this increases the chance of having hock lesions when the hocks collide with the bedding surface or cubicle fittings more often. Alternatively, hock lesions may predispose the cows to be lame in the future as when the cows have lesions on the hock, they may change their walking posture, rising and lying behaviours, and eventually increase the risk of lameness. There are no published longitudinal studies to establish the directionality of the relationship between lameness and hock lesion.

The aims of this study were to investigate i) the transition of hair loss on left and right hocks from one visit to the next visit, ii) the association between lameness and the presence of hair loss on the hock and other related factors in a longitudinal study.

4.2 Materials and methods

4.2.1 Data set and study methodology

Three farms with dairy cattle housed in cubicles with mattresses and bedded with sawdust located in Nottinghamshire (n=2) and Northamptonshire (n=1) were recruited to the study. Heifers on Farm 1 and Farm 2 were housed in a straw yard until calving. Heifers on Farm 3 were housed on straw until 12 months of age and then were moved to cubicle housing bedded with sawdust on mattresses. About three weeks before parturition, they were moved back to a straw housing until they calved. Heifers on Farm 1 were turned out to grass during Sep 2008-Oct 2008 and May 2009-Oct 2009 while heifers on Farm 2 and Farm 3 were housed continuously. All farms were visited by the researcher (SLP) with an assistant on approximately the same date (± 12 days) of every month from September 2008 until March 2010. Approximately 20 to 30 Holstein-Friesian heifers which calved between July 2008 and July 2009 were selected from each herd and observed monthly. Any animals that were sick at calving or during the study period and any animals which were kept in straw yard housing for longer than 2 months were excluded from the study. Height and girth of the selected heifers were observed on the initial visit. For each visit, body condition score, mobility score, cleanliness score and the severity of hock lesions were observed. The severity of hock lesions were measured according to the hair loss and ulceration condition on both hocks separately using four point categorical scales (score 0-3) (Whay et al., 2003; Huxley and Whay, 2006). The layout and dimensions of the housing were

recorded on the initial visit, while the mean volume of sawdust cubicle bedding on each farm was recorded during each visit. Milk records were accessed from National Milk Records with farmer consent.

4.2.2 Statistical analysis

The data was screened for errors and missing values, and then frequency tables were created. Comparison of hair loss and ulceration conditions between left and right hock were made respectively. Associations between lesions (hair loss and ulceration) on the left and right hock were tested using Chi square test. The transitions of hair loss on both left and right hock from one visit to the next visit were observed respectively. The proportion of animals with occurrence and resolution of lameness were calculated and used as the predictors in the model. Occurrence of lameness was defined as a change from non-lame (score 0 or 1 at prior visit (t_{-1})) to lame (score 2 or 3 at current visit (t)) whereas resolution of lameness was defined as a change from lame at current visit (t) to non-lame at next visit (t_{+1}). Descriptive analyses were conducted in Stata version 12 (StataCorp, USA).

4.2.2.1 Logistic regression model to investigate risk factors of hair loss on the hocks

The outcome variable was the hair loss score with no hair loss (score 0) and the presence of hair loss (score 1 / 2 / 3). A logistic regression model with two hierarchical levels with repeated measure of month at level 1 nested within

animal at level 2 was built. Each explanatory variable with a Wald statistic p -value ≤ 0.10 were retained and taken forward into the final model. Variables with a significance value less than or equal to Wald statistic p -value ≤ 0.05 were retained in the final model.

Model specifications were

$$\text{Logit}(\pi_i) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_i X_i + u_k$$

$$u_k \sim N(0, \sigma_k^2)$$

Outcome $\sim$ Bernoulli (n_i, π_i) with individual data $n_i=1$ for all probability (π_i)

where π_i is the presence/absence of hair loss at month (i), β_0 is the intercept, $\beta_1, \beta_2, \dots, \beta_i$ were the coefficients of explanatory variables of $X_1, X_2, \dots, X_i$ respectively and u_k represents random effect of animal level which were assumed to follow a normal distribution with mean zero and variance σ_u^2 . The model was created in MLwiN version 2.25 (Centre for Multilevel Modeling, University of Bristol) and estimation was carried out by quasi-likelihood methods and followed by Markov chain Monte Carlo (MCMC) methods with 500,000 iteration and a burn in of 5,000. The chain mixing and stability were then evaluated visually (a detailed of diagnosis method is on Chapter 2.4.2) (Browne, 2009).

The explanatory variables that were tested in the analysis (Table 4.1) were: (i) farm variables; (ii) left/right; (iii) five categories of lameness at previous and current visit (t_{-1}, t); (iv) five categories of lameness at current and next visit (t, t_{+1}); (v) three categories of cleanliness score (the more dirty hocks had a

higher cleanliness score); (vi) four categories of last record of milk yield (kg);
(vii) three categories of body condition score (BCS).

Table 4.1 List of explanatory variables used in a logistic regression model to investigate hock hair loss

Variables	Categories
Farm	1
	2
	3
Left/right	Left
	Right
Lameness (t_{-1}, t) (Resolution)	Non-lame at previous and current visit (0,0)
	Lame at previous and current visit (1,1)
	Lame at previous and recovered at current visit (1,0)
	Non-lame at previous and being lame at current visit (0,1)
	Others (not under above 4 categories)
Lameness (t, t_{+1}) (Occurrence)	Non-lame at current and next visit (0,0)
	Lame at current and next visit (1,1)
	Non-lame at current and becoming lame at next visit (0,1)
	Lame at current and recovered at next visit (1,0)
	Others (not under above 4 categories)
Cleanliness score (more dirty hocks had higher cleanliness scores)	0-5
	6-7
	8-11
Last record of Milk Yield (kg)	<21.8
	21.8-27.2
	27.3-32.1
	>32.2
BCS	1.5
	2
	2.5-4.5

4.3 Results

4.3.1 Descriptive analysis

Eighty five heifers were selected from three farms. There were 855 monthly animal observations conducted. A total of 94 observations (15 heifers) were excluded from the study due to insufficient follow-up observations ($n \leq 4$ observations). Out of the remaining 70 heifers (761 observations), there were 23, 30 and 17 animals from Farms 1, 2 and 3 respectively. The median (IQR) number of monthly animal observations for Farms 1, 2 and 3 were 13 (12-14), 9 (7-10), and 11 (10-13) respectively. For each observation, the severity of hock lesions (hair loss and ulceration) was recorded for both left and right hocks separately. Out of 1522 records (761 records from each hocks), there were 22 and 25 missing records for left and right hocks respectively. The remaining 1475 records (739 records for left hocks and 736 records for right hocks) were used in the analysis.

The mean volume of sawdust bedding per cubicle (litre) is illustrated in Figure 4.1. The mean volume of bedding on Farm 3 was consistent (0.6-0.8l per cubicle) throughout the study period except in January 2009 when it reached a maximum (1.7l per cubicle) and the minimum was 0.4l per cubicle in December 2009. The maximum (minimum) mean volume of bedding for Farm 1 and Farm 2 were 2.0l (0.6l) per cubicle and 2.7l (0.0l) per cubicle respectively. Due to missing records, the mean volume of bedding was not used in the further analysis.

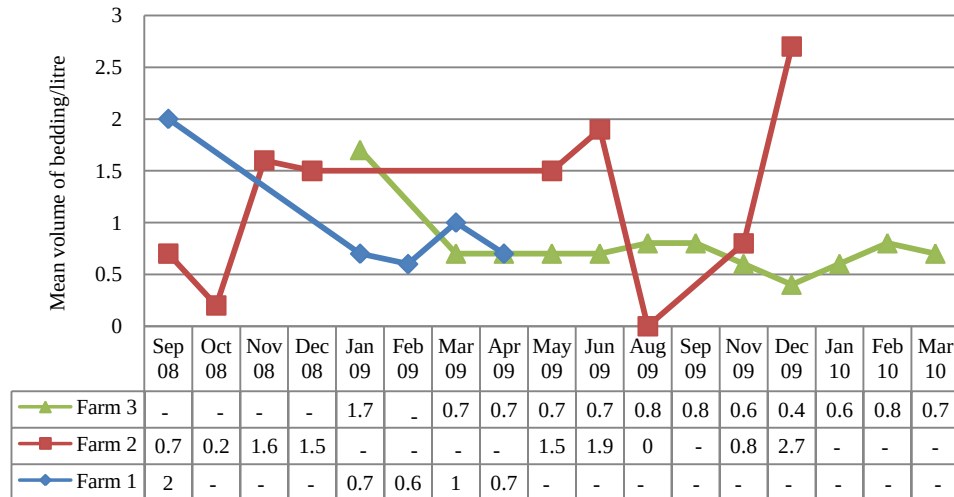


Figure 4.1 The mean volume (litre) of cubicle bedding (sawdust) that was recorded throughout the study period

4.3.2 Comparison of hair loss and ulceration between left and right hocks at the current visit (*t*)

There were approximately 12% (91) of cows with no hair loss (score 0) on both hocks while 78% (570) had hair loss (score 1 / 2 / 3) on both hocks. Hair loss on the left hock was associated significantly with hair loss on the right hock ($p<0.001$). Approximately 4% (29) of cows had hair loss on the right hock and no hair loss on left hock, while 6% (40) of cows had hair loss on left hock and no hair loss on the right hock.

Approximately 67% (487) of cows had no ulceration on either hock whereas 12% (88) of cows had ulceration (score 1 / 2 / 3) on both hocks. Ulceration on the left hock was associated significantly with the right hock ($p<0.001$). Approximately 10% (74) of cows had an ulcerated right hock and no ulceration on the left hock while 11% (81) of cows had an ulcerated left hock

and no ulceration on the right hock. The distribution of hair loss and ulceration on both left and right hocks are presented in Table 4.2.

Table 4.2 The distributions of hair loss and ulceration on both left and right hocks

Left hock	Right hock			Left hock	Right hock		
	No hair loss	Hair loss	Total		No ulceration	Ulceration	Total
No hair loss	91	29	120	No ulceration	487	74	561
%	12	4	16	%	67	10	77
Hair loss	40	570	610	Ulceration	81	88	169
%	6	78	84	%	11	12	23
Total	131	599	730	Total	568	162	730
	18	82	100		78	22	100

4.3.3 Transitions of lameness: occurrence and resolution

Out of 181 transitions, there were 82 resolutions (lame to non-lame) and 99 occurrences (non-lame to lame). Table 4.3 elucidates the number of transitions in each explanatory variable. About 12% (12), 56% (54) and 32% (31) of observations of cows with BCS 1.5, 2 and 2.5-4.5 transitioned from non-lame to lame at the next visit (occurrence) respectively whereas 5% (4), 59% (48) and 37% (30) of observations cows transitioned from lame to non-lame (resolution) respectively.

The percentage of cow observations in different cleanliness score categories for both occurrences and resolutions were similar (showed in Table 4.3). For cow observations in four different milk yield categories (less than 21.8kg, 21.8-27.2kg, 27.3-32.1kg and more than 32.1kg), in 23% (22), 15% (14), 40% (38) and 23% (22) of observations cows had transitioned from non-lame to

lame by the next visit (occurrence) respectively, whilst in 20% (16), 38% (30), 33% (26) and 10% (8) of observations cows transitioned from lame to non-lame by the next visit (resolution) respectively.

Table 4.3 The distribution of lameness transitions by variable: occurrence and resolution

Variables	Categories	Transition				Total
		Occurrence		Resolution		
		Freq.	%	Freq.	%	
BCS	1.5	12	12	4	5	16
	2	54	56	48	59	102
	2.5-4.5	31	32	30	37	61
Cleanliness score	0-5	31	32	31	38	62
	6-7	49	50	33	40	82
	8-11	18	18	18	22	36
Last record of milk yield (kg)	<21.8	22	23	16	20	38
	21.8-27.2	14	15	30	38	44
	27.3-32.1	38	40	26	33	64
	>32.1	22	23	8	10	30

4.3.4 Transitions of hair loss on the left and right hocks

The transitions of ulcerations were not explored further due to insufficient numbers of ulcerations observed. Table 4.4 describes the transitions of hock lesion score for both left and right hocks separately. Approximately 58% and 53% of cows with no lesions at the current visit had hair loss (score 1-3) on the left and right hock at the next visit respectively whereas 42% and 47% of cows with no lesions remained with no lesions at the next visit respectively. For left (right) hocks with score 1, 2 and 3 at the current visit, 36% (42%), 43% (41%) and 63% (59%) of these remained at the same score at the next visit respectively. There were 50% (46%) and 29% (31%) of left (right) hocks with score 1 and 2 which got more severe (increased to a higher score) by the

next visit respectively. Approximately 14% (13%) and 28% (28%) of left (right) hocks with score 1 and 2 had resolved (decreased to a lower score) by the next visit respectively. A total of 37% (41%) of left (right) hocks with score 3 had resolved by the next visit.

Table 4.4 Transition of hair loss between visits for both left and right hocks

Total 3 farms	Next visit				Total 3 farms	Next visit			
	Left hock with hair loss					Right hock with hair loss			
Current visit	No change	Increase	Decrease	Total	Current visit	No change	Increase	Decrease	Total
Score 0	36	49		85	Score 0	47	53		100
%	42	58		100	%	47	53		100
Score 1	46	63	18	127	Score 1	52	57	16	125
%	36	50	14	100	%	42	46	13	100
Score 2	70	47	45	162	Score 2	72	54	48	174
%	43	29	28	100	%	41	31	28	100
Score 3	130		76	206	Score 3	106		73	179
%	63		37	100	%	59		41	100
Total	282	159	139	580	Total	277	164	137	578
%	49	27	24	100	%	48	28	24	100

4.3.5 Logistic regression model to investigate risk factors for hair loss on the hocks

A list of significant variables associated with hair loss is presented in Table 4.5. Cows that were lame at the previous visit and recovered at the current visit (OR: 6.32, CI: 1.59-25.15) or cows that continued to be lame (OR: 4.79, CI: 1.09-21.09) at the current visit had higher odds of having hair loss on the hocks compared with cows that were non lame at the previous and current visit respectively. The odds of having hair loss for cows producing between 21.8-27.2 kg (OR: 2.21, CI: 1.18-4.12), 27.3-32.1kg (OR: 3.36, CI: 1.58-7.16)

Table 4.5 List of significant variables in a logistic regression model investigating hair loss on the hocks

Variable	Categories	Freq.	Coeff.	Odd ratios	95%C.I.	<i>p</i> -value
Intercept		1475	0.64			
Farm	1	593	Ref.			
	2	508	1.85	6.37	2.59 15.62	<0.001*
	3	374	1.64	5.17	1.84 14.50	<0.001*
Left/right	Left	739	Ref.			
	Right	736	-0.26	0.77	0.50 1.18	0.23
Cleanliness score	0-5	513	Ref.			
	6-7	739	0.37	1.44	0.86 2.41	0.16
	8-11	211	0.88	2.41	1.08 5.39	0.03 *
Last record of Milk Yield (kg)	<21.8	326	Ref.			
	21.8-27.2	327	0.79	2.21	1.18 4.12	0.01 *
	27.3-32.1	320	1.21	3.36	1.58 7.16	<0.001*
	>32.2	321	0.95	2.59	1.16 5.80	0.02 *
Lameness (t_{-1}, t)	Non-lame at previous and current visit (0,0)	677	Ref.			
	Lame at previous and current visit (1,1)	211	1.57	4.79	1.09 21.09	0.04 *
(Resolution)	Lame at previous and recover at current visit (1,0)	82	1.84	6.32	1.59 25.15	0.01 *
	Non-lame at previous and being lame at current visit (0,1)	102	0.24	1.27	0.27 5.93	0.76
	Others (not under above 4 categories)	274	-0.22	0.80	0.43 1.51	0.50
Lameness (t, t_{+1})	Non-lame at current and next visit (0,0)	660	Ref.			
	Lame at current and next visit (1,1)	213	0.47	1.60	0.42 6.17	0.49
(Occurrence)	Non-lame at current and being lame at next visit (0,1)	99	0.17	1.19	0.50 2.84	0.70
	Lame at current and recover next visit (1,0)	81	-0.18	0.83	0.17 4.09	0.82
	Others (not under above 4 categories)	264	-0.66	0.52	0.26 1.03	0.06

* *p*-value ≤ 0.05 , Ref.: reference, Freq.: frequency, C.I.: credible interval
Random variability between cows: 1.15 (0.47).

and 32.2-48.0kg (OR: 2.59, CI: 1.16-5.80) were higher compared with cows producing 3-21.7kg of milk respectively. Cows with a cleanliness score between 8-11 had higher odds of having hair loss compared with lower cleanliness score between 0-5 (OR: 2.41, CI: 1.08-5.39). Farm 2 (OR: 6.37, CI: 2.59-15.62) and Farm 3 (OR: 5.17, CI: 1.84-14.50) had higher odds of having hair loss on the hocks compared with Farm 1 respectively.

4.4 Discussion

This study demonstrated that 78% of cows had hair loss on both hocks and 12% had no hair loss on either hock respectively, which is slightly lower compared with a cross-sectional study of 76 UK farms which reported that 83% of cows had lesions on both hocks (Potterton et al., 2011b). Only 4-6% of the cows had hair loss on a single hock. The findings suggest that cows usually have lesions on both hocks together rather than one hock, supporting the findings of previous studies which showed that cows with hair loss on one hock are more likely to have hair loss on the other hock (Weary and Tazkun, 2000; Potterton et al., 2011b). In the case of ulcerated hocks, there were 12% and 67% of cows that had ulceration and no ulceration on both hocks respectively. These findings are similar to a UK study that reported 16.5% of hocks had ulceration (Potterton et al., 2011b). There were significant associations of hair loss and ulceration on left and right hocks respectively, where cows were more likely to have (or not have) lesions on both hocks. Previous studies have found that cows spend equal time lying on both sides

(Phillips et al., 2003; Forsberg et al., 2008), therefore the percentage of abrasion occurring on both hocks are likely to be equal.

When considering transition between categories of hair loss, cows with hair loss on the hocks either mild (score 1 or 2) or severe (score 3) were more likely to remain in the same condition or be more severe in the next month. This suggests that hair loss is a chronic lesion and does not resolve after a month; or much less likely that hair loss might resolve and redevelop rapidly within a month, allowing the same score to be obtained between 2 consecutive visits. However, as the observation period was approximately monthly by the same observer, although this reduced inter-observer bias the dynamics of hair loss lesions was hard to ascertain. The observer visited the farms approximately once per month and the length of time between visits varied, at approximately between 18 and 42 days (± 12 days). These variations could have introduced some bias however, assuming there was some randomness to visits not dependent on the development of hock lesions this is unlikely. There is also possibility that scoring system was used did not capture the lesions accurately as suggested in previous chapter (Chapter 3), resulting no significant dynamic changes of transitions between visits.

The current findings suggested that cows which are lame in the previous month (whether they remain lame or recover by the current month) have a higher risk of having hair loss on the hock in the current month compared with cows which are not lame at the previous or current month. This implies that lameness is leading to the development of hair loss in the next month. Lame

cows spend longer time lying and frequently change their rising behaviour (Chapinal et al., 2009; Ito et al., 2010). This may increase the risk of lesions developing on the hock. Another possible reason is that lame cows may suffer from foot lesions which influence them to change their behaviours to protect their foot. They may preferentially neglect their hocks, hence the risk of hock lesion development increases. Although this study elucidated that lameness precedes cows having hock lesions in the next month, the confidence interval of the resolution predictor was large (CI:1.59-25.15), perhaps due to lack of power and variability due to other unknown factors not captured in the study.

There was no significant association between the risk of hock lesions and lameness occurrence; in other words, hair loss on the hock does not lead to lameness. One possible explanation is that the outcome variable in this model was hair loss on the hock and not a severe hock lesion such as ulceration or wounds. It might be that hair loss on the hock does not have serious consequences for cows so they do not change their walking, lying and rising behaviours, so they do not increase their risk of becoming lame in the future. Severe hock lesions may cause cows to be lame in the future as the painful feeling from severe hock lesions may cause the cows to change their postures and behaviours to protect their hocks (Huxley and Whay, 2006). Alternatively, cows with severe lesions on the hock may change their walking posture, rising and lying behaviours, and eventually increase the risk of lameness. Further investigation is needed to explore whether severe hock lesions increase the risk of cows becoming lame in the future.

The current study indicated that dirtier cows have a higher risk of having hair loss on the hock compared with cleaner cows, the finding is similar to result from a study conducted in Ontario (Zurbrigg et al., 2005b), nevertheless, the result is contrasted with previous published results which indicated that dirt had a protective effect on the hocks (Potterton et al., 2011a). Animal cleanliness itself is likely to be linked with other factors, for instance it is related to the condition of housing, environment, management factors and husbandry practices. Furthermore, animal cleanliness might be linked with other unidentified variables which were not measured in this or other studies. Therefore, it is hardly surprising that the relationship between cleanliness score and hair loss on the hocks is complex. Even if current information cannot provide an explanation of the relationship between these variables, farmers should always keep their animals as clean as possible since dirtier animals have a higher risk of lameness, mastitis and lower milk quality (Mülling et al., 2006; Kelly et al., 2009; Sant'anna and Paranhos da Costa, 2011).

Mean milk yield was associated positively with the risk of having hair loss on the hock, a finding similar to the cross-sectional studies of Rutherford et al. (2008) and Sogstad et al. (2006). Cows with higher milk yield potentially have a bigger udder and body size (Lin et al., 1987). This may cause the cows to widen the distance between the limbs while walking or lying down, and lead to protruding parts of the body, such as hocks, to crash into the cubicle fittings more often, and thereby increase the risk of lesions developing on the hocks. Furthermore, cows with higher milk yield are more likely to be lame (Green et

al., 2002) and lame cows spend longer time lying down and increase the risk of having lesions on the hocks (Chapinal et al., 2009; Ito et al., 2010). Cows with higher milk yield also have a higher risk of being dirtier cows and cleanliness score has been reported as a risk factor for hock lesions (Zurbrigg et al., 2005b; Nyman et al., 2007). The direct impact of hock lesions on milk yield is an unanswered question; it is possible that the pain and discomfort caused by hock lesions may decrease dry matter intake, thereby affecting milk production. Further investigation is needed to investigate the causal relationships between hock lesions, lameness, cleanliness score and milk yield.

Compared with Farm 2 and Farm 3, cows on Farm 1 had a lower risk of having hair loss on the hock. One possible explanation is cows on Farm 1 were turned out to grass whereas cows on Farm 2 and Farm 3 were housed continuously. Decreasing the duration of exposure to lying surface may lower the risk of hock lesion, a theory which is supported by other cross-sectional studies (Keil et al., 2006; Potterton et al., 2011a). However, as herd management, housing system and features in cubicles were not fully recorded in the current study, other potential causal factors could not be confirmed.

4.5 Conclusion

This is the first study to establish the causal relationship between the lameness and hock lesions. Despite certain limitations, this study suggests that lameness precedes the development of hair loss on the hock, however, there could be other factors that influence the risk of having hair loss on the hock that could

not be determined with the data collected from this study. Cleanliness score and milk yield are associated positively with the risk of having hair loss on the hock.

CHAPTER 5: UNRAVELLING THE ASSOCIATION BETWEEN LAMENESS AND BODY CONDITION SCORE IN DAIRY CATTLE USING A MULTISTATE MODELLING APPROACH

5.1 Introduction

Lameness is one of the most costly and serious welfare concerns in dairy cattle (Enting et al., 1997; Kossaibati and Esslemont, 1997; Kossaibati et al., 1999; Cha et al., 2010). There is a significant impact of lameness on milk production, reproductive performances and it results in higher culling rates (Rajala-Schultz and Grohn, 1999; Green et al., 2002; Bicalho et al., 2007; Peake et al., 2011; Sulayeman and Fromsa, 2012). Lameness causes feelings of discomfort and long duration of pain could lead to dairy cattle altering their gaits (Whay et al., 1998; O'Callaghan, 2002).

In the UK, an average herd prevalence of lameness of 36% (range: 0 to 79%) has been reported (Barker et al., 2010; Leach et al., 2010). However, the herd prevalence of lameness varies depending on the farm housing systems, type of base and bedding material and structure of cubicle (Sogstad et al., 2005; Espejo et al., 2006; Haskell et al., 2006; Rutherford et al., 2009). Those farms that housed cows in cubicles had a higher lameness prevalence compared with those housed in straw yards or tie stalls (Sogstad et al., 2005; Haskell et al., 2006; Rutherford et al., 2009). Comfortable lying surface, organic bedding materials and a longer cubicle lying area are associated with a lower herd prevalence of lameness (Dippel et al., 2009; Rutherford et al., 2009). There are

cow-level risk factors for lameness, including age, number of parities and body condition score. The older and higher parity cows generally have a higher risk of lameness (Wells et al., 1993; Sogstad et al., 2005; Haskell et al., 2006; Dippel et al., 2009; Rutherford et al., 2009). Cross-sectional studies have reported that there is an association of lameness with poor body condition score (Espejo et al., 2006; Dippel et al., 2009; Hoedemaker et al., 2009).

Body condition score is a body energy assessment and one of the health indicators (Gillund et al., 2001; Kellogg, 2010). Several studies have reported that a greater BCS is associated with a higher risk of ketosis whereas a lower BCS is associated with lower milk yield, higher culling rate, lower reproductive performance and higher risk of displaced abomasums and endometritis (Markusfeld et al., 1997; Gillund et al., 2001; Berry et al., 2007a; Hoedemaker et al., 2009).

In a cross-sectional study, Bicalho et al. (2009) reported that lame cows are more likely to have a thinner digital cushion compared with non-lame cows and reported a significant positive association between BCS and thickness of the digital cushion, where the cows with a low BCS had a thinner digital cushion compared with cows with higher BCS. The digital cushion is a layer between the bone (third phalanx) and corium tissue which acts as part of the suspension apparatus in dairy cattle, thus thinning of the digital cushion may impair the supporting and shock absorbing functions, thereby increasing the risk of lameness (Bicalho et al., 2009). Hence, it is hypothesized that losing

BCS could influence the cows to change from non-lame to lame due to thinning of the digital cushion. Green et al. (2013) in a longitudinal study conducted on one farm found that cows with low BCS ($BCS < 2.5$) in the last 0-2 months and greater than 2-4 months had a higher risk of treatment for lameness in a 30 day period. Being conducted on one farm limits generalizability from this study and also there was no exploration of the dynamics of change of BCS on both the occurrence of and recovery from lameness.

A multilevel multistate discrete time event history model could be used to investigate the effect of factors on the likelihood of transitions between states (e.g. disease/healthy) in longitudinal data. Their use is becoming more common in exploring complex dynamics of diseases on farms (Kaler et al., 2010; Reader et al., 2011; Nielsen et al., 2012) as they avoid the limitation of fitting a separate model for each transition (Steele et al., 2004) accounting for the correlation that might exist between the transitions due to heterogeneity. The aims of this study were to investigate i) the effect of change in BCS and other risk factors on the transitions from non-lame to lame and lame to non-lame states in cows in a longitudinal study ii) the correlation between both transitions.

5.2 Materials and methods

5.2.1 Data set and study methodology

The data were collected from four herds, where Herd 1 and Herd 2 housed cows on mats and sawdust, Herd 3 housed cows on the mats with straw and Herd 4 housed cows on deep sand. All four herds are located within a 20 mile radius of Salisbury, Wiltshire. The annual rolling mean herd sizes of 138-359 cows in milk and the 305 days yield was 5889-9171 per cow. The majority of cows were Holstein x Friesians and the dairy cattle calving between July 2010 and June 2011 were selected from each herd. One observer (JAW) visited all the herds every 13-15 days from July 2010 until December 2011. For every visit, the body condition score (BCS) and mobility score (MS) of selected cows were recorded. Body condition score (BCS) was scored according to Edmonson et al. (1989) using a scale of 1-5 with increments of 0.25. Mobility score (MS) was scored according to Whay et al. (2003) on a scale from 0 to 3. Other information such as parity, age, days of lactation and month of latest calving of selected cows were recorded.

5.2.2 Statistical analysis

Cows with no information for parity, age, days of lactation, month of latest calving and BCS at calving (0-15 days post partum) were excluded from the dataset (91 cows). If cows had missing visits in the middle of the observation period, the remaining observations following the missing visit were excluded.

Only cows with at least 5 observations were used in the analysis (59 cows were excluded). The BCS was grouped into four categories: group 1 (BCS G1 1.00-1.75), group 2 (BCS G2 2.00-2.25), group 3 (BCS G3 2.50-2.75) and group 4 (BCS G4 3.00-5.00) while MS was categorized into two groups: non-lame (MS 0 and 1) and lame (MS 2 and 3). Descriptive analyses were done in Stata version 12 (StataCorp, USA).

5.2.2.1 Defining states, transitions and episodes

There were two states in the multilevel multistate model: non-lame (MS 0 and 1) and lame (MS 2 and 3). For each animal, there were two possible transitions, which transitioned from the non-lame to lame state or vice versa. The cow was either in recovered (lame to non-lame state) or lame (non-lame to lame state) transition. For each transition, the duration of time was calculated until another transition occurred and this was considered an episode.

5.2.2.2 Multilevel multistate discrete time event history model

A multilevel multistate discrete time event history model was set up to investigate the changes of the transitions between lame and non-lame states and vice versa in dairy cattle. A cow was in either non-lame (coded 0) or lame state (coded 1) for each observation. For each episode, the time interval was categorized in discrete periods (15 days) and the number of time interval (t) was measured as $t = 1, 2, 3 \dots$ up to the maximum observed time. The response

variable was the binary indicator of event occurrence. The event was when a cow transitioned from lame to non-lame or non-lame to lame in the next visit. The event indicator was coded as 1 or 0 depending on the occurrence of an event: if no event occurred, zero was coded; one was coded if an event occurred (non-lame to lame state or vice versa). For instance, if a cow transitioned from the lame state to the non-lame state at the fifth visit, then, the discrete time interval was {1,2,3,4,5} and the event change was {0,0,0,0,1}.

The hazard of an event in a state i at time t within episode j for cow k was

$$h_{ijk(t)} = \Pr\{y_{ijk(t)}=1 | y_{ijk(t-1)}=0\}$$

where i indexes the state, j indexes episode and k indexes dairy cattle.

A two level of the model with level 2 represents cows and level 1 depicts time interval within episodes for a state was built. With the logit link function, the model took the form:

$$\text{Logit}[h_{ik(t)}] = \beta_0 + \alpha_i(t) + \beta X_{ik(t)} + u_k$$

$$u_k \sim N(0, \sigma_u^2)$$

Where β_0 is a state specific intercept, $\alpha_i(t)$ is an effect of duration which is a piecewise constant step function of time interval with four categories of each state, $\beta x_{ik(t)}$ refers to the fixed or time varying effects of a vector of covariates for each state and u_k represents the random effect of cow level for each state which were assumed to follow a multivariate normal distribution with covariance and non-zero correlation between random effects. The model was created in MLwiN version 2.25 (Centre for Multilevel Modeling, University of

Bristol) and estimation was carried out by quasi-likelihood methods, followed by Markov chain Monte Carlo (MCMC) methods with 500,000 iteration and a burn in of 5,000. The chain mixing and stability were then evaluated visually (a detailed diagnosis method in Chapter 2.4.2) (Browne, 2009).

5.2.2.3 Covariates (predictor variables) used in the model

The covariates with fixed effects used in the analysis were: parity group (1st, 2nd, 3rd and >3rd), herd (Herd 1, 2, 3 and 4), month of calving (January-March, April-June, July-September and October-December) and BCS at calving (BCS G1- 1.00-1.75, BCS G2- 2.00-2.25, BCS G3- 2.50-2.75 and BCS G4-3.00-5.00). Days of lactation and changes of BCS at the current visit compared with calving were included in the model as time-varying effects. Days of lactation referred to the days which compared the current visit with the latest calving date and then categorized into three categories: 0-90 days, 91-120 days and more than 120 days. Likewise, the changes of BCS at current visit compared with calving were categorized into five categories:

- a. No changes (C1)
- b. Decrease in 2 or 3 categories of BCS compared with calving (C2) (e.g, BCS G4 drop to BCS G2 or BCS G1)
- c. Decrease in 1 category of BCS compared with calving (C3) (e.g, BCS G4 drop to BCS G3)
- d. Increase in 1 category of BCS compared with calving (C4) (e.g, BCS G2 increase to BCS G3)

- e. Increase in 2 categories of BCS compared with calving (C5) (e.g, BCS G2 increase to BCS G4).

The first visit of each cow was excluded from the dataset, as the BCS of the first visit (BCS at calving) was used as the reference for the following visits.

5.3 Results

5.3.1 Descriptive analysis

There were a total of 8351 observations obtained from 731 cows. The last and first visit of each cow was excluded leaving 6889 observations remaining in the final dataset. These included 110 (1100 observations), 329 (2953 observations), 166 (1626 observations) and 126 (1210 observations) cows from Herd 1, 2, 3 and 4 respectively. There were 214 (29%), 145 (20%), 113 (16%) and 259 (35%) cows in parity 1, 2, 3 and more than 3 respectively. A total of 166 (23%), 115 (16%), 258 (35%) and 192 (26%) cows calved within Jan-Mar, Apr-Jun, Jul-Sep and Oct-Dec respectively. The frequencies of variables at the cow level by herd are presented in Table 5.1.

Out of 1635 episodes, 1042 were non-lame episodes and 593 were lame episodes. Approximately 50% (519/1042) of non-lame episodes transitioned to the lame state and 81% (483/593) of lame episodes ended with a transition to the non-lame state. For transitions from the non-lame to the lame state, approximately 65% occurred within 45 days, 84% within 90 days and the

remaining (16%) occurred after 90 days. About 88% of transitions from the lame to the non-lame state occurred within 45 days, 97% within 90 days and about 3% occurred after 90 days.

Table 5.1 The frequency of the predictor variables at the cow level by herd

Herd	1		2		3		4		Total
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.
Cows	110	15	329	45	166	23	126	17	731
Parity group									
1	25	23	100	30	48	29	41	33	214
2	15	14	48	15	55	33	27	21	145
3	19	17	50	15	21	13	23	18	113
>3	51	46	131	40	42	25	35	28	259
Month of calving									
Jan-Mar			81	25	49	30	36	29	166
Apr-Jun			83	25	18	11	14	11	115
Jul-Sep	84	76	82	25	58	35	34	27	258
Oct-Dec	26	24	83	25	41	25	42	33	192
BCS at calving									
BCS G1 (1.00-1.75)			16	5	3	2	2	2	21
BCS G2 (2.00-2.25)	23	21	116	35	46	28	41	33	226
BCS G3 (2.50-2.75)	81	74	163	50	105	63	72	57	421
BCS G4 (3.00-5.00)	6	5	34	10	12	7	11	9	63

Freq.: frequency, %: percentage

Mean and 95% standard deviation of body condition score by days of lactation (15 days) is presented in Figure 5.1. BCS decreased from calving until approximately 45 days of lactation and increased gradually until the end of lactation, although there was a plateau in the middle of lactation.

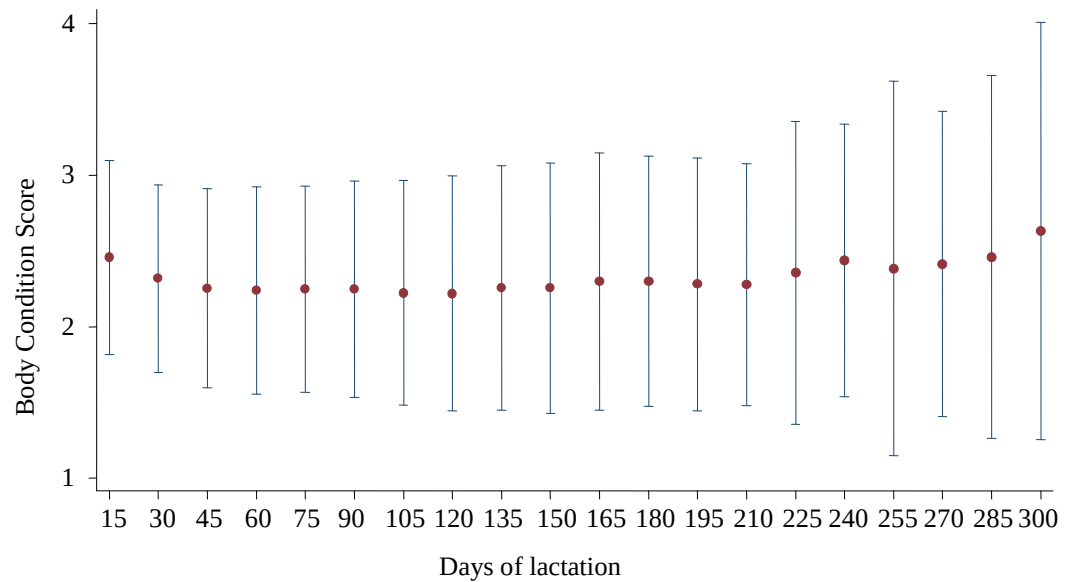


Figure 5.1 Mean and 95% standard deviations of body condition score by days of lactation

5.3.2 Multilevel multistate model

5.3.2.1 Fixed effects (covariates/predictor variables)

The number of observations for each covariate and final multilevel model with odds ratio (OR) and 95% of credible intervals (CI) are presented in Table 5.2 and Table 5.3 respectively. The cows with BCS G1 (1.00-1.75) and BCS G2 (2.00-2.25) at calving had a significantly higher probability of transition from the non-lame to the lame state (OR: 7.73, CI: 3.37-17.71; OR: 1.54, CI: 1.11-2.14) and lower probability of transition from the lame to the non-lame state (OR: 0.21, CI: 0.08-0.52; OR: 0.31, CI: 0.19-0.51) compared with the cows with BCS G3 (2.50-2.75) respectively.

When comparing the BCS at the current visit with the BCS at calving; cows where BCS decreased by 2 or 3 groups (e.g. BCS G4(3.00-5.00) changed to

BCS G2(2.00-2.25) or G1(1.00-1.75) and/or BCS G3(2.50-2.75) changed to G1(1.00-1.75)) had a higher probability of transition from the non-lame to the lame state (OR: 2.07, CI: 1.30-3.29) and lower probability of transition from lame to non-lame (OR: 0.44, CI: 0.23-0.82) compared with cows with no change in BCS. Similarly, cows where BCS decreased 1 group from any BCS G4(3.00-5.00), G3(2.50-2.75) or G2(2.00-2.25) had a lower probability of transition from the lame to the non-lame state compared with cows where BCS did not change (OR: 0.66, CI: 0.44-0.98); there was no significant effect of this change in the transition from non-lame to lame.

Cows in BCS G1(1.00-1.75), G2(2.00-2.25) or G3(2.5-2.75) that increased one category/group at the current visit had a lower probability of transition from the non-lame to the lame state (OR: 0.53, CI: 0.33-0.83) and a higher probability of transition from the lame to the non-lame state (OR: 2.49, CI: 1.37-4.53), compared with the cows with no change in BCS.

The probability of cows transitioning from the non-lame to the lame state was lower the longer a cow remained in non-lame state (OR: 0.53, CI: 0.40-0.71; OR: 0.33, CI: 0.21-0.51). In contrast, the longer the cow was in the lame state, the lower the probability (OR: 0.42, CI: 0.18-0.96) of transition from the lame to the non-lame state (i.e. they were more likely to remain lame). Cows in 91-120 days of lactation (OR: 1.64, CI: 1.17-2.29) and >120 days of lactation (OR: 2.11, CI: 1.58-2.82) were more likely to change from the non-lame to the lame state compared with the cows in ≤ 90 days of lactation. Cows within 91-120 days were less likely to change from the lame to the non-lame state

compared with the cows in ≤ 90 days of lactation (OR: 0.67, CI: 0.44-1.00). Cows that calved between Oct-Dec had a higher probability of transition from lame to non-lame state compared with the cows that calved during Jan-Mar (OR: 1.79, CI: 1.05-3.06) whereas the month of calving did not have a significant effect on transition from the non-lame to the lame state.

Cows in parity 3 and above had a higher probability (OR: 3.00, CI: 1.95-4.60; OR: 3.51, CI: 2.39-5.17) of transition from the non-lame to the lame state and a lower probability (OR: 0.50, CI: 0.25-1.00; OR: 0.36, CI: 0.19-0.67) to transition from the lame to the non-lame state compared with cows in parity 1. Cows in parity 2 did not show any significant difference in both transitions compared with the cows in parity 1.

Herd 2 (OR: 3.18, CI:1.98-5.11) and Herd 3 (OR: 1.94, CI:1.19-3.15) had a significantly higher probability of transition from the non-lame to the lame state compared with Herd 1; Herd 2 had a significantly lower probability of transition from the lame to the non-lame state compared with Herd 1 (OR: 0.23, CI:0.10-0.53).

Table 5.2 Frequency of observations for each covariate/predictor variable in the multilevel multistate model by state (lame/non-lame)

Covariates	Categories	State			
		Non-lame (%)		Lame (%)	
Duration spent in state (days)	≤45	2062	(38)	974	(70)
	46-90	1618	(29)	240	(17)
	≥90	1819	(33)	176	(13)
Herd	1	1028	(19)	72	(5)
	2	1989	(36)	964	(69)
	3	1402	(26)	224	(16)
	4	1080	(20)	130	(9)
Days of lactation	0-90 days	3136	(57)	684	(49)
	91-120	923	(17)	238	(17)
	>120	1440	(26)	468	(34)
Month of calving	Jan-Mar	1104	(20)	398	(29)
	Apr-Jun	671	(12)	251	(18)
	Jul-Sep	2211	(40)	484	(35)
	Oct-Dec	1513	(28)	257	(18)
Parity group	1	1818	(33)	145	(10)
	2	1239	(23)	159	(11)
	3	809	(15)	278	(20)
	>3	1633	(30)	808	(58)
BCS at calving	BCS G3 (2.50-2.75)	3413	(62)	549	(40)
	BCS G1 (1.00-1.75)	74	(1)	104	(7)
	BCS G2 (2.00-2.25)	1517	(28)	640	(46)
	BCS G4 (3.00-5.00)	495	(9)	97	(7)
BCS (Comparing current visit with calving)	No change	2860	(52)	574	(41)
	Decrease by 2,3 groups	341	(6)	210	(15)
	Decrease by 1 group	1801	(33)	517	(37)
	Increase by 1 group	467	(8)	87	(6)
	Increase by 2 groups	30	(1)	2	(0.1)

Table 5.3 Multilevel multistate model of factors associated with transition of cows from the non-lame to the lame and the lame to the non-lame state in dairy cows

Transitions		Non-lame to lame ^{a,b,c}					Lame to non-lame ^{a,b,c}				
Covariates	Categories	Freq.	OR.	95% C.I.		<i>p</i> -value	Freq.	OR.	95% C.I.		<i>p</i> -value
Duration spent in state (days)	≤45	339	Ref.				427	Ref.			
	46-90	98	0.53	0.40	0.71	<0.001	43	0.64	0.40	1.04	0.07
	≥90	82	0.33	0.21	0.51	<0.001	13	0.42	0.18	0.96	0.04
Herd	1	55	Ref.				49	Ref.			
	2	272	3.18	1.98	5.11	<0.001	247	0.23	0.10	0.53	<0.001
	3	115	1.94	1.19	3.15	0.01	113	0.64	0.28	1.47	0.29
	4	77	1.36	0.82	2.28	0.24	74	0.83	0.34	2.01	0.68
Days of lactation	0-90 days	284	Ref.				267	Ref.			
	91-120	84	1.64	1.17	2.29	<0.001	66	0.67	0.44	1.00	0.05
	>120	151	2.11	1.58	2.82	<0.001	150	0.72	0.50	1.03	0.07
Month of calving	Jan-Mar	132	Ref.				130	Ref.			
	Apr-Jun	81	0.71	0.45	1.11	0.13	70	1.19	0.67	2.12	0.55
	Jul-Sep	184	0.95	0.66	1.38	0.79	164	0.81	0.50	1.33	0.41
	Oct-Dec	122	0.82	0.56	1.20	0.31	119	1.79	1.05	3.06	0.03
Parity group	1	88	Ref.				80	Ref.			
	2	78	1.26	0.83	1.91	0.28	76	1.08	0.52	2.25	0.83
	3	109	3.00	1.95	4.60	<0.001	95	0.50	0.25	1.00	0.05
	>3	244	3.51	2.39	5.17	<0.001	232	0.36	0.19	0.67	<0.001

continued

Table 5.3 (continued) Multilevel multistate model of factors associated with transition of cows from the non-lame to the lame and the lame to the non-lame state in dairy cows

Transitions		Non-lame to lame ^{a,b,c}					Lame to non-lame ^{a,b,c}				
Covariates	Categories	Freq.	OR.	95% C.I.	p-value		Freq.	OR.	95% C.I.	p-value	
BCS at calving	BCS G3 (2.50-2.75)	266	Ref.				242	Ref.			
	BCS G1 (1.00-1.75)	29	7.73	3.37	17.71	<0.001	24	0.21	0.08	0.52	<0.001
	BCS G2 (2.00-2.25)	173	1.54	1.11	2.14	0.01	173	0.31	0.19	0.51	<0.001
	BCS G4 (3.00-5.00)	51	0.92	0.56	1.51	0.74	44	1.51	0.73	3.10	0.27
BCS	No change	253	Ref.				207	Ref.			
(Compare current visit with calving)	Decrease by 2,3 groups	64	2.07	1.30	3.29	<0.001	62	0.44	0.23	0.82	0.01
	Decrease by 1 group	169	1.12	0.86	1.46	0.41	165	0.66	0.44	0.98	0.04
	Increase by 1 group	31	0.53	0.33	0.83	0.01	48	2.49	1.37	4.53	<0.001
	Increase by 2 groups	2	0.50	0.07	3.37	0.48	1	5.85	0.12	276.25	0.37

^a Intercept (coefficient (standard error)): Non-lame to lame: -3.80 (0.34); Lame to non-lame :2.44 (0.61)

^b Random variability between cows: Non-lame to lame : 0.80 (0.27) ; Lame to non-lame: 0.87 (0.36)

^c Covariance : -0.32(0.25)

Freq.: frequency, OR.: odd ratios, C.I.: credible interval, p-value.: significant value, Ref.: reference

5.3.2.2 Random effects

Estimated random effects covariance matrices for the models with duration effects only and duration with all covariates are presented in Table 5.4. For the model with duration effects only, there was a moderate negative correlation (-0.76) between the probability of both transitions from non-lame to lame and lame to non-lame. It indicated that the cows with a high (low) probability of switching from non-lame to lame had a low (high) probability of changing from the lame to the non-lame state. Alternatively, the cows with a longer (shorter) duration of the non-lame state were likely to have a shorter (longer) duration in the lame state. The correlation between both transitions after adding covariates was not significant at the 5% level. The model converged visually with stable chain mixing.

Table 5.4 Random effects covariance matrix for a multilevel model looking into transitions from the non-lame to the lame and the lame to the non-lame state in cows

Model	Coefficient	SE
Duration effects only		
Non-lame to lame	2.45*	0.55
Lame to non-lame	1.63*	0.65
Covariance between both transitions	-1.51*	0.58
Correlation between random effects	-0.76	
Duration+ covariates		
Non-lame to lame	0.80*	0.27
Lame to non-lame	0.87*	0.36
Covariance between both transitions	-0.32	0.25
Correlation between random effects	-0.38	

*Significant at 5% level, SE: standard error.

5.4 Discussion

The key result from this study is that loss of BCS increased a cow's probability of becoming lame and decreased its likelihood of recovery; this was after controlling for body condition score at calving. Furthermore, cows with a greater decrease of BCS had a higher (lower) probability of transition from non-lame to lame (lame to non-lame) compared with cows that had a relatively lower decrease in BCS. Previous study has demonstrated that BCS is associated with the thickness of the digital cushion (Bicalho et al., 2009). The results from the current study support the hypothesis that a greater loss of BCS leads to a higher risk of lameness due to possibly thinner digital cushion but also suggest that this impact of loss of BCS possibly prolongs to the chance of recovery. The digital cushion is thought to absorb part of the load and protecting the corium, thus lowering the incidence of claw lesion and lameness (sole ulcers and white line disease) (Räber et al., 2004; Räber et al., 2006; Bicalho et al., 2009). Alternatively, thin cows have lower social rank and are therefore less able to compete with other cows for resources such as lying space and access to feed (Hohenbrink and Meinecke-Tillmann, 2012). Being out competed in the environment could put them at greater risk of becoming lame and make them less likely to recover once a lameness event occurs.

The current study supports the findings of Green et al. (2013) who proposed that low BCS (BCS <2.5) measured 60 days before the 30 days risk period were associated with a higher risk of treatment for lameness. However the current study extends the finding by Green et al. (2013), firstly by enhancing

the generalizability of the findings by looking into 4 different herds compared to one. Secondly, by looking at the dynamics of changes in BCS and lameness, the current study suggests that a) in addition to low BCS being a risk for lameness, any significant loss of BCS has an added detrimental effect on occurrence and recovery from lameness in a 15 day period b) thinner cows at calving who gain BCS had a lower chance of becoming lame and a higher chance for recovery compared with their counterparts who stay thin. All these findings enhance the importance of regular monitoring and maintenance of BCS on farms.

Cows in parity 3 and greater were more likely to become lame and less likely to recover in the current study. These animals are more likely to have experienced lameness in previous lactations, previous work has demonstrated that this increases the chance to having lameness again in the future (Hirst et al., 2002). Alternatively, thickness of the digital cushion is also influenced by age, number of parities and days of lactation (Räber et al., 2004; Bicalho et al., 2009), these factors also had a significant effect on lameness transitions in the final model of current study. The amount of fat in the digital cushion is reported to be different according to parity; the thickness of the layer increases until third parity and then decreases (Räber et al., 2004; Bicalho et al., 2009). Cows in parity 3 and greater were more likely to become lame and less likely to recover in the current study.

Between 91-120 days of lactation, current results suggest that cows were more likely to be lame and less likely to recover from lameness; this might be

attributable to the reason that during this period the digital cushion is in its thinnest condition (Bicalho et al., 2009). Likelihood of lameness was also significantly higher among cows >120 days in lactation; one explanation might be that the digital cushion in these cows is probably not as thick as cows in early lactation although the digital cushion is still on its way to get thicker (Bicalho et al., 2009). Alternatively, injuries to the corium around the time of parturition and in early lactation take a period of time to cause lameness and hence are more likely to be seen in later lactation.

The duration that cows spent in the particular state is important and substantially influences the risk of lameness and recovery (Reader et al., 2011). Current results suggest that cows that have been lame for longer have less chance to recover. Equally, cows that are non-lame for longer have less chance to become lame. This could be due to the effect of cow heterogeneity or genetic resistance to lameness, where cows with inherently thinner digital cushion will have a higher risk of lameness and lower recovery compared with other cows. Other possible explanations could be lame cows generally have insufficient nutrition, lower feed intake and less feeding time (Bach et al., 2007; Kilic et al., 2007) influencing the recovery, or it could be due to some failures in efficacy of treatments. Since lameness data was only available as mobility scores, there was no information on the causes of lameness or what treatments were given. Reader et al. (2011) found that cows with a history of past treatment for sole ulcer and digital dermatitis were more likely to become lame compared with non-treated cows, suggesting that treatment may not be fully healing the lameness problem.

Based on the results presented here, cows in Herd 1 had a higher chance of recovery and a lower chance of lameness compared with Herd 2 and Herd 3 respectively. Comparing the percentage of cows in BCS categories at calving, the majority of cows (79%) from Herd 1 were in BCS G3 and BCS G4, whereas there were only 60%, 70% and 66% of counterparts in these categories in Herds 2, 3 and 4 respectively. Cows in Herd 1 had relatively better BCS which might be due to unknown factors, however, unfortunately herd management, housing system and data on other potential factors were not (fully) recorded in the current study. Including the factors related to housing system and management practices in future studies could be helpful to further explore this area.

This study was conducted with one observer (JAW) who received initial training and made all BCS and MS score measurements to avoid inter-observer bias. Even though the records come from four herds which were not selected randomly, it was a dataset of cows with range of body condition score and mobility score and the associations between change in BCS on lameness occurrence and recovery remained after controlling for herd effect, therefore there is no reason to suspect these do not represent a typical UK dairy.

5.5 Conclusion

In conclusion, the study suggests that loss of BCS and increase in BCS could influence the risk of being lame and the chance of recovery from lameness, possibly due to the thickness of the digital cushion. Regular monitoring and

maintenance of a consistent BCS on farms could be a helpful tool for reducing lameness. Finally parity, days of lactation and month of calving also affected both lame and non-lame transitions.

CHAPTER 6: ASSOCIATION BETWEEN BODY CONDITION SCORE, LAMENESS AND SELECTED REPRODUCTIVE PERFORMANCE INDICATORS IN DAIRY CATTLE

6.1 Introduction

Poor fertility has been considered as one of the largest economic losses in the dairy herds (Kossaibati et al., 1999; Gonzalez-Recio et al., 2004). A decline in reproductive performance has been reported worldwide including, the United Kingdom (Royal et al., 2000), Spain (Lopez-Gatius, 2003; Gonzalez-Recio et al., 2004) and the United States (Lucy, 2001). Over the past decades, a decline in a first service conception rate, prolonged days from calving to conception and lower conception rate and other infertility problems have been reported (Hillers et al., 1984; Domecq et al., 1997; Royal et al., 2000; Lopez-Gatius, 2003; Gonzalez-Recio et al., 2004). There are certain difficult to summarize and identify the factors associated with low reproductive because of its multifactorial nature.

The impact of lameness on fertility has been well documented, where numerous studies have reported that lame cows are more likely to have a lower successful rate of first service, lower hazard ratio of conception rate, longer days of calving to conception, longer days of calving to first service and longer calving interval (Collick et al., 1989; Argaez-Rodriguez et al., 1997; Suriyasathaporn et al., 1998; Melendez et al., 2003; Bicalho et al., 2007; Kilic et al., 2007; Machado et al., 2010). The relationship between lameness

and infertility is unequivocal, however, the reasons behind the impact of lameness on fertility remain unclear. Feelings of discomfort and a long duration of pain resulting from lameness could lead dairy cattle to have longer duration of lying, less feeding time and lower dry matter intake, resulting in the cows losing weight and experiencing a negative energy balance which could influence follicular development and ovulation performance (Whay et al., 1998; Galindo and Broom, 2002; O'Callaghan, 2002; Bach et al., 2007; Ito et al., 2010). Lamé cows have a higher risk of reproductive disorders, including a higher risk of delayed cyclicity (Melendez et al., 2003; Garbarino et al., 2004), lower ovulation rate (Morris et al., 2009), lower intensity of oestrus (Dobson et al., 2008) and signs of oestrus were less likely to be observed (Peeler et al., 1994), all leading to poor fertility performance.

Stress from lameness is suggested to reduce fertility by interfering with normal follicular activity, such as a reduction in the amounts of gonadotrophin-releasing hormone (GnRH) and luteinising hormone (LH) leading to lower oestradiol production by slowing follicular growth and reducing the expression of oestrus behaviour (Dobson and Smith, 2000; Dobson et al., 2008). Increased stress also influences the immune system of dairy cattle (greater ratio of neutrophil and lymphocyte and CD14 expression) which has impacts on fertility (O'Driscoll et al., 2009; Tomlinson et al., 2012).

There are a few studies which have reported no association between lameness and fertility (Cobo-Abreu et al., 1979; Dohoo and Wayne Martin, 1984; Barkema et al., 1994; Melendez et al., 2003; Sogstad et al., 2006; Peake et al.,

2011) even though the majority of studies have demonstrated a negative association. Furthermore, most studies investigated the effect of a single lameness event on fertility due to ease of availability of such data at farm level. There is limited published data exploring how the different severities of lameness and duration of lameness, including repeated lameness events, impact on different fertility parameters.

Similar to lameness, body condition score has been reported to have either a negative impact or no impact on reproductive performance. For example several authors (Markusfeld et al., 1997; Suriyasathaporn et al., 1998; Kim and Suh, 2003; Lopez-Gatius et al., 2003; Hoedemaker et al., 2009; Machado et al., 2010) reported that cows with low BCS had lower reproductive performance whereas others (Ruegg and Milton, 1995; Domecq et al., 1997; Heuer et al., 1999; Jilek et al., 2008) have found no association. Similar to lameness, the physiological mechanisms behind the impact of low BCS on fertility remain unclear. One possible explanation is that cows with low BCS, or loosing body condition, experience negative energy balance; this condition influences ovulation performance, quality of oocytes and regulation of gene expression, thereby affecting pregnancy (Lopez-Gatius et al., 2003; Kadokawa and Martin, 2006; Roche et al., 2009).

There are several studies (Barkema et al., 1994; Domecq et al., 1997; Suriyasathaporn et al., 1998; Loeffler et al., 1999; Kilic et al., 2007; Hoedemaker et al., 2009) that have investigated the effect of BCS and/or lameness on conception at the first artificial insemination (AI) but there are no

published studies investigating the effect of these factors with the likelihood of each AI leading to pregnancy. Understanding the risk factors associated with the likelihood of each AI leading to pregnancy is important to improve the success rate of each AI.

A significant positive association between BCS and lameness has been reported in a few studies (Espejo et al., 2006; Dippel et al., 2009; Hoedemaker et al., 2009) and is also reported in Chapter 5 of this thesis. Understanding the complex dynamic relationships between BCS, lameness occurrence and fertility in longitudinal studies is crucial to help us improve fertility in dairy herds.

The aims of this study were to investigate in a longitudinal study the effects of both lameness occurrence and BCS on i) the likelihood of an artificial insemination (AI) leading to a pregnancy (model 1), ii) the probability of conception and days to conception (model 2 and 3).

6.2 Materials and methods

6.2.1 Data set and study methodology

Cows that calved between 3 January 2010 and 29 June 2011 in four selected herds were included in the study. All four herds are located within a 20mile radius of Salisbury, UK. The annual rolling mean herd sizes ranged from 138-359 cows in milk and the 305 days yield ranged from 5889-9171 kg per cow.

Herd 1 and Herd 2 housed cows on mats with sawdust, Herd 3 housed cows on the mats with straw and Herd 4 housed cows on deep sand. The majority of cows were Holstein x Friesians and this study was conducted for 18 months, from 14 July 2010 until 30 December 2011. The observer visited the herds every 13-15 days, the body condition score (BCS) and mobility score (MS) of selected cows were recorded at every visit after they calved. Body condition score was scored according to Edmonson et al. (1989) on a scale of 1-5 with increment of 0.25 and mobility score was assessed according to Whay et al. (2003) on a scale from 0 to 3. Other information such as parity, age, days of lactation, month of calving and clinical events were recorded.

Fertility records and first recording of milk yield including day of artificial insemination (AI), number of AIs, pregnancy diagnosis results, days in milk, milk yield (kg), milk protein (%), milk fat(%) and somatic cell count (cell/ml, categorized as ≤ 200 or >200), were obtained from on-farm software or the farm's milk recording company. The recording of milk yield less than or equal to five days post calving was discarded as described in Madouasse et al. (2010). The pregnancy diagnosis was assessed by ultrasonography from 30 days after service.

Cows with incomplete information (e.g., no calving information and fertility event records) or absent records (e.g., no records until end of study) were excluded from the study. Fertility events which occurred after the first successful AI or after 200 days of lactation were excluded from the dataset.

Reproduction performance indicators used in the analysis were:

- a. Likelihood of AI leading to pregnancy
- b. Number of AI: number of services received before conception, or to the end of the study (200 days post calving)
- c. Ca-Con: days from calving to conception, or to the end of the study (for those cows which did not conceive)
- d. Likelihood/probability of conception: likelihood/probability of cows successfully conceiving

6.2.1.1 Covariates (predictor variables) used in the model

The covariates with fixed effects used in the analysis were: herd (Herd 1, 2, 3 and 4), month of calving (January-March, April-June, July-September and October-December), parity group (1st, 2nd, 3rd and >3rd), days from calving to AI (G1 (group 1)- 0-45 days, G2 (group 2)- 46-90 days, G3 (group 3)- 91-135 days, G4 (group 4)- 136-180 days, G5 (group 5)- >180 days) and first recording of milk yield (milk yield (kg), milk protein (%), milk fat(%) and somatic cell count (cell/ml, categorized as ≤ 200 or >200)).

BCS was grouped into four categories: group 1 (BCS G1 1.00-1.75), group 2 (BCS G2 2.00-2.25), group 3 (BCS G3 2.50-2.75) and group 4 (BCS G4 3.00-5.00) while MS was categorized into two groups, non-lame (MS 0 and 1) and lame (MS 2 and 3). A wide range of BCS, MS and lameness variables were tested in the analysis (as described in 6.2.2.1, 6.2.2.2 and 6.2.2.3).

6.2.2 Statistical analysis

6.2.2.1 Data structure for model 1 (logistic regression model)- To investigate the effect of lameness and BCS on the likelihood of an AI leading to a pregnancy

A logistic regression model (refer to Chapter 2.2 for detailed methodology) was used to investigate the effects of lameness and BCS on the likelihood of an AI leading to a pregnancy. In the dataset, each record represented an AI, so that cows could have multiple records if they had more than one AI, for instance, there were 3 records for a cow which was served 3 times (AI).

The outcome variable was successful AI leading to pregnancy (coded 1) or failure of AI (coded 0). Due to the large proportion of cows having less than or equal to 3 AI (80%), a single level logistic regression model with cow level was built. Variables with a significance value less than or equal to Wald statistic $p\text{-value} \leq 0.05$ were retained in the final model.

The model was written as:

Outcome $\sim$ Bernoulli (n_i, π_i) with individual data $n_i=1$ for all probability (π_i)

$$\text{Logit}(\pi_i) = \log\left(\frac{\pi_i}{1-\pi_i}\right) = \beta_0 + \beta_1 X_1 + \dots + \beta_i X_i$$

where π_i is the probability of an AI leading to a pregnancy, β_0 is the intercept, β_1 and β_i were the coefficients of covariates of X_1 and X_i respectively. Data was analysed using Stata version 12 (StataCorp, USA). The model was created

in MLwiN version 2.25 (Centre for Multilevel Modelling, University of Bristol) and estimation was carried out by quasi-likelihood methods and followed by Markov chain Monte Carlo (MCMC) methods with 500,000 iteration and a burn in of 5,000. The chain mixing and stability were evaluated visually (refer to Chapter 2.4.2 for detailed methodology) (Browne, 2009).

Covariates with fixed effects (6.2.1.1) and a range of lameness and BCS variables were initially explored in the analysis. A list of the lameness and BCS variables used for modelling are described below,

- Lameness variables
 - a. Lameness status at calving and at each AI (≈ 15 days)
 - b. Lameness status for 2 visits before each AI (≈ 30 days)
 - c. Total number of MS3 scores in the 3 visits before each AI (G1-no MS 3, G2- 1 MS 3, G3-2 or 3 MS 3)
 - d. Number of lameness events in the 3 visits before each AI (G1- never lame for 3 visits, G2- lame for 3 visits, G3- lame for 2 visits, G4- lame for 1 visit)
 - e. Changes between MS at each AI compared with calving
- BCS variables
 - a. BCS at calving and each AI (≈ 15 days)
 - b. Average BCS at the 2 visits before each AI (≈ 30 days) and 3 visits before each AI (≈ 45 days)
 - c. Changes between BCS at each AI compared with calving

6.2.2.2 Data structure for model 2(Cox proportional hazard model)– To investigate the effect of lameness and BCS (fixed effects) on the probability of conception and days to conception

A Cox proportional hazard model (refer to Chapter 2.3.3.1 for detailed methodology) was used to investigate the effects of BCS and lameness (fixed effects) on the probability of conception and Ca-Con. For the Cox model approach, one record per cow was used in the analysis.

The dependent variable ('Ca-Con') was days from calving to conception, or to the end of the study. The Ca-Con period for the cows who conceived was the interval between the date of calving and the date of successful AI, whereas the cows who failed to conceive at 200 days (the end of the study) were censored. The failure variable (censor variable) indicated whether a cow had conceived successfully (coded 1) or had not conceived (coded 0) within the study period. The hazard at time t for each cow i was :

$$h_i(t) = h_0(t)\exp(\beta_i X_i)$$

The Cox model with natural log was expressed as:

$$\text{Log}[h_i(t)] = \log[h_0(t)] + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_i X_i$$

Where $\beta_1, \beta_2, \dots, \beta_i$ were the coefficients of explanatory variables of $X_1, X_2, \dots, X_i$ respectively. Kaplan Meier survival curves and log-rank test were used to test the equality of hazard function across two or more groups of categorical covariates. All covariates with a $p \leq 0.20$ in univariable analysis retained for multivariable model building. A covariate with $p \leq 0.05$ was retained in the

final model. After the final model was fitted, the proportional hazard assumption was tested based on Schoenfeld's global test. Model fit was tested by Cox-Snell residual plot.

Similar fixed effect covariates (6.2.1.1) with various lameness and BCS variables were used in the analysis, the latter are described below.

- Lameness variables
 - a. Lameness status at calving and last AI
 - b. Total number of MS 3 scores in the 2 visits and 3 visits after calving (G1-no MS 3, G2- 1 MS 3, G3-2 or 3 MS 3)
 - c. Total number of MS3scores in the 3 visits before the last AI (G1-no MS 3, G2- 1 MS 3, G3-2 or 3 MS 3)
 - d. Lameness events in the 3 visits before the last AI (G1-Neverlame for 3 visits, G2- Lame for 3 visits, G3- lame for 2 visits, G4- lame for 1 visit)
- BCS variables
 - a. BCS at calving and last AI
 - b. Average BCS at the first 2 and 3 visits after calving
 - c. Average BCS at the 2 visits and 3 visits preceding the last AI

6.2.2.3 Data structure for model 3 (multilevel discrete time event history model)- To investigate the effects of lameness and BCS (time varying effects) on the likelihood of conception

A multilevel discrete time event history model (refer to Chapter 2.3.3.2 for detailed methodology) was used to investigate the effects of lameness and BCS (time varying effects) associated with the probability of conception occurs during the time interval (t), given that no event has occurred in the previous time interval ($t-1$). The data were converted to the person-period format for each cow, with one record per discrete time unit. The length of the discrete time interval (t) was 15 days and the records were expanded until the last successful AI or 195 days of lactation (up to the 13th discrete time unit) for those cows who failed to conceive. A total of 5637 records were used in the analysis.

A two level logistic regression model was built: level 2 represented the cow, level 1 was the time interval. The binary response y_{ij} is:

$$y_{ij} = \begin{cases} 1 & \text{if cow } j \text{ was conceived at time } i, \\ 0 & \text{if cow } j \text{ was not conceived at time } i. \end{cases}$$

The hazard of conception (π_{ij}) was:

$$\pi_{ij} = \Pr(\text{cow } j \text{ conceives at time } i) = \Pr(y_{ij}=1)$$

$$\text{Logit}(\pi_{ij}) = \beta_0 + \alpha_i(t) + \beta X_{ij} + u_{ij}$$

$$u_{ij} \sim N(0, \sigma_u^2)$$

where $\beta 0_i$ is a state specific intercept, $\alpha_i(t)$ is a effect of duration which is a piecewise constant step function of time interval, βX_{ij} refer a vector of coefficients for covariates and , u_{ij} was random effect of cow level, which to reflect the variation among cows with σ^2_u the variance of the normal distribution. The model was created in MLwiN version 2.25 (Centre for Multilevel Modelling, University of Bristol) and estimation was carried out by quasi-likelihood methods and followed by Markov chain Monte Carlo (MCMC) methods with 500,000 iteration and a burn in of 5,000. The chain mixing and stability were evaluated visually (Browne, 2009). A Chi square test or Fisher's exact test (observation less than 5) was used to test an association between 2 variables.

The similar fixed effect covariates (6.2.1.1) with various lameness and BCS variables were investigated, the latter are described below.

- Lameness variables
 - a. Lameness status at calving, current visit and previous visit
 - b. Changes in lameness status at each visit compared with calving
 - c. Change in lameness status at each visit compared with previous visit
 - d. Lameness events for 3 visits, including the current visit and the 2 previous visits (G1- neverlame for 3 visits, G2- lame for 3 visits, G3- lame for 2 visits, G4- lame for 1 visit)
 - e. Total number of MS3 scores in the 2 visits including the current and previous visit and 3 visits including the current visit and the 2 previous visits(G1 -no MS 3, G2- 1 MS 3, G3- 2 or 3 MS 3)

- BCS variables
 - a. BCS at calving, current visit and previous visit
 - b. Average BCS for 2 visits (current visit and previous visit) and 3 visits(current visit and previous 2 visits)
 - c. Changes to BCS at each visit compared with calving
 - d. Changes to BCS at each visit compared with the previous visit

6.3 Results

6.3.1 Descriptive analysis

The number of cows in Herd 1, 2, 3 and 4 were 147, 323, 170, 131 respectively. Forty one cows were excluded from the analysis due to incomplete information such as no calving information and fertility event records. There were 52 non pregnant cows that left the study before 200 days of lactation, these were also excluded from the dataset. The final dataset used in the analysis contained a total of 678 cows (Herd 1-126 cows; Herd 2-293; Herd 3-141; Herd 4-118).

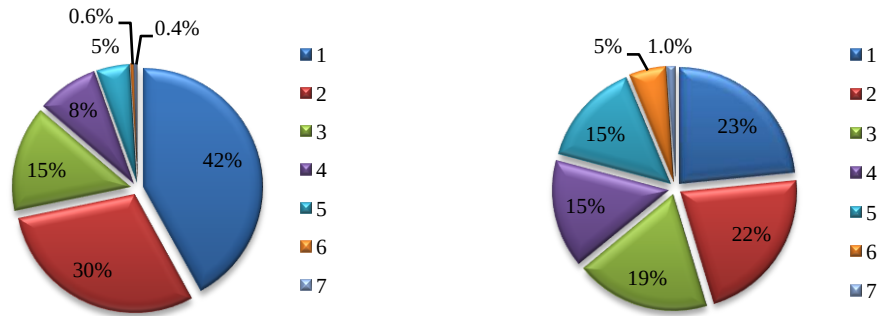
Out of 678 cows, the number of cows who conceived during the study period was 538 (79%). The percentage of cows that conceived in Herd 1, 2, 3 and 4 were 122 (97%), 225 (77%), 89 (63%) and 102 (86%) respectively. The frequencies of cows in herd, by parity and month of calving are presented in Table 6.1.

Table 6.1 The frequency of the variables at cow level for cows who conceived and cows who failed to conceive within 200 days

Variables	Categories	Not conceived		Conceived	
		Freq.	Percent	Freq.	Percent
Herd	1	4	3	122	23
	2	68	49	225	42
	3	52	37	89	17
	4	16	11	102	19
Parity group	1	45	32	164	30
	2	32	23	106	20
	3	18	13	85	16
	>3	45	32	183	34
Month of calving	Jan-Mar	29	21	116	22
	Apr-Jun	24	17	65	12
	Jul-Sep	60	43	196	36
	Oct-Dec	27	19	161	30
BCS at calving	G1 1.00-1.75	3	2	18	3
	G2 2.00-2.25	47	34	154	29
	G3 2.50-2.75	76	54	315	59
	G4 3.00-5.00	11	8	37	7
	Missing	3	2	14	3
Lameness status at calving	Non-lame	110	79	452	84
	Lame	27	19	72	13
	Missing	3	2	14	3

Freq.:frequency

The median number of AI for the cows who conceived and failed to conceive were 2 (IQR: 1-3) and 3 (IQR: 2-5) respectively. The maximum number of AI for all cows was 7. There were 226 (42%), 160 (30%), 78 (15%), 45 (8%), 24 (4%) and 5 (1%) cows who conceived at the first AI, 2nd AI, 3rd AI, 4th AI, 5th and 6-7th AI respectively. For the 140 cows who failed to conceive, there were 26 (19%), 32 (23%), 23 (16%), 22 (16%), 26 (19%) and 11 (8%) that had received 1, 2, 3, 4, 5 and 6-7 AI before the end of study respectively (Figure 6.1).



a. Cows that conceived (538 cows)

b. Cows that failed to conceive (192 cows)

Figure 6.1 Distribution of number of AI for cows that conceived and cows that failed to conceive within 200 days of lactation respectively

The Ca-Con period is presented in Figure 6.2. Approximately 15%, 29%, 19%, 15% and 22% of cows conceived at less than 60, 61-90, 91-120, 121-150 and 151-200 days of lactation respectively.

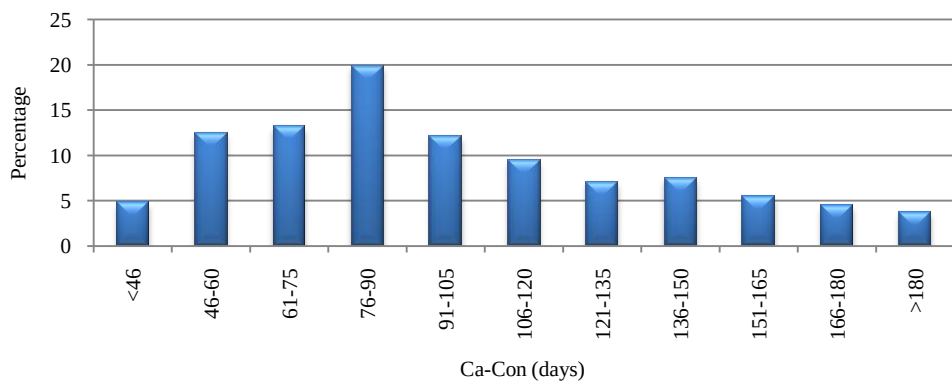


Figure 6.2 The distribution of Ca-Con period (days) for the cows who conceived (538 cows)

6.3.2 Model 1(logistic regression model)- To investigate the effect of lameness and BCS on the likelihood of an AI leading to a pregnancy

Lameness and BCS variables as described in the methodology were tested in the model showed no significant effect on the likelihood of an AI leading to a pregnancy. Only Herd and days after calving were retained in the final model (Table 6.2).

Herd 2 (OR: 0.24, CI: 0.17-0.35), Herd 3 (OR: 0.15, CI: 0.10-0.22) and Herd 4 (OR: 0.25, CI: 0.17-0.37) had a significantly lower likelihood of an AI leading to pregnancy compared with Herd 1. For days from calving to AI, the probability of an AI leading to a pregnancy increased until 180 days.

Table 6.2 Logistic regression model of covariates associated with likelihood of an AI leading to pregnancy in dairy cows

Covariate	Freq.	OR.	95% C.I.		p-value
Herd					
1	188	Ref.			
2	686	0.24	0.17	0.35	<0.001*
3	372	0.15	0.10	0.22	<0.001*
4	311	0.25	0.17	0.37	<0.001*
Days from calving to AI					
G1 0-45	97	Ref.			
G2 46-90	707	1.79	1.08	2.96	0.02 *
G3 91-135	441	1.83	1.09	3.07	0.02 *
G4 136-180	245	2.47	1.43	4.28	<0.001*
G5 >180	67	1.77	0.86	3.64	0.12

Freq.: frequency, OR.: odd ratios, C.I.: credible interval, *: significant value ≤ 0.05 , Ref.: reference

6.3.3 Model 2 (Cox proportional hazard model)- To investigate the effect of lameness and BCS (fixed effects) on the probability of conception and days to conception

Variables with $p \leq 0.20$ in the univariable analysis were herd, month of calving, average BCS at the 3 visits before the last AI, total number of MS 3 scores in the 3 visits before the last AI, lameness events for 3 visits before the last AI, milk protein % at first milk recording and milk yield at first recording (L) (Table 6.3). These variables were taken forward into the multivariable analysis.

Table 6.3 List of covariates with $p \leq 0.20$ associated with the probability of conception in the univariable analysis

Covariate	Freq.	HR.	95%C.I.		<i>p</i> -value	
Herd						
1	126	Ref.				
2	293	0.45	0.36	0.57	<0.001	*
3	141	0.30	0.22	0.39	<0.001	*
4	118	0.62	0.47	0.80	<0.001	*
Parity						
1	209	Ref.				
2	138	0.92	0.72	1.18	0.51	
3	103	1.09	0.84	1.42	0.50	
>3	228	1.05	0.85	1.29	0.68	
Month of calving						
Jan-Mar	145	Ref.				
Apr-Jun	89	0.92	0.68	1.25	0.60	
Jul-Sep	256	0.97	0.77	1.22	0.79	
Oct-Dec	188	1.21	0.95	1.53	0.12	
Average BCS at the 3 visits before last AI						
BCS G3 2.50-2.75	244	Ref.				
BCS G1 1.00-1.75	49	0.69	0.49	0.97	0.04	*
BCS G2 2.00-2.25	344	0.76	0.64	0.92	<0.001	*
BCS G4 3.00-5.00	10	0.66	0.31	1.41	0.28	
<i>continued</i>						

Table 6.3 (continued) List of covariates with $p \leq 0.20$ associated with the probability of conception in the univariable analysis

Covariate	Freq.	HR.	95%C.I.	p-value	Covariate
Total number of MS 3 scores in the 3 visits before last AI					
No MS 3	575	Ref.			
1 MS 3	46	0.81	0.57	1.16	0.25
2 and 3 MS 3	26	0.51	0.27	0.96	0.04 *
Lameness events for 3 visits before last AI					
Never lame for 3 visits	449	Ref.			
Lame for 3 visits	53	0.59	0.42	0.82	0.002*
Lame for 2 visits	52	0.54	0.38	0.78	0.001*
Lame for 1 visit	93	0.89	0.69	1.15	0.37
Milk recording variables					
Days in first milk recording	678	1.00	0.99	1.00	0.42
Milk yield at first recording (L)	621	0.99	0.99	1.00	0.15
Milk protein % at first milk	648	1.84	1.37	2.46	<0.001 *
Milk fat % at first milk recording	648	1.02	0.92	1.12	0.73
Milk lac % at first milk recording	648	0.92	0.53	1.59	0.77
SCC (x000) at first milk					
≤200	556	Ref.			
>200	91	0.92	0.72	1.19	0.54

Freq.: frequency, HR.: hazard ratio, C.I.: credible interval, *: significant value ≤ 0.05 , Ref.: reference

There were two lameness variables that were significant in the univariable analysis. Since the lameness variables were strongly correlated, two separate models were built to have individual coefficients. Two final multivariable models with hazard ratio (HR) and 95% of credible intervals (CI) are presented in Table 6.4 (model 2A) and Table 6.5 (model 2B) respectively. The Kaplan Meier Survival curve is presented in Figure 6.3. Schoenfeld's global test showed that both final models satisfied the proportional hazard assumption. Cox-Snell residual plots showed that the final model 2A fits the data well and 99.4% of data fits well in the final model 2B. Omitting 0.60% (4 outlier observations) of the data did not influence the parameter estimates,

therefore it was concluded that the final model 2B fits the data well (Figure 6.4).

Compared with Herd 1, Herd 2 (HR: 0.41, CI: 0.31-0.54), Herd 3 (HR: 0.26, CI: 0.19-0.35) and Herd 4 (HR: 0.53, CI: 0.39-0.71) had a lower probability of conception. Cows that calved between Jul-Sep had 27% less probability of conception compared with cows that calved between Jan-Mar (HR: 0.73, CI: 0.56-0.96).

Cows that had 2 or 3 MS 3 scores in the 3 visits before the last AI had a 49% less chance of conception (HR: 0.51, CI: 0.30-0.86) compared with cows with no MS 3 scores (model 2A, Table 6.4). Cows with no MS 3 scores, 1 MS 3 score and 2-3 MS 3 scores had a mean Ca-Con of 118 days (median 103 days), 125 days (median 110 days) and 159 days (median 171 days) respectively.

Cows that were lame for 3 visits (HR: 0.64, CI: 0.45-0.92) and lame for 2 visit out of 3 visits before the last AI (HR: 0.58, CI: 0.40-0.83) had a lower chance of conception compared with cows which were never lame during the 3 visits before the last AI respectively (model 2B, Table 6.5). The cows that were never lame during the last 3 visits, lame for 1 visit, lame for 2 visits and lame for 3 visits before the last AI had a mean Ca-Con period of 115 days (median 101 days), 119 days (median 102 days), 144 days (median 161 days) and 143 days (median 140 days) respectively.

The average BCS at the 3 visits before the last AI did not meet the proportional hazards assumption for Cox modelling (Steele et al., 2004), therefore, the average BCS at the 3 visits before the last AI was excluded from the analysis and was explored in model 3 as a time varying covariate.

Table 6.4 Model 2A: Cox proportional hazard model of covariates associated with probability of conception (including total number of MS 3 scores in the 3 visits before the last AI)

Covariate	Freq.	HR.	95%C.I.		<i>p</i> -value
Herd					
1	126	Ref.			
2	293	0.41	0.31	0.54	<0.001*
3	141	0.26	0.19	0.35	<0.001*
4	118	0.53	0.39	0.71	<0.001*
Month of calving					
Jan-Mar	145	Ref.			
Apr-Jun	89	1.03	0.75	1.42	0.85
Jul-Sep	256	0.73	0.56	0.96	0.02 *
Oct-Dec	188	1.09	0.85	1.40	0.48
Total number of MS 3 scores in the 3 visits before the last AI					
No MS 3	575	Ref.			
1 MS 3	46	0.90	0.62	1.30	0.57
2 and 3 MS 3	26	0.51	0.30	0.86	0.01 *

Freq.: frequency, HR.: hazard ratio, C.I.: credible interval, *: significant value ≤ 0.05 , Ref.: reference

Table 6.5 Model 2B: Cox proportional hazard model of covariates associated with probability of conception (including number of lameness events in the 3 visits before the last AI)

Covariate	Freq.	HR.	95% C.I.		<i>p</i> -value
Herd					
1	126	Ref.			
2	293	0.43	0.33	0.57	<0.001*
3	141	0.26	0.19	0.35	<0.001*
4	118	0.54	0.40	0.72	<0.001*
Month of calving					
Jan-Mar	145	Ref.			
Apr-Jun	89	1.05	0.76	1.43	0.78
Jul-Sep	256	0.72	0.55	0.93	0.01 *
Oct-Dec	188	1.06	0.83	1.36	0.64
Lameness events in the 3 visits before last the AI					
Never lame 3 visits	449	Ref.			
Lame 3 visits	53	0.64	0.45	0.92	0.02 *
Lame 2 visits	52	0.58	0.40	0.83	0.003 *
Lame 1 visit	93	1.04	0.81	1.34	0.77

Freq.: frequency, HR.: hazard ratio, C.I.: credible interval, *: significant value ≤ 0.05 , Ref.: reference

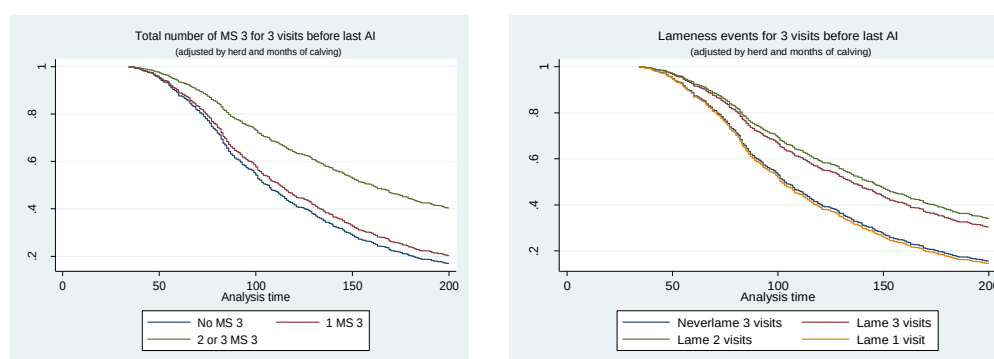
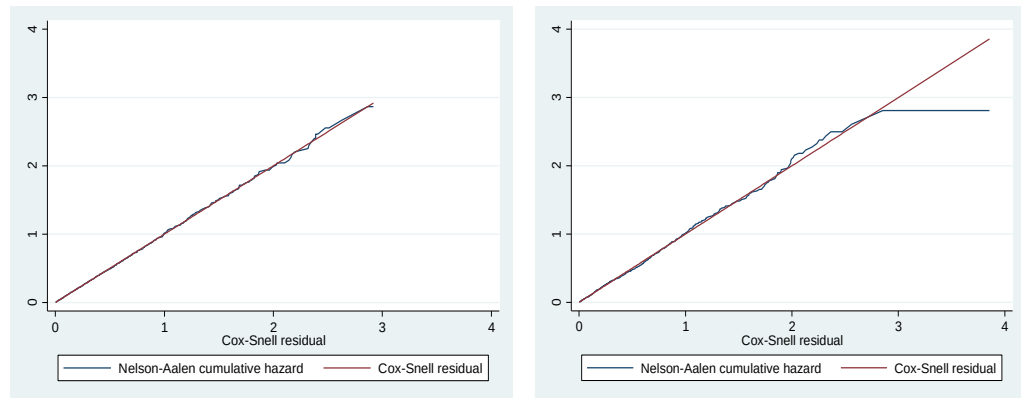


Figure 6.3 Kaplan Meier Survival curves for total number of MS 3 scores and lameness events in the 3 visits before the last AI after adjusting for herd and months of calving



Model 2A

Model 2B

Figure 6.4 Cox-Snell residual plots showing the model fits of Cox proportional hazard model 2A and Cox proportional hazard model 2B

6.3.4 Model 3 (multilevel discrete time event model)- To investigate the effect of lameness and BCS (time varying effects) on the likelihood of conception

Since the average BCS for 3 visits was highly correlated with the total number of MS3 scores in 3 visits, these variables could not be added to the same model, therefore two final multivariable models were built. These models with odds ratio (OR) and 95% of credible intervals (CI) are presented in Table 6.6 (model 3A) and Table 6.7 (model 3B). The association between the average BCS and total number of MS 3 scores in 3 visits is presented in Table 6.8 and Figure 6.5.

Herd 2 (OR: 0.42, CI: 0.27-0.66), Herd 3 (OR: 0.23, CI: 0.13-0.41) and Herd 4 (OR: 0.46, CI: 0.27-0.79) had a significantly lower likelihood of conception compared with Herd 1. For duration from calving, the likelihood of conception increased with increased duration from calving until 200 days. Cows that

calved between Jul-Sep had a lower likelihood of conception compared with cows that calved between Jan-Mar (OR: 0.51, CI: 0.32-0.80).

For average BCS for 3 visits, cows in BCS G1 (BCS1.00-1.75)(OR: 0.58, CI: 0.34-0.97) and BCS G2 (BCS 2.00-2.25) (OR: 0.66, CI: 0.48-0.91) had a lower likelihood of conception compared with cows in BCS G3 (BCS 2.50-2.75) (model 3A, Table 6.6). Cows with 2-3 MS 3 scores in 3 visits had a lower likelihood of conception (OR: 0.36, CI: 0.16-0.83) compared with cows with no MS 3 scores (model 3B, Table 6.7).

For the association between average BCS and total number of MS3 scores, there was a significant association between the two (p -value<0.001). There were 19%, 6%, 1% and 0% of cows in BCS G1, BCS G2, BCS G3 and BCS G4 had 2-3 MS 3 scores respectively, whereas 61%, 86%, 97% and 96% of the cows in BCS G1, BCS G2, BCS G3 and BCS G4 had no MS 3 scores respectively (Table 6.8).

Table 6.6 Model 3A: Multilevel discrete time event model of covariates associated with likelihood of conception (including average BCS for 3 visits)

Covariate	Freq.	OR.	95% C.I.		<i>p</i> -value
Herd					
1	819	Ref.			
2	2515	0.42	0.27	0.66	<0.001*
3	1387	0.23	0.13	0.41	<0.001*
4	916	0.46	0.27	0.79	<0.001*
Duration from calving (days)					
Duration 0-45	2031	Ref.			
Duration 46-75	1237	3.30	1.91	5.69	<0.001*
Duration 76-105	921	7.01	3.58	13.70	<0.001*
Duration 106-135	633	6.63	2.91	15.06	<0.001*
Duration >136	815	8.57	3.23	22.74	<0.001*
Parity					
1	1736	Ref.			
2	1193	1.16	0.77	1.73	0.48
3	834	1.50	0.97	2.33	0.07
>3	1874	1.23	0.85	1.78	0.27
Month of calving					
Jan-Mar	1230	Ref.			
Apr-Jun	753	0.92	0.58	1.46	0.72
Jul-Sep	2160	0.51	0.32	0.80	<0.001*
Oct-Dec	1494	0.83	0.56	1.23	0.35
Average BCS for 3 visits					
BCS G3 2.50-2.75	1407	Ref.			
BCS G1 1.00-1.75	474	0.58	0.34	0.97	0.04 *
BCS G2 2.00-2.25	1846	0.66	0.48	0.91	0.01 *
BCS G4 3.00-5.00	54	2.25	0.97	5.24	0.06

Freq.: frequency, OR.:Odd ratio, C.I.: credible interval, *: significant value $\leq$ 0.05, Ref.: reference
Random variability between cows: 1.44 (0.24).

Table 6.7 Model 3B: Multilevel discrete time event model of covariates associated with likelihood of conception (including total number of MS 3 scores in 3 visits)

Covariate	Freq.	OR.	95% C.I.		<i>p</i> -value
Herd					
1	819	Ref.			
2	2515	0.36	0.20	0.65	<0.001*
3	1387	0.17	0.06	0.42	<0.001*
4	916	0.34	0.17	0.68	<0.001*
Duration from calving (days)					
Duration 0-45	2031	Ref.			
Duration 46-75	1237	3.67	1.76	7.64	<0.001*
Duration 76-105	921	9.30	2.73	31.66	<0.001*
Duration 106-135	633	10.27	2.04	51.63	<0.001*
Duration >136	815	15.33	2.05	114.77	0.01 *
Parity					
1	1736	Ref.			
2	1193	0.99	0.61	1.61	0.98
3	834	1.34	0.80	2.25	0.27
>3	1874	1.15	0.75	1.74	0.52
Month of calving					
Jan-Mar	1230	Ref.			
Apr-Jun	753	0.90	0.52	1.57	0.72
Jul-Sep	2160	0.48	0.26	0.89	0.02 *
Oct-Dec	1494	0.83	0.51	1.34	0.44
Total number of MS 3 scores in 3 visits					
No MS 3	3293	Ref.			
1 MS 3	287	0.66	0.37	1.16	0.15
2 and 3 MS 3	201	0.36	0.16	0.83	0.02 *

Freq.: frequency, OR.:Odd ratio, C.I.: credible interval, *: significant value ≤ 0.05 , Ref.: reference
Random variability between cows: 1.49 (0.25).

Table 6.8 Association between average of BCS and total number of MS 3 scores for 3 visits

Average BCS for 3 visits	Total number of MS 3 scores for 3 visits								
	0	%	1	%	2-3	%	Missing	Total	%
BCS G1 1.00-1.75	290	61	93	20	91	19		474	8
BCS G2 2.00-2.25	1579	86	163	9	101	6		1843	33
BCS G3 2.50-2.75	1369	97	29		9	1		1407	25
BCS G4 3.00-5.00	52	96	2	4	0	0		54	1
Missing							1854	1854	33
Total	3290	58	287	5	201	4	1854	5632	100

%;percentage

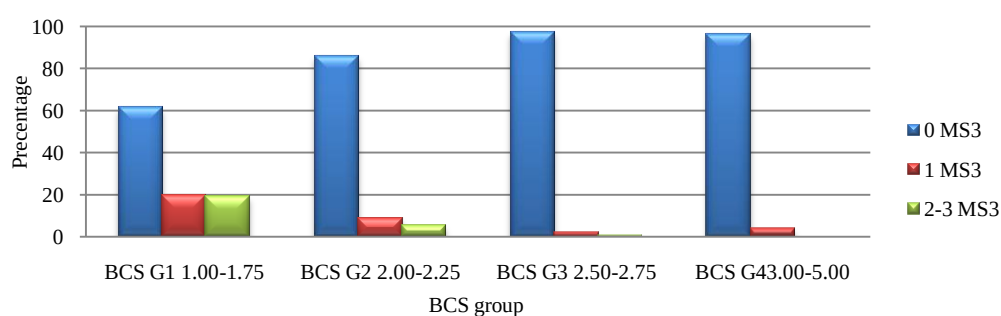


Figure 6.5 Association between average of BCS and total number of MS 3 for 3 visits

6.4 Discussion

The study results show that lameness and poor BCS have no effect on the likelihood of an AI leading to pregnancy (6.3.2-model 1) though both lameness and poor BCS have effect on the Ca-Con period and likelihood of conception (overall) (6.3.3-model 2 and 6.3.4-model 3). The relationship between lameness and fertility is well documented, however, the current study suggests that the impacts of lameness might depend on the chronicity of lameness (duration of lameness). The current study (model 2 and model 3) demonstrated that cows with 1 lameness recording or 1 MS 3 within the 3 visits had no effect on the probability of conception and the Ca-Con interval,

whereas cows with at least 2 lameness occurrences or 2 MS 3 within the 3 visits had a lower probability of conception and a longer Ca-Con period. One possible explanation is cows with chronic lameness (prolonged lameness or repeated lameness occurrences) are suffering longer duration of pain, more severe stress and lower feed intake resulting in more severe impacts on reproductive performance and immune function, eventually leading to lower probability of conception and longer Ca-Con interval. Alternatively, cows in chronic lameness might have lameness due to claw horn lesions whereas cows in short period of lameness (single lameness recording within several assessments) might be resulted from other causes of lameness such as infectious disorder, leg injuries and other cause of less severity. One study in UK reported that cows with claw horn lesions (sole ulcer and white line disease) had longer duration of pain (lower nociceptive threshold) (Whay et al., 1998) represented by repeated lameness recordings). This suggests that duration of lameness in cows resulting from different causes of lameness may have different (pain) impacts on fertility. Previous studies have found that cows with different causes had different probability to conceive, for instances, cows with claw horn lesions and multiple lesions were less likely to conceive compared with healthy cows (Collick et al., 1989; Hernandez et al., 2001; Machado et al., 2010), whereas lame cows with interdigital necrobacillosis or papillomatous digital dermatitis (PDD) were reported to have no significant differences compared with healthy cows (Hernandez et al., 2001). The current study did not record the causes of lameness, further research is suggested to investigate whether different causes of lameness resulting in repeated lameness recordings have different impacts on fertility. Another possible

explanation could be that there was an observer error in scoring mobility however given one observer scored all the cows its likely to be more reproducible unless there was a non-random bias, which seem unlikely.

The current study failed to find any significant effect of lameness based on a single lameness recording (e.g. lameness status at current visit, previous visit and before AI). Using a single lameness recording might introduce some unaccountable bias as dairy cattle have a higher chance of transition from non-lame to lame or lame to non-lame between two consecutive observations (Reader et al., 2011). Reader et al. (2011) also reported that cows with two similar mobility score recordings were more likely to remain at the same mobility score; this evidence suggested that repeated lameness recordings should be the standard method of assessment rather than a single lameness recording. A single lameness recording is able to provide a description of the overall lameness situation in the whole dairy herds at a specific time point (e.g. prevalence of lameness), but it provides little information on the condition of cows throughout the lactation. In the UK, farmers generally record lameness once or twice per year (Reader et al., 2011). In order to monitor the condition of cows, regular mobility assessment should be carried out on dairy farms, ideally once per month based on the recommendations from DairyCo (DairyCo., 2014b).

It is not surprising that the average BCS for the previous 3 visits did not meet the assumption of proportional hazard (6.3.3-model 2) as an effect of BCS should be time dependent variable, where cows have different BCS at varying

time points. Cows usually have lower BCS in early lactation due to the period of negative energy balance following calving and the BCS will then increase until the end of lactation. Due to BCS being a time dependent variable, a discrete time event model (6.3.4-model 3) was used to investigate the effects on the likelihood of conception. From this model, the results showed that lower average BCS (BCS 2.00-2.25 and BCS 1.00-1.75) in 3 visits (≈ 45 days) had a lower probability of conception compared with cows in normal BCS (BCS 2.50-2.75). Cows in low BCS were associated with lower feed intake, higher risk of metabolic diseases, longer duration of the anovulatory phase, lower quality of oocytes and abnormal hormone levels; these resulting cows to have lower likelihood of conception (Loeffler et al., 1999; Lopez-Gatius et al., 2003; Kadokawa and Martin, 2006; Hoedemaker et al., 2009).

Another possible explanation was cows in lower BCS were more likely to be lame, possibly due to cows in lower BCS having thinner digital cushion (Bicalho et al., 2009). Cows in low BCS are more likely to have pain and stress resulted from lameness leading the cows to have lower probability of conception. This has been demonstrated in Chapter 5.

To the author's knowledge, this is the first study to investigate the effects of lameness and BCS on the likelihood of an AI leading to pregnancy. The current findings suggest that there is no significant difference in the likelihood of an AI leading to pregnancy (likelihood of pregnancy after receiving an insemination) between lame cows (and those with low BCS) and non-lame cows (model 1) but overall, they have a lower likelihood of pregnancy and a

longer Ca-Con interval (model 2 and model 3). There are a number of possible explanations of these results. Firstly, this was possibly due to the impacts of lameness and poor BCS on the early stage of the oestrous cycle, such as changes in oestrogen, gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) level i.e. the normal reproductive cycle; consequently the lame cows (and those with low BCS) may have been less likely to express oestrus and hence less likely to receive an insemination. Successful reproduction starts with a good oestrus cycle (e.g., oestrus behaviours), followed by placement of fertile spermatozoa (AI or bull) into the uterus at the correct time point and finally the cows must have a successful conception and a normal pregnancy (Hughes, 1984). Previous studies have demonstrated that lameness and poor BCS have impact on the signs, intensity and number of oestrus behaviours (Peeler et al., 1994; Dobson et al., 2008; Walker et al., 2008). If lame cows in this study were less likely to express oestrus and less likely to be served, it is logical to assume they would have a lower overall likelihood of pregnancy and a longer calving to conception period, despite the fact that the conception rate of individual AI events between lame and non-lame cows being the same.

Another possible explanation is that either conscious or subconscious bias on the part of the farmers may have influenced their decision to give inseminations when they detected the signs of oestrus behaviours. For example, cows may have come into oestrus equally but farmers might have decided to give (or not give) inseminations based on the cows' previous health records, current condition or mobility score. Alternatively, farmers might

consciously or subconsciously use different types of semen on cows depending on observable conditions such as condition score and lameness. These conscious or subconscious decisions may introduce some unaccountable bias which has influenced the likelihood of an AI leading to pregnancy between the two groups.

Lastly, the data analysed provided no record of whether hormone injections (holding injections) such as GnRH were given to the cows. Farmers may have provided GnRH 'holding injections' to certain cows (especially for those deemed 'unhealthy' with low BCS or poor mobility) in order to increase the successful rate of AI. If such treatments were given unequally it could have led to there being no significant difference in the likelihood of an AI leading to pregnancy between lame (and those with low BCS) and non-lame (and those with normal BCS) cows. Any of these factors could have reduced the chances to be served and consequently lead to lower likelihood of pregnancy and a longer Ca-Con interval despite the fact that the likelihood of an individual AI leading to a pregnancy was not different between lame (and animals with lower body condition) and non-lame cows.

Days from calving to AI and herd had significant effects on the likelihood of an AI leading to pregnancy. It is suggested that the timing of AI given to dairy cattle is important (Barrel, 1984), the current study showed that cows which received an AI more than 45 days after they calved had a higher likelihood of AI leading to pregnancy compared with cows which received an AI within 45 days after calved. This finding is similar to Macmillan(1984) which reported

cows that received an insemination within 40DIM had a lower conception rate (nearly 20% lower) compared with cows that were inseminated after 40DIM (Macmillan, 1984). This might be due to the fact that in the early stage of lactations cows are usually in negative energy balance (NEB), where cows are more likely to have lower energy intake, low body condition score, imbalance hormone levels and metabolic problems (Coffey et al., 2002; Kadokawa and Martin, 2006; Ahmed et al., 2010; Kellogg, 2010), resulting in impaired follicular development and lower rates of ovulation (Kadokawa and Martin, 2006; Morris et al., 2011). A uterine contamination around calving or in early lactation might have reduced the probability of pregnancy and as a result the cows had a lower probability of pregnancy in early lactation (Sheldon et al., 2006; Walsh et al., 2011).

Cows in Herd 1 had a higher likelihood of an AI leading to pregnancy, better BCS, lower prevalence of lameness and also the highest (60%) successful rate of first service conception compared with the other herds. This herd effect could be due to management practices, housing systems, insemination methods (competence of the technician), timing of AI given, method of heat detection, quality of semen used and other herd specific factors, unfortunately these factors were not recorded or analysed in this study. Including factors related to housing system, management practices and other potential influences in future studies could be helpful to further explore these findings.

6.5 Conclusion

The study results suggest that cows with chronic lameness (longer duration of lameness) and low BCS had a lower probability of conception and a longer Ca-Con period compared with healthy cows, however these factors don't influence the likelihood of an individual AI leading to pregnancy; these findings suggest that farmers should maintain the cows in healthy condition to increase the chance to be served (higher chance and intensity of oestrus behaviours) and thus increasing an overall likelihood of pregnancy.

CHAPTER 7: GENERAL DISCUSSION AND FUTURE DIRECTIONS

7.1 Introduction

Over the past few decades, many researchers around the world have worked on lameness in dairy cattle to find solutions to minimize its prevalence and reduce its impacts on health, productivity and welfare. However, based on the prevalence and incidence levels of lameness that have been reported in the UK (Chapter 1, Table 1.1 and Table 1.2), the studies show an increase in lameness over time, with prevalence rising from 21% to 36% although there is a wide range across studies. The difference in levels of lameness could be attributed to differences in measures used to define and record lameness in these studies or true differences due to the impact of differences in management systems. Increases in herd sizes and the recent production trends in the UK dairy industry (Archer et al., 2010a) have also led to increased demand from cows and thus an increase in the risk of production diseases, including lameness. However, one of the major factors underlying the lack of decrease in lameness levels is that the aetiology of lameness, especially for certain lesion types, such as sole ulcer, white line disease, digital dermatitis is still not fully understood. The complex underlying relationships between lameness, poor BCS and other welfare problems are unclear. This could lead to a lack of successful control and prevention strategies which is perhaps further compounded by a lack of intervention studies on various control measures and a full understanding of barriers of uptake of lameness control on farm (Bell et al., 2009; Leach et al., 2013).

To devise a successful prevention strategy for lameness, a first step is to understand the aetiology of lameness. Hock lesions are one of the most common leg injuries in the dairy industry (Busato et al., 2000; Weary and Tazskun, 2000) and have been suggested as one of the causes of lameness (Huxley and Whay, 2006). However the cause and effect of the relationship between them is poorly understood. Among the three main presentations, lesions on the hock (hair loss, ulceration and swelling), hair loss is the most prevalent on farms (Huxley et al., 2004; Potterton et al., 2011a). Most studies have looked into risk factors for the presence and absence of these lesions with the assumption of progression from hair loss to swelling. Potterton et al. (2011a) investigated risk factors for the presence or absence of these three hock lesions separately and based on the identification of unique risk factors for these presentations challenged the assumption of a linear progression of lesions from hair loss to swelling and/or ulceration. In Chapter 3, risk factors were investigated for area of hair loss, these included locomotion score, cleanliness, milk yield, body condition score, duration of winter housing, stall base and bedding materials. This study suggests that some identified risk factors e.g. application of hygiene products, length of time mats and mattresses have been used and some features of freestall design, for the presence/absence of hair loss which have been reported previously (Potterton et al., 2011a) do not contribute to an increasing area of hair loss. Even though there are no published studies investigating the relationship between the degree of hair loss and the welfare of dairy cattle, it is logically to assume that cows with severe hair loss have poorer welfare, and Livesey et al. (2002) also suggested that reducing hair loss could be also beneficial for reducing severe

lesions. Further studies investigating the effects of the increased area of hock lesions on nociceptive threshold could be conducted to investigate these aspects along with investigation of development of these lesions. Also, the risk factors that have been reported in this thesis for the area of hair loss need further testing in an experimental longitudinal study, as the data in the current study were obtained from a cross-sectional study. Despite the identification of the ‘primary’ risk factors for increased area of hair loss as reported in this thesis, the ‘secondary’ risk factors including farmers’ perception of hock lesions, their awareness and willingness to implement intervention also have to be investigated as this will determine the success of intervention strategies (Whay, 2007). Another key result from the study on hock hair loss, suggested that ordinal scoring systems may not be capturing the severity of hock lesions accurately, when comparison of the scores given on a categorical scale were made to the lesion area measured on the hock maps. Hock maps, though inherently subjective, can capture relatively large amounts of details such as severity of lesions, regions affected and lesion size which could improve our understanding of the aetiology of hock lesions. Further studies are required to test the intra- and inter- reliability of data captured on hock maps, especially to warrant their use for research purposes (e.g. continuous monitoring of hock lesions over time).

In Chapter 4, the temporal association between hock hair loss and lameness was investigated. There are two general hypotheses on the association between hock lesions and lameness; lame cows spend longer time lying and frequently change their rising behaviour (Chapinal et al., 2009; Ito et al., 2010), therefore

increasing the risk of lesions developing on the hock; alternatively, lesions on the hock may be painful and make the cows change their walking posture, rising and lying behaviours, and eventually increase the risk of lameness. The results of this chapter suggest that lameness is leading to the development of hair loss on the hock and not vice-versa. In addition to hair loss, further research is now required to investigate the temporal association of lameness with the other two main presentations of lesions including ulceration and swelling. It may be that more severe hock lesions, such as ulceration, might cause cows to be lame because of the pain and discomfort resulting from severe hock lesions, or because cows change their postures and behaviours to protect their hocks (Huxley and Whay, 2006), resulting in animals becoming lame in the future.

In Chapter 5 and Chapter 6, the relationship between lameness and body condition score and their impact on fertility was investigated. Notably, the relationship between lameness and body condition score appears complex; previous studies have reported a positive association between lameness and poor body condition score (Espejo et al., 2006; Dippel et al., 2009; Hoedemaker et al., 2009). The results of Chapter 5 suggest that the increase and decrease of BCS (compared to BCS at calving) have effects on both the risk of lameness and the likelihood of recovery; this is the first study to report the effect of dynamic changes in BCS on both the occurrence and recovery of lameness. The findings support the hypothesis that cows which lose BCS mobilize fat from the whole body including the digital cushion, resulting in an increased risk of lameness. Furthermore, it is possible that pain may result

from the distal phalanx (PIII) sinking and pinching the nerves in the corium because of thinning of the digital cushion; consequently lame cows with BCS loss may have longer duration of pain, more severe pain and lower feed intake, and thus have less chance to recover compared with cows with no change in BCS. To confirm these hypotheses, future longitudinal studies on the observation of thickness of the digital cushion together with BCS and MS of cows are required. Based on the findings from this thesis, maintaining the target body condition of cows may be one of the key preventive measures to reduce lameness, but this has to be confirmed by further work. Additionally body weight measurements should be included in investigations of the association between body condition score, lameness and digital cushion thickness to provide a better understanding of the underlying complex relationships, as a large proportion of the body weight of dairy cattle is supported by digital cushion (Räber et al., 2004). Therefore it is logical that changes to body weight may apply certain pressure and impact on the digital cushion, resulting in cows becoming lame. In the meantime increasing farmers' awareness about the importance of regular BCS monitoring would be beneficial to improve animal health and welfare.

In Chapter 6, results agreed with previous studies that lameness and poor body condition score have significant impacts on fertility. The further investigations conducted in this thesis suggest that the impact of lameness on fertility depends on the chronicity of lameness. Cows which are lame for longer are more likely to have a lower probability of conception and a longer Ca-Con period. This may be because cows which are lame for longer have a longer

duration of pain, more severe stress and lower feed intake. Alternatively, cows with chronic lameness are more likely to have lower BCS, and cows in low BCS are more likely to have lower probability of conception. Even though the impact of lameness on fertility is unequivocal; results from Chapter 6 suggests that the effects of lameness and body condition score have no significant impact on the likelihood of each AI leading to pregnancy. Combining both findings above, suggests that the main reason lame cows have a lower probability of pregnancy is that lameness influences the expression of oestrus (Dobson and Smith, 2000; Dobson et al., 2008). If lame cows are less likely to express oestrus and less likely to be served, they will have a lower overall likelihood of pregnancy and a longer Ca-Con period. However, decision making by farmers may have influenced the results. In this study there was no information available about decision making, either subconscious or conscious, concerning when farmers decide to serve a cow. Secondly, there is no record of what efforts or any methods the farmers applied to improve the success rate of each AI, such as holding injections (hormone injections) or other ‘special’ supplements, particularly to those ‘unhealthy’ lame cows. Further work is required in order to understand owner perceptions to lame cows and the decision that farmers make when they give inseminations.

7.2 Statistical methods

Few different statistical modelling have been used in this thesis (Chapter 3-6) and the choice of which modelling technique to use was depending on the outcome and type of studies. In chapter 3, multilevel linear model was used

because of the hierarchical data with the log transformed outcome. The variability associated with each level of nesting is accounted for in the analysis. One assumption of this model is that outcome should be normally distributed. There are several methods to achieve normal data transformation such as transform the data into cubic form (y^3), logarithmic form ($\ln y$), square form (y^2), reciprocal form ($1/y$), square root form ($\sqrt{y}$) and etc. There are two points have to be considered when deciding on the method of transformation; first, the outcome after transformation must achieve normal distribution. Secondly it should be an appropriate way to interpret the model with transformed outcome (e.g., back transform).

In Chapter 4, the two level logistic regression model was used because the outcome of interest was presence or absence of a lesion at particular time t . Since the dataset has lot of missing observations the transitions between no hock lesion to hock lesion and vice versa could not be analyzed using the similar complex modelling used in Chapter 5 to give more understanding of the aetiology of hock lesions.

In this thesis, complex statistical modelling including multilevel multistate, discrete time event history modelling was used to investigate the existing datasets to unravel associations and achieve the aims of the study. For instance, using multilevel multistate modelling (Chapter 5) provided an understanding of the relationship between the risk of lameness and its recovery (transitions between non-lame and lame state). The work identified the heterogeneity that exists among cows with lameness, e.g. cows that have a

higher risk of lameness will have less chance to recover and the duration of lameness influences the risk of lameness. This joint modelling of transitions allowed for adjusted odds ratios for risk of recovery and lameness for each variable to make better comparisons on their contribution to each transition (i.e lame to non-lame, non-lame to lame). This would not be possible using traditional modelling approaches, looking at risk factors for each transition. However, there are a number of disadvantages of using this complex model. First, to be able to run these models there must be no missing records for the outcome of interest for each object (59 cows were excluded), as missing data in transitions means a row-wise deletion of that variable. It is often hard to achieve such complete datasets especially in field based trials. An alternative to deal with missing data is conducting MCMC imputation using Bayesian methods, however, the drawbacks of using this method in complex modelling is that are intensively computational and convergence is hard to achieve (Horton and Lipsitz, 2001). Secondly, for running multistate models the data must be expanded and restructured into the person period format before analysis. This is time consuming, and the large dataset finally obtained by restructuring, needs advanced computer equipment (processor and storage) for analyzing the data and analysis can take several hours for the models to converge (up to 8 hours was used for running this model in the study).

The usefulness of these models could be further exploited with very large datasets as one could explore in more detail transitions between multiple states instead of only two states as used in this thesis. For example, the researcher can investigate factors associated with the likelihood of cows transitioning

from the MS 1 state to the MS 2 state, the MS 1 state to the MS 3 state etc. Moreover, the wealth of data in large datasets could be investigated using the same methodology for risk of transitions between lesion types and the probability of recovery from different lesions or competing risks for different lesions.

In Chapter 6, two different models including Cox model and discrete time event history model were used in the same data because the simple Cox model could not be used to handle the time varying variables. The advance Cox model (stratified Cox model) or discrete time event model is suggested to solve this problem. There were two main reasons why a discrete time event model was finally chosen; the easiness of interpretation of results using this model and there was a danger of low number of observation for each stratified level in stratified Cox model which could have led to the bias estimation. In the dataset, there were 52 cows that left the study without a recorded reason. Availability of large datasets with the records of the causes (such as death, sick, culled or sold) of why cows were censored from the study, could have been further explored by expanding the multistate modelling to develop a competing risk model which could help to examine the probability of reasons for cull/leaving the study in the same model, helping to understand whether the effects of covariates are similar for all events or they vary by specific events (Steele, 2005).

There are a few key points which users must be aware of while constructing their models; firstly, screen the data explicitly (e.g., errors, missing values,

outliers); secondly, chose an appropriate statistical model (e.g., depending on the outcome of interest and type of studies); thirdly, check the assumptions underlying that particular modelling approach (e.g, normality, constant effect of variables); fourthly, ensure the model that is created is biologically reasonable; fifthly, assess the goodness of fit of the model (e.g., residual plots) and finally interpret the model correctly (e.g., odd ratios). Undoubtedly, statistical models can help us to explain part of the information in the data, but there remains some unexplained variation which cannot be fully explored even with complex statistical techniques, including multilevel multistate discrete time event models which have been used in this thesis.

7.3 Limitations

The datasets used were collected from either cross-sectional or longitudinal observational studies; these studies could not establish the cause and effect of the relationships though they can give a measure of the strength of associations. Also, the direct comparison between two groups of animals could not be made in these studies due to the fact that animals might be influenced by other factors such as herd management practices, husbandry factors, housing system or other unidentified factors not fully recorded by the observer.

All the visits in the four studies analysed were conducted by a single observer (SLP for Chapter 3 and 4; WAJ for Chapter 5 and 6), but the intra-observer reliability was not formally evaluated. The observers who collected the data

received training to reduce the risk of misclassification, and one could expect having a single observer for longitudinal data dismisses the chance of inconsistency that could exist among multiple observers which would have influenced the study results (McHugh, 2012).

In Chapter 3, there were missing data for hock maps (486 hocks), mainly due to cows with dirty hocks which meant the hock condition could not be accurately recorded (399 hocks). This may have introduced bias. These missing hocks may represent a certain subset of animals, possibly representing the group of very dirty animals, resulting in a smaller number of animals in the 'very dirty' category. A small number of hock maps (12) could not be used due to technical difficulties. Another disadvantage of using hock maps is despite the detail they provide on lesions they are time consuming to complete; the observer may sometimes not get enough time to record the lesions on the hock maps due to movement of the animal. Furthermore, hand drawn hock maps have an element of subjectivity; validity could be tested by comparison against some automated measurement of lesions and by measuring the inter- and intra- observer reliability.

In Chapter 4, the observer recorded the hock lesion condition approximately once per month but the length of time between visits varied between 18 and 42 days; this variation may have introduced bias to the study as the transition or progression of hock lesions may have occurred within a month, for instance, hair loss might resolve and redevelop rapidly within a month, resulting in the same score being recorded between two consecutive visits.

The lameness data in Chapter 5 and Chapter 6 was only available as mobility scores, there was no information on the causes of lameness or what treatments were given. Particularly in Chapter 6, there was no record of the insemination methods, timing of AI, method of heat detection, quality of semen used and other factors which might potentially influence the study results.

7.4 Conclusions and future works

7.4.1 Chapter 3- Area of hair loss in dairy cows with hock lesions: a study of its risk factors and correlation to categorical scale on 76 dairy farms in the Midlands region of the United Kingdom

Out of 94 risk factors, six factors were significantly associated with area of hair loss on hocks in dairy cows: locomotion score 3, poor cleanliness (score 10-18), mean milk yield 25.1-58.1kg, poor body condition score (1-1.5), duration of winter housing greater than or equal to 41 days and some combinations of stall base and bedding materials. A longitudinal study of the development of hock lesions and identification of risk factors associated with each lesion type could be useful to improve our understanding of the aetiology of hock lesions thus helping to devise a prevention strategy to improve animal welfare.

The secondary finding from this study suggests that an ordinal categorical scale for hair loss in its current form is not accurately capturing the severity (area) of lesions. Hock maps are a useful tool for monitoring the development

and transitions of hock lesions over time and could be employed in longitudinal studies.

7.4.2 Chapter 4- Investigations into the association of lameness with hair loss on the hocks in dairy cattle in a longitudinal study

The results suggest that lameness leads to the development of hair loss on the hock, not vice-versa. However, hock lesions measured in the study in the thesis were based on the hair loss condition on the hock and this is considered not as severe as ulcerations or wounds. It may be that severe hock lesions lead to lameness because of the associated severity. Future randomised controlled trials are required to understand the underlying relationship between lameness and hock lesions in order to develop a successful control strategy for lameness.

Cows with hair loss on the hocks either mild (score 1 or 2) or severe (score 3) were more likely to remain in the same condition or be more severe in the next month. There is limited information about how fast hock lesions develop and transition over time. Future work could include observing lesions more different time window (weeks) in order to understand the development and resolution of hock lesions.

Investigations on the relationship between hock lesions and animal behaviour is another area that could be pursued in further studies using more detailed measurement of the severity of hock lesions with hock maps. Cows with hock

lesions are logically assumed to have a higher risk of abnormal rising and lying behaviours as hock lesions cause pain and feeling of discomfort (Huxley and Whay, 2006).

7.4.3 Chapter 5- Unravelling the association between lameness and body condition score in dairy cattle using a multistate modelling approach

Cows with a greater decrease of BCS (compared to BCS at calving) had a higher probability of becoming lame and a lower probability of recovering. On the contrary, an increase of BCS from calving was associated with a lower probability of cows moving from the non-lame to the lame state and a higher probability of moving from the lame to the non-lame state. It is hypothesised that this was possibly due to the changes of thickness in the digital cushion. Thus further studies observing the thickness of the digital cushion together with measurements of body condition, body weight and lameness of cows would be useful in order to explore the relationships between them.

7.4.4 Chapter 6- Association between body condition score, lameness and selected reproductive performance indicators in dairy cattle

Results suggested that there is no significant impact of lameness and BCS on the likelihood of an AI leading to a pregnancy. However, cows with chronic lameness had a lower probability of conception and a longer Ca-Con interval; cows with lower average BCS have a lower probability of conception and they are more likely to be lame. The causes and treatments of lameness are

suggested to be collected along with MS and BCS measurements in a future study to provide a better understanding of the extent of impact of lameness on fertility. Furthermore, understanding owner perceptions and the decision making of farmers could be useful for designing strategies to improve health and fertility of dairy cows.

7.5 Final conclusions

This PhD thesis has added to our understanding of the complex inter-relationships between lameness, hock lesions, body condition and fertility in dairy cattle. The results of Chapter 4 have suggested that hock lesions are not the cause of lameness. This does not mean to say that hock lesions are not important as their impacts to welfare are clear, management practices including a reduction in the days of housing and an increase in lying comfort (Chapter 3) are proposed to farmers to reduce the risk of hock lesions.

Chapter 6 has suggested that body condition and lameness have impacts on the overall probability of fertility, possibly due to cows with poor body condition and lameness having lower intensity and less oestrus behaviour events. Therefore they are less likely to be served and have a lower probability of fertility and more days from calving to conception. Chapter 5 has suggested that maintaining cows in optimum body condition could reduce the risk of lameness events occurring and increase the chance of recovery from lameness if it does occur. Monitoring the body condition of cows and improving nutritional managements are recommended to farmers to reduce the risk of lameness and to improve fertility performance.

Future research requirements have been identified (7.1 and 7.4) to improve our understanding of the aetiology of lameness, poor body condition, hock lesions and infertility. This work is required to help in the development of strategies to reduce these costly diseases on farm and improve the health and welfare of dairy cows.

This thesis has used a variety of statistical modelling techniques to achieve the overall aims of each chapter, however there are certain limitations to each approach. Even though the statistical techniques employed could not fully explain the data analysed, choosing the correct analysis and following the right procedures to construct the models can increase our understanding of the data that is collected from dairy farms. Discrete time event history and multilevel multistate modelling that were used in this thesis are becoming important in the epidemiology field as they can help researchers to handle event history data (Chapter 5 and Chapter 6). These data are generally complex, and may have features such as repeated events (e.g. repeated occurrence of lameness throughout the study), multiple origin states (e.g. non-lame or lame at the start of lactation), multiple transitions of diseases (e.g. non-lame to lame and lame to non-lame), different timing of occurrence and types of censoring. The use of complex modelling techniques (Chapter 5 and Chapter 6) can help to handle these features and provide information on the complex dynamics of diseases on farms.

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Appendix 1 Locomotion scoring systems reported in the literatures

Reference	Scale	Description
(Manson and Leaver, 1988)	1.0	Minimal abduction/adduction without uneven gait or tenderness
	1.5	Slight abduction/adduction, without uneven gait or tenderness
	2.0	Presence of abduction or adduction, uneven gait, perhaps tenderness
	2.5	Presence of abduction or adduction, uneven gait and tenderness
	3.0	Slight lameness but not affect behaviour
	3.5	Obvious lameness, difficult turning but not affect the behaviour
	4.0	Obvious lameness, difficult turning, the behaviour is affected
	4.5	Difficulty in rising, walking, the behaviour is affected
	5.0	Extreme difficulty in rising, difficult walking, adverse effect on the behaviour
(Tranter and Morris, 1991)	0	No abnormality of gait
	1	Lameness hardly noticeable
	2	Slightly lame
	3	Markedly lame
	4	Affected limb not weight bearing
(Wells et al., 1993)	0	Gait abnormality not visible at walk, not reluctant to walk
	1	Mild variation from normal gait at walk; intermittent gait asymmetry or mild bilateral or quadrilateral restriction in free movement
	2	Moderate and consistent asymmetry or symmetric gait abnormality, but able to walk without continuous stimulation
	3	Marked gait asymmetry or severe symmetric abnormality
	4	Recumbent
(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- the cow stands and walks with a level-back posture. Her gait is normal
	2	Mildly lame- The cow stands with a level-back posture but develops an arched back posture while walking. Her gait remains normal
	3	Moderately lame- An arched-back posture is evident both while standing and walking. Her gait is affected and is best described as short-striding with one or more limbs
	4	Lame- An arched-back posture is always evident and gait is best described as one deliberate step at a time. The cow favors one or more limbs/feet
	5	Severely lame- The cow additionally demonstrates an inability or extreme reluctance to bear weight on one or more of her limbs/feet

continued

Reference	Scale	Description
(Winckler and Willen, 2001)	1	Normal gait
	2	Uneven gait (stiff, very careful)
	3	Short striding gait with one limb
	4	Short striding gait with more than one limb or strong reluctance to bear weight on one limb
	5	Does not support on one limb or strong reluctance to put weight on two or more limbs, holding a limb up whenever possible
(Cook, 2003)	1	Non-lame- Walks rapidly and confidently, making long strides with a level back
	2	Slight lameness- Walks more slowly making shorter strides with an arched back. Stand with a level back and do not favour a leg. Difficult to detect any weight transfer from affected limb.
	3	Moderate lameness- Often thin, walks slowly making deliberate short steps with an arched back, and frequent stops. Weight transfer will cause sinking of the dew-claws of the unaffected hind limb. Encounters difficulty turning. Stands with an arched back and frequently lifts affected foot
	4	Severe lameness- Usually very thin. Moves very slowly, making frequent stops to reset affected limb, which is only partially weight bearing. Frequently salivate due to grinding of teeth. Extreme difficulty turning. Stands and walks with pronounced arched back
(Whay et al., 2003)	0	Sound
	1	Abnormal locomotion/ perhaps tender footed
	2	Lame
	3	Severely lame
(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, 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, 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, joints show signs of stiffness, asymmetric gait, 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, 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 flexi on with very hesitant and deliberate strides, asymmetric gait, inability to bear weight on one or more limbs

Appendix 2 Description of scoring systems used to assess hock lesions in dairy cattle from published studies

Reference	Scoring system	Brief description
(Gustafson, 1993)	Categorical (5 categories) Score 0-4 Score 0-no lesion Score 1-bare pale area Score 2-bare red area Score 3-occurrence of serum, sore scab Score 4-open infected wounds	Based on hair loss and ulceration condition
(Wechsler et al., 2000)	Categorical (4 categories) Hairless patches less than 2cm in diameter Hairless patches more than 2cm in diameter Scabs or wounds less than 2cm in diameter Scabs or wounds more than 2cm in diameter	Based on hair loss and ulceration condition
(Weary and Taszkun, 2000)	Binary- score 1 or score 2 Score 1- area of hair loss less than 10cm ² with no evidence of skin breakage Score 2- broken skin, dark scab, or area of hair loss greater than 10cm ² .	Based on hair loss and ulceration condition
(Chaplin et al., 2000)	Categorical (5 categories) Score 0-5 Score 0-no lesion Score 1-bare pale area Score 2-bare red area Score 3-occurrence of serum, sore scab Score 4-open infected wounds Score 5-swelling and or adventitious bursae(fluid filled sac on hock)	Based on hair loss, swelling and ulceration condition * This system was extended from Gustafson (1993) scoring system.
(Vokey et al., 2001)	Categorical (3 categories) Score 0-2 Score 0-smooth Score 1-roughened Score 2-missing Categorical (3 categories) Score 0-2 Score 0-absent Score 1-slight swelling, still able to feel the lateral tarsal joint Score 2-severe swelling, unable to feel the lateral tarsal joint	Based on hair loss condition Based on swelling condition
(Livesey et al., 2002)	Categorical(3 categories) Score 0-no lesion Score 1-minor abnormality- hair loss Score 2-severe-other abnormalities Abnormalities including hair loss, skin abrasion, and subcutaneous swelling and enlargement of the hock joint or associated with synovial structures	Based on hair loss, swelling and ulceration condition
(Regula et al., 2004)	Categorical (4 categories) Score 0-3 Score 0-no alteration Score 1-hair less patch, but skin unaltered Score 2-reddening and or swelling of skin Score 3-open wound or abscess	Based on hair loss and ulceration condition

continued

Reference	Scoring system	Brief description
(Rutherford et al., 2008)	Binary -sound or damaged Sound-no hair damage or evidence of reduction in hair length that had not progressed to reveal bare skin Damaged- hair loss had progressed to leave bare patches skin or an abrasion was present	Based on hair loss and ulceration condition
(Lombard et al., 2010)	Categorical (3 categories) Score 1-3 Score 1-no hair loss or swelling on both hocks Score 2-hair loss but no swelling Score 3-hair loss, swelling or draining lesions	Based on hair loss and swelling condition * Developed by Cornell University and can be downloaded from http://www.ansci.cornell.edu/prodairy/pdf/hockscore.pdf
(Husfeldt and Endres, 2012)	Categorical (3 categories) Score 1-3 Score 1-no lesion Score 2-mild lesion-hair loss Score 3-severe lesion-swollen hock with or without hair loss	Based on hair loss and swelling condition

Appendix 3 Hock map

Left lateral	Left point of hock	Left medial
Right lateral	Right point of hock	Right medial

Farm ref. no. _____
 Visit no. _____
 Animal ID _____
 Pen no. _____

Notes

Hair loss left: _____
 Ulceration left: _____
 Hair loss right: _____
 Ulceration right: _____

Key

Ruffled hair/ Partial hair loss
 Complete hair loss
 Ulceration