Furthering our Understanding of the Aetiopathogenesis of Claw Horn Lesions

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Abstract

Claw horn lesions (CHL – sole haemorrhage, sole ulceration, and white line disease) are a prevalent and recurrent cause of lameness in dairy systems globally. These are conditions that compromise the health and welfare of dairy cattle and can challenge the economic viability of a dairy enterprise. Despite our understanding of the importance of these lesions, our knowledge surrounding their pathogenesis remains poor. It has previously been identified that lameness begets lameness and that CHLs are correlated with pathological change to the anatomy of the bovine distal limb. In Chapter 3 it was identified that dairy cattle with a lifetime history of lameness were more likely to have a lower volume of digital cushion in their lateral claws at slaughter. This association and others were explored using a retrospective cohort study. This study involved the hind hooves of 102 Holstein cattle culled from a research herd being scanned using magnetic resonance imaging (MRI). Animal variables including lameness, CHL, body condition score (BCS) were collated for the duration of the animal's lactating lifetime. The volume and total fat fraction of the digital cushion were calculated using a m-DIXON quant scan sequence. Animals with a history of lameness, CHL occurrence and low BCS were more likely to have less digital cushion in their lateral claws at cull. It was postulated that a weaker genetic and developmental foundation to the digital cushion structure led to certain animals being predisposed to CHLs due to the function of this structure being compromised. These animals then experienced a lifetime of lameness and CHLs which led to the metabolism of digital cushion to facilitate inflammatory pathways. The histology of these digital cushions isolated a vast range in collagen content and adipocyte size in Chapter 4. Animals with a history of lameness in the four weeks before cull tended to have digital cushions with a higher proportion of Type 3 collagen in comparison to Type 1, which is indicative of scarring and inflammatory disease. It was hypothesised that these animals had a higher level of matrix metalloproteinase (MMP – an inflammatory mediator) action local to the hoof which can degrade (and cause the turnover of) collagenous tissues.

The treatment and prevention of lameness derived from CHLs may play a vital role in protecting the functional anatomy of the hoof. If this anatomy can be protected, then it is believed that the animal is less likely to experience CHLs in the future. From previous research and that presented in Chapters 3

and 4, the inflammatory aetiology of the CHLs is hypothesised to be a driving force behind the pathological change that occurs. Further to this, there is a growing body of evidence to suggest that inflammation experienced by the dairy cow around the transition period (and the degree to which it exists) can be highly influential on the future health status of the animal. The impacts of transition period inflammation on lameness have never been examined before. Indeed, non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to be important in improving cure rates when used as part of a treatment regimen for CHLs. This has been demonstrated through single outcome measures, however their efficacy in the long-term management of lameness within the dairy herd has never been examined. Chapters 5 and 6 examine the efficacy of NSAID administration in the treatment and prevention of lameness through the implementation of a three-year long randomised controlled trial (RCT). Holstein cattle within the study herd were subjected one of four treatment groups for the entirety of the study period with lameness scoring and treatment administered on a fortnightly basis and a blinded outcome score occurring fortnightly. Chapter 5 asses the impacts of the treatment regimens on the risk of lameness and culling in heifers that calved into the study herd after January 2018. These animals were subjected to treatment regimens from first calving through until the end of the study period or culling. From this study, it was identified that animals that received NSAID at first and subsequent calvings, and at lameness events were at a significantly reduced risk of being scored as lame or culled when compared to those that received no NSAID at calving or lameness. It was hypothesised that systemic inflammation at calving can initiate pathological change to distal limb anatomy which predisposes an animal to CHL onset. Furthermore, the effect size and robust nature of the RCT gives strength to the case for routine administration of NSAID at calving for the purposes of lameness management. The effect size observed presents a substantial opportunity to reduce the prevalence of lameness in the global dairy herd substantially.

Chapter 6 examined the effects of NSAID administration as above, but in a cohort of adult dairy cattle that had already calved once prior to study initiation. Contrary to Chapter 5, no effect of NSAID administration at calving or lameness was observed on the risk of an animal being scored as lame or culled from the herd. It could be suggested that these animals have already had some degree of

pathological change to distal limb anatomy associated with either transition or lameness which has led to a predisposition to future lameness which cannot be undone. This predisposition may counteract any benefits associated with NSAID administration as a treatment or prevention, meaning that with regards to long term lameness management, there is little if any positive prospect for animals that have been previously lame or calved without NSAID administration. This thesis, however, does not diminish the importance of the pain management associated with NSAID administration.

This thesis furthers our understanding of pathological change to distal limb anatomy and optimal treatment and prevention regimens for lameness in dairy cattle. Presented within Chapter 5 and 6 is the longest running RCT conducted to date investigating lameness treatments with longitudinal outcomes. This has provided a robust evidence base for informed, impactful management practices to be implemented, and has provided evidence for further hypothesis to be framed around.

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Abbreviations

AMS	Automated Milking Systems
aTSE	Adiabetic Turbo Spin Echo
BCS	Body Condition Score
C	Control
CHLs	Claw Horn Lesions
DD	Digital Dermatitis
DIM	Days In Milk
DMI	Dry Matter Intake
EDPET	Early Detection, Prompt Effective Treatment
H&E	Haemotoxylin & Eosin
mDIXON	Modified DIXON
MMP	Matrix Metalloproteinase
MRI	Magnetic Resonance Imaging
MUFA	Mono-Unsaturated Fatty Acids
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PFA	Paraformaldehyde
PSR	Picrosirius Red
RCT	Randomised Controlled Trial
S	Select

SARA	Sub-Acute Ruminal Acidosis
SH	Sole Haemorrhage
SST	Sole Soft Tissues
SU	Sole Ulcer
WLD	White Line Disease

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1 Introduction and Literature Review

1.1 The Challenge of Lameness

Lameness is a debilitating multifactorial condition affecting dairy and beef cattle worldwide which can be identified visually as an altered gait associated with pain on locomotion (Bicalho et al., 2009). Lameness scoring is an important tool for farmers to be able to identify and assess any changes in gait and/or pain associated with lameness (Whay et al., 2003; Reader et al., 2011). Multiple systems of lameness scoring exist worldwide, all of which aim to assess the ability of the animal to walk without pain. Scoring methods typically attribute a lameness score to the individual being assessed, these scores are a reflection of the animals ability to move without pain and allow for the identification of lame or sound animals (Manson and Leaver, 1988; Sprecher et al., 1997; AHDB Dairy, 2020; for a more fulsome description of lameness scoring, please refer to Chapter 1.4.2.). It is estimated that approximately one in three lactating animals in the UK dairy herd are lame at any given time, however the prevalence between farms ranges vastly, highlighting a disparity between individual farm businesses and the lameness prevalence at a local level (Barker et al., 2010; Griffiths et al., 2018; Randall et al., 2019a). The UK prevalence is not dissimilar to those reported for the USA and Canada within which similar production systems are reported, with approximately one in five lactating cows being lame in Canada (Solano et al., 2016) and one in four in the USA (Foditsch et al., 2016). Like the UK, individual farms in the USA and Canada can have a vastly different lameness prevalence with some farms having less than 10% and others having more than 60% of their milking herd as lame (Foditsch et al., 2016; Solano et al., 2016).

Pain associated with locomotion presents a substantial welfare challenge to the animal (Whay et al., 2003), with the ability to display normal behaviours being prevented through pain. Whay and Shearer (2017) described how lameness directly impacts the five freedoms which are commonly used as a tool to evaluate animal welfare. The five freedoms and the way in which lameness impacts them are described in the points below:

- Freedom from hunger or thirst - Animals suffering from lameness demonstrate different feeding (Barker et al., 2018) and drinking (Stokes et al., 2012) behaviours to their sound counterparts with lame animals commonly having a lower dry matter intake (DMI) and spending less time at the feed bunk (Bach et al., 2007) and water troughs (Stokes et al., 2012).
- Freedom from discomfort – It is generally understood that dairy cattle suffering from lameness experience difficulties getting in and out of stalls meaning that they are commonly reluctant to rest in a conventional stall design (Whay and Shearer, 2017), the lying time of dairy cattle can also vary substantially when compared to their sound counterparts with some severely lame animals not able to rest in stalls appropriately (Walker et al., 2008).
- Freedom from pain, injury, and disease – Lameness is understood to be an extremely painful condition regardless of the cause. Animals suffering from this disease are in a state of hyperalgesia, meaning that animals experience extreme pain even from a relatively small noxious stimulus (Whay et al., 1998).
- Freedom to express normal behaviour – Animals suffering from lameness have a vastly different time budget when compared to their non-lame counterparts (Walker et al., 2008; Gomez and Cook, 2010; Weigele et al., 2018). A time budget is described as a breakdown of the 24-hours in a day with respect to what behaviours/tasks are displayed through this period. Animals suffering from lameness have a larger proportion of their time budget assigned to the task of milking, primarily due to animals taking longer to walk from their assigned housing unit/paddock to the milking parlour (Espejo et al., 2006; Main et al., 2010). Additionally, lame cows tend to be lower in the hierarchical social structure of the group in which they are housed, making them subject to “bullying” behaviour (Galindo and Broom, 2002).
- Freedom from fear and distress – Severe lameness and the pain associated with it is a clear compromise of this freedom (Whay et al., 1998). When animals are treated for lameness, it has been shown that cortisol levels increase (a common sign of stress in livestock), this is further exacerbated by the movement of an animal through a handling system (Cook et al., 2009) into a hoof trimming crush (Janßen et al., 2016).

The Five Domains concept of animal welfare also can be used to clearly illustrate how lameness has a substantial negative impact on animal welfare. The Five Domains encompass the nutritional, environmental, and health related challenges an animal may face within their lives and combines it with behavioural interactions they may have with humans or other animals to create the fifth domain of the animal's mental state which is directly related to the animal's overall welfare standard. Lameness clearly impacts on all of these domains in a fashion similar to the Five Freedoms as described by Whay and Shearer (2017). Further to the Five Freedoms and Five domains, the Three Circles is another means of assessing animal welfare. These three circles describe an animal's basic health and ability to function, its ability to live in a "natural state", and its emotional state of wellbeing. Again, from the evidence described previously, it is clear how lameness impacts on the three circles model for assessing animal welfare.

The welfare challenges associated with lameness are vast, with farmers being stewards of the animals that they care for, it is clear that further knowledge and understanding is required to empower them to tackle this condition in an impactful manner. The challenge with delivering impactful solutions for conditions such as lameness is commonly associated with a lack of understanding around the losses associated with the disease and the costs incurred through its treatment.

1.2 Losses Associated with Lameness in the Herd

Alongside the obvious welfare challenges associated with the pain of lameness, the economic impacts of this condition are commonly understated (Huxley, 2013). The direct costs associated with the treatment of the condition are evident to the producer, namely the price of equipment (hoof trimming crush, hoof knives, orthopaedic hoof blocks, etc) , the cost of a contracted hoof trimmer or the time of in house staff, and any form of pain relief or antimicrobial treatment required (Dolecheck et al., 2018). However, it is the indirect losses derived from lameness are commonly understated (Bruijnis et al., 2010; Dolecheck et al., 2019) . These indirect losses are described below.

1.2.1 Milk Yield

Many studies have examined the effect lameness has on the milk yield of individual animals. Upon initial inspection of a paper published by Green et al. (2002) it would appear that lameness has a positive relationship with milk yield. Lame animals within that study appeared to have a higher 305d milk yield in comparison to their counterparts who were never lame. The authors from this paper explained that lameness is a disease of high production (i.e. high yielding animals are more likely to become lame), with the milk yields of high merit animals being suppressed following lameness. Reader et al. (2011) identified that the milk yield of an individual dropped before they became visibly lame and Randall et al. (2016) identified that more severe hoof lesions were associated with a decreased milk daily yield. Bach et al. (2007) found that lame animals housed with an automated milking system (AMS) had a reduced milk yield in comparison to their sound counterparts and were also more likely to be late in visiting the milking system, they suggested that the reduced feed intake observed in that study, and an increased time between visits to the AMS were responsible for a decreased daily milk yield. Many papers have been published with an aim of assessing the effects of lameness on milk yield, however interpreting them should be done with caution as challenges arise due to different definitions of lameness being used (such as the use of lameness scoring systems vs lesion identification, such as in Reader et al. (2011) and Randall et al. (2016) respectively), and different means of summarising milk yields losses (Bruijnis et al., 2010; Randall et al., 2016). Furthermore, the assessment of the impact of lameness on milk production also needs to consider the predisposition high yielding animals have to the development of lameness. Animals that sit on a higher production plane are much more likely to become lame than their lower producing herd mates, meaning that at lameness onset, these animals are likely to be suppressed onto a lower productive plane. What has been categorically displayed is that lameness is a disease of high production (Green et al., 2014) which has negative consequences on milk yield, with losses ranging between 270kg and 570kg (summarised by Huxley (2013)).

1.2.2 Reproductive Efficiency

It has been demonstrated that lame cows have a reduced reproductive efficiency irrespective of management system and metric used to measure reproductive performance (Somers et al., 2015; Remnant et al., 2019; O'Connor et al., 2020b). Lameness has clearly been shown to extend calving interval (Enting et al., 1997) and the return to service interval (Remnant et al., 2019). It has been demonstrated that lame animals are less able to express oestrus behaviour (meaning that the recognition of oestrus by farm staff or automated system is much less likely to occur) (Walker et al., 2008) and have a reduced cyclicity when compared to their sound counterparts (Remnant et al., 2019; Omontese et al., 2020).

1.2.3 Culling

Lame animals tend to be culled earlier in life and lactation (Cook, 2020; O'Connor et al., 2020b). The reasons behind culling may also be influenced by lameness. Reproductive performance is the most common reason for culling however, the occurrence of lameness may also mean that animals are culled for reproductive failures that have originally been driven by lameness occurrence (Cook, 2020). This means that the value of animals being culled for lameness purposes may be substantially underrepresented.

1.2.4 Feed Efficiency

Animals suffering from lameness demonstrate different feeding behaviours in comparison to their sound counterparts (Llonch et al., 2018). In a longitudinal study, Bach et al. (2007) locomotion scored cattle twice weekly and recorded DMI and feeding behaviour. In that study, it was found that animals suffering from an increased lameness score spent less time eating and had a reduced DMI. This reduced DMI resulted in a reduced daily milk yield and would in turn lead to a reduction in BCS. The associations between lameness and BCS are complex, with lameness being a debilitating disease and a disease of debilitated cattle (Bicalho et al., 2009). Thin cows are more likely to go lame, however it is conversely true that lame cows are likely to become thin and lose BCS (Bicalho et al., 2009; Green et al., 2014; Newsome et al., 2017a; b). When animals lose BCS, it is typically due to being in a state of negative energy balance which in turn presents a large range of metabolic challenges for the animal

meaning that they are not as efficient in performance metrics as they may have once been (Wankhade et al., 2017).

1.2.5 The Total Costs – an "Iceberg" Effect?

In a review published by Huxley (2013) it was estimated that the total cost of a single case of lameness was between £131-£425. If these values are compared with the most recently reported prevalence values for the UK (~33%) (Griffiths et al., 2018; Randall et al., 2019a) it becomes apparent that lameness is associated with a large loss of potential income for the individual farm and the wider dairy industry.

If the nature of lameness is considered (irrespective of cause), it becomes apparent that the most substantial loss of lameness may not lie within the losses of milk yield etc. from a single event. The majority of an animal's risk of becoming lame is derived from a previous lameness event, namely, lameness begets lameness (Newsome et al., 2016; Randall et al., 2018a). When accounting for the costs of a disease such as this, how accurate is it to encapsulate the hidden costs of future lameness events? It may be more pertinent in the future to examine the costs of this disease across an animal's life to calculate the costs of a single event, accounting for the chronicity of the condition.

Additional to the economic and welfare-based challenges presented by lameness, the condition can also challenge citizen perceptions of the industry. This condition is easily observed by an untrained member of the public (Garcia et al., 2015). A large proportion of the consumers of dairy products are now becoming more aware of the origin of their foodstuffs, with a particular focus on the welfare of the cow being at the forefront of this (Wolf and Tonsor, 2017). With this in mind, alongside the previously mentioned challenges, urgent action is required to reduce the prevalence of lameness across the worldwide dairy herd to not only improve the welfare of dairy cattle and improve the productivity of dairy businesses, but also to maintain and improve a strong consumer base to ensure future viability of the industry (Wolf and Tonsor, 2017).

1.3 The Claw Horn Lesions

The pain associated with lameness most commonly originates from the hoof and is typically caused by what are categorised as infectious and non-infectious causes (Murray et al., 1996; Barker et al., 2010). Other sources of lameness can occur higher in the limb, from fractures or due to influences on the nervous system (Kirkwood et al., 2018). Both the infectious and non-infectious causes most commonly affect the hind hooves of the dairy cow (Murray et al., 1996). Infectious causes of lameness include digital dermatitis and interdigital necrobacillosis (Barker et al., 2010). Of these two conditions digital dermatitis is the most common and is caused by a treponeme infection in the interdigital space or above the heel bulbs in any location on the distal limb (Cheli and Mortellaro, 1974; Murray et al., 1996; Barker et al., 2010). Although infectious causes of lameness present a substantial challenge to the mobility of the global dairy herd, the topic of this thesis is the claw horn lesions (CHLs). As the name suggests, the term CHLs captures a range of conditions affecting the hoof capsule.

1.3.1 Introduction to Claw Horn Lesions

CHLs present as a range of different conditions affecting the horn of the hoof capsule, briefly; (1) Sole haemorrhage (SH) is visualised as a reddening of the sole horn through the incorporation of haemorrhage into horn production; (2) Sole ulceration (SU) is understood to be a later stage of the SH disease process , it is visualised as the focal cessation of horn production which can be accompanied by the protrusion of underlying tissue in severe cases (Van Amstel and Shearer, 2006; Shearer et al., 2012); (3) White line disease (WLD) is classified as the incorporation of haemorrhage into the white line region that connects the sole horn to the wall horn, or the separation of the sole horn and wall horn completely at the white line (Shearer and van Amstel, 2011; Shearer et al., 2012). WLD can be identified by the same characteristics as SH or SU but located at the white line junction of the hoof, typically separation at the white line junction can be visualised from the plantar aspect of the sole when WLD is present (Murray et al., 1996). CHLs are highly prevalent amongst the global dairy herd (Leach et al., 2012; Solano et al., 2016). The pain associated with the presence of CHLs (Whay et al., 1998) and the economic costs (Huxley, 2013) associated with them present a critical challenge to the

future viability of the dairy industry (Wolf and Tonsor, 2017). However, what is more challenging about these diseases is their aetiopathogenesis; these are chronic conditions that are now believed to impede the cow for the remainder of her life (Newsome et al., 2016). To understand the aetiopathogenesis of these conditions, the internal functional anatomy of the hoof must first be understood.

1.3.2 Claw Horn Lesion Aetiology

Claw horn lesions are associated with a failing of the functional anatomy of the hoof, namely the suspensory apparatus (Lischer et al., 2002; Tarlton et al., 2002; Knott et al., 2007) and the digital cushion (Bicalho et al., 2009; Newsome et al., 2017a; Griffiths et al., 2020). The suspensory apparatus describes the laminar attachments which bind the inner aspect of the dorsal wall to the distal phalanx, suspending the bone within the hoof capsule (Lischer et al., 2002). The digital cushion is comprised of three parallel cylinders of fat which extend proximally beneath the distal phalanx from the heel towards the toe, this structure acts to dampen the compressive forces associated with locomotion and standing (Bicalho et al., 2009) (for a more fulsome description of the structure of the digital cushion and the suspensory apparatus, please refer to Chapter 1.3.3).

The suspensory apparatus and digital cushion normally act to dissipate the force associated with hoof-strike away from the sensitive germinal epithelium, particularly under the most caudal aspect of the pedal bone where the “typical ulcer site” occurs (Lischer et al., 2002; Räber et al., 2004). The typical ulcer site is where SH and SU most commonly occur (Van Amstel and Shearer, 2006), the aetiology of SH and SU are discussed below. The typical ulcer site is situated where the initial point of hoof-strike occurs (zone four, Figure 1-1), this point also happens to co-exist in line with the most caudal aspect of the distal phalanx. This creates a “pinch point” where the sole corium is exposed to the maximum force load from the interaction between the internal anatomy of the hoof and the floor (Van Der Tol et al., 2002, 2003). Upon failure or overload of the digital cushion and / or suspensory apparatus (the causes of which can be vast), contusions occur in the vascularised corium due to excessive compression of the sole corium. This leads to the incorporation of haemorrhage (i.e. SH) into the newly forming horn creating a poorer quality horn which is more susceptible to further

damage (Lischer et al., 2002; Newsome et al., 2016). The incorporation of haemorrhage in the form of SH can occur in zones four five and six as visualised by Figure 1-1. This contusion and incorporation of haemorrhage is understood to be associated with localised inflammation at the site of trauma (Newsome et al., 2016, 2017b). If the SH goes untreated and horn production continues to be disrupted, then horn production can eventually cease, leading to the localised interruption of sole horn formation which can eventually be seen as a full thickness deficit in the sole and the protrusion of flesh through the horn referred to as a SU (Van Amstel and Shearer, 2006).

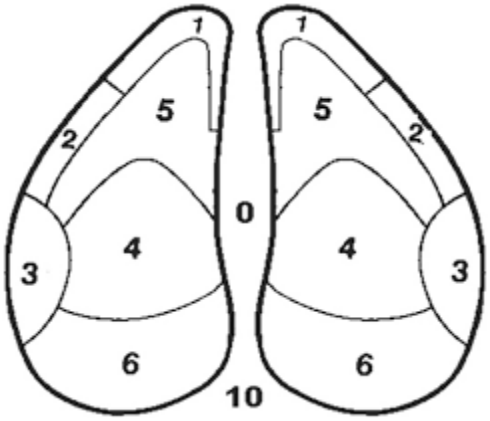


Figure 1-1 The hoof map. Zones of the distal surface of the claw, as recommended at the 6th Symposium on Diseases of the Ruminant Digit, Liverpool 1990. Zones 1, 2 and 3 are regions of the white line, zone 4 is the sole region, 5 the toe and 6 the heel. Zone 0 is the interdigital space and 10 the heel bulbs.

WLD has a similar pathogenesis to SU and SH, however the process through which this occurs is less well understood with an even smaller evidence base (Shearer and van Amstel, 2017). WLD disease occurs in the white line, which is visualised as zones one, two and three in 1-1. A lot of the understanding of WLD is based on observational research and statements which suggest that WLD originates by the same means as SU and SH, however the causes of the contusions caused within the white line have different origins (Tarlton et al., 2002; Shearer and van Amstel, 2017). What is currently understood is that WLD is associated with poor walking surfaces and with sharp twists turns and bends in the walkways and housed environment (Shearer and van Amstel, 2017). These shearing forces are associated with the incorporation of haemorrhage into the production of horn (Shearer and van Amstel, 2017). Associated with this incorporation is a weaking of the white line, eventually leading to separation of the wall and sole. (Shearer and van Amstel, 2017). If weakening of the white

line occurs, this can lead to this region being susceptible to perforation by objects from the environment (Greenough, 2007; Shearer and van Amstel, 2017). Once separation of the white line occurs, the horn will not re-join and foreign material can continue to track through to the corium leading to infection and abscess formation of the hoof until complete separation of this horn occurs and indeed, the exposure of flesh through the wall horn can follow(Greenough, 2007; Shearer and van Amstel, 2017). What is clear is that the traditional view of debris from walking surfaces being the sole cause of white line is not the case (although they can be a substantial contributory factor and can exacerbate the condition significantly). What appears to initiate WLD is a weakening of the white line horn, the inappropriate management of this and external factors such as debris and walking surfaces in combination(Shearer and van Amstel, 2017).

Lesions at the toe (such as toe ulcers or toe necrosis) have different risk factors attributed to them in comparison to lesions that occur at the typical sole ulcer site (Kofler, 2017). These lesions are found in the toe triangle which is classified as regions one and five in 1-1. The main risk factors associated with toe lesions include excessive wear and over trimming; these risk factors are both associated with thin soles which are understood to be a predisposing condition for toe lesions (van Amstel and Shearer, 2008; Kofler, 2017). If an animal has thin soles, they are lacking in the protective functionality of the sole horn in the toe triangle which protects the sole corium from external pressures (Bell, 2015). If these pressures are too great in the toe triangle, then contusions can occur in this area in a similar fashion to SH/SU and WLD pathogenesis. Indeed, the white line can separate or sole ulceration can occur due to thin soles or sole trauma in the toe triangle (Kofler, 2017).

The lateral claws of the hind limbs are the most common site for CHLs to occur (Barker et al., 2010), it is understood that this is because the majority of weight and force is exerted on the lateral claws of the hind hooves at standing (Van Der Tol et al., 2002, 2004) and during locomotion (Van Der Tol et al., 2003) . It is important to note that when failure of functional anatomy is described, it refers to the structures inability to offer adequate protection to the sole corium. This can be brought about through internal mechanisms specific to the digital cushion or suspensory apparatus, through excessive pressures exerted on the hoof of the cow derived from the environment in which she is managed, or a

combination of the two (Newsome et al., 2016). These pressures may be so great, that the internal anatomy of the hoof is unable to cope irrespective of their condition, resulting in the formation of CHLs. A summary diagram of the aetiology proposed by Newsome et al. (2016) is presented in Figure 1-2 which summarises the key inputs into CHL pathogenesis.

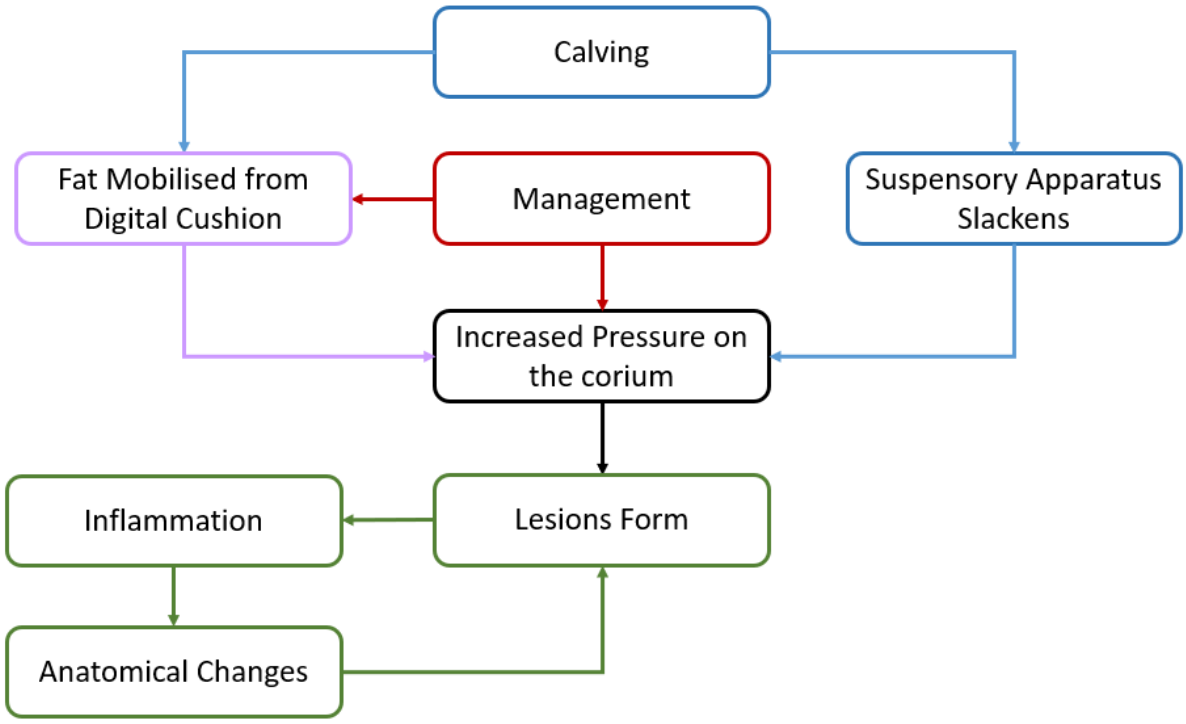


Figure 1-2 A summary diagram of the aetiology proposed by Newsome et al. (2016). The blue regions denote effects of calving, the red management, and the green the "downwards spiral" of pathological change associated with CHL onset.

1.3.3 Descriptive Anatomy

The hoof of the dairy cow functions to dissipate the forces associated with locomotion and standing away from the vascularised sole corium located under the distal phalanx (Newsome et al., 2017a; b). This is achieved by spreading the weight load into the dorsal and axial hoof walls and the heel bulb (Lischer et al., 2002; Räber et al., 2004). This prevents trauma to the sole corium and thereby minimises an animal's risk of developing CHLs (Bicalho et al., 2009). The primary structures involved with this function are the suspensory apparatus and the digital cushion (Lischer et al., 2002; Räber et al., 2004; Newsome et al., 2017a; b).

The suspensory apparatus can also be described as the laminae or the laminar corium which bind the distal region of the inner dorsal hoof wall to the distal phalanx (Lischer et al., 2002). The suspensory

apparatus is well developed on the abaxial wall and less so on the axial wall (with longer interdigital ligaments present), predisposing the flexor tuberosity to exert more pressure on the sole corium at what is commonly described as the "typical ulcer site" (Lischer et al., 2002).

Beneath the distal phalanx exists the digital cushion (also termed the supportive apparatus); a vital structure in the hoof capsule with respect to protecting the sensitive sole corium (Räber et al., 2004; Bicalho et al., 2009). This modification of the subcutis layer of the integument is understood to dissipate the forces of hoof-strike away from the sole corium beneath the flexor tuberosity across the sole horn and horn walls (Newsome et al., 2017b). This structure consists of three parallel cylinders (axial, abaxial and middle cushions) of connective tissue containing depots of adipose tissue which extend from the heel bulb proximally towards the apex of the distal phalanx (Räber et al., 2004; Newsome et al., 2017a; b, visualised in Figure 1-3). Animals with a thinner digital cushion are more likely to experience CHLs and lameness than those with thicker digital cushions due to a reduced force dissipating capacity (i.e. thicker digital cushions appear more able to dissipate the forces associated with standing and locomotion) (Bicalho et al., 2009; Newsome et al., 2017a; b; Griffiths et al., 2020). Numerous studies have demonstrated this relationship using trans-sole ultrasonography (a method that involves capturing an image of the sole soft tissues (SST) - namely the corium and digital cushion at the midline of the plantar aspect of the hoof at the typical ulcer site using an ultrasound scanner (Bicalho et al., 2009; Newsome et al., 2017a; b; Griffiths et al., 2020).



Figure 1-3 The plantar view of a hind hoof with the sole removed by band saw. The digital cushion can be visualised as the yellow regions that begin at the heel and extend proximally towards the toe.

Räber et al. (2004) conducted a descriptive dissection study of the digital cushion in which subjective observations of fat were made. Within that study, it was observed that the front medial and hind lateral claws contained less fat than their lateral and medial counterparts, respectively. Further to this, it appeared that heifers and animals of a parity greater than three presented a lower fat score than animals in their second or third parity. This study was elaborated by the same group in 2006 when the fat composition of the digital cushion was described. This study detailed how animals of a second to third parity had considerably more quantifiable fat in comparison to heifers or animals of a parity greater than three. Within that study, an emphasis placed on a slightly higher quantity of mono-unsaturated fatty acids (MUFAs) in the digital cushion in comparison to control fat samples. Further to this, the study found that the digital cushions of heifers comprised of a higher proportion of arachidonic acid in comparison to adult cattle, this fatty acid is a key component in various inflammatory pathways (Calder, 2006) that could be associated with the formation of CHLs. This finding leads to the suggestion that the digital cushion is not fully developed by the time an animal enters the milking herd as a primiparous heifer, and in fact animals appear to have a more developed and mature digital cushion in their second to third lactations. Supplementary to this is the work conducted by Gard et al. (2015) in which two-month-old calves were randomly allocated to a treatment group to assess the effect of exercise and terrain on the development of the digital cushion. Upon culling at 6 months old, the right fore and hind hooves were imaged through a magnetic resonance imaging (MRI) scanner to assess the volume and surface area of the digital cushion. It was concluded that animals exercised over an alternative terrain had a higher volume and surface area of digital cushion in comparison to their control group counterparts. This suggests that the development of the digital cushion is adaptive and could have a vital role to play in the prevention of CHLs.

Beneath the distal phalanx exists the subcutis (the digital cushion, which has already been described) alongside the dermis and epidermis. The dermal and epidermal layers are also described as the sole corium. As previously described, the subcutis extends until the distal-most third of the distal phalanx (Hendry et al., 1997). In this most distal section of bone, the dermal layer of the corium attaches directly to the distal phalanx, meaning that no protection from the subcutis is offered in this region

(Hendry et al., 1997; Newsome et al., 2017a). The epidermal layer is comprised of epidermal cells that undergo keratinisation at the cell layer which interfaces with the sole horn (Hendry et al., 1997). This sub-layer is known as the stratum basale, also termed the stratum germinativum or the "germinal epithelium". The germinal epithelium is the site responsible for horn production through cellular proliferation; As the epithelial cells proliferate distally, they undergo keratinisation to form tubular and inter-tubular horn (Hendry et al., 1997). The dermis functions as a highly vascularised tissue to supply metabolites and oxygen via diffusion to the ever-proliferating epidermal layer.Figure 1-4 Figure 1-4 gives a visual representation of the location of anatomic structures described within this section.

1.3.4 Laminitis

It has been previously postulated that dairy cattle suffer from laminitis (Vermunt, 1992; Vermunt and Greenough, 1994; Greenough, 2007) as a result of feeding a carbohydrate rich diet (typically sourced from concentrate feeds). The laminitis hypothesis is one that is well established in the pathogenesis of equine lameness; however, this model has little evidence to prove it is a contributory factor in the aetiology of CHLs in dairy cattle, despite it being one of the most prevalent theories for the aetiology of disease. The laminitis hypothesis stated that dairy cattle being fed a high dose of carbohydrates would suffer from sub-acute ruminal acidosis (SARA) leading to a systemic inflammatory response. This inflammatory response would lead to the activation of metalloproteinases which can have systemic implications for the animal due to their circulation throughout the blood stream. This

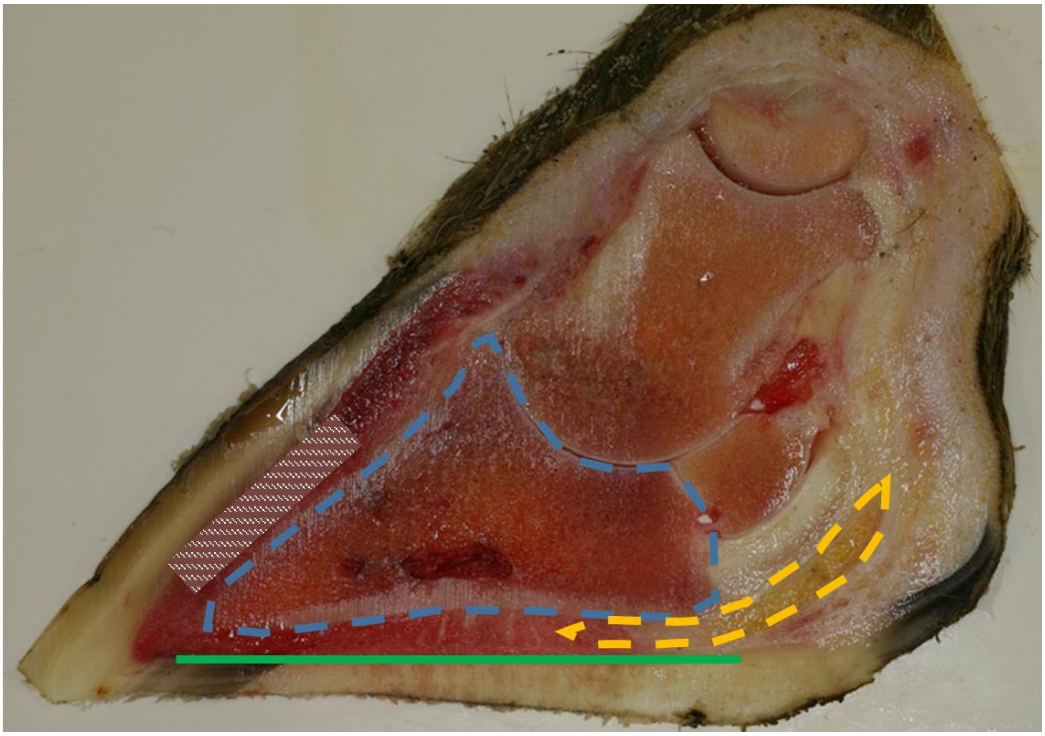


Figure 1-4 A cross section of the claw of a dairy cow. The blue hashed line represents the pedal bone, the white striated region represents the supportive apparatus, the yellow hashed region represents the digital cushion, and the green solid line represents the germinal epithelium. The region between the blue and green regions represents the corium.

hypothesis claims that the suspensory apparatus is particularly vulnerable to these matrix metalloproteinases, resulting in inflammation of the laminae. This inflammation of the laminae led to the displacement of the pedal bone and the compression of the sole corium. This compression causes contusions in the corium, ultimately leading to the incorporation of haemorrhage and disruption of horn production. In the equine model, this leads to the compression and rotation of the pedal bone, whereas the proposed model in dairy cattle is suggested to lead to a predisposition to SH and SU or other conformation challenges in the hoof. Numerous studies have been designed to induce laminitis in dairy cattle to provide evidence towards carbohydrate overload being a contributory factor to the pathogenesis of CHLs (reviewed by Randall et al., 2018b). Several of these attempts have led to acute illness in the study animals (Thoefner et al., 2005; Ding et al., 2020) whilst being unable to demonstrate a clear link between carbohydrate overload and the development of CHLs (Randall et al., 2018b). These studies have commonly concluded that animals experiencing a carbohydrate overload experience discomfort on locomotion, however this could be due to the discomfort associated with ruminal acidosis. A consistent flaw in many of these studies exists in their ability to complete an appropriate method of data analysis, frequently not accounting for important confounding factors or

the hierarchical nature of their data structure (Randall et al., 2018b). Furthermore, many of the studies examining the histological impacts of carbohydrate overload on the suspensory apparatus rely on small numbers of animals, meaning that the study does not have sufficient statistical power to definitively address the hypothesis in question (Randall et al., 2018b). The evidence base for SARA and sub-clinical laminitis is very limited not only in terms of breadth of evidence, but also the power of the limited evidence available. In recent years, the lameness research community has made a decisive shift away from laminitis as a primary pathogenic pathway causing CHLs.

1.3.5 The Calving Effect

Changes do occur to the suspensory apparatus around the time of calving, however. The mechanism of this is driven by the systemic effects of hormones associated with the birthing process. Tarlton et al. (2002) demonstrated that the suspensory apparatus slackens, thereby losing structural integrity, in what appears to be a fibrogenic process. It was hypothesised that this is due to the systemic circulation of the hormone relaxin. Relaxin is linked to the increased laxity of connective tissue required for a calf to pass through the birth canal (Sherwood, 2004). If relaxin has systemic effects, then the suspensory apparatus could be susceptible to its action, meaning that the structure would not be able to suspend the pedal bone within the hoof capsule effectively (Tarlton et al., 2002). These changes due to systemic hormone secretion coincide with a time when the hoof of the dairy cow (especially the freshly calved heifer) are experiencing a large number of environmental challenges (Knott et al., 2007; Randall et al., 2016). Knott et al. (2007) demonstrated how the suspensory apparatus of heifers being housed within stall-based yard with a concrete floor were impaired in their functionality when compared to their counterparts who were housed on loose straw yards. This slackening of the suspensory apparatus reaches its nadir at the onset of lactation, meaning that not only is the suspensory apparatus compromised due to housing conditions, but the pressure it has to support will be greater due to an increased amount of time spent standing (typically on concrete) due to milking (Tarlton et al., 2002; Knott et al., 2007).

1.3.6 The Challenged Digital Cushion

Numerous studies have demonstrated that the digital cushion (Bicalho et al., 2009; Newsome et al., 2017a; b; Griffiths et al., 2020) is compromised by the onset of lactation. It is understood that the primary mechanism of this association is due to the loss of body condition score derived from the high energy demand of milk production in early lactation i.e. the period of time when dietary intake cannot keep up with the growing energy demands of increasing milk production (Bicalho et al., 2009; Green et al., 2014; Newsome et al., 2017a; b). As animals lose BCS, they metabolise adipose tissue to meet the energy demand (Wankhade et al., 2017). This systemic metabolism of adipose tissue also occurs within the hoof capsule, with fatty acids from the digital cushion being mobilised to help meet energy demand (Green et al., 2014; Newsome et al., 2016, 2017a; b). As these fatty acids are mobilised, the thickness of the digital cushion decreases, thereby impairing the structures ability to support the pedal bone and protect the corium from excessive forces (i.e. the thinner digital cushion resulting from BCS loss leads to a predisposition to CHLs) (Newsome et al., 2016, 2017a; b). This hypothesis has been further reinforced by studies demonstrating that BCS loss predisposes lameness (Green et al., 2014; Randall et al., 2015). These longitudinal studies have used repeated measures of animals over time to investigate risk factors for lameness; Green et al. (2014) and Randall et al. (2015) both clearly identified that body condition loss or animals being of a lower body condition both increased an animals risk of becoming lame. Randall et al. (2015) clearly explained animals of a BCS <2 were more likely to develop mild or severe lameness. Green et al. (2014) described how a low BCS can precede lameness by >2-4 months and offered an explanation to the findings of Reader et al. (2011) that animals drop milk yield before they become lame due to the BCS loss associated with both conditions. Further to the assessment of lameness score against BCS, several studies have examined the SST thickness with respect to BCS and stage of lactation. Both Bicalho et al. (2009) and Newsome et al. (2017a; b) demonstrated the relationship between the thickness of the corium and digital cushion (as measured by trans-sole ultrasonography) and the stage of lactation, BCS of the animal and the risk of lameness. Those animals of a lower BCS tended to have thinner SST thickness in comparison to those of a higher BCS, the thickness of the SST also reduced as an animal

progressed through its lactation, reaching its nadir at approximately 120 days in milk (DIM). Further to these findings, it has been described how animals with a thinner digital cushion are at a higher risk of developing future CHLs. Newsome et al. (2017a; b) elaborated on the description provided by Bicalho et al. (2009) through the use of longitudinal measurements of SST thickness throughout an animals lactation. Within those studies animals that developed a SU or severe SH through their lactation originally had thinner SST than their counterparts who did not.

Newsome et al. (2017b) also demonstrated that once a SU of severe SH had developed, the SST tended to be thicker which the authors of that study suggested may be due to an inflammatory response. The impacts of inflammation on the digital cushion are still to be determined. It is possible the fatty acids (most notably the high levels of arachidonic acid described by Räber et al. (2006)) contained within the digital cushion could be utilised in the inflammatory process (Calder, 2006; Contreras et al., 2017a; b), resulting in the metabolism of digital cushion fat to generate mediators to the inflammation pathway. This process of inflammation is also associated with the deposition of scar tissue (Ogawa, 2017); the impacts of which on the structures ability to dampen compressive and concussive forces are unknown. This formation of scarring may be a result of, or additional to, the remodelling of adipose tissue. Upon the mobilisation of fatty acids either due to adipose metabolism or localised inflammation the cellular structure of adipose tissue can undergo “remodelling” resulting in a vastly different cellular structure potentially impacting on its functionality (Contreras et al., 2017 a; b). This systemic remodelling of adipose tissue has been demonstrated to occur during the periparturient period, with systemic adipose tissue inflammation and metabolism occurring in line with this (Contreras et al., 2017a; b). Insulin resistance could also have a key role to play in the metabolism of the digital cushion. Systemic inflammation in adipose and muscle tissues can induce insulin resistance which in turn drives a reduction in BCS (Bradford et al., 2015). This reduction in BCS is the result of systemic adipose metabolism which could include the adipose tissue in the digital cushion. If the digital cushion is metabolised then the individual in question is at a higher risk of developing CHLs, thereby increasing the animal’s lameness score. Further to this, Fabbri et al. (2020) described the differences in appearance between the SST of healthy claws and those with CHLs when

visualised utilising trans-sole ultrasonography. These differences and the repercussions associated with them present an area for future research; the impact of SST thickness on CHL occurrence is now well described, what is less well known is the impacts of CHLs on the structure and function of the digital cushion. It is apparent is that the digital cushion is a structure which is vital in the prevention of CHLs, meaning an opportunity may be present to influence its structure to benefit the future health of the animal's hoof.

1.3.7 The Role of Inflammation

Supplementary to the literature described in Chapter 1.3.2 (which nominates inflammation as a driving mechanism through which CHLs develop) exists a randomised controlled trial (RCT) which further support this hypothesis (Thomas et al., 2015). Within that study, the importance of non-steroidal anti-inflammatory drugs (NSAIDs) with respect to optimising treatment outcomes is described. Animals that received a therapeutic trim, orthopaedic block, and a three-day course of NSAIDs had an improved treatment outcome than their herd mates that received a therapeutic trim and orthopaedic block without NSAIDs. This would suggest that dampening an inflammatory response is important in optimising the treatment outcomes given, lending to the hypothesis that the CHLs have an inflammatory pathogenesis. Contradictory to this, research conducted by Laven et al. (2008) identified no benefit to the routine administration of NSAIDs as part of a lameness treatment protocol. However, challenges existed in both studies; in the work published by Thomas et al. (2015) only the lame limb was lifted and treated (which then acted as a confounding effect for contralateral limb lameness), five technicians were used to treat lame animals, none were reported to have received any specific training in therapeutic hoof trimming other than their specific veterinary qualifications, and indeed, the administering technician had a significant effect on the treatment outcome of that individual case of lameness. The published work conducted by Laven et al. (2008), similar to the work conducted by Thomas et al. (2015) does not control for the lameness history of the animal, or indeed the severity of lesion presented. As we currently understand that lameness begets lameness due to pathological change to distal limb anatomy (see below, Newsome et al., 2016), the lameness history of these animals could be a substantial confounder for any results presented from the study. Further to

this, the work conducted by Laven et al. (2008) relied on farmer identification of lame animals. The producer perception of lameness has already been shown to be poor, with farmers commonly only picking out late stage lameness (Leach et al., 2010; Ranjbar et al., 2020). This would mean that this study (by design) likely lended itself to assessing the use of NSAIDs in the treatment of late-stage lameness. Despite the challenges around the design of these studies, further investigations are warranted before their findings are accepted or dismissed.

The purported inflammation associated with CHLs is a poorly understood area of their aetiology. Newsome et al. (2016) outlined the potential pathogenic mechanisms through which CHLs develop. Central to this was the negative impact that the inflammation associated with trauma to the soft tissues could have a long-term detrimental effect on the functional anatomy of the hoof of the dairy cow. Within that study, new bone formations on the plantar aspect of the distal phalanx (most commonly seen on the caudal plantar area) were identified on animals that had a lifetime history of lameness. Tsuka et al. (2012) identified that new bone growth on the plantar aspect of the distal phalanx was positively correlated with the age of the animal and were more prevalent amongst animals culled with sole ulcers. Newsome et al. (2016) identified that the development of this "exostosis" is positively correlated with a lifetime history of lameness, meaning that animals who spent more of their life as lame tended to have larger exostoses on the plantar aspect of the pedal bone. Both Tsuka et al. (2012) and Newsome et al. (2016) identified that the development of this bone could predispose cattle to future CHL occurrence. This bone formation develops most prominently at the initial point of hoof-strike (the caudal plantar aspect of the distal phalanx) where concussive forces are at their greatest, meaning that corium "pinching point" between the pedal bone and horn could be exacerbated substantially. Newsome et al. (2016) went on to describe how the inflammation associated with the aetiology could rely on the fatty acids located in the digital cushion as mediators for the associated biochemical process. They suggested that this would lead to a thinning of the digital cushion, again predisposing the dairy cow to future CHL occurrence. This hypothesis leads to the suggestion that dairy cows may never truly recover from a CHL, even if the proliferation of new bone formation on the distal phalanx and the utilisation of digital cushion is small, it may still act as a predisposing factor

to the development of future CHLs. What is apparent is that the effective “cure rate” of CHLs is improved substantially with the use of NSAIDs (Thomas et al., 2015). This improved cure rates with the use of NSAID was demonstrated by use of an RCT conducted by Thomas et al. (2015) in which animals received a combination of treatments to evaluate their efficacy with respect to CHLs. This study, in combination with the aetiology outlined by Newsome et al. (2016) suggests that treatment with NSAIDs is not only the way to improve CHL cure rates amongst the dairy herd, but also reduce recurrence rates. The importance of previous lameness events and their contribution towards future lameness events has been described extensively in a study conducted by Randall et al. (2018a) in which the proportion of risk for a current lameness event can largely be attributed to a previous lameness event (more so than BCS loss). Further understanding the long-term impacts of utilising NSAIDs in the treatment and prevention of CHLs may provide a vital insight into the chronicity cycle associated with the CHLs.

A new area of research is emerging investigating the genetic component underpinning the development of the digital cushion. Stambuk et al. (2020) identified specific genes which were associated with digital cushion thickness in Holstein and Jersey cattle. These genes held specific functions pertaining to fat deposition, bone development and keratinocyte function. Furthermore, Oikonomou et al. (2014) estimated the heritability of digital cushion thickness, displaying a moderate heritability index. The aforementioned work leads to the suggestion that certain animals could be genetically predisposed to future CHLs, causing a lifetime of repeat lameness bouts. This predisposition could be caused by animals not having the genetic capacity to during the development period cultivate a fully functional digital cushion. A less functional digital cushion would not be able to appropriately dissipate the concussive forces of hoof strike as effectively, predisposing the animal to CHLs.

Complimentary to the genetic component of the ability of the digital cushion to function properly is the developmental component which occurs in early life. Research has been conducted by Gard et al. (2015) to examine the effect of exercise and terrain on the development of the digital cushion in calves. This involved two-month-old calves being exercised daily over rough terrain for 4 months. At

six months old the animals were culled, and the right fore and hind hooves were collected and passed through a MRI scanner to ascertain digital cushion volume. Calves in the exercise group experienced a 37% increase in digital volume compared to the control calves. This study further highlights the unknown impact of the rearing period on the development of an optimal digital cushion structure. Further research is required to determine if the differences observed by Gard et al. (2015) correspond firstly to differences in the structure and composition of the digital cushion in an animals adult life, and what the observed increase in volume and surface area means for an animals future risk of lameness (although it is likely to have a positive effect on diminishing the risk of CHL development).

The published literature surrounding the genetic and developmental components of the digital cushions structure when read in combination could suggest that certain individuals are predisposed to lameness when entering the milking herd. If there is no genetic selection for traits that improve the structure of the digital cushion, and animals do not undergo any form of "pre-conditioning" before they enter the milking herd (as described in Gard et al., 2004), then the digital cushion of the animal may not develop sufficiently, meaning that the forces associated with hoof-strike are not dissipated and CHLs are experienced as a result of this.

1.4 Claw Horn Lesion Management

The optimisation of treatment regimens for CHLs has been slow when compared to other endemic diseases affecting the dairy cow such as mastitis and reproductive conditions (Randall et al., 2018b). This could in part be due to a lack of understanding around the breadth of the challenge faced by the global dairy industry (Cutler et al., 2017). Additionally, this could also be due to the lack of pharmaceutical sales associated with the treatments required for CHLs, meaning that funding required for trial work to test the efficacy of various treatments isn't available as no sales of products result from the trials. Even when comparisons are made between digital dermatitis treatment/prevention in comparison to CHL treatment/prevention, the difference in volume of literature available is substantial. At present there is a basic understanding of the optimal treatment regimens for the CHLs developed by a small number of intervention studies (Laven et al., 2008; Thomas et al., 2015, 2016; Stilwell et al., 2019; Thomsen et al., 2019). These papers are primarily investigating the use of

NSAIDs, therapeutic blocks and hoof trimming (Laven et al., 2008; Thomas et al., 2015, 2016), with a few others including the use of anaesthetic (Stilwell et al., 2019) and different housing conditions (Thomsen et al., 2019). All of the aforementioned papers look purely at recovery rates, no longitudinal data is utilised when examining the effects of CHL treatment. Additionally, the literature providing the evidence base for hoof trimming (whether it be therapeutic or preventative) does not provide a strong description of optimal preventative and curative strategies (Bell, 2015). Further research investigating long term treatment outcomes will be vital for the future improvement of animal welfare standards and help to further the efficiency of production systems globally. Below the evidence for the current principles of lameness detection and treatment are discussed and reviewed.

1.4.1 Farm Level Risk Factors

Several cross-sectional studies have been conducted worldwide to examine farm level risk factors for a higher CHL prevalence in the dairy herd. The main focus of this thesis is around the pathogenesis of CHLs and so a brief description of the farm level risk factors associated with their onset are described. The reader is encouraged to refer to the cited literature for a more comprehensive understanding of CHL risk factors. In the grazing herd the pertinent risk factors included long walking distance to and from the parlour, poor track maintenance, low space per animal in the collecting yard and feed face, and wet weather conditions to which the animals are exposed (Burow et al., 2014; Hund et al., 2019; O'Connor et al., 2020a). Within the housed environment, particular risk factors to note include abrasive/uneven walking surface and low-lying times (Dippel et al., 2009; Barker et al., 2010). Across both grazing and housed systems, the maintenance of an appropriate BCS (Green et al., 2014) and the routine maintenance of hoof shape (Griffiths et al., 2018) and the rapid treatment of lameness (Barker et al., 2010) also appear as significant contributory factors in the management of lameness.

1.4.2 Lameness Detection

The assessment of gait is typically utilised to identify pain on locomotion (i.e. lameness), with a range of methods and descriptions being used to characterise this (Thomsen et al., 2008; Afonso et al., 2020). Within the UK, this most commonly takes place as cows are exiting the milking parlour across a solid, level surface (AHDB Dairy, 2020). From this assessment, most scoring systems will attribute

a numerical value to semi-quantify the impact of the pain experienced upon locomotion (Afonso et al., 2020). Different scoring systems utilise varying methods of gait assessment and as a result, comparisons between them can prove challenging. Least of all because different values across scoring systems may not have a comparable counterpart in another. In the United Kingdom, the four-point AHDB lameness scoring system is the most commonly utilised scoring system in a commercial setting (AHDB Dairy, 2020).

The subjective nature of lameness scoring provides a challenge for comparing scientific literature and on-farm estimates of lameness (Thomsen et al., 2008; Cutler et al., 2017; Afonso et al., 2020). The use of different observers between studies means that the perceived pain threshold required for an animal to be considered as lame will be different relative to who is lameness scoring (Cutler et al., 2017; Afonso et al., 2020). Afonso et al. (2020) examined the range of lameness scoring methods in published research and the respective incidence rates reported. In that review, the authors described how the use of different methods of lameness detection (and the subjectivity associated with them) makes cross-examination of studies difficult, particularly with reference to attributing costs to a condition or disease. The two most commonly utilised (in the UK) scales described by Manson and Leaver (1988) and AHDB Dairy (2020) are described in

Table 1-1 and Table 1-2 respectively (Afonso et al., 2020).

Manson and Leaver (1988) Score	Description
1.0	Minimal abduction/adduction, no unevenness of gait, no tenderness.
1.5	Slight abduction/adduction, no unevenness or tenderness.
2.0	Abduction/adduction present, uneven gait, perhaps tender.
2.5	Abduction/adduction present, uneven gait, tenderness of hooves.
3.0	Slight lameness, not affecting behaviour.
3.5	Obvious lameness, some difficulty in turning, not affecting behaviour pattern.
4.0	Obvious lameness, difficulty in turning, behaviour pattern affected.
4.5	Some difficulty in rising, difficulty in walking, behaviour pattern affected.
5.0	Extreme difficulty in rising, difficulty in walking, adverse effects on behaviour pattern.

Table 1-1 The lameness scoring system described by Manson and Leaver (1988)

AHDB Dairy (2020) Score	Description
0	Walks with even weight-bearing and rhythm on all four hooves, with a flat back. Long, fluid strides possible.
1	Steps uneven (rhythm or weight-bearing) or strides shortened; affected limb or limbs not immediately identifiable.
2	Uneven weight-bearing on a limb that is immediately identifiable and/or obviously shortened strides (usually with an arch to the centre of the back).
3	Unable to walk as fast as a brisk human pace (cannot keep up with the healthy herd). Lame leg easy to identify – limping; may barely stand on lame leg(s); back arched when standing and walking. Very lame.

Table 1-2 The lameness scoring system described by AHDB Dairy (2020)

At present, there is a vast range in the frequency of lameness scoring being conducted annually on farms within Great Britain, not all milk contracts audit lameness as part of their on-farm welfare assessments. This irregularity of scoring presents a challenge to making progress to reduce the current lameness prevalence in the UK. The importance of regular lameness scoring is that it allows for the early detection of lame animals to allow for rapid treatment at the initial onset of disease. Groenevelt et al. (2014) described how animals that received treatment immediately after being scored as lame had a much better outcome than those that received a delayed treatment (which relied on identification outside of lameness scoring). The authors highlighted the importance of fortnightly lameness scoring and treatment in the reduction of lameness on a herd and individual animal level. Supplementary to this, a randomised controlled trial conducted by Thomas et al. (2016) found that irrespective of the treatment used, the outcome for chronically lame animals (or indeed animals with a delayed treatment) was poor. The authors highlighted that any delay in treatment would reduce the likelihood of a lameness cure.

Eriksson et al. (2020) identified that to appropriately identify a case of lameness, animals should be lameness scored on a weekly basis and explained that this would allow for the appropriate classification of mildly lame animals. In that study the authors suggested that mildly lame animals would need to be scored as such twice consecutively for them to be considered as lame. This would ensure that so called “over-scoring” does not occur i.e., sound animals are not being mis-classified.

However, given the previous studies describing the importance of early intervention in a case of lameness, the approach described by Eriksson et al. (2020) may not be appropriate. The aforementioned literature highlights the importance of regular, frequent lameness scoring and rapid treatment following the identification of lameness which is the key underpinning of the early detection and prompt effective treatment (EDPET) principle.

In recent years, there has been a movement to automate the lameness score process using various means. The automation of lameness scoring could change the speed at which animals are treated, allowing for more positive treatment outcomes as a result. These automated methods have included the use of accelerometer and gyroscopic data (Ito et al., 2010), thermography (Alsaaod and Büscher, 2012), video recognition software (Hansen et al., 2018) and force/pressure plate technology (Mokaram Ghotoorlar et al., 2012). The flaw behind these systems exists in their reliance on lameness score data to develop the algorithms required to automatically assign a cow a lameness category, meaning that a reliance on human perception still exists. There is no objective determination of lameness on which they are founded. The strength of these systems, however, is that they may offer a consistent means of reporting lameness amongst a dairy herd (Bell and Walker, 2016). This would help to remove the challenging subjective nature of lameness scoring within a herd which is pertinent when multiple technicians are available nationwide and globally to provide this service. A strong level of consistency may allow for more accurate benchmarking of lameness score data amongst farms nationwide and globally. The other exciting opportunity behind these methods of scoring are their ability to develop daily reports in which animals may be selected for treatment at the earliest point of lameness detection (Bell and Walker, 2016). This is important for two reasons; 1) it allows for the treatment of disease and the alleviation of pain at its earliest onset, 2) the improved treatment outcomes with respect to CHLs and digital dermatitis due to early intervention (Groenevelt et al., 2014; Bell and Walker, 2016; Thomas et al., 2016) . Barriers are present which prevent farmers accessing these systems, primarily a lack of understanding around the importance of lameness appears to be a significant contributory factor towards farmer decision making around lameness detection

alongside understanding the cost of the system and the cost of lameness (Bennett et al., 2014; Bell and Walker, 2016; Van De Gucht et al., 2017).

1.4.3 Hoof Trimming

Hoof trimming serves two purposes; 1) The routine maintenance of hoof shape to prevent lameness and 2) the investigation and treatment of lameness (Toussaint Raven, 1971; Stoddard and Cramer, 2017). This maintenance of hoof shape and treatment of lameness most commonly follows the implementation of the "Dutch five-step method" (as described in Toussaint Raven (1971)). The traditional Dutch five-step method is described in two stages; steps one to three describe routine maintenance of hoof shape and steps four and five cover the treatment of CHLs. To carry out the Dutch five-step method, the animal must be securely and properly restrained, with the leg held in a secure accessible position to view the sole of the hoof. Step one involves the trimming of toe length to 7.5cms on the "template claw" and the trimming of sole horn to achieve a 5mm step at the toe, this is the medial claws on the hind hooves and the lateral claws on the front hooves. The template claw is where the toe length and sole depth are established to match the non-template claw for the purposes of balance, which takes place in step two. Step two involves the removal of toe length and sole depth to balance the non-template claw with the template claw. Step three describes the removal of horn at the typical sole ulcer site; this involves using hoof knives to dish out the area described as the typical ulcer site (zone four, 1-1). Typically, the template claw receives a smaller model than the non-template claw. If a lesion or painful claw has been identified in steps one through three, then the operator should move on to complete steps four and five, if this is not the case then the hoof trimming process is complete for that animal. Step four describes the alleviation of pressure from the diseased area of the claw and is typically achieved by the application of an orthopaedic hoof block to the other claw on that hoof if possible (i.e., if no disease is present on that claw). Orthopaedic hoof blocks come in a wide variety of shapes, sizes and designs and can be made from a variety of materials (typically plastic, rubber or wooden) and are adhered to the hoof using a bonding agent. Step four can also include the removal of horn from the affected area to create a height difference between the two claws to ensure that the diseased region of the claw does not bear large portions of weight. Step five

involves the removal of all loose and diseased horn from the affected claw, within this process all horn that has separated or become ridged as part of the development of the CHL should be removed to prevent the later exacerbation of the condition.

Supplementary to the traditional Dutch five step method, two adaptations have been offered to update the current method of trimming to better suit the modern animal. The first of these is being more conservative with how much horn is removed from the toe length. In a study conducted by Archer et al. (2015), it was recommended that a toe length of 90mm (when trimming toe length to a point) should be used for animals over four years of age and 85mm for animals in their first four years of life. In that study the authors highlighted that trimming to the traditional 75mm would leave approximately 50% of animals trimmed short and as a result would have been in immediate pain or gone on to develop CHLs as a consequence of inappropriate hoof angle and poor sole depth. Secondly, Stoddard (2018) identified that the utilisation of a wider modelling technique (used in step three) reduces the presence of CHLs in the next lactation when applied to heifer dry off trims. This technique involves the removal of hoof horn at the typical ulcer site in a much deeper and wider fashion (whilst avoiding cutting into the corium) leaving an 18mm gap between the dorsal wall and the outer most edge of the model itself. Within that study, the effects were only observed in animals enrolled in their first lactation, i.e., animals of parity two or greater at enrolment did not receive any benefits from the application of a wider model. This could be due to the pathological changes associated with a history of lameness as described by Newsome et al. (2016) resulting in a predisposition to lameness in future lactations as observed by Randall et al. (2018a). If bony proliferations occur on the pedal bone due to previous lameness, then the animal is already predisposed to future lameness meaning that any intervention to reduce CHLs administered could effectively be masked by this predisposition.

Conflicting evidence exists as to the efficacy of the Dutch five-step method in its ability to prevent future lameness. Several studies have been conducted to investigate the potential benefits of preventative hoof trimming on lameness occurrence with conflicting results. Manske et al. (2002), Fjeldaas et al. (2006) and Hernandez et al. (2007) all reported a positive effect of hoof trimming on

preventing future bouts of lameness (although the study published by Fjeeldas et al. (2006) only reported this in tie stall barns). Conversely, Van Hertem et al. (2014) found that following routine hoof trimming an increase in lameness score was observed. Daros et al. (2019) found that multiparous animals that received a dry off trim were at a higher risk of becoming lame in their second lactation than those in their first lactation, with the authors suggesting that a routine preventative trim at dry off may not be appropriate for all dairy cows. In a randomised controlled trial described by Thomas et al. (2015), the operator treating the animal for lameness was a significant variable, with respect to the treatment outcome (i.e. some operatives had better treatment outcomes than others), suggesting that between operators, the ability and skill required to trim a cows hoof will vary. It is becoming widely accepted within the industry that operators who are trimming cows hooves need to be appropriately trained and audited, with an understanding that over trimming can cause poor balance within the hoof and leave an insufficient sole depth at the toe, predisposing the cow to lesions (Kofler, 2017). When the research around hoof trimming technique is examined, there is very little robust evidence available and a large amount of opinion published (Bell, 2015). Although opinion has a role in driving research, it is not what leads to advancements in understanding of disease pathogenesis and management and so caution is advised when alternative methods of hoof trimming are implemented as the evidence base behind any method is currently weak. However, all of the studies reviewed in this thesis have described the Dutch five-step method as their primary method of trimming. This method is understood amongst the research literature to be current “best practice” despite a lack of updates to the method in recent years. This is an area where a large amount of progress needs to be made, not only in terms of understanding what best practice hoof trimming is, but also how best to train individuals to implement this on farm.

1.4.4 Early Detection and Prompt Effective Treatment

The early detection and prompt effective treatment (EDPET) principle in its most simple construct is described as the early detection of a disease, followed by the prompt and effective treatment of the condition. In the case of lameness, this takes place through regular lameness scoring to allow for the identification of newly lame animals whilst the condition causing the lameness is in its primacy. The

prompt effective treatment takes the format described by Thomas et al. (2015) whereby a therapeutic trim, orthopaedic block and a three day course of NSAID are administered to the lame individual. Both Groenevelt et al. (2014) and Thomas et al. (2015, 2016) have provided a strong evidence base supporting the importance of the EDPET principle being implemented with respect to lameness.

Despite the current strong evidence base underpinning the EDPET principle, its implementation on farm with respect to lameness has been poor at best. Numerous barriers exist in the implementation of lameness control programmes on farm, most of which are driven by farmer perception of lameness. Firstly, it has been clearly demonstrated that farmers are likely to underestimate the prevalence of lameness on their unit (Leach et al., 2010; Ranjbar et al., 2020). Another barrier to investment in lameness control programmes is the lack of understanding around the true cost of the condition. As described previously lameness is a condition associated with hidden losses rather than direct losses or costs (such as the disposal of milk in the case of clinical mastitis). When hidden losses are incurred it becomes difficult to quantify accurately the cost of a condition on an individual unit, meaning that making an investment of either time or capital into a control programme is difficult to justify financially and assessing the return on investment impractical (Leach et al., 2010). However, a growing evidence base is present to suggest that not only is lameness control important for the welfare of the cattle and the financial viability of the unit, but also for the prevention of chronic lameness in the herd, the costs of which will likely far outweigh the cost of a single case of lameness.

Newsome et al. (2016) began to explain the potential importance of implementing the EDPET principle in combination with NSAIDs with respect to lameness aetiology; they described how the development of new bone on the most caudal aspect of the pedal bone is linked to animals having a history of lameness. Within that study it was proposed that the inflammatory aetiology associated with CHLs is responsible for this pathological change. From this it was hypothesised that animals would respond best to treatment when the EDPET principle is implemented in combination with the use of NSAIDs. It was hypothesised that this would disrupt the inflammatory aetiology associated with CHLs, meaning that the development of new bone on the caudal plantar aspect of the pedal bone may be minimised if not prevented. This could result in animals that do not have a CHL predisposition due

to this contributory factor. It could be taken that this breaks the chronicity cycle associated with CHLs and leads to the prevention of chronic lameness or chronic CHLs, in turn reducing the repeat cases of lameness across an animal's lifetime. This hypothesis, however, is yet to be tested using a robust RCT using longitudinal measures of lameness scores.

1.5 Aims and Conclusions

This review has evaluated the current literature and highlighted key knowledge gaps in the understanding of the chronicity cycle associated with CHLs and most importantly, the lack of evidence as to how this cycle can be prevented or minimised. The current understanding of the pathogenesis of CHLs has recently expanded to understand the role of inflammation and the detrimental effects that this can have on the pedal bone and the potential impacts it may have on the digital cushion. Previous literature highlights the importance of NSAIDs in the treatment of lameness when an animal is treated without delay. Any form of delay or a lack of NSAID in the treatment protocol reduces the chance of a positive treatment outcome. Key studies involved in the recent advancements in understanding examine the failing of the suspensory apparatus (associated with calving, not SARA) and the failing of the digital cushion (and its associations with BCS and proximity to calving). These have shown that the suspensory apparatus slackens in the periparturient period, and that digital cushion thickness is compromised in the time between the onset of lactation and peak daily yield being reached. It has been hypothesised that this change is due to adipose metabolism and inflammation. This inflammation may have negative impacts on the digital cushion's ability to successfully dissipate the concussive forces associated with locomotion and the compressive forces exerted by standing. A substantial portion of the recently published research, when taken in combination, challenges the hypothesis that sub-clinical laminitis is a primary contributory factor in the pathogenic pathway responsible for the onset of CHLs. The furthering of our understanding of the pathogenic mechanisms responsible for CHLs is vital for the future success of the dairy industry, with a high demand for efficient, high welfare production systems. If the UK (and worldwide) dairy industry is unable to break the chronicity cycle associated with CHLs, then it is unlikely that the future expansion and intensification of the industry will remain ethical (as large numbers of animals

will be experiencing pain due to lameness). With this respect the development of optimum treatment regimens and prevention methods must be rapidly adopted.

The aims of this thesis were to (1) investigate the impact of a lifetime history of CHLs and lameness on the structure of the digital cushion (2) develop our understanding of the long-term use of NSAIDs in the treatment and prevention of lameness. The first aim was addressed using MRI facilities to quantify the volume of digital cushion in the hindlimbs of dairy cattle at cull (as described in Chapter 3) and using histopathological analyses to understand the nature of any differences observed (as described in Chapter 4). The second aim was addressed through the implementation of a 34-month RCT investigating the long-term outcomes of treatment regimens using NSAIDs at different life events when applied across a single commercial herd (as described Chapters 5 and 6).

Understanding the impacts of CHLs on the digital cushion and the role of NSAIDs in the long-term treatment and management of these conditions is vital to help improve cow welfare globally. Alongside improving the current welfare standards of dairy systems globally, the data and resources generated from this thesis will hopefully allow future hypotheses to be designed, tested, and accepted or rejected to allow for the further refinement of lameness management on farm.

1.6 COVID – 19 Impact Statement

The work presented throughout this thesis has been challenged by the COVID-19 global pandemic in numerous ways. At all times, the team administering the research have prioritised our health and safety whilst minimising the effects of the pandemic on this body of research. It must be noted that the supervisory team involved in this project have been supportive and encouraging of healthy working habits in a time when pressures were high. The composition of this thesis document has been challenged by the pandemic through a shift away from meeting in person and a reliance on personal IT equipment. Below it is noted where the pandemic has impacted on different aspects of the studies described in this thesis.

1.6.1 Chapter 3

This body of work was in the writing stage when the national lockdown was instigated in the UK. It has made organising discussions surrounding the writing and review process challenging and has led to an increased time editing this section of work than would have normally been experienced. This is purely due to the inability to meet face-to-face and an over-reliance on technology.

1.6.2 Chapter 4

The work presented in this chapter is no doubt the section of this PhD which has been most heavily impacted by the pandemic. As our dissection work was complete (for the purpose of sample collection) and the sectioning and staining began, the UK entered a state of national lockdown (March 2020). This led to a four-month delay to re-entry to the lab. When re-entry was permitted it was with a limited capacity; access to facilities was permitted on specific days at dictated times, our working environment had changed substantially, and a limited number of our team was permitted entry to the lab for the purposes of social distancing. This has meant that a substantial body of work presented within this thesis was delayed by four months, adding time pressures to the end of this process. Of the sectioning, staining, microscopy and image analysis, ~80% of this was completed in the final six months of this PhD programme. The adipocyte size data which contained within Chapter 4 was not completed as a result, and so preliminary descriptive analysis is presented.

1.6.3 Chapters 5 and 6

The RCT presented in Chapters 5 and 6 was also hindered in its administration through the course of the pandemic. Three primary technicians administered the treatments for the RCT outside of the pandemic, two treating lameness and one recording treatment data. At the onset of the lockdown, it was decided that one of the operators would not travel to the farm for the duration to ensure that the RCT had one technician to administer some study administration if the other two were required to self-isolate. During this time, all data recording was completed by the two operators administering treatment. To minimise the risk of operators having to self-isolate concurrently, the two technicians administering treatment stopped working in tandem and treated animals on a “shift” basis, with one operator treating animals in the morning and the other in the afternoon using separate equipment to

minimise the risk of transmission. One of the operators administering the study was in isolation for a period. The purchasing of consumables for the trial administration also presented frequent challenges with suppliers facing issues in sourcing certain products required.

2 General Methods

2.1 Introduction

All methods described within the following chapter have been used extensively throughout the research required to complete this thesis. Where methodologies have differed in the following chapters, any variations are described thoroughly with relevant references attached if necessary.

2.2 Chapters 3 and 4

Two studies were conducted utilising cadaver material sourced from SRUC's Crichton Royal Farm. These two studies are presented in Chapters 3 and 4 which describe the use of MRI scanning to assess the impacts of lameness in the animal's life on digital cushion volume, and the association between lameness in the animal's life and the content of collagen contained within the digital cushion. Below, the farm management and data collection pertinent to the studies conducted are described.

2.2.1 Farm Management

Animals were housed at one of two facilities (Langhill or Acrehead) where milking took place three times a day. The Langhill herd is one of the most intensively monitored and studied research herds in the UK. Data collection pertinent to the current study is reported below, please see Pryce et al. (1999), Chagunda et al. (2009) and Randall et al. (2015) for a more fulsome description of herd management. Whilst part of the same enterprise, the Acrehead herd is less intensively monitored and has more of a commercial focus.

2.2.2 Langhill

The 200 cow Langhill herd was continuously housed year-round with access to rubber matted stalls. The flooring between the stalls and the feed face was grooved concrete. The herd was run on a long term 2X2 factorial trial where animals of either a control (C) or select (S) genetic line (Pryce et al., 1999) were managed on one of two feed systems. These nutritional systems included high forage:low forage (Pryce et al., 1999), homegrown:by-product (Chagunda et al., 2009), or a high energy:low energy system (Smith et al., 2019) during the study period. Target milk yields were between 8,500

and 12,000Kg per cow per year dependent upon the management system to which the animal belonged.

Up until April 2017, animals were lameness scored weekly by trained and experienced technicians utilising a five-point scale (Manson and Leaver, 1988). After this date animals were scored on a fortnightly basis. Animals presenting as a single score four or five (described as “obvious lameness affecting behaviour” or “severe lameness with extreme difficulty walking” respectively) or twice consecutively as a score three (described as “lameness that does not affect behaviour”) were considered lame and received treatment by a veterinarian or a trained member of farm staff as described by Randall et al. (2015). The herd was attended bi-annually by a professional hoof trimmer to maintain hooves identified as overgrown by farm staff. Animals were run through a hoof bath containing 5% copper sulphate at each midday milking during weekdays (Monday-Friday) to control digital dermatitis. BCS was captured weekly, adopting the scoring system described by Mulvany (1977) using a 5-point scale with increments of 0.25.

2.2.3 Acrehead

Unless removed for management purposes or to maintain herd size, animals remained at Langhill for 4 lactations before being moved to the Acrehead herd. At the Acrehead site, the flooring between the stalls was slatted concrete and the feed face floor was grooved concrete. Data collection at Acrehead followed different protocols; within this herd animals were lameness scored every 4-6 weeks and BCS was collected on an irregular basis. Only animals culled from the Langhill herd had a comprehensive lameness and BCS data set for the time preceding slaughter.

2.3 Chapters 5 and 6

For the studies in Chapters 5 and 6 to be conducted, a single commercial farm was recruited. On this unit, all heifers that were calving into the herd, and the adult cattle that had calved at least once were recruited. The studies described in Chapters 5 and 6 were run concurrently, that is to say that the heifers and adult cattle (described in Chapters 5 and 6 respectively) were lameness scored and treated as one unit. For the purposes of statistical analysis, the two cohorts were then separated and described

individually. The description of the datasets collated from these groups of cattle are described in Chapters 5 and 6, respectively. Farm management, study administration and data collection are described below.

2.3.1 Farm Management

The study population utilised in Chapters 5 and 6 comprised a cohort of Holstein cattle within a commercial dairy herd. The herd consisted of 490 milking cows and was located in North Nottinghamshire, England. The farm was selected based on locality to the University of Nottingham and willingness of the farm owners to conduct the study. Once enrolled, animals were monitored throughout the study period which was completed between 8th January 2018 and 23rd October 2020.

All cows were continuously housed with access to stalls containing a rubber matted surface and bedded with a blend of sawdust and lime. Stalls were bedded twice daily. The flooring throughout the animal environment was grooved concrete which was cleaned by mechanical scraping with a tractor twice daily. Lactating cows were housed in one of three barns depending on stage of lactation; early lactation, mid lactation and late lactation yards were stocked at 90, 95 and 105 respectively, in terms of cows per 100 stalls. All cows were milked three times a day through a rotary parlour and the mean 305-day milk yield was ~11,600kg.

All cows received a routine maintenance hoof trim at each drying-off according to the protocol described by Toussaint Raven (1971) with the modifications described by Archer et al. (2015) and Stoddard (2018) in which toe length is preserved and a wider model is taken respectively. Lactating cows were walked through a hoof bath containing 2% formalin at each milking to control digital dermatitis (Jacobs et al., 2019).

2.3.2 Lameness Scoring

To identify lame animals throughout the study (and therefore those eligible for treatment) all lactating animals were lameness scored by one of two trained technicians on a fortnightly basis (±48hours) with two other technicians blinded to treatment group scoring fortnightly to generate a blinded outcome lameness score. The lameness scoring system described by Thomas et al. (2015), outlined in Table 2-1, was utilised. Lameness scoring was conducted by trained technicians who were subjected to annual audits to assess agreeability (as

determined by Cohen's Kappa and Gwet's AC1) and re-training was conducted if needed. Lameness scores of 2 and 3 were divided into 2a, 2b, and 3a, 3b respectively to reflect the range of mobility described within each category of the conventional AHDB scoring system described in

Table 1-1. This system was adopted to enable scorers to identify and differentiate between uneven steps and asymmetry which would identify an animal as lame easily and accurately.

Score	Description
0	Walks with even weight bearing and rhythm on all 4 hooves, with a flat back. Long fluid strides possible.
1	Steps uneven (rhythm or weight bearing or strides shortened, affected limb or limbs not immediately identifiable).
2a	Mild asymmetry in hind-limb movement. Decreased stride length on affected limb and slightly decreased stance duration with a corresponding increase in limb flight velocity on the nonaffected side. Walking velocity remains normal. Back may be raised.
2b	Moderate asymmetry in hind-limb movement. Decreased stride length on affected limb and a distinct decrease in stance duration. Limb flight on the nonaffected limb is correspondingly faster and the overall walking velocity is reduced. Back usually raised.
3a	Severe asymmetry in hind-limb movement. Marked decrease in stride length on affected limb and very short stance duration. Limb flight on nonaffected limb rapid and walking velocity reduced such that cow cannot keep up with healthy herd. Back raised.
3b	Minimal or non-weight bearing on affected limb. Back raised. Reluctant to walk without encouragement.

Table 2-1 A description of the adapted AHDB lameness scoring system first described by Thomas et al. (2015) and utilised in a thirty-four-month long randomised controlled trial investigating the efficacy of non-steroidal anti-inflammatory drugs in the treatment and prevention of lameness and the subsequent risk of culling.

2.3.3 Lameness Treatment

Lameness scoring for the identification of animals for treatment, was conducted by one of two trained technicians who were assessed in annual audits to evaluate level of agreement (as determined by Cohen's Kappa and Gwet's AC1) and re-training was conducted if needed. To identify lame animals throughout the study (and therefore those eligible for treatment) all lactating animals were lameness scored on a fortnightly basis (±48hours). The lameness scoring system described by Thomas et al. (2015) was used, as outlined in Table 2-1. Animals identified as lame (Scores 2 or 3) were treated within 72 hours by one of three trained operators. Three trained operators undertook all lameness treatments; hoof trimming protocols and operators were reviewed on an annual basis by a specialist external hoof care auditor (NJB) to ensure consistency and best practice in trimming technique was maintained. Animals were trimmed using an approach based on the Dutch Five-Step Method (Toussaint Raven, 1971) with the adaptations described by Archer et al. (2015) and Stoddard (2018). Both the lame limb and the contralateral limb were examined in all animals. A painful claw was identified by lesion presence or the use of hoof testers. Where their application was considered necessary as part of the treatment protocol (identified as appropriate by the presence of one healthy and one diseased claw within a hoof, i.e. diseased claws weren't blocked) , orthopaedic blocks were applied using a two-part adhesive (Mini Moo Gloo, Integra Adhesives Inc, British Columbia, Canada) following the cleaning and drying of the healthy contralateral claw. Blocks were applied such that the diseased region of the claw was lifted from the ground by a minimum of 2cm. Blocks were not applied to diseased claws. Animals suffering from severe lesions were referred for veterinary intervention following initial treatment which would involve a preliminary trim and application of an orthopaedic block if required. Animals requiring veterinary intervention would receive treatment under local anaesthetic with recordings being made in line with the study protocol. If an animal were in Treatment Groups 2, 3 or 4, the first lameness treatment dose of NSAID was administered in the hoof trimming crush at the end of the assessment and treatment protocol. Two Velcro leg bands were applied to the left hind legs of animals denoting a requirement for further NSAID administration. Different coloured leg bands were used to indicate the days of future treatment e.g., blue indicated a

future treatment was required on Tuesday. Once animals had been milked in the afternoon of the two following days, subsequent doses of NSAID were administered and a leg band removed to denote the animal had been treated that day. The animal's ID was cross checked against a list of individuals requiring treatment for each day to ensure all animals received their allocated dose of NSAID at the correct timings.

Following lameness treatment, animals entered a treatment refractory period of 28 days to allow time for recovery, beginning on the day of lameness diagnosis. Animals could only be re-treated within this refractory period if they were deemed to be severely lame (score ≥ 3a) by farm staff or study technicians. This additional treatment would entail a therapeutic trim and application of an orthopaedic block to the sound claw.

Lame animals identified outside of the fortnightly lameness scoring that were not in the refractory period had treatments determined by the protocol for the group to which they belonged. That is, animals *always* received the same treatment protocol throughout the study, regardless of how or when they were identified for treatment. All hoof blocks were removed 24 days (±48 hours) after application if they were still present. Any blocks that fell off before this point were not re-applied unless required for an interim lameness treatment as described above.

2.3.4 Lameness Outcome Scoring

To ensure no bias arose from knowledge of treatments, all study animals were scored for lameness as an outcome measure on a fortnightly basis throughout the study, by two trained, independent lameness scorers (i.e., the herd was scored twice fortnightly by different observers, once to identify animals for treatment and once as an outcome measure for the study). These outcome lameness scores were used in the statistical analyses to evaluate differences between treatment group. The operators conducting the outcome assessment were not associated with any other aspects of the study, were blinded to treatment group protocols and allocation, and conducted the herd lameness score independent to any other aspects of study administration. Cows with orthopaedic blocks were lameness scored during the time the block was in place and it was noted that the block was present, however, this score was not used in the final dataset. The outcome lameness scorers were subjected to

annual audits to evaluate level of agreement (as determined by Cohen's Kappa and Gwet's AC1) and re-training was conducted if needed.

2.3.5 Lameness Scoring Training and Auditing

The four lameness scorers underwent training before study initiation. This training initially relied on the use of online resources for the scorers to familiarise themselves with the scoring system being used. Following this, 5 practical sessions took place, wherein a minimum of 40 cows would be scored and discussed to ensure continuity of lameness diagnosis. All training sessions pre-study initiation were delivered by JW. At each session, time was allowed not only for the scoring of cows, but also to facilitate discussion about the location to score in and good cattle handling technique. Before study onset, a Cohen's Kappa and Gwet's AC30 value was calculated to assess the level of intra-observer agreeability between the four lameness scorers. This was deemed to be acceptable if it was above 0.6 for both metrics, this was the case for the pre-study training.

Assessment of agreeability and retraining took the following format. Annually, an external hoof care auditor (Dr Nick Bell) assessed the lameness scoring methods used. This was completed using "classroom" style discussion and practical scoring sessions. During these audits, all scorers would score the same cattle to assess the intra-observer agreeability level using Cohen's Kappa and Gwet's AC30 as above. Re-calibration was required once due to a lower agreeability level from one scorer (who remained anonymous). This took the format of lameness scoring and discussion on farm, followed by a re-assessment using the statistical metrics stated previously for all scorers.

2.3.6 Hoof Trimming Auditing

The three technicians (JW, DB, NB) delivering the lameness treatments had already received training from established providers which are recognised as "the current standard" in the UK dairy industry. All hoof trimming training taught the Dutch Five-Step Method. Implementation of this on farm took the format of the Dutch Five Step Method, however two adaptations were implemented to follow the evidence base regarding trimming technique. These adaptations were described by Archer et al. (2015) and Stoddard (2018). Briefly, to achieve a suitable hoof angle and avoid the over-trimming of

cows, Archer et al. (2015) recommended a toe length of 85-90mm would be most appropriate for adult cattle. Furthermore, Stoddard (2018) recommend the use of a deeper-wider model on the lateral claws of the hind hooves.

3 A History of Lameness and Low Body Condition Score is Associated with a Reduced Digital Cushion Volume, Measured by Magnetic Resonance Imaging, in Dairy Cattle.

3.1 Introduction

The CHLs, principally sole haemorrhage, sole ulcer and white line disease, are highly prevalent amongst the global dairy herd (Leach et al., 2012; Solano et al., 2016). CHLs are associated with a failing of the functional anatomy of the hoof, primarily the suspensory apparatus (Lischer et al., 2002; Tarlton et al., 2002) and the digital cushion (Bicalho et al., 2009). The hoof of the dairy cow carries and dissipates the concussive forces associated with locomotion through the suspensory apparatus (which suspends the pedal bone within the hoof capsule from the dorsal wall) (Lischer et al., 2002) and the digital cushion which is situated beneath the pedal bone (Lischer et al., 2002; Räber et al., 2004). It has been suggested that when the functionality of these structures is impaired, extra concussive force is exerted on the horn producing germinal epithelium within the corium, leading to the incorporation of haemorrhage into the newly forming horn and ultimately to the disruption of horn production and the formation of CHLs (Newsome et al., 2017b).

The digital cushion appears to play a key role in force dissipation. Dissection studies investigating the structure of the digital cushion in relation to age provided important characterisation of its structure (Räber et al., 2004, 2006). The digital cushion is comprised of three parallel cylinders of adipose tissue containing a high proportion of connective tissue. Räber et al. (2006) first described how differences arise within the individual cushions and between animals of different parities. In that study, the digital cushions of animals in their second or third parity contained more fat than those of heifers. The digital cushions of animals in their fourth or above parity contained more connective tissue than their younger counterparts.

Numerous studies have investigated the association between BCS, the thickness of the digital cushion and lameness onset. Previous research has demonstrated that maintaining dairy cattle at a BCS > 2.5

(out of 5) minimises the risk of CHLs requiring treatment (Green et al., 2014). Additional to this, an eight year study of a single herd showed that cattle with a BCS < 2 were more likely to be lameness scored as either lame or severely lame (Randall et al., 2015). It was hypothesised by both studies that their respective findings were due to a positive association between BCS and the thickness of the digital cushion, meaning that animals with a higher BCS have a thicker digital cushion. Increased digital cushion thickness has been suggested to offer a better ability to dissipate the concussive forces associated with hoof-strike, thereby minimising the risk of CHL development (Bicalho et al., 2009).

A number of studies such as those conducted by Bicalho et al. (2009) and Newsome et al. (2017a; b) have examined the thickness of the sole soft tissues (namely the corium and the digital cushion) using trans-sole ultrasonography. These studies have highlighted the importance of the association between BCS and the thickness of the sole soft tissues, with an increase in BCS being associated with an increased sole soft tissue thickness. However, Räber et al. (2004) previously described how the middle cushion (the common target of trans-sole ultrasonography) frequently does not overlap with the most caudal aspect of the pedal bone, meaning that efforts to visualise the thickness of the digital cushion in this location, may result in the thickness of the corium alone being captured. Up until now, these studies have focused on the interaction between BCS, soft tissue thickness and subsequent lameness/CHL occurrence without exploring the associations between the occurrence of CHLs and the physical structure of the digital cushion.

A study conducted by Newsome et al. (2016) illustrated the associations between lameness and pathological change to the pedal bone. Lameness and CHL occurrence during life were found to be positively associated with the presence of new bone formation on the caudal aspect of the pedal bone (the area where the initial point of hoof-strike occurs) in the hind hooves of cull dairy cattle. It was suggested that once formed, the presence of this osteoma could predispose animals to future CHL development, leading to a “downwards spiral” of lameness and CHLs.

At present, there are large gaps in the understanding of the aetiology of CHLs with limited knowledge of the interactions between lameness events and the structure of the digital cushion. Newsome et al. (2016) postulated that the inflammatory nature of CHL pathogenesis could lead to the utilisation of

the fatty acids in the digital cushion as inflammatory mediators and / or the development of scar tissue in or around the digital cushion. If correct, this could alter the size, structure and functionality of the digital cushion and reduce its ability to dissipate the concussive forces of hoof-strike.

The detailed aetiopathogenic mechanisms which lead to the formation of CHLs in cattle remain poorly understood. Whilst modern imaging modalities such as MRI have previously been used to study the effect of terrain and exercise on the development of the digital cushion in calves (Gard et al., 2015), they have not been used to examine associations between the digital cushion and lameness. In recent years, MRI protocols and hardware have been developed to allow for the quantification and better visualisation of adipose tissues using mDIXON quant packages (Kise et al., 2017). The output of these protocols allows for the volume of a tissue and the fat fraction within said tissue to be accurately calculated. The study presented within this chapter was designed to investigate associations between animal level variables (including lameness history) and the volume of, and fat content within, the digital cushion in adult dairy cattle at cull. The null hypothesis was that the digital cushion volume within the hind hooves of dairy cows at cull was not associated with CHL or a history of lameness during the lactating lifetime of the animal. The secondary objective of the study was to quantify the volume and fat fraction contained within the digital cushion in a sample of culled dairy cows.

3.2 Materials and Methods

3.2.1 Study Design

A retrospective cohort study was designed and conducted to investigate the association between animal level variables and the volume of the digital cushion at cull and is reported in accordance with the STROBE guidelines (von Elm et al., 2008; STROBE, 2020). The study was conducted with permission from The University of Nottingham, School of Veterinary Medicine and Science ethics committee (Reference Number 1913 161208).

3.2.2 Study Herd and Herd Management

Aspects of herd management pertinent to the current study are described in Chapter 2.2, a brief description is given below. Holstein cattle culled from the SRUC's research herd were considered the

study units. These animals were housed continuously and milked three times daily at either the Langhill or the Acrehead sites. Animals housed at the Langhill facility were managed across a 2X2 factorial trial design where animals belong to one of two genetic lines and nutritional systems. Animals were lameness scored on a weekly basis using the scale described by Manson and Leaver (1988) until April 2017, after which point animals were scored on a fortnightly basis. A comprehensive description of the lameness scoring system utilised is described in Chapter 2.2. Data collection at the Langhill site was much more comprehensive than that at the Acrehead site where data collection was limited to irregular BCS recordings and monthly lameness scoring. At Acrehead, the animals were managed on a more commercial basis, with variations in data collection, diet composition and management system, dependent upon the trial to which the animal was enrolled.

3.2.3 Sample Collection – Hind Hooves at Slaughter

Immediately after slaughter, the distal hindlimbs of all animals were recovered at the slaughterhouse by a single cut at the hock joint and labelled with the animal and limb identification. Hindlimbs were stored locally at -20 ˚C before being transported to the University of Nottingham at -20˚C, every 4-6 months. To minimise storage volume and facilitate positioning within the MRI scanner, the hoof was removed from the remainder of the limb using a single cut approximately 2cms above the coronary band whilst frozen. Hooves were maintained at -20 ˚C within labelled polythene packaging until required for scanning.

3.2.4 Hoof MRI Scanning Protocols

Hooves were thawed at room temperature for 24 ± 3 hours prior to scanning. The MRI protocol was performed using a Philips 3T Ingenia scanner (Philips, Best, Netherlands) based at the Sir Peter Mansfield Imaging Centre (University of Nottingham), with body transmit coil and anterior ds body coil with posterior ds bed coil. Hooves were scanned as corresponding hind hoof pairs, within their labelled packaging, with a 50ml water vial placed next to the right hoof for orientation. All scans were acquired on the coronal plane. Two scanning protocols were utilised:

- T1 weighted adiabetic turbo spin-echo (aTSE) sequence (Coronal plane, 2D acquisition repetition time (TR) = 630ms, echo time (TE) = 15ms, flip angle = 90˚, slice thickness = 2.5mm, interslice gap = 0.3mm, scan duration = 7mins 5secs
- Modified DIXON (mDIXON) Quant sequence (Coronal plane, 2D acquisition, TR = 2ms, TE = 8ms, flip angle = 10˚, slice thickness = 1mm, interslice gap = -0.5mm, scan duration = 4mins 1sec)

The principles of the aTSE and mDIXON scan protocols are described in Hennig et al (1986) and Kise et al (2017) respectively.

Scans were exported as Digital Imaging and Communications in Medicine (DICOM) files and anonymised for image analysis to calculate digital cushion volume and fat content. To calculate digital cushion volume (mls), regions of interest (ROIs) were drawn on individual cushions using MATLAB (The Mathworks Inc.) and volume was computed from the number of voxels within a ROI. Fat Fraction maps were created on the scanner using the mDIXON Quant package (Philips, Best, Netherlands, NL), ROIs were then drawn over the cushion of interest and histogram analysis completed to compute the modal fat fraction with full width half maximum to assess skew. From the modal fat percentage, the volume of fat within each cushion was derived.

3.2.5 Data Handling and Descriptive Analysis

Available herd data encapsulated the time spent within the milking herd since first calving (i.e., no dry-period or pre-first calving data was available). Animal data were exported from the farm database for the period 10th April 2010 – 27th September 2018 into Microsoft Excel files (Microsoft Corp., 2016). All data handling and manipulation was carried out using Microsoft Excel 2016 and RStudio V1.2.5033 (RStudio Team 2019, RStudio: Integrated Development for R. RStudio, Inc., Boston, MA). Data handling, screening and manipulation included exploration of the data to identify missing values or errors. Timeframes utilised in the analysis were based on the date of an animal's lameness score giving a "week of score" to which other data would be appended. Data collected between lameness scores were adjoined to the "week of score" at the beginning of the respective seven- or fourteen-day period which existed between the scores. Descriptive statistics were used to investigate the

distribution of datapoints and to assist in the construction of explanatory variables for statistical models. Associations between cushion volume, claw and hoof location were investigated using scatterplots, histograms, and Mann-Whitney U-tests.

3.2.6 Statistical Modelling

Multivariable linear regression models were constructed to investigate the associations between lameness history and digital cushion volume. The outcome variable was the total volume (mls) of digital cushion contained within the lateral claws of both hind hooves at cull, which was calculated by summing the volumes of the axial, middle and abaxial cushion contained within the left and right hind hoof's lateral claws. Linear regression models were constructed in R Studio taking the format:

$$Y_i = \beta O + \beta_i X_i + \cdots + eO_i,$$

$$[\mathrm{e}O_i] \sim N(O, \sigma_e^2)$$

Where Y_i was the outcome variable of the i^{th} cow, X_i represented the explanatory variables for the i^{th} cow. β_i was the coefficient for the respective explanatory variables for the i^{th} cow, βO the intercept value and eO_i the residual error term, with an assumed normal distribution with mean = 0 and variance = σ_e^2. All explanatory variables were tested within the model initially using univariate linear regression models to explore the associations between individual variables and the outcome variable. Final models were constructed with a forward stepwise approach with variables being retained in the model when $P < 0.05$. A forward stepwise approach was used to test confounders and remove them where appropriate.

Model fit was assessed by visual assessment of residuals and the removal of outlying datapoints (large influence or high leverage) to check their impact on model parameters. Histograms of residuals and Cook's D plots were utilised for this visualisation (Cook, 1977). If coefficients remained statistically similar (coefficients <5% difference) upon outlier removal with the same biological interpretation, then model fit was deemed adequate.

Three final models were constructed following the testing of all variables. These three models were constructed to explore variables which were unlikely to confound one another (i.e. lameness history, CHL recordings and lameness before slaughter) and explain a good proportion of variation presented within the population (Models 3A, 3B and 3C):

- Model 3A explored the associations between the mean lameness score and BCS recorded in the 4 weeks before cull (as lameness scores and BCS are placing a continuous outcome into a categorical one), and the volume (mls) of digital cushion present in the lateral claws at slaughter.
- Model 3B explored the associations between the animal's full lactating lifetime history of lameness and BCS, and the volume (mls) of digital cushion present in the lateral claws at slaughter.
- Model 3C explored the associations between the number of CHLs requiring treatment across the animal's full lactating lifetime, and the volume (mls) of digital cushion present in the lateral claws at slaughter.

Explanatory variables to explore the associations between events in the first and last lactations as well as the lactating lifetime of the animal were calculated as follows; number of CHL treatments recorded (either as a continuous or categorical (0-1, 2-6, 7+) variable), proportion of lameness scores sound (i.e. score 0 – 2; proportion was calculated by summing the number of lameness scores recorded as sound and dividing by the total number of recorded scores), proportion of BCS >3 (proportion was calculated by summing the number of BCS recorded >3 and dividing by the total number of recorded scores), the animals average liveweight (in Kgs as derived by averaging all recorded liveweights for the period described) and daily milk yield (calculated by averaging the daily milk yield in litres for the period described), age at cull (in months), days in milk (DIM) at cull, and the genetic line to which the animal belonged (C or S) and the feed trial to which the animal belonged throughout its lifetime (higher or lower plane of nutrition). The DIM at which an animal first became lame, and the average BCS and lameness score in the four weeks before cull were also calculated. Animals could only be

included in the models if a complete dataset for all variables was present up to seven days before cull. Model fit was evaluated by visual assessment of residuals and the removal of outlying datapoints (large influence or high leverage) to check their impact on model parameters; if coefficients remained statistically similar (<5% difference) with the same biological interpretation, then model fit was deemed adequate.

3.2.7 Validation of Scanning Protocols

Prior to study initiation, 18 hind hooves from clinically normal dairy cattle were collected from a local slaughterhouse and assigned a unique identification number. Within 10 hours post-slaughter the hooves were subjected to the aTSE and mDIXON MRI scanning protocols described above. Within 4 hours of scanning, the hooves were frozen at -20˚C for a period between 7 and 12 days. Hooves were then thawed for 24 ± 3 hours prior to being scanned for a second time using the same MRI protocols. Hooves were re-labelled with a new unique identification so operators, including the technician carrying out image analysis were blinded to animal identification.

The correlation between the volumes of digital cushion in each hoof pre- and post-freezing was evaluated using Pearson's correlation coefficient.

3.3 Results

3.3.1 Validation of Scanning Protocols

The digital cushion volumes measured in the "fresh" hind hooves were highly correlated to those in the "freeze-thawed" hind hooves with a correlation coefficient of 0.92. It was concluded that freeze thawing had a negligible effect on the estimation of digital cushion volume.

3.3.2 Animal Dataset

204 hind hooves were MRI scanned from 102 animals culled from the Langhill (57 animals) and Acrehead (45 animals) herds. The scans from four animals (one culled from the Acrehead site, three from the Langhill site) contained large artefacts within the images and were discarded, meaning the outcome variable was available for 196 hind hooves from 98 animals. Of the animals scanned, the mean parity at cull was 3.0 and the mean age at slaughter was 63 months.

3.3.3 Descriptive Statistics

Table 3-1 describes the results derived from the analysis of all 196 hind hooves. The median (min – max) digital cushion volumes for lateral and medial claws were 16.10mls (0.00 – 29.86mls) and 19.42mls (3.53 – 35.44mls) respectively. Median volume of digital cushion fat for lateral and medial claws were 5.36mls (0.00 – 16.82mls) and 5.89mls (1.01 – 20.52mls) respectively (Figure 3-2, Figure 3-3). The combined volume of digital cushion in the lateral claws was less than the medial claws in 72 of the 98 animals (*$P < 0.001$,* Figure 3-41*)*. Additionally, the volume of fat derived from the modal fat fraction was highly correlated with the volume of digital cushion (*$R^2 = 0.77$;* Figure 3-4). No significant differences were observed between the volume of digital cushion contained within the right hind limb and the left hind limb.

	Claws	Mean	Median	IQR	Min	Max
Total Digital Cushion Volume (mls)	Lateral	15.82	16.10	9.10	0.00	29.86
	Medial	19.32	19.42	10.42	3.53	35.44
Digital Cushion Fat Volume (mls)	Lateral	5.66	5.36	3.12	0.00	16.82
	Medial	6.42	5.89	4.10	1.01	20.25

Table 3-1 Descriptive data for the volume of lateral and medial digital cushions and their respective fat fractions from a sample of 196 hind claws from the hind hooves of 98 cull dairy cows. Animals were culled from the Langhill and Acrehead Herds at the Royal Crichton research dairy herd (SRUC Dumfries). Measurements were ascertained using a mDIXON Quant sequence with a 3-Tesla MRI scanner based at the Sir Peter Mansfield Imaging Centre (University of Nottingham).

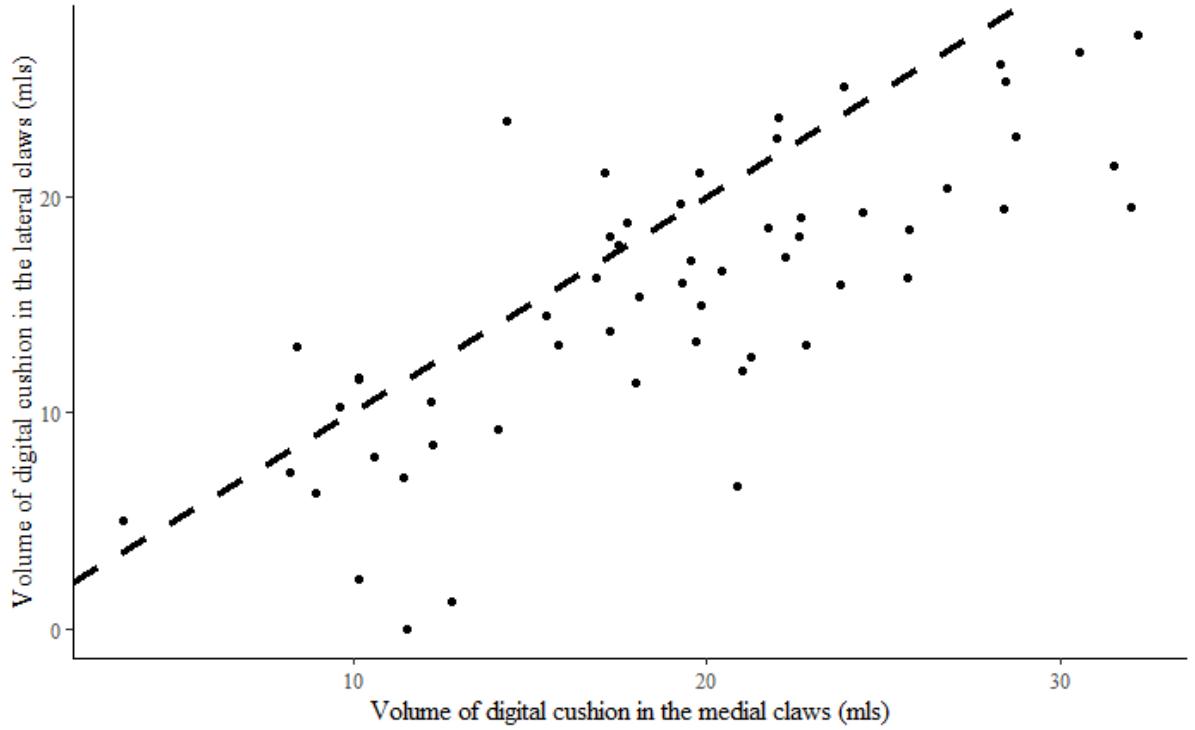


Figure 3-1 A scatterplot of the volume of lateral and medial digital cushions from a sample of 228 hind claws from the hind hooves of 57 cull dairy cows. Animals were culled from the Langhill Herd at the Royal Crichton research dairy herd (SRUC Dumfries) and selected for analysis if a complete mobility history was present. Measurements were ascertained using a mDIXON Quant sequence with a 3-Tesla MRI scanner based at the Sir Peter Mansfield Imaging Centre (University of Nottingham). The hashed line represents the point at which the lateral and medial claws have an equal digital cushion volume. 14 of the 57 animals had more volume of digital cushion in their lateral claws in comparison to their medial counterparts.

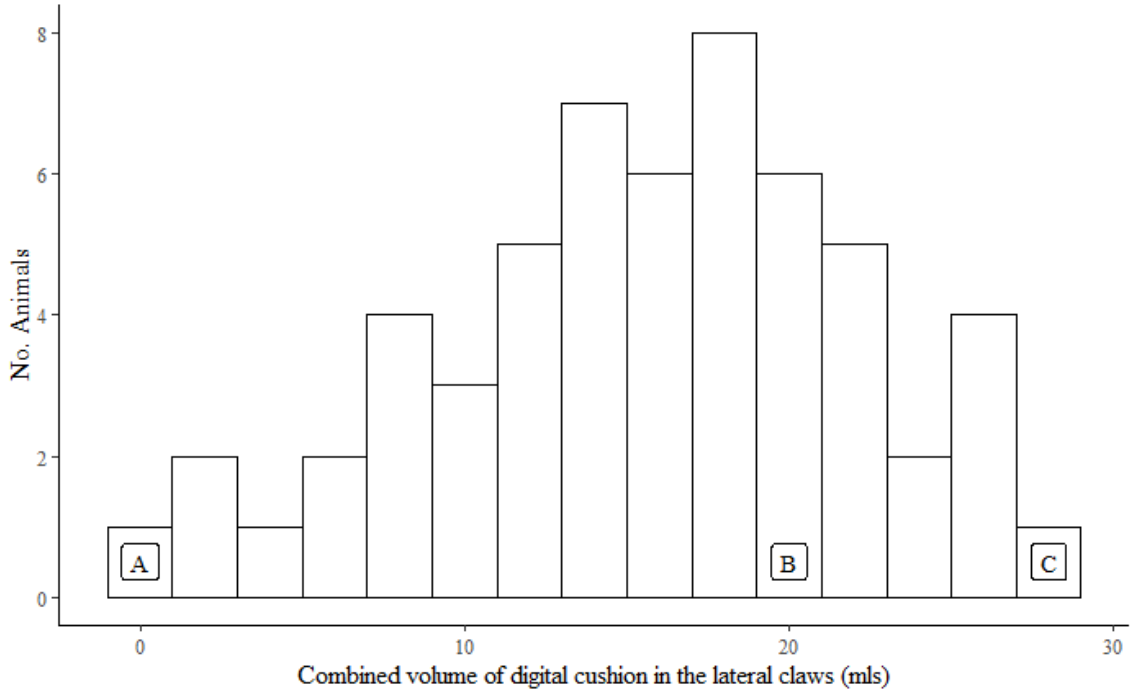


Figure 3-2 A histogram of the combined volume of digital cushions in 104 lateral claws from the hind hooves of 57 cull dairy cows. Animals were culled from the Langhill Herd at the Royal Crichton research dairy herd (SRUC Dumfries) and selected for analysis if a complete mobility history was present. Measurements were ascertained using a mDIXON Quant sequence with a 3-Tesla MRI scanner based at the Sir Peter Mansfield Imaging Centre (University of Nottingham).

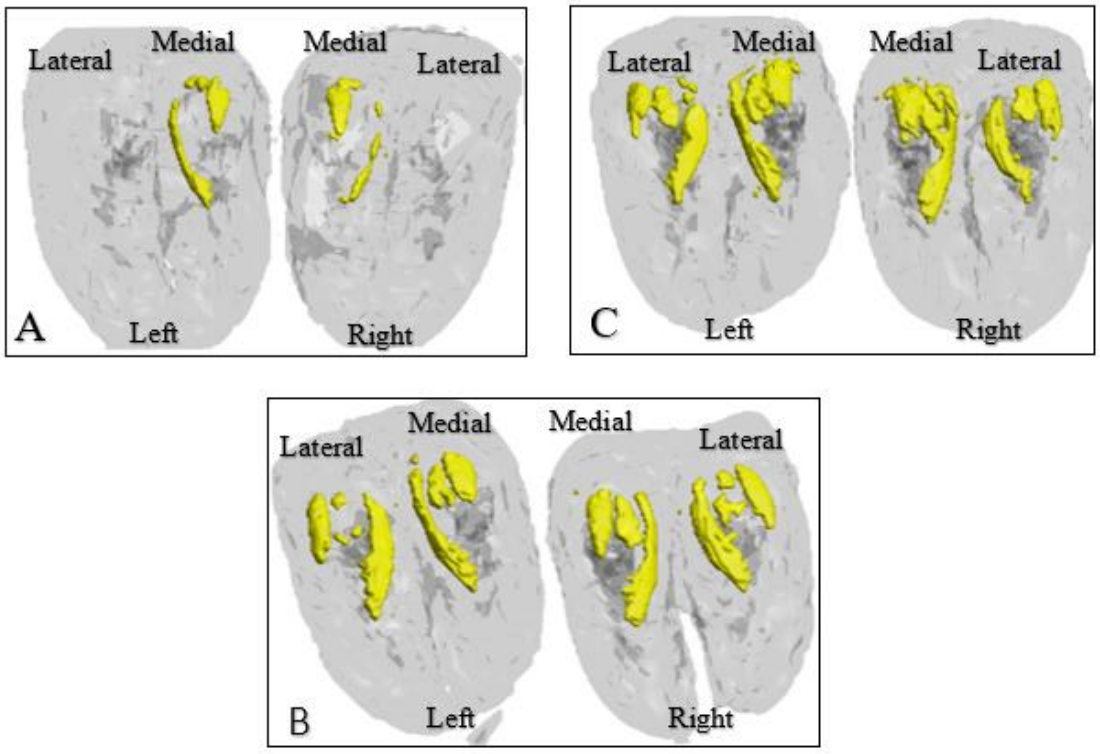


Figure 3-3 Reconstructions of the mDIXON Quant sequence MRI scans for animals represented in Figure 3-2. Animals were culled from the Langhill Herd at the Royal Crichton research dairy herd (SRUC Dumfries) and selected for analysis if a complete mobility history was present. Scans were ascertained using a 3-Tesla MRI scanner based at the Sir Peter Mansfield Imaging Centre (University of Nottingham). The reconstructions view the hoof-capsule from the plantar aspect, with the yellow regions denoting digital cushion and the grey areas representing other tissues within the hoof. Volume differences between B and C can be explained through a difference in thickness as well as surface area when viewing solely from the plantar aspect. The differences in cushion volume could represent a predisposition to future lameness as well as the potential consequences of CHLs.

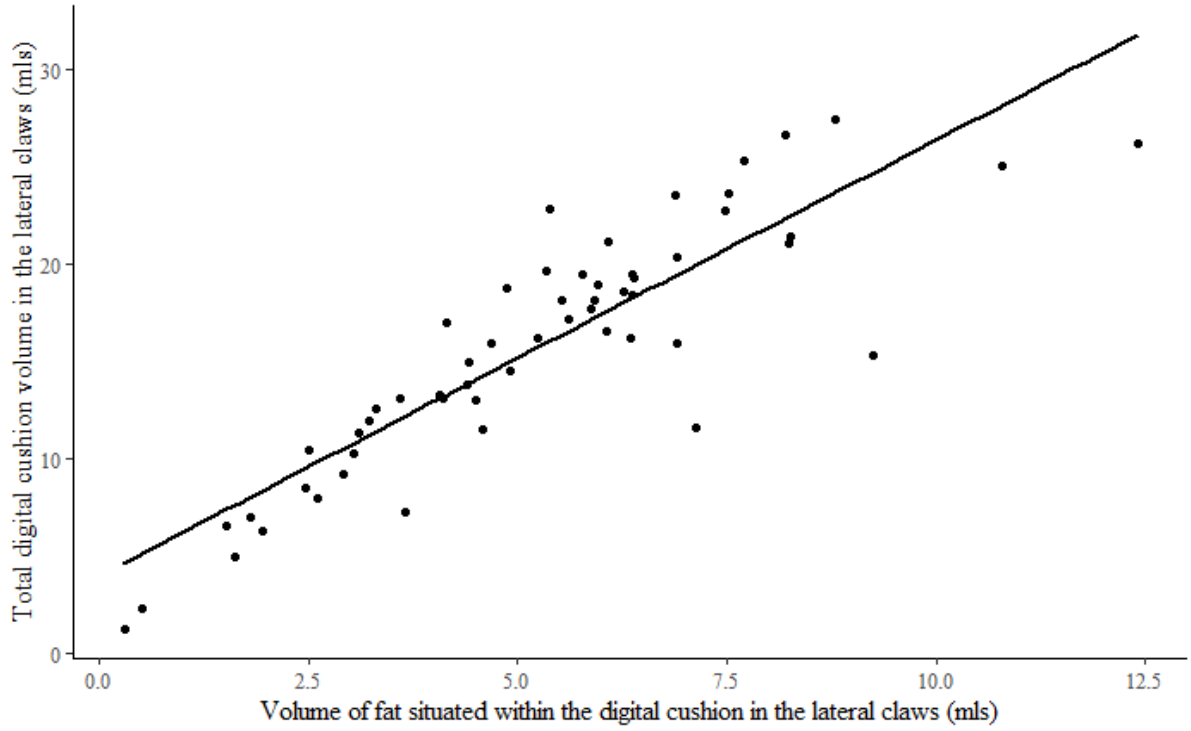


Figure 3-4 A scatterplot of the combined volume of digital cushions contained within the lateral claws and the respective volume of fat within those cushions from a sample of 104 lateral claws from the hind hooves of 57 cull dairy cows. Animals were culled from the Langhill Herd at the Royal Crichton research dairy herd (SRUC Dumfries) and selected for analysis if a complete mobility history was present. Measurements were ascertained using a mDIXON Quant sequence with a 3-Tesla MRI scanner based at the Sir Peter Mansfield Imaging Centre (University of Nottingham). As the volume of fat contained within the cushion increases, the volume of the entire cushion increases.

3.3.4 Statistical Modelling

Data from 57 animals with a complete history of lameness and BCS data until a maximum of seven days before cull was available to construct all models. Of these 57 animals, 23 had at least one treatment for a CHL recorded during their lactating lifetime. All animals within the final models were culled only from the Langhill herd. Table 3-2 describes the explanatory variables constructed for animals retained within the models, with the models themselves being described in Table 3-3, Table 3-4, and Table 3-5. No explanatory variables from the first lactation remained in the final models.

	Mean	Median	Max	Min
Lactation number at cull	2.4	2.0	5.0	1.0
Age at cull	51.4	51.0	92.0	26.0
Proportion of scores submitted as sound (%)	75.0	79.7	100.0	0.0
Proportion of scores submitted as BCS > 3 (%)	2.5	0.0	28.1	0.0
Average daily weight (Kg)	584.0	585.0	697.1	443.2
Average daily yield (L)	27.5	27.7	40.6	16.2
Number of CHLs treated	1.3	0.0	18.0	0.0
Average lameness score four weeks before cull	2.2	2.0	5.0	0.0
Average BCS four weeks before cull	2.00	2.00	3.50	1.75

Table 3-2 Summary of explanatory variables constructed for 57 animals culled directly from the Langhill Herd at the Royal Crichton research dairy herd (SRUC Dumfries). These animals were selected based on the presence of a complete lameness history. The hind hooves of these animals were passed through a three Tesla MRI scanner based at the Sir Peter Mansfield Imaging Centre (University of Nottingham) using a mDIXON Quant sequence to ascertain outcome variables of fat volume and digital cushion volume. Explanatory variables were constructed based on animal data made available from the farm database and encapsulated the time spent within the milking herd since first calving.

3.3.4.1 Model 3A. Associations between lameness scores in the four weeks before cull, CHLs and the volume of digital cushion

Increased mean lameness score in the 4-week period before cull was associated with a reduced volume of digital cushion in the lateral claws at slaughter. For every point increase in mean lameness score a decrease of 2.8mls in digital cushion volume was observed (*P = 0.02, 95% CI: -4.1 - -1.5mls*). Animals with a higher mean BCS in the 4-week period before cull had a significantly increased volume of digital cushion at cull. For every point increase in the mean BCS before cull, an associated increase in digital cushion volume of 2.56ml was observed (*P = 0.02, 95% CI: 1.0- 4.1mls*). In addition to the lameness status (measured by mean lameness score) of the animal in the 4 weeks before cull, the treatment of a CHL at any point during the animal's lactating lifetime was significantly associated with digital cushion volume; for every CHL treatment recorded there was an

associated decrease in digital cushion volume of 0.62ml at the end of life (*P < 0.001, 95% CI: -1.0 - -0.2mls*). Body weight was significant in the final model; for every additional Kg of liveweight an associated increase in digital cushion volume of 0.05ml was observed (*P<0.001, 95% CI: 0.02 – 0.07mls).*

Fifty three percent of the variation observed in digital cushion volume within the dataset was explained by the model. Model fit was assessed by visual assessment of residuals and Cooks D bar plot to identify individual datapoints identified as outliers (Cook, 1977). One animal was identified as an outlier and was removed from the dataset for investigation into influence on the model coefficients. The coefficient remained similar (<5% difference) and biologically plausible. The individual was retained within the model as it was not deemed to substantially effect model outputs or fit. Residuals were normally distributed and so model fit was deemed to be good (Figure 3-5, Figure 3-6).

Model 3A. Outcome variable – Combined volume of digital cushion in the lateral claws

	Coefficient	CI (95%)	P-Value
Intercept	17.4		
No. CHL treatments	-0.6	-1.0 - -0.2	<0.001
Mean lameness score 4 weeks before cull	-2.8	-4.1 - -1.5	0.002
Mean BCS 4 weeks before cull	2.6	1.0 – 4.1	0.002
Mean liveweight	0.05	0.02 – 0.07	<0.001
R^2	0.53		

Table 3-3 Outputs from a linear regression model investigating the association between lameness and the volume of digital cushion in the lateral claws of the hind limbs at slaughter. The outcome variable was the combined volume of digital cushion in the lateral claws. The volume of digital cushion was determined from a sample of 104 lateral claws from the hind hoovest of 57 cull dairy cows. Animals were culled from the Langhill research herd at SRUC's Crichton Royal Farm (Dumfries) and selected for analysis if a complete mobility history was present. Explanatory variables were constructed based on animal data made available from the farm database and encapsulated the time spent within the milking herd since first calving. Model 3A explores the associations between lameness in the four weeks before cull and the volume of the digital cushion at cull.

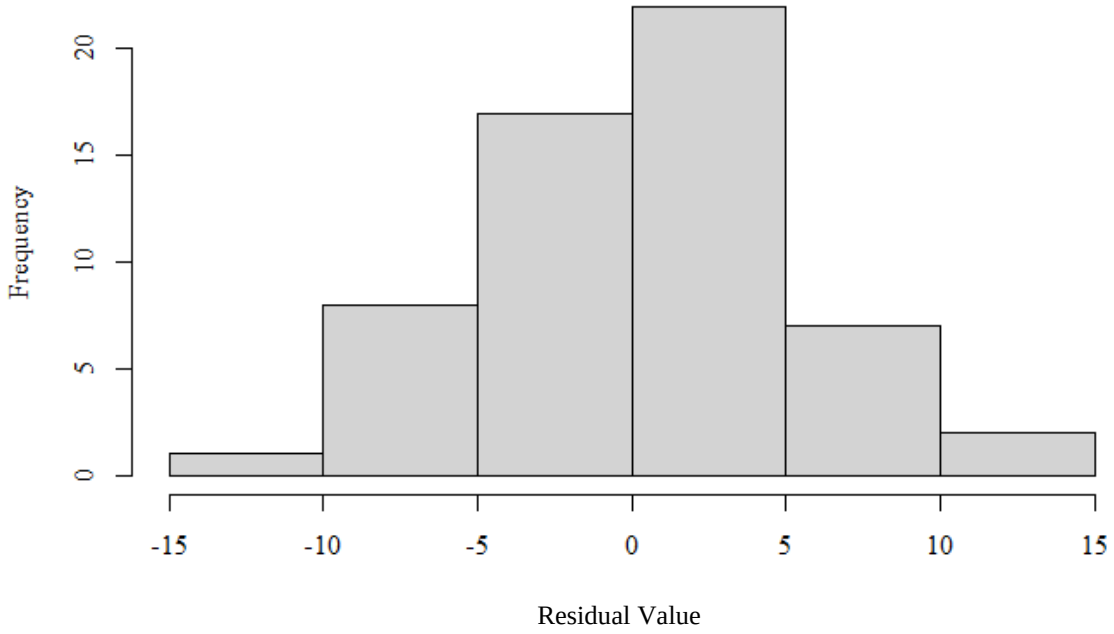


Figure 3-5 A plot of the residuals generated from Model 3A. Model 3A explores the associations between lameness in the four weeks before cull and the volume of the digital cushion at cull. The residuals were normally distributed and model fit was deemed to be good.

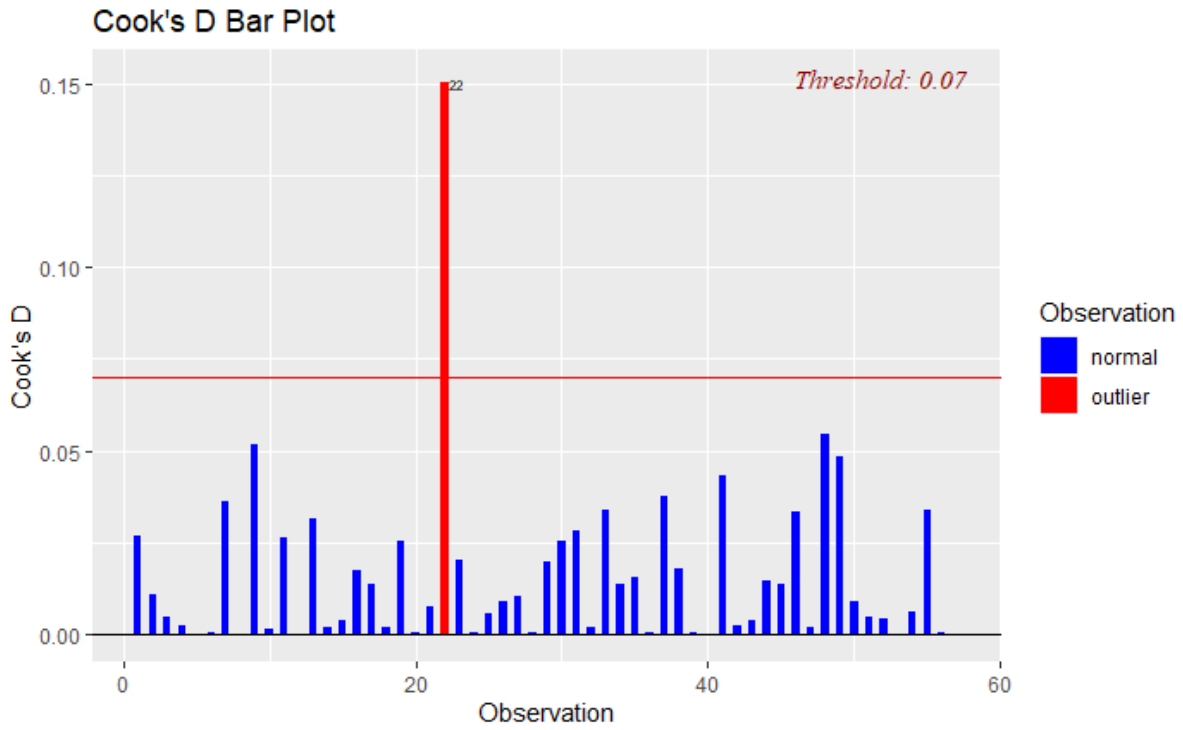


Figure 3-6 A Cook's D bar plot assessing the model fit of Model 3A. Model 3A explores the associations between lameness in the four weeks before cull and the volume of the digital cushion at cull. This plot isolates Datapoint 22 as an outlier. To investigate its effect on model fit this datapoint was removed and the model reconstructed. Coefficients remained similar (<5% difference) and biologically plausible. For the final models presented within this chapter, Datapoint 22 was retained within the dataset.

3.3.4.2 Model 3B. Associations between the lactating lifetime lameness scores, and the volume of digital cushion

Animals with an increased proportion of lameness scores recorded as sound during the lactating lifetime had a higher volume of digital cushion in the lateral claws at slaughter. Every percentage point increase in sound scores was associated with an increase of digital cushion volume in the lateral claws at slaughter of 0.08mls (*P = 0.02, 95% CI: 0.01 – 0.15mls*). Animals with a higher proportion of BCS > 3 recorded across their lactating lifetime tended to have an increased volume of digital cushion at cull. For every percentage increase in the proportion of BCS > 3, an associated increase in digital cushion volume of 0.31mls was observed (*P = 0.06, 95% CI: -0.02 – 0.65mls*). Animals with a higher bodyweight had a significantly increased volume of digital cushion at cull. For every Kg increase in the mean lactating lifetime bodyweight, an associated increase in digital cushion volume of 0.03mls was observed (*P = 0.02, 95% CI: 0.000 – 0.006*). Finally, DIM at cull was significant in the final model. For every day later in lactation at which an animal was culled, an associated increase in digital cushion volume of 0.01ml was observed (*P = 0.02, 95% CI: 0.00 – 0.02mls).*

Forty percent of the variation in digital cushion volume observed within the dataset was explained by the model. Model fit was assessed by visual assessment of residuals and Cooks D bar plot to identify individual datapoints identified as outliers (Cook, 1977). One animal was identified as an outlier and was removed from the dataset for investigation into influence on the model coefficients. The coefficient remained similar (<5% difference) and biologically plausible. The individual was retained within the model as it was not deemed to substantially effect model outputs or fit. Residuals were normally distributed and so model fit was deemed to be good (Figure 3-7, Figure 3-8).

Model 3B. Outcome variable – Combined volume of digital cushion in the lateral claws

	Coefficient	CI (95%)	P-Value
Intercept	5.6		
Proportion of life spent sound	0.08	0.01 – 0.15	0.02
Proportion of life spent at BCS>3	0.31	-0.02 – 0.65	0.06
Mean liveweight	0.03	0.00 – 0.006	0.03
DIM at cull	0.01	0.00 – 0.02	0.02
R^2	0.40		

Table 3-4 Outputs from a linear regression model investigating the association between lameness and the volume of digital cushion in the lateral claws of the hind limbs at slaughter. The outcome variable was the combined volume of digital cushion in the lateral claws. The volume of digital cushion was determined from a sample of 104 lateral claws from the hind hooves of 57 cull dairy cows. Animals were culled from the Langhill research herd at SRUC's Crichton Royal Farm (Dumfries) and selected for analysis if a complete mobility history was present. Explanatory variables were constructed based on animal data made available from the farm database and encapsulated the time spent within the milking herd since first calving. Model 3B explores the association between the proportion of lameness scores submitted as sound throughout the animals lactating life and the volume of digital cushion at cull.

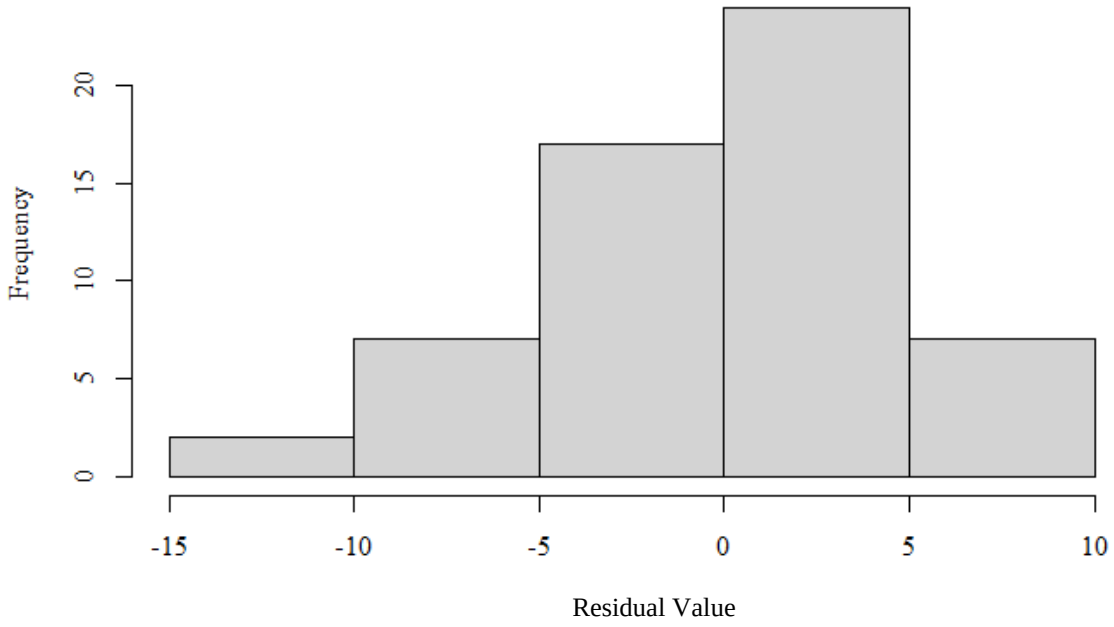


Figure 3-7 A plot of the residuals generated from Model 3B. Model 3B explores the association between the proportion of lameness scores submitted as sound throughout the animals lactating life and the volume of digital cushion at cull. The residuals were normally distributed and model fit was deemed to be good.

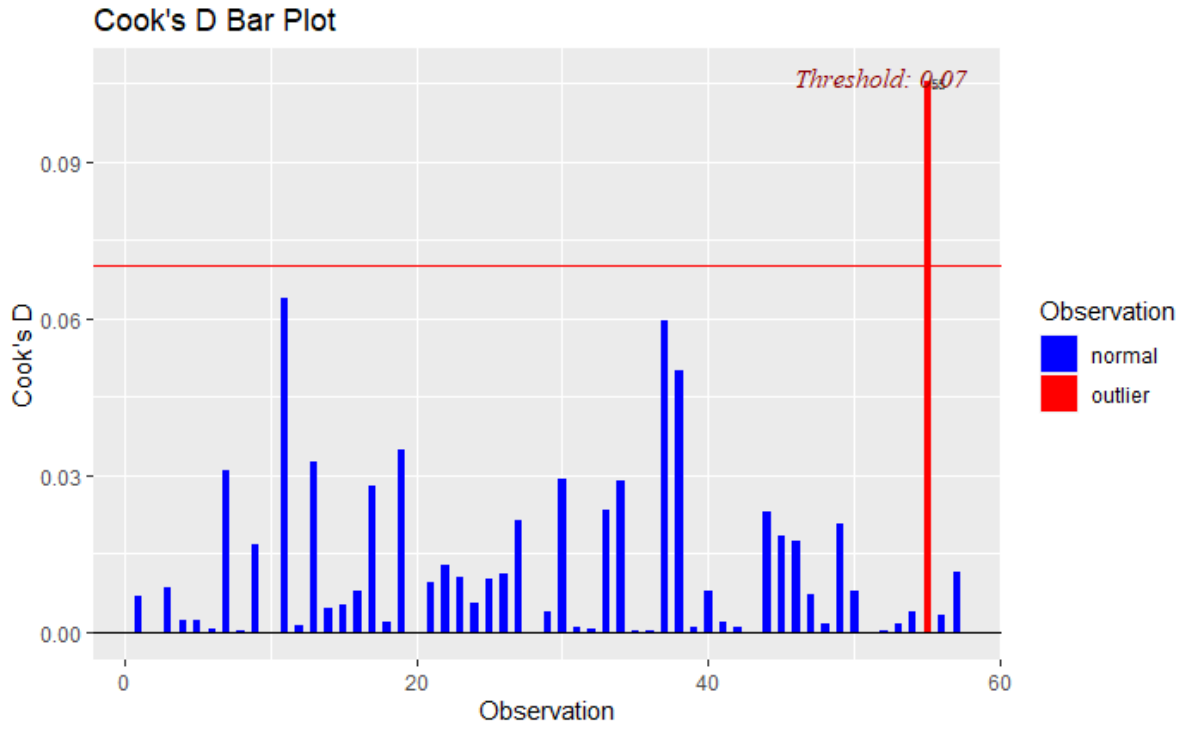


Figure 3-8 A Cook's D bar plot assessing the model fit of Model 3B. Model 3B explores the association between the proportion of lameness scores submitted as sound throughout the animals lactating life and the volume of digital cushion at cull. This graph isolates Datapoint 55 as an outlier. To investigate its effect on model fit this datapoint was removed and the model reconstructed. Coefficients remained similar (<5% difference) and biologically plausible. For the final models presented within this chapter, Datapoint 55 was retained within the dataset.

3.3.4.3 Model 3C. Associations between lactating lifetime CHL treatments and the volume of the digital cushion

Animals with recordings for CHL treatments during their lactating lifetime had a reduced volume of digital cushion in the lateral claws at slaughter. Each CHL treatment was associated with a decrease of 0.89ml in digital cushion volume in the lateral claws at slaughter (*P > 0.001, 95% CI: -1.2 - -0.6mls*). Animals with a higher average bodyweight throughout their lactating lifetime had a significantly increased volume of digital cushion at cull. For every Kg increase in average bodyweight, an associated increase in digital cushion volume of 0.04mls was observed (*P = 0.001, 95% CI: 0.02 – 0.07mls*). Finally, DIM at cull had a significant effect on digital cushion volume in the final model. For every day later in lactation at which an animal was culled, an associated increase in digital cushion volume of 0.01ml was observed (*P < 0.001, 95% CI: 0.01 - 0.02mls).*

Fifty four percent of the variation in digital cushion volume observed within the dataset was explained by the model. Model fit was assessed by visual assessment of residuals and Cooks D bar plot to identify individual datapoints identified as outliers (Cook, 1977). One animal was identified as an outlier and was removed from the dataset for investigation into influence on the model coefficients. The coefficient remained similar (<5% difference) and biologically plausible. The individual was retained within the model as it was not deemed to substantially effect model outputs or fit. Residuals were normally distributed and so model fit was deemed to be good (Figure 3-9, Figure 3-10).

Model 3C. Outcome variable – Combined volume of digital cushion in the lateral claws

	Coefficient	CI (95%)	P-Value
Intercept	13.3		
No. CHL treatments	-0.9	-1.2 - -0.6	<0.001
Mean liveweight	0.04	0.02 – 0.07	0.001
DIM at cull	0.01	0.01-0.02	<0.001
R^2	0.54		

Table 3-5 Outputs from a linear regression model investigating the association between lameness and the volume of digital cushion in the lateral claws of the hind limbs at slaughter. The outcome variable was the combined volume of digital cushion in the lateral claws. The volume of digital cushion was determined from a sample of 104 lateral claws from the hind hooves of 57 cull dairy cows. Animals were culled from the Langhill research herd at SRUC's Crichton Royal Farm (Dumfries) and selected for analysis if a complete mobility history was present. Explanatory variables were constructed based on animal data made available from the farm database and encapsulated the time spent within the milking herd since first calving. Model 3C explores the association between the number of CHLs recorded throughout the animals lactating life and the volume of digital cushion at cull.

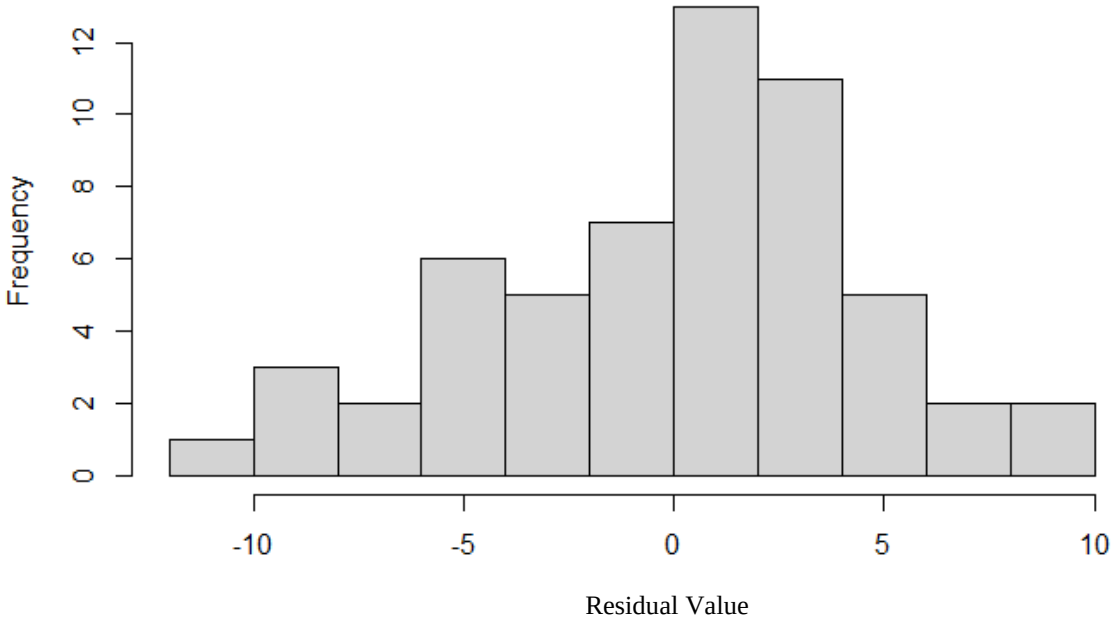


Figure 3-9 A plot of the residuals generated from Model 3C. Model 3C explores the association between the number of CHLs recorded throughout the animals lactating life and the volume of digital cushion at cull. The residuals were normally distributed and model fit was deemed to be good.

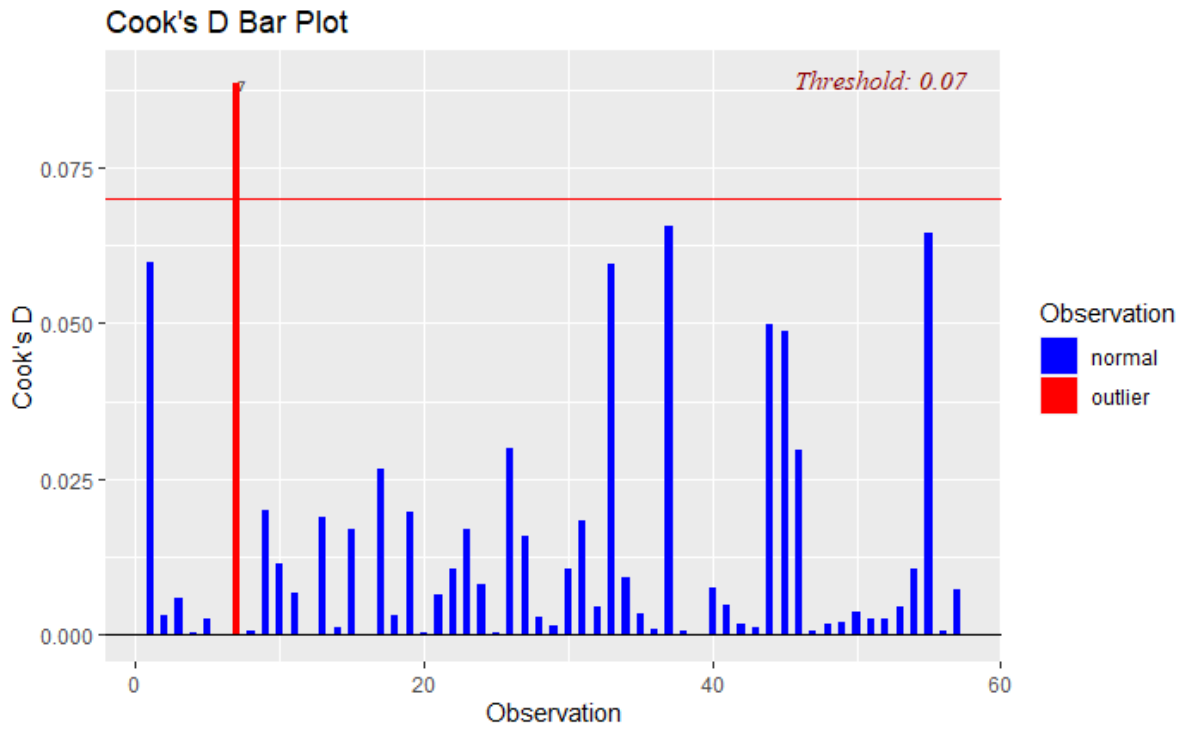


Figure 3-10 A Cook's D bar plot assessing the model fit of Model 3C. Model 3C explores the association between the number of CHLs recorded throughout the animals lactating life and the volume of digital cushion at cull. This graph isolates Datapoint 7 as an outlier. To investigate its effect on model fit this datapoint was removed and the model reconstructed. Coefficients remained similar (<5% difference) and biologically plausible. For the final models presented within this chapter, Datapoint 7 was retained within the dataset.

3.4 Discussion

3.4.1 Summary of Results

This is the first study to describe the volume and fat fraction of the digital cushion, using MRI, in a large sample of adult dairy cattle. This study identified a substantial range in the volume of digital cushion present within the lateral claws of animals at cull (0 – 29.86ml). Animals experiencing a history of lameness (either described as CHL treatments, or by proportion of lameness scores submitted as lame) in their lactating life, or an increased mean lameness score in the four weeks before slaughter were more likely to have a reduced volume of digital cushion in the lateral claws at cull. Additionally, animals with a higher proportion of BCS>3 submitted throughout their lactating life or having an increased mean BCS in the four weeks before cull were more likely to have an increased volume of digital cushion in the lateral claws at cull.

3.4.2 Causality

Due to the nature of the design of this study, the direction of causality within the associations between lameness and digital cushion volume identified cannot be ascertained from the data. Either animals could have had a reduced volume of digital cushion when they entered the milking herd, which predisposed them to CHLs during adult life, or the animals could suffer from CHLs, and the disease process resulted in a reduction in digital cushion volume. Either explanation could describe the associations identified at slaughter and are discussed below. The variation in digital cushion volume observed within the current study may be the effects of a combination of factors occurring throughout the animal's life. These factors have been categorised as genetic, developmental and disease derived.

3.4.3 Genetic Foundation and the Development of the Digital Cushion

Genetic foundations linked to the structure of the digital cushion have been described previously. Oikonomou et al. (2014) described how BCS and digital cushion thickness have a moderate heritability, with animals that are genetically predisposed to maintaining BCS at a more stable level are also predisposed to a lower risk of becoming lame. Further to this, Stambuk et al. (2020) isolated genetic markers associated with digital cushion thickness, with those linked to fat deposition, bone

growth and keratinocyte function being highlighted as genes of importance. These studies lead to the conclusion that some animals are genetically predisposed to having a thinner/impaired digital cushion. If thinner digital cushions are, as previously identified, a risk factor for disease, these animals may experience more CHLs and lameness during life. This could lead to the end-of-life associations identified.

Alongside the genetic component, the rearing period has been shown to play a role in the development of the digital cushion. Gard et al. (2015) demonstrated that exercise over rough terrain can increase the volume of digital cushion in four-month-old calves. This leads to the proposition that it may be possible to pre-condition hooves before first calving to reduce the risk of claw horn disease by increasing the volume of the digital cushion (and potentially by other as yet unidentified changes to claw architecture). The ability to genetically select for a thicker digital cushion, alongside "conditioning" the hoof using exercise on hard surfaces, could lead to animals having a digital cushion which is more able to dissipate the forces of hoof-strike, thereby reducing the risk of CHLs in adult life. However, if animals have a genetic predisposition to a reduced digital cushion volume and their hooves have not been "conditioned" as youngstock then they are more likely to experience CHLs in adult life due to a reduced ability of the digital cushion to dissipate concussive forces.

3.4.4 Hypothesised Impacts of Lameness on the Digital Cushion

The predisposition of animals to CHLs, from the previously mentioned genetic and developmental inputs, could lead to degradation of the structure of the digital cushion in the animal's adult life. CHLs are understood to follow a pathogenic pathway which is reliant on localised inflammation (Newsome et al., 2016). The process of inflammation can utilise fatty acids as mediators and initiators (Contreras et al., 2017a). These fatty acids could be sourced from the adipocytes local to the site of disease, in this case those situated in the digital cushion. It has been demonstrated that the mobilisation of fatty acids from adipose tissue results in the effective remodelling of the cellular structure (Contreras et al., 2017a; b), which could in turn lead to a reduced volume of adipose tissue. It is also noteworthy that the volume of digital cushion, and percentage of fat contained within the tissue were highly correlated in our study, suggesting that a reduction in fatty acids contained within the tissue leads to its reduction

in volume. This remodelling and reduction in fatty acid quantity could impede the digital cushion's ability to dissipate the concussive forces associated with hoof-strike, thereby further predisposing the animal to future CHL development. This creates a self-perpetuating cycle where lameness impedes the functionality of the digital cushion to beget future lameness.

The remodelling of adipose tissue and release of inflammatory mediators can also occur during adipose metabolism, which has been shown to be associated with animals losing body condition (Contreras et al., 2017a; b). Furthermore, the transition period has also been associated with an increase in systemic inflammation and adipose metabolism (Bradford et al., 2015; Contreras et al., 2017a; b). This inflammation and adipose metabolism could result in the digital cushion decreasing in volume, thereby reducing its ability to function normally, and predisposing the animal to future CHLs. The time around the transition period is a potential intervention point to minimise the risk of the digital cushion being adversely influenced, thereby preventing subsequent CHL development. It is possible that the development of CHLs and reduction in BCS could impede the digital cushion's future ability to function from the earliest stage of the process of inflammation, through the previously described self-perpetuating cycle. This reduction in digital cushion volume could present as the earliest stage of pathological change in the hoof of the dairy cow. Even mild lameness could lead to a reduction in digital cushion volume, potentially explaining the predisposition of heifers to future lameness if they suffer from CHLs in their first lactation (Randall et al., 2016). The hypothesis that inflammation degrades the structure of the digital cushion presents an opportunity to investigate the ability for anti-inflammatory drugs to minimise an animal's risk for CHL development through administration at times of lameness or systemic inflammation.

3.4.5 The Chronicity of Claw Horn Lesions

Furthermore, it could be that an animal may never truly recover from a CHL due to the permanent deterioration of the digital cushions structure. Within Model A, when accounting for lameness status of the animal in the four weeks before cull, the historic treatment of a CHL during the animals lactating life was negatively associated with digital cushion volume. This suggests that the differences in digital cushion volume observed in this study are chronic in nature, with historic CHLs presenting a

long-term challenge to the structure of the digital cushion. Upon inspection of diseased claws post mortem, Lischer et al. (2002) found that the adipose tissue contained within the digital cushion of animals with sole ulcers was replaced largely by connective tissue indicative of scarring. The means of the current study measuring the volume of digital cushion would lead to animals with a high degree of connective collagenous tissue in their digital cushions being reported as having little to no digital cushion. The effect of a high digital cushion connective tissue content on the functionality of the tissue needs to be explored, however a clear link to historic and present pathology has been identified. It has been demonstrated in various species that inflammation and trauma can lead to the remodelling and deposition of collagen fibres (Bonnans et al., 2014). The remodelling and deposition of these fibres may permanently inhibit the digital cushion's ability to dissipate force, further predisposing the animal to future CHLs. The study by Lischer et al. (2002) in combination with the present research could be taken to suggest that CHLs have a lasting impact on the structure of the digital cushion. Furthermore, Newsome et al. (2016) identified that animals with a history of lameness tended to have large developments of bone on the most caudal aspect of the pedal bone. The impact of the development of this bone on the digital cushion (in that it develops into the position of the digital cushion) is unknown, but it could be postulated that the changes associated with lameness are long-term on this structure. This long-term change in structure could lead to the impediment of the digital cushions ability to function optimally. The authors hypothesise that the digital cushion may never truly recover from the inflammatory process associated with CHLs, thereby predisposing animals to future CHL onset. Further research investigating optimal treatment regimens in "newly lame" animals could prove essential for reducing the global lameness prevalence by breaking of the chronicity cycle hypothesised in this thesis.

3.4.6 Implications for Future Research

Animals suffering from lameness previously may be predisposed to future lameness through the reduction in digital cushion volume observed in the current study, and the development of bone on the most caudal aspect of the pedal bone observed by Newsome et al. (2016). These pathological changes in the hoof will predispose an animal to CHLs, meaning that intervention or observational studies

investigating CHL presence should focus on the use of animals naïve to CHLs. This will ensure the true effects of an intervention or risk factor can be accounted for, without the unknown effects of previous lameness creating “background noise” in terms of lameness prevalence and recurrence. The use of trans-sole ultrasonography is common in research investigating the structure of the digital cushion. However, based on our results, the authors question the accuracy (the degree to which the result of a measurement, conforms to the correct value) and precision (how close the repeated measured values are to each other) of trans-sole ultrasonography. Many animals had little digital cushion in their lateral claws at slaughter, with the middle cushion commonly the smallest cushion by volume or not present at all. Räber et al.(2004) described how the middle cushion does not overlap the most caudal aspect of the pedal bone. When the method of trans-sole ultrasonography commonly described in research publications (Bicalho et al., 2009; Newsome et al., 2017a; b; Fabbri et al., 2020) is considered, the “typical ulcer site” is commonly visualised by using the flexor tuberosity along the midline of the plantar aspect of the claw as a landmark. This would capture an image of the middle fat pad of the digital cushion. However, when the lack of middle cushion identified and the irregularity of the middle cushion overlapping with the pedal bone is considered, it becomes apparent that trans sole ultrasonography at this location may result in the capturing of the thickness of the corium situated under the flexor tuberosity alone. This may result in the over-estimation of digital cushion thickness if no digital cushion is present whatsoever. This is pertinent when it is considered that trans-sole ultrasonography cannot distinguish between the soft tissues located within the hoof. Kofler et al. (1999) validated the method of trans-sole ultrasonography in acquiring sole soft tissue thickness measurements, however it must be considered firstly that only healthy digits were imaged (meaning that they may have had intact and healthy digital cushions) and secondly that purely the thickness of the combined corium and digital cushion were taken (i.e., there was no differentiation between the two).Given the concerns that have been identified and its increasing use in the research literature, further work is urgently required to robustly validate trans-sole ultrasonography as an accurate and precise measure of digital cushion thickness.

3.4.7 Study Limitations

There are several limitations which should be considered when interpreting the findings of our study. Firstly, and as previously stated, the direction of causality cannot be attributed from a retrospective study such as this. The associations described should be interpreted with care and it is recommended that they are used to inform hypotheses for future prospective intervention studies. Secondly, the study population examined was based on a convenience sample of 102 animals from a single UK dairy research centre. The 57 animals selected for further analysis were only those with complete lameness data up to the point of cull, which may have created a bias in the population studied. It is not possible to comment on the extent of any potential bias or the impact of that potential bias on the results, however, no factors which would suggest the animals studied are not representative of intensively managed dairy cattle were identified. The use of cadaver material should also be considered when interpreting our results. From the validation work carried out initially, however, it appears that the influence of the freeze-thaw cycle on the digital cushion volume was minimal which suggests these results are likely to remain applicable to live animals.

3.4.8 Conclusions

The volume of digital cushion in the lateral claws of the hind limbs varies substantially (0 – 29.86ml) between adult dairy cattle. A large proportion of the observed variation appears associated with the lameness history of the animal. This chapter hypothesises that this association may arise initially from animals having a different genetic potential for digital cushion development. This development can be aided or impeded during the rearing period to help “condition” the hoof to prevent future CHL development. The development of the digital cushion in early life then “sets the scene” for the animal’s future mobility, through either a predisposition or antipathy to CHL development. The authors hypothesise that the inflammatory CHL pathogenesis utilises fatty acids from the digital cushion. The findings of this study also question the validity of using trans-sole ultrasonography to identify digital cushion thickness. Several animals in this study had little to no digital cushion in the lateral claws, meaning that attempts to capture digital cushion thickness using trans-sole ultrasonography may prove inaccurate due to an inability of this imaging method to distinguish

between corium and digital cushion. Future research focussing on minimising any reduction in digital cushion volume through effective treatment and prevention strategies presents an opportunity to reduce the lameness prevalence amongst the global dairy herd.

4 Lameness Immediately Prior to Culling is Associated with a Higher Proportion of Type 3 Collagen within the Digital Cushion

4.1 Introduction

The digital cushion can be described as three parallel cylinders of connective tissue containing adipose depots (Räber et al., 2004). This structure extends beneath the distal phalanx dorsally from the bone's most caudal aspect and exists to protect the corium (wherein exists the germinal epithelium) from the concussive forces of hoof strike (Räber et al., 2004; Newsome et al., 2017b). If the digital cushion is unable to appropriately dissipate the concussive forces of hoof strike, contusions can occur resulting in the disruption of horn production (Newsome et al., 2016). This disruption can lead to the formation of CHLs which are a highly prevalent group of lameness causing conditions occurring globally in the developed dairy herd (Leach et al., 2012; Solano et al., 2016). CHLs present a substantial economic (Huxley, 2013) and welfare (Whay and Shearer, 2017) challenge to the future viability of the global dairy industry.

Previous studies have investigated the associations between BCS and the thickness of the SST (which is purported to include the digital cushion thickness) using trans-sole ultrasonography (Bicalho et al., 2009; Newsome et al., 2017a; b; Griffiths et al., 2020). These studies described positive associations between BCS and the SST thickness (as animals gained BCS, the thickness of the SST increased), suggesting that as animal lose BCS, fat is mobilised systemically, including from the digital cushion. A thinner digital cushion has been linked to an increased risk of lameness (Bicalho et al., 2009; Newsome et al., 2017b; Griffiths et al., 2020). Indeed, this has been supported by longitudinal studies which have demonstrated that BCS loss precedes lameness (Green et al., 2014; Randall et al., 2015). The literature suggests that thinner digital cushions are less able to dissipate the concussive forces of hoof strike, meaning that contusions within the hoof capsule are more likely to occur leading to the formation of CHLs (Bicalho et al., 2009; Newsome et al., 2016, 2017a; b).

Further studies have dissected the digital cushions from hooves of cadaver dairy cattle to relate histological measures of the structure to animal variables (Hiss-Pesch et al., 2019; Newsome et al.,

2021). Those studies identified that the digital cushions of lower BCS animals contained smaller adipocytes than those of higher BCS animals. Furthermore, Räber et al. (2004) identified variations between the digital cushions of older animals against their younger counterparts. The authors identified that the digital cushions of heifers contained mainly loose connective tissue. Animals in their second or third parity contained significantly more fat and animals in their fourth or greater lactation had a large amount of connective tissue and little adipose (Räber et al., 2004). Newsome et al. (2016) suggested that the deposition of connective tissue may be linked with fat being mobilised from the digital cushion to facilitate the inflammation associated with CHL pathogenesis, however this is yet to be proven. Räber et al. (2006) also identified differences between the fatty acid composition of the digital cushions across parities with the digital cushions belonging to heifers containing more arachidonic acid than animals in their second or greater parity. These studies suggest that an animal's age and BCS are linked to the structure and function of the digital cushion, however the impact of lameness and CHLs on the digital cushion remain unknown. Interestingly, despite all of the studies carried out to date, there is still information not known about the basic anatomical and histological structures, including collagen content, of the digital cushions themselves in both healthy and non-healthy animals.

Adding to the challenge of the CHLs is the chronicity cycle that is associated with them; CHLs beget future CHLs (Newsome et al., 2016; Randall et al., 2018a). The occurrence of CHLs has been linked to pathological change affecting the pedal bone (it is hypothesised that local inflammation is responsible for these changes) which is understood to predispose animals to future CHLs (Tsuka et al., 2012; Newsome et al., 2016).

Chapter 3 identified associations between digital cushion structure and a lifetime history of lameness; namely animals that experienced a longer period of time with an increased lameness score, or animals that had a history of CHLs were more likely to have a reduced volume of digital cushion within their lateral claws at cull. This thesis hypothesised that the observed reduction could already be in part present at the beginning of the animals lactating lifetime due to genetic and developmental predispositions. This low volume of digital cushion would likely predispose the animal to future

CHLs. Indeed, it has already been demonstrated that there are key genetic (Oikonomou et al., 2014; Stambuk et al., 2020) and developmental (Gard et al., 2015) elements inputting into the future structure of the digital cushion. The potential increase in occurrence of CHLs driven by this predisposition could lead to the further reduction of digital cushion volume by using fatty acids in inflammatory pathways. This utilisation of fatty acids from adipose tissue has been shown to lead to the "remodelling" of the tissues cellular structure (Contreras et al., 2017a; b). The extent of this remodelling in the digital cushion remains to be observed, and the implications it may have on functionality of the digital cushion are still to be understood.

The collagen content of a given tissue can be quantified using Picrosirius Red (PSR) staining (for an example image, please see Figure 4-7). This stain has been developed to identify different collagenous fibres by either a red, green, or yellow stain when visualised under polarized light. A birefringence of red/yellow is indicative of Type 1 Collagen, whereas a weaker green birefringence is indicative of Type 3 Collagen (Junqueira et al. 1979; Montes and Junqueira 1991). The presence of Type 3 Collagen has been strongly associated with inflammatory diseases and the tissue remodelling associated with them (Montes and Junqueira 1991).

Despite the understood importance of the digital cushion, little research has been conducted investigating the impact of lameness and CHLs on its structure and function. Whilst substantial efforts have been made to understand the mechanisms through which BCS is linked to the digital cushion, little progress has been made by the way of understanding the impacts of lameness and CHLs on this structure. The current study was designed to investigate the associations between animal variables (including lameness history) and the collagen content and adipocyte size contained within the digital cushion in adult dairy cattle at cull as identified by PSR staining. The null hypothesis was that the collagen content and adipocyte size contained within the digital cushions within the hind hooves of adult dairy cows at cull was not associated with a history of lameness and CHLs during the animal's lactating lifetime.

4.2 Materials and Methods

4.2.1 Study Design

A retrospective cohort study was designed and conducted to investigate the association between animal level variables and the adipocyte structure and collagen content of the digital cushion at cull, and is reported in accordance with the STROBE guidelines (von Elm et al., 2008; STROBE, 2020). The study was conducted with permission from The University of Nottingham, School of Veterinary Medicine and Science ethics committee (Reference Number 1913 161208).

4.2.2 Study Herd and Herd Management

Aspects of herd management pertinent to the current study are described in Chapter 2.2 and are the same as Chapter 3.

4.2.3 Sample Collection and Storage

All cadaver material was collected as described in Chapter 3.2.3. The hind hooves selected for this study underwent the same freeze-thaw cycle for the MRI scanning to take place as described in Chapter 3.2.4. Once scanned the hooves were stored at -20˚C within 4 hours of the scanning taking place. The hooves were removed from -20˚C conditions for a minimum of 24 hours and a maximum of 31 hours.

4.2.4 Dissection Protocol

To expose the digital cushion for further dissection, slices were removed from the sole of the still frozen (stored at -20˚C) hoof along the coronal plane using a bandsaw until the digital cushion was visible (as seen in Figure 4-1**)**. After the digital cushion was exposed, the samples were left at room temperature for 12 ± 4 hours to thaw. Individual cushions were removed and cut into cuboidal pieces measuring approximately 5mm X 10mm X 10mm (height, width, length respectively) and placed into scintillation vials containing 4% para-formaldehyde (PFA) for 12 hours ± 3 hours at 5˚C. Vials were labelled as follows (1) Animal ID, (2) Limb ID (L-Left, R-Right), (3) Claw ID(L-Lateral, M – Medial), (4) Cushion ID (A-Abaxial, B-Middle, C-Axial), (5) Sample number (which varied for each animal/cushion depending on the cushion size).



Figure 4-1 A cross-section of a right hind hoof demonstrating the cut utilised and described in Chapter 4.2.4. This cut was utilised to access each individual digital cushion for separate dissection in a cohort of 54 animals. The samples derived from this process underwent PSR and HandE staining.

4.2.5 Processing Protocol

Once removed from the initial 4% PFA fixative solution, the samples underwent the following fixation/processing protocol. Samples were: (1) submerged in 1X phosphate buffer saline (PBS) and stored for 7-14 days (min – 7 days, max – 14 days) at 5˚C, (2) dehydrated in increasing concentrations of ethanol (70 % for 1.5 hours at room temperature, 90 % for 72 hours at 5 °C, 100 % for 3 hours at room temperature [×2]), (3) immersed in xylene for three hours [x2], and (4) embedded in paraffin wax at 60°C.

4.2.6 Sectioning Protocol

Serial tissue sections were cut at 9µm thickness using a microtome (Leica Microsystems (UK) Ltd., Milton Keynes, UK) with a N35 long duration stainless steel microtome blade (FEATHER®, Osaka, Japan) and mounted onto polysilinated glass microscope slides (Fisher Scientific Ltd., UK). All sections were cut on the sagittal plane. The slides were stored at room temperature before being stained with either haematoxylin and eosin or picrosirius red.

4.2.7 Haematoxylin and Eosin Staining Protocol

Sections were (1) deparaffinised in xylene for four minutes, (2) rehydrated through an ethanol series (100%, 90% then 70%; four minutes each) and rinsed with distilled water (dH_2O), (3) placed into haematoxylin (Sigma-Aldrich, UK) for two and a half minutes and rinsed with tap water. The sections were then (4) immersed in 1% industrial methylated spirit and then 10% ammoniated water for 15 seconds each, rinsed in dH_2O and then (5) placed in eosin (Sigma-Aldrich, UK) for four minutes and rinsed again in dH_2O. The sections were then (6) dehydrated through an ethanol series (70%, 90%, 100%; four minutes each), (7) placed in xylene for four minutes and (8) mounted using DPX mountant and a glass coverslip.

4.2.8 Picrosirius Red Staining Protocol

Sections were (1) de-parrafinised in xylene for four minutes, and (2) rehydrated through an ethanol series (100%, 90%, 70%; five minutes each). (3) Picrosirius red solution (Abcam, UK, ab150681) was applied to completely cover the tissue and cover film applied for 1.5 hours. The sections were then (4) rinsed in two changes of acetic acid solution followed by (5) one rinse with 100% ethanol. Sections were (6) dehydrated in two changes of 100% ethanol for five minutes each, (7) placed in xylene for four minutes and (8) coverslips were mounted using DPX.

4.2.9 Microscopy and Sampling Techniques

Systematic random sampling (Mayhew and Burton, 1988) was utilised to capture images of one tissue section from each slide. This method ensured coverage of each specimen without duplication of the same area being photographed twice. One slide was photographed per cushion dissected. H&E-

stained samples were examined and photographed using light microscopy (DM5000 B, Leica, Germany) with a minimum of 12 images being acquired where possible, to allow for adequate cell recruitment. The PSR stained samples were viewed and photographed under polarised light microscopy (DM5000 B, Leica, Germany) with five images being captured where possible. All images were exported in the .tiff format with a 100µm scale bar attached for reference. All microscopy was carried out by a single, blinded trained operator.

4.2.10 Image Analysis

Images were processed using Fiji software (Schindelin et al., 2012). The area and perimeter of individual adipocytes were ascertained from the H&E-stained samples using the "Measure and Label" macro, taking the format described in Figure 4-2.

```
macro "Measure and label" {
    lineWidth = 2;
    run("Measure");
    run("Line Width...", "line="+lineWidth);
    // setForegroundColor(255,255,0); // yellow
    run("Draw", "slice");
    run("Label");
}
```

Figure 4-2 A description of the macro ran through the FIJI software to enable analysis of cell perimeter and area. This macro enables the technician to draw around the cell perimeter and from that the cell was labelled, and the area and perimeter of the shape drawn was calculated.

Images were processed in a random order. Measurements were made by one of two trained technicians following the same standard operating procedure to ensure no systemic error occurred whilst tracing adipocyte perimeters. A minimum of 200 cells per sample were measured, as per the gold standard of systematic random sampling. The contrast was enhanced for the H&E images using the "enhance contrast" function in Fiji with the pixels enhanced by 0.3% to allow for better determination of adipocyte boundaries. Once 200 cells were measured by the technician, the remaining cells on the current image were measured to ensure no measurement bias, and no further images for that section were analysed. All adipocyte data was exported to Microsoft Excel (Microsoft Corp., 2016).

The analysis of the PSR images relied on two macros designed to analyse the amount of "red" and "green" colour in the image, both of which are specific to Type 1 and Type 3 collagen respectively.

These macros are displayed in Figure 4-3, the colour thresholds described in these macros are the same as those described by Zerbinati and Calligaro (2018). The macros functioned to identify and quantify the number of pixels relating to each of the two wavelengths of colour identified.

```
// select the red channel, which has the best contrast
 run("RGB Stack");
 setSlice(1);
 // change scale
 run("Set Scale...", "distance=132 known=100 pixel=1 unit=um");
 // erase scale bar
 setBackgroundColor(255, 255, 255);
 makeRectangle(1019, 906, 320, 44);
 run("Clear", "slice");
 run("Select None");
 // set threshold
 setAutoThreshold();
 getThreshold(min, max)
 setThreshold(0, 34);
 // measure area and area fraction
 run("Set Measurements...", "area area_fraction limit display redirect=None decimal=3");
 run("Measure");
 selectWindow("Results");
```

```
// select the green channel, which has the best contrast
 run("RGB Stack");
 setSlice(2);
 // change scale from inches to microns
 run("Set Scale...", "distance=132 known=100 pixel=1 unit=um");
 // erase scale bar
 setBackgroundColor(255, 255, 255);
 makeRectangle(1019, 906, 320, 44);
 run("Clear", "slice");
 run("Select None");
 // set threshold
 setAutoThreshold();
 getThreshold(min, max)
 setThreshold(44, 210);
 // measure area and area fraction
 run("Set Measurements...", "area area_fraction limit display redirect=None decimal=3");
 run("Measure");
 selectWindow("Results");
```

Figure 4-3 The two macros ran in FIJI to analyse the proportion of red collagenous tissue (left image) and green collagenous tissue (right image). Images were analysed in the .tiff format. These macros separated out the pixels that could be attributed to a wavelength of colour, and then count the number of non-colour pixels vs coloured pixels to give an area and proportion of specifically coloured pixels. These macros were used on all PSR stained tissue sections.

Once the total pixels of each respective colour had been collated, the proportion of pixels defined as "red" or "green" was calculated by dividing the total area of pixels for either red or green by the total area of coloured pixels.

Data retrieved from both image analysis protocols was stored in Microsoft Excel Spreadsheets for the purposes of data cleaning and analysis.

4.2.11 Data Handling and Manipulation

Available herd data encapsulated the time spent within the milking herd since first calving (i.e., no dry-period or pre-first calving data was available). Animal data were exported from the farm database for the period 10th April 2010 – 27th September 2018 into Microsoft Excel files (Microsoft Corp., 2016). All data handling and manipulation was carried out using Microsoft Excel 2016 and RStudio V1.2.5033 (RStudio Team 2019, RStudio: Integrated Development for R. RStudio, Inc., Boston, MA). Data handling, screening and manipulation included exploration of the data to identify missing values or errors. Timeframes utilised in the analysis were based on the date of an animal's lameness score giving a "week of score" to which other data would be appended. Data collected between lameness scores were adjoined to the "week of score" at the beginning of the respective seven- or fourteen-day

period which existed between the scores. Descriptive statistics were used to investigate the distribution of datapoints and to assist in the construction of explanatory variables for statistical models.

4.2.12 Statistical Modelling

A multivariable linear regression model was constructed to investigate the associations between lameness history, digital cushion volume and collagen content within the digital cushion. The outcome variable was the proportion (%) of collagen that could be attributed as Type 1 within the lateral claws of both hind hooves at cull, which was calculated by averaging the proportion of Type 1 collagen within the axial, middle and abaxial cushion contained within the left and right hind hoof's lateral claws. This, as a function, also described the proportion of Type 3 collagen as it is the proportion of Type 1 in comparison to Type 3 that is described. Linear regression models were constructed in R Studio taking the format:

$$Y_i = \beta O + \beta_i X_i + \cdots + eO_i,$$

$$[\mathrm{e}O_i] \sim N(O, \sigma_e^2)$$

Where Y_i was the outcome variable of the i^{th} cow, X_i represented the explanatory variables for the i^{th} cow. β_i was the coefficient for the respective explanatory variables for the i^{th} cow, βO the intercept value and eO_i the residual error term, with an assumed normal distribution with mean = 0 and variance = σ_e^2. All explanatory variables were tested within the model initially using univariate linear regression models to explore the associations between individual variables and the outcome variable. Final models were constructed with a forward stepwise approach with variables being retained in the model when $P < 0.05$.

Model fit was assessed by visual assessment of residuals and the removal of outlying datapoints (large influence or high leverage) to check their impact on model parameters. Histograms of residuals and Cook's D plots were utilised for this visualisation (Cook, 1977). If coefficients remained statistically similar (<5% difference) upon outlier removal with the same biological interpretation, then model fit was deemed adequate.

One final model was constructed (Model A):

- Model 4A explored the associations between the mean lameness score and BCS recorded in the 4 weeks before cull, and the proportion of collagen that is Type 1 within the digital cushion present in the lateral claws at slaughter.

4.3 Results

4.3.1 Animal Dataset

648 individual digital cushions were dissected from hind hooves of 54 animals culled from the Langhill herd, a description of the cushion locations is given in Table 4-1. A total of 49 digital cushions were deemed irretrievable from this sample (i.e., no digital cushion was able to be dissected out from the claws), and 16 were lost due to handling and processing errors meaning that a total of 583 cushions were dissected from 216 claws from 108 hind hooves belonging to 54 animals. Of the animals dissected, the mean parity at cull was 2.4 and the mean age at slaughter was 53 months. From the 583 digital cushions, 2,915 photographs were taken for the purpose of analysis.

Claw ID	Cushion ID		
	Abaxial	Middle	Axial
Left Lateral	52	48	53
Left Medial	55	45	53
Right Lateral	48	44	48
Right Medial	46	44	47

Table 4-1 The total number of digital cushions made available for staining and microscopy following dissection and processing. These samples dissected from a cohort of 54 animals culled from the Langhill herd based at SRUC's Crichton Royal Farm. These animals were selected based on their comprehensive lameness history.

4.3.2 Descriptive Statistics – Picrosirius Red

Table 4-2 describes the results derived from the analysis of all 108 hind hooves. The median (min – max) proportion of Type 1 Collagen contained within the lateral and medial claws was 56.2% (23.6–83.8%) and 59.6% (13.3 – 92.7%) respectively (Figure 4-4), and thereby the median (min – max) proportion of Type 3 Collagen contained within the lateral and medial claws was 43.8% (16.2–76.4%) and 40.4% (7.3 – 86.7%) respectively. The collagen content of the digital cushions in the lateral claws was made up of more Type 3 Collagen than Type 1 in 35 animals. (*P > 0.05;*Figure 4-5, Figure 4-7*)*. Additionally, the volume of digital cushion, as derived from the mDIXON Quant sequence described in Chapter 3, was not strongly correlated with the proportion of Type 1 collagen contained within the respective cushions ($R^2 = 0.05$;Figure 4-6). No significant differences were observed between the proportion of Type 1 collagen contained within the right hind limb and the left hind limb (*P = 0.84*).

	Claw	Mean	Median	Interquartile range	Minimum	Maximum
Proportion (%) of Type 1 collagen	Lateral	56.2	54.9	6.5	23.6	83.8
	Medial	59.6	57.7	8.1	13.3	92.7

Table 4-2 Descriptive statistics of the proportion of Type 1 collagen contained within the digital cushions of the lateral and medial claws. The proportion of Type 1 collagen in each claw was derived from the average collagen content across the three cushions contained within that respective capsule. These samples were dissected from a cohort of 54 animals culled from the Langhill herd based at SRUC's Crichton Royal Farm. These animals were selected based on their comprehensive lameness history.

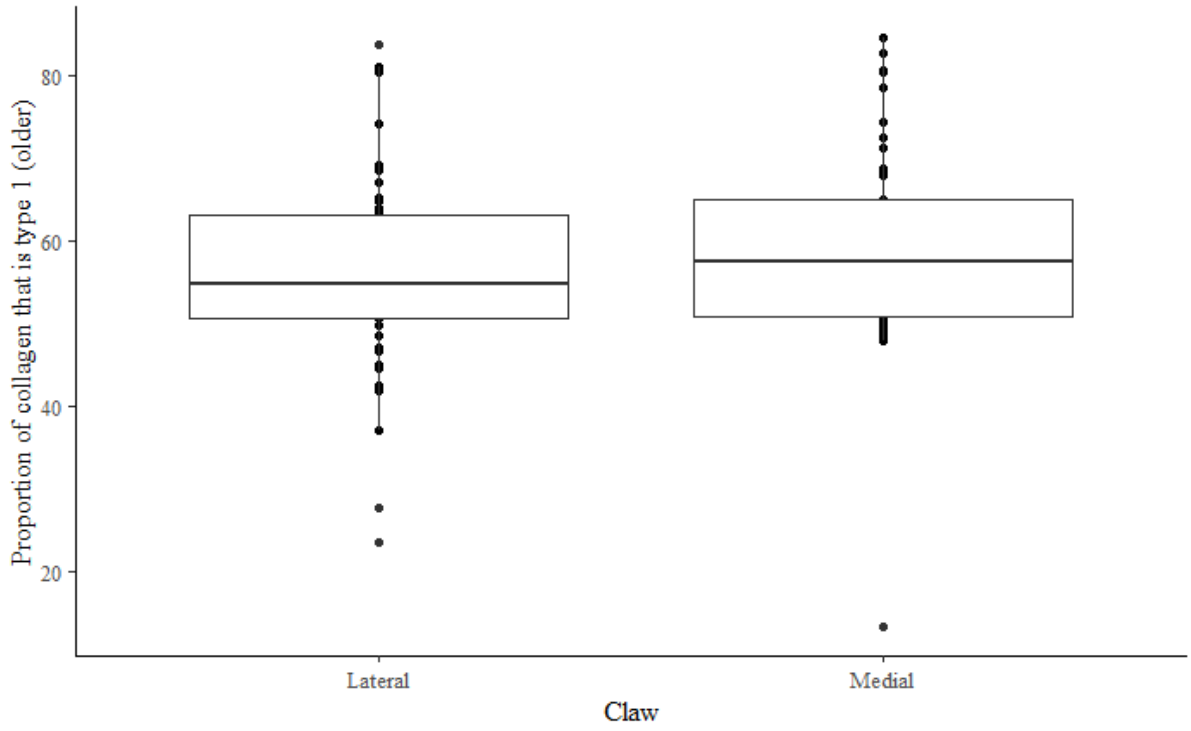


Figure 4-4 A boxplot illustrating the difference in Type 1 collagen content in the lateral and medial claws. The proportion of type one collagen in each claw was derived from the average collagen content across the three cushions contained within that respective capsule. These samples were dissected from a cohort of 54 animals culled from the Langhill herd based at SRUC's Crichton Royal Farm. These animals were selected based on their comprehensive lameness history.

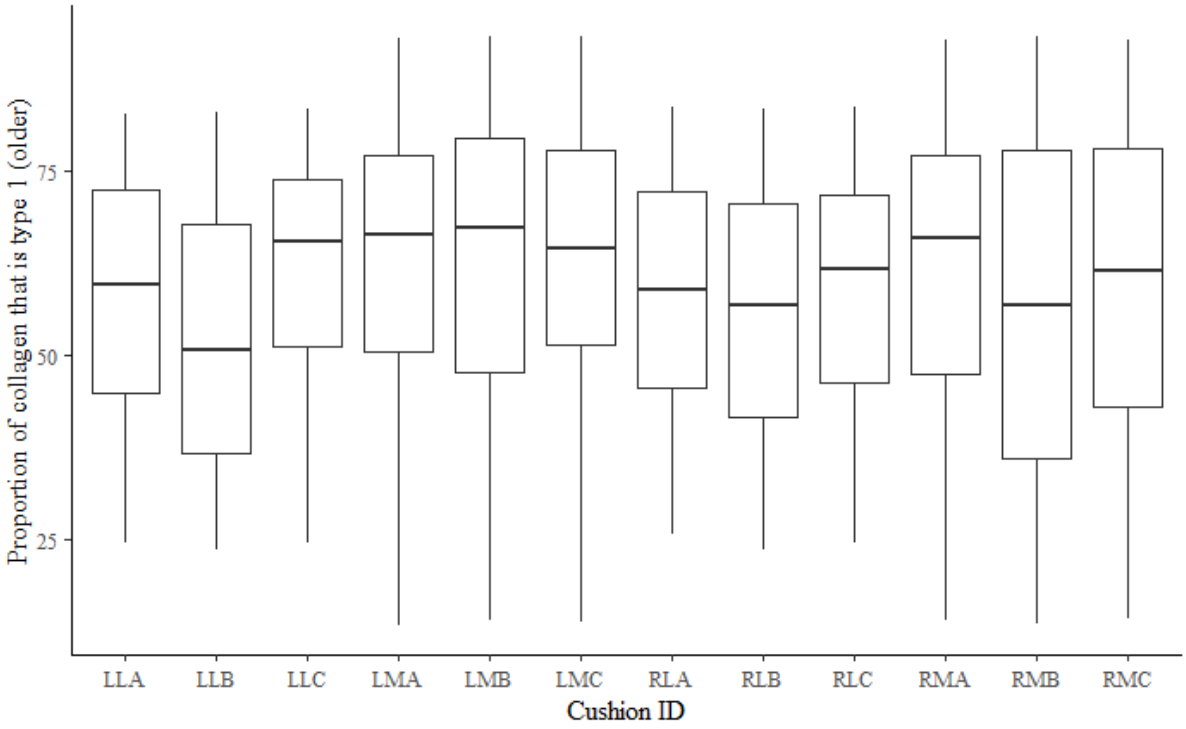


Figure 4-5 A boxplot illustrating the difference in Type 1 collagen content between the different individual digital cushions. The proportion of type one collagen in each cushion was derived from the average collagen content across four photomicrographs taken for that respective cylinder of the digital cushion. The location of each cushion is denoted as follows; 1) Hoof Location (L = left, R = Right), 2) claw location (L = Lateral, M = Medial) and cushion location (A = abaxial, B = middle, C = axial), for example LLC would represent the digital cushion dissected from the left lateral axial site. These samples were dissected from a cohort of 54 animals culled from the Langhill herd based at SRUC's Crichton Royal Farm. These animals were selected based on their comprehensive lameness history.

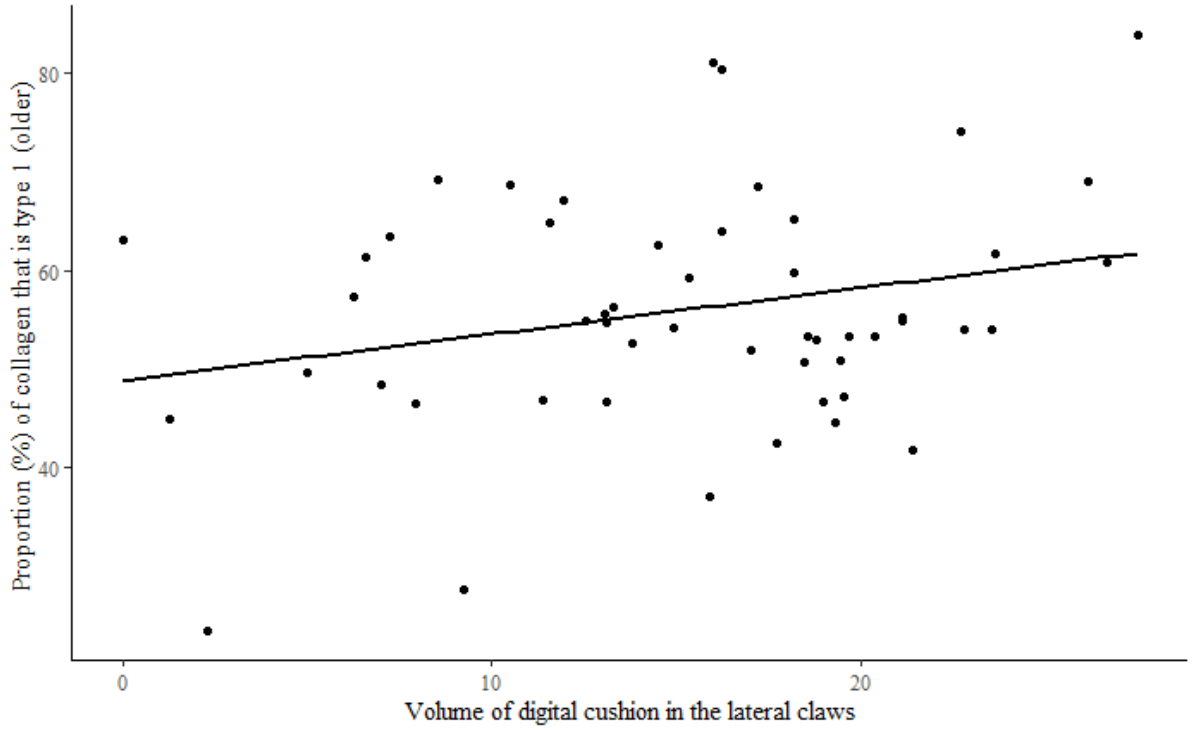


Figure 4-6 A scatterplot illustrating the poor correlation between combined volume of digital cushion within the lateral claws and the proportion of collagen that is Type 1 in the lateral claws. The proportion of Type 1 collagen in each claw was derived from the average collagen content across the three cushions contained within that respective capsule. The volume of digital cushion was calculated from an MRI scan of the respective hoof using an mDIXON Quant sequence that is described in Chapter 3. These samples were dissected from a cohort of 54 animals culled from the Langhill herd based at SRUC's Crichton Royal Farm. These animals were selected based on their comprehensive lameness history.

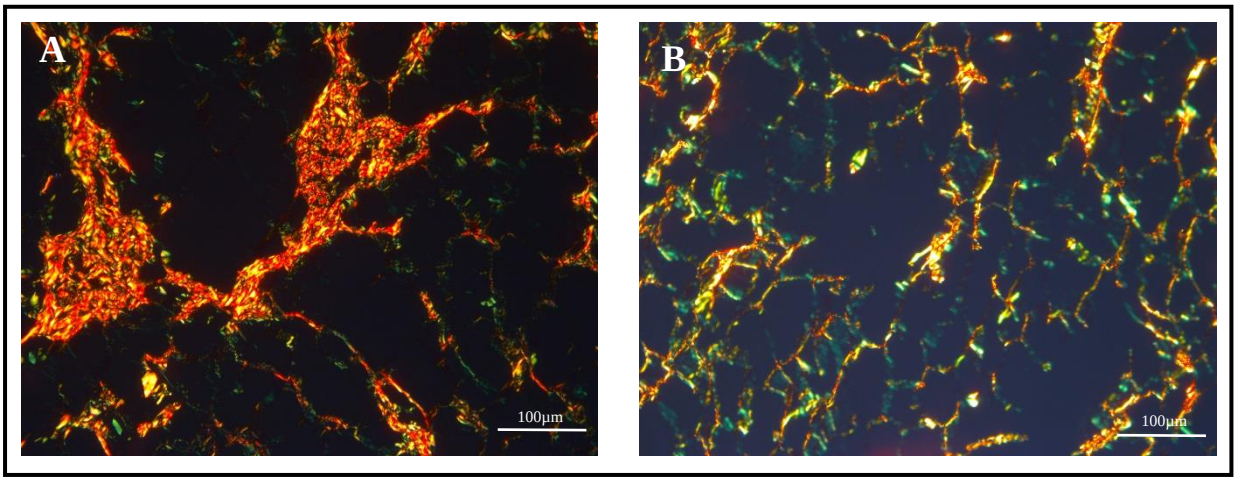


Figure 4-7 Photomicrographs illustrating collagen abundance in the digital cushion in sound (A) and lame (B) animals before cull. A) Middle cushion of a left lateral claw of an animal that had a mean lameness score of 1.8 in the four weeks before cull. Type 1 collagen content (denoted as red filaments) was greater than the Type 3 collagen (denoted as green filaments) content. B) Middle cushion of a left lateral claw of an animal that had a mean lameness score of 4.3 in the four weeks before cull. In this image the Type 3 collagen content was greater than the Type 1.

4.3.3 Statistical Modelling – Picrosirius Red

Histological data from 54 animals with a complete history of lameness and BCS data until a maximum of seven days before cull was available to construct all models. Of these 54 animals, 22 had at least one treatment for a CHL recorded during their lactating lifetime.

describes the explanatory variables constructed for animals retained within the models, with the model itself being described in Table 4-4.

	Mean	Median	Max	Min
Lactation number at cull	2.4	2.0	5.0	1.0
Age at cull	52.8	51.0	92.0	27.0
Proportion of scores submitted as sound (%)	73.8	79.1	100.0	0.0
Proportion of scores submitted as BCS > 3 (%)	2.6	0.0	28.1	0.0
Average daily weight (Kg)	584.1	585.7	680.1	443.2
Average daily yield (L)	27.7	27.7	40.6	16.2
Number of CHLs treated	1.9	0.0	18.0	0.0
Average lameness score four weeks before cull	2.3	2.1	5.0	0.0
Average BCS four weeks before cull	2.3	2.2	3.5	1.4

Table 4-3 Summary of explanatory variables constructed for 54 animals culled directly from the Langhill Herd at the Royal Crichton research dairy herd (SRUC Dumfries). These animals were selected based on the presence of a complete lameness history. Hind hooves were passed through a three Tesla MRI scanner based at the Sir Peter Mansfield Imaging Centre (University of Nottingham) using a mDIXON Quant sequence before having all retrievable digital cushion dissected from them. Variables were constructed based on animal data made available from the farm database and encapsulated the time spent within the milking herd since first calving.

4.3.3.1 Model 4A. Associations between lameness scores in the four weeks before cull, CHLs and the proportion of Type 1 collagen in the digital cushion

Increased mean lameness score in the 4-week period before cull was associated with a reduced proportion of Type 1 collagen in the digital cushion in the lateral claws at slaughter. For every point increase in mean lameness score an absolute decrease of 3.9% in Type 1 collagen in the digital cushion was observed (*P = 0.05, 95% CI: -0.1 - -7.72%*). Due to the method by which this proportion of Type 1 collagen was calculated, the inverse would be the case for Type 3 collagen proportion (i.e. an absolute increase in the proportion of Type 3 collagen of 3.9% would be expected with every point increase in the mean lameness score in the four weeks before slaughter).In addition to the lameness status (measured by mean lameness score) of the animal in the 4 weeks before cull, the volume of the digital cushion in the lateral claws remained within the model as a potential confounder of results, for every ml increase in combined digital cushion volume, the proportion of Type 1 collagen increased by an absolute of 0.3% (*P = 0.2, 95% CI:* -0.2 - 0.8).

Fourteen percent of the variation observed in digital cushion volume within the dataset was explained by the model. Model fit was assessed by visual assessment of residuals and Cooks D bar plot to identify individual datapoints identified as outliers (Cook, 1977). Six animals were identified as outliers and were removed from the dataset for investigation into influence on the model coefficients. The coefficient remained similar (<5% difference) and biologically plausible. The animals were retained within the model as they were not deemed to substantially effect model outputs or fit. Residuals were not normally distributed and so model fit was deemed to be poor (Figure 4-8, Figure 4-9).

Model 4A. Outcome variable – Average proportion of Type 1 collagen in the digital cushion of the lateral claws

	Coefficient	CI (95%)	P-Value
Intercept	62.3		
Volume of digital cushion in the lateral claws	0.3	-0.2 - 0.8	0.23
Mean lameness score 4 weeks before cull	-3.9	-0.1 - -7.72	0.05
R^2	0.14		

Table 4-4 Outputs from a linear regression model investigating the association between lameness and the proportion of collagenous tissue in the lateral claws of the hind limbs at slaughter that is Type 1 collagen. The outcome variable was the proportion Type 1 collagen from total collagenous tissue (types 1 and 3) in 108 lateral claws from the hind hooves of 54 cull dairy cows. Animals were culled from the Langhill research herd at SRUC's Crichton Royal Farm (Dumfries) and selected for analysis if a complete mobility history was present. Explanatory variables were constructed based on animal data made available from the farm database and encapsulated the time spent within the milking herd since first calving. Model 4A explores the associations between lameness in the four weeks before cull and the proportion of collagenous tissue in the digital cushion that is comprised of Type 1 collagen at cull.

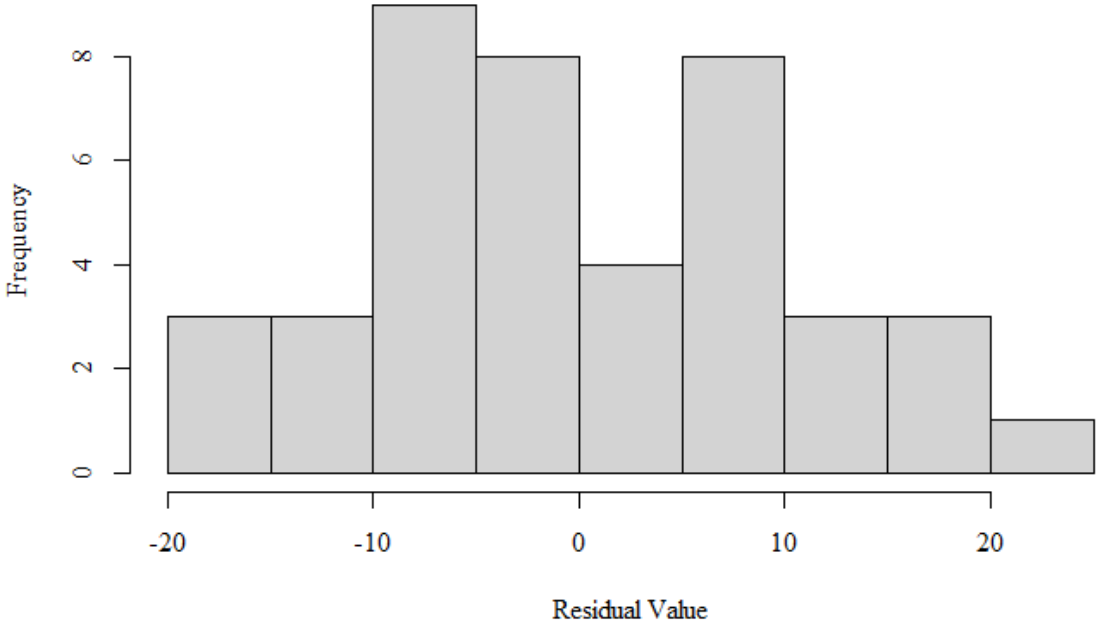


Figure 4-8 A plot of residuals derived from Model 4A. Model 4A explores the associations between lameness in the four weeks before cull and the proportion of collagenous tissue in the digital cushion that is comprised of Type 1 collagen at cull. The residuals were not normally distributed and so model fit was deemed as poor.

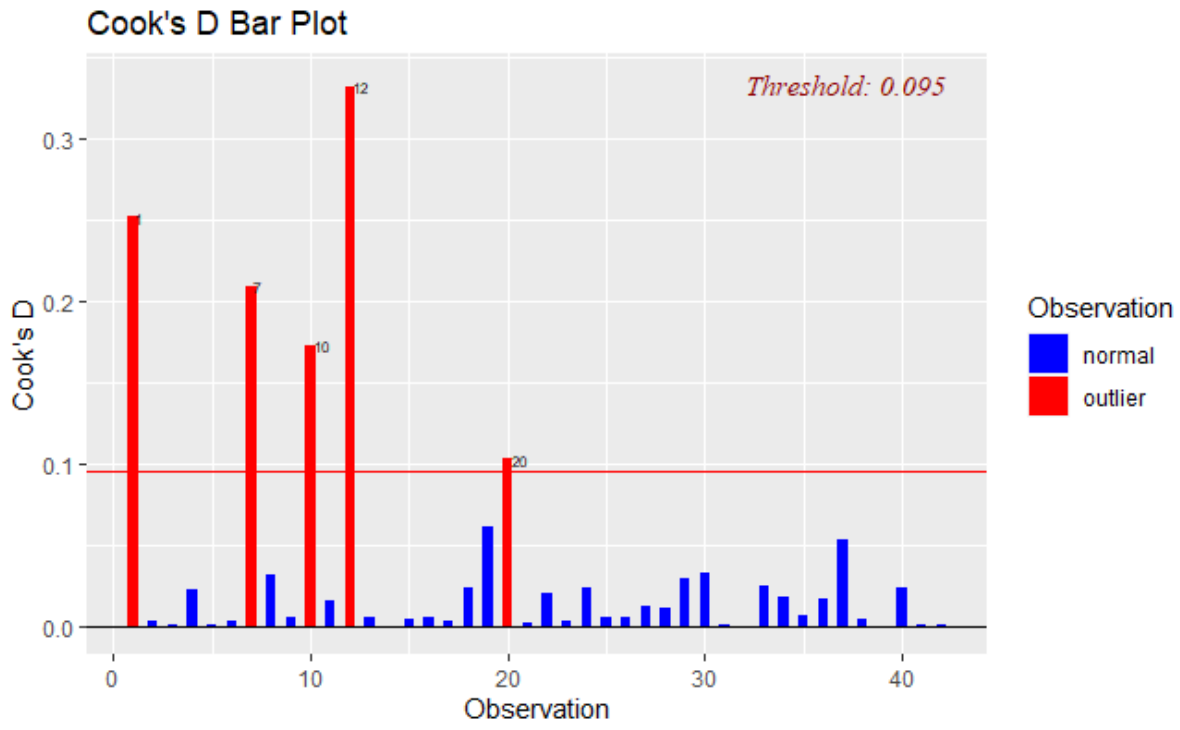


Figure 4-9 A Cook's D bar plot assessing the model fit of Model 4A. Model 4A explores the associations between lameness in the four weeks before cull and the proportion of collagenous tissue in the digital cushion that is comprised of Type 1 collagen at cull. This plot isolates Datapoints 1, 7, 10, 12 and 20 as outliers. To investigate their effects on model fit these datapoints were removed and the model reconstructed. Coefficients remained similar (<5% difference) and biologically plausible. For the final models presented within this chapter, these datapoints were retained within the dataset.

4.3.4 Descriptive Results – Haematoxylin and Eosin Pilot Study

A sub-set of samples from 13 animals were made available for a pilot descriptive analysis of cell size and perimeter. This section of Chapter 4 has been heavily impacted by the COVID-19 global pandemic. For a description of the impact of the national lockdown and associated challenges please see Chapter 1.6.2.

Table 4-5 describes the results derived from the analysis of 26 claws from 13 animals. The median (min – max) adipocyte area present within the lateral and medial claws was 1765.8µm (705.2 - 3607.8µm) and 1784.6µm (939.0 - 3622.1µm) respectively (Figure 4-10, Figure 4-12). The median (min – max) adipocyte perimeter present within the lateral and medial claws was 167.0µm (92.5 – 238.1µm) and 166.6µm (122.3 – 240.3µm) respectively (Figure 4-11, Figure 4-13). The adipocyte area of the digital cushions in the lateral claws was larger than the medial claws in 7 animals.

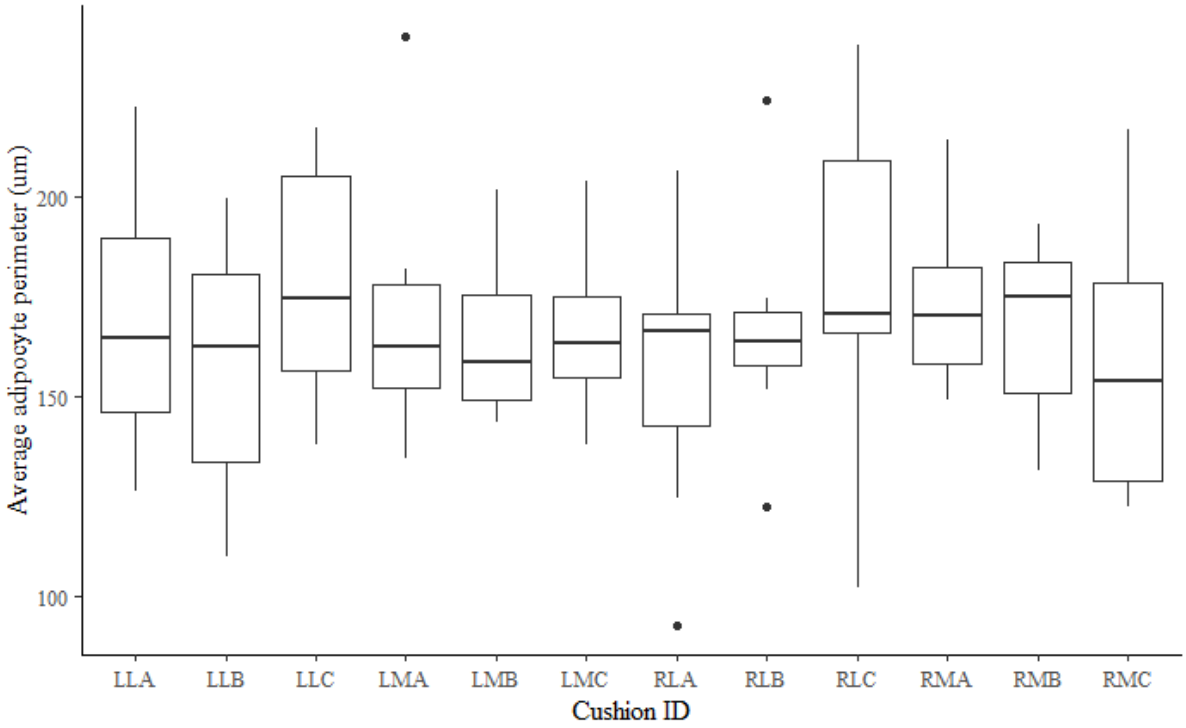


Figure 4-10 A boxplot illustrating the difference in adipocyte perimeter (um) between the different individual digital cushions. The average cell area was determined by calculating the average cell area for each cushion where >200 adipocytes had been measured for that individual site. The location of each cushion is denoted as follows; 1) Hoof Location (L = left, R = Right), 2) claw location (L = Lateral, M = Medial) and cushion location (A = abaxial, B = middle, C = axial), for example LLC would represent the digital cushion dissected from the left lateral axial site. These samples were dissected from a cohort of 13 animals culled from the Langhill herd based at SRUC's Crichton Royal Farm. This represents pilot data from a more comprehensive dataset.

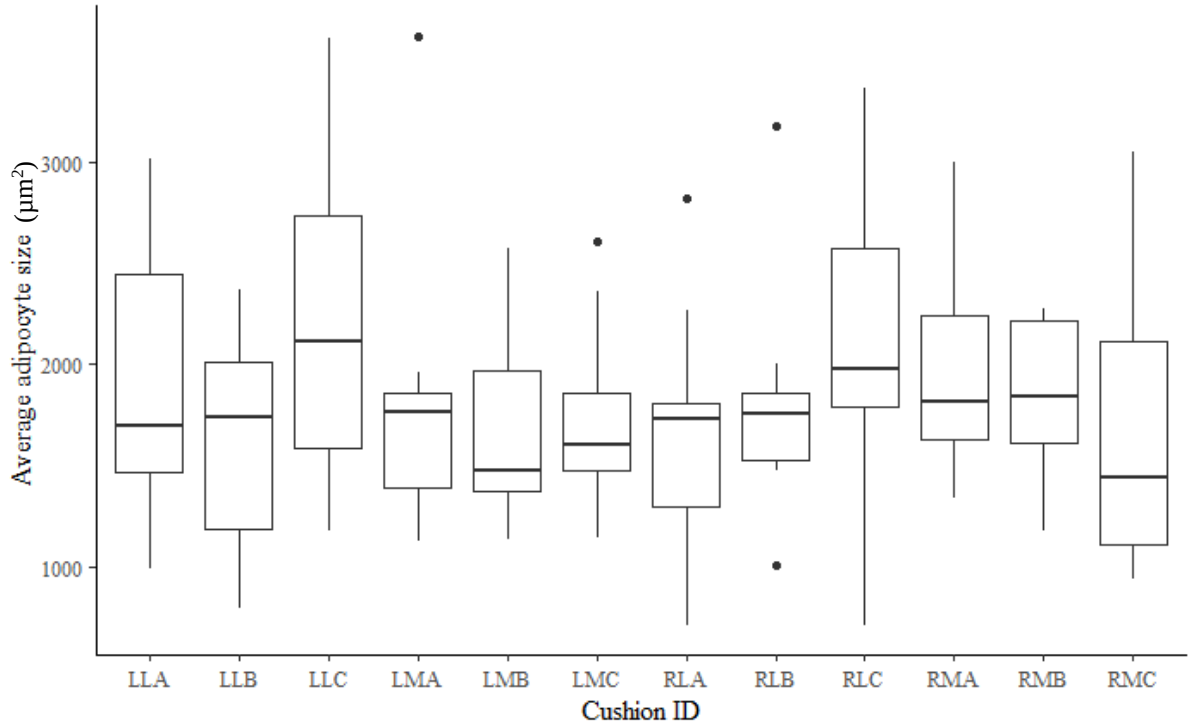


Figure 4-11 A boxplot illustrating the difference in adipocyte area (um) between the different individual digital cushions. The average cell area was determined by calculating the average cell area for each cushion where >200 adipocytes had been measured for that individual site. The location of each cushion is denoted as follows; 1) Hoof Location (L = left, R = Right), 2) claw location (L = Lateral, M = Medial) and cushion location (A = abaxial, B = middle, C = axial), for example LLC would represent the digital cushion dissected from the left lateral axial site. These samples were dissected from a cohort of 13 animals culled from the Langhill herd based at SRUC's Crichton Royal Farm. This represents pilot data from a more comprehensive dataset.

	Cushion ID	Mean	Median	Interquartile range	Minimum	Maximum
Adipocyte Perimeter (µm)	Lateral	168.0	167.0	35.4	92.5	238.1
	Medial	166.7	166.6	33.7	122.3	240.3
Adipocyte area (µm²)	Lateral	1841.2	1765.8	825.9	705.2	3607.8
	Medial	1854.3	1784.6	817.0	939.0	3622.1

Table 4-5 Descriptive statistics of the proportion of Type 1 collagen contained within the digital cushions of the lateral and medial claws. The proportion of Type 1 collagen in each claw was derived from the average collagen content across the three cushions contained within that respective capsule. These samples were dissected from a cohort of 54 animals culled from the Langhill herd based at SRUC's Crichton Royal Farm. These animals were selected based on their comprehensive lameness history.

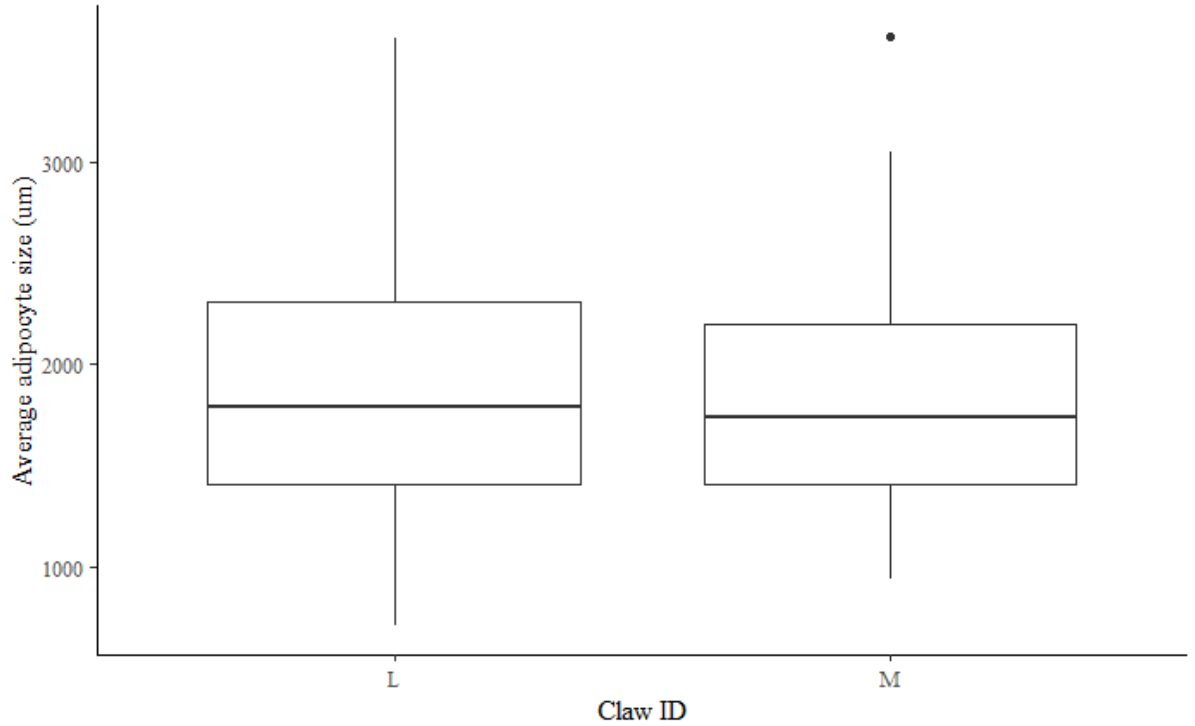


Figure 4-12 A boxplot illustrating the difference in adipocyte area (um^2) between the lateral (L) and medial (M) claws. The cell area was determined by calculating the average cell area for each cushion if >200 adipocytes had been measured for that individual site, an average of this was taken for each animal's lateral and medial claw. These samples were dissected from a cohort of 13 animals culled from the Langhill herd based at SRUC's Crichton Royal Farm. This represents pilot data from a more comprehensive dataset.

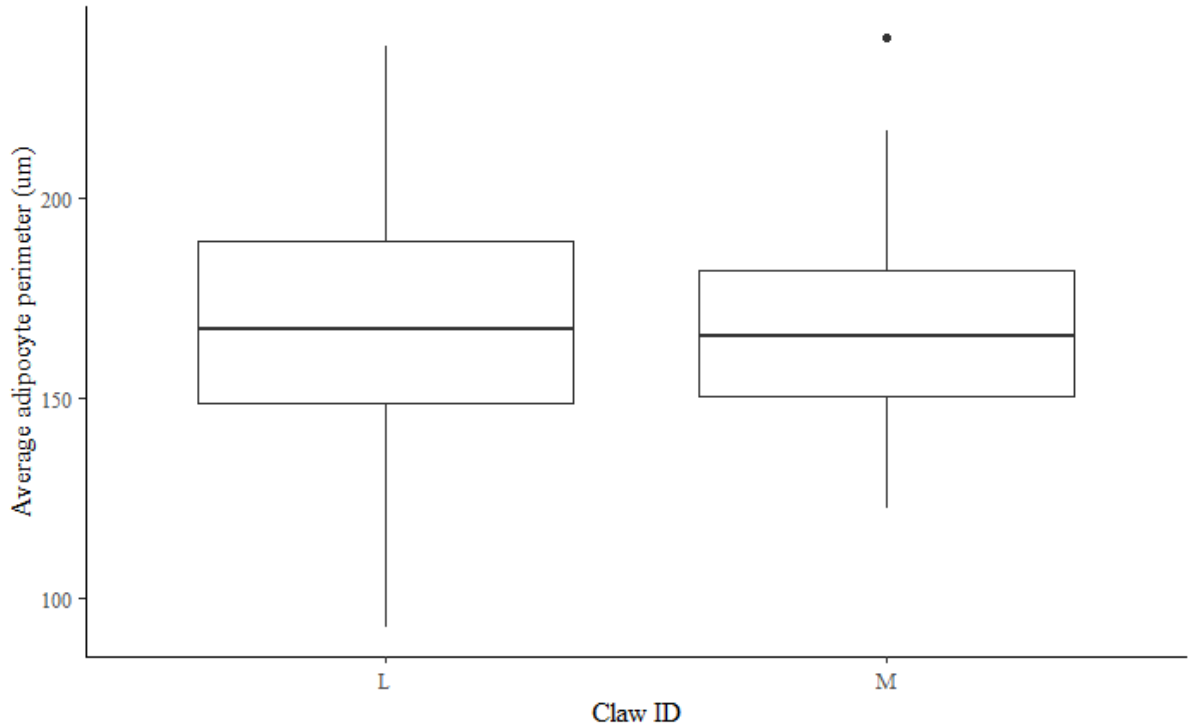


Figure 4-13 A boxplot illustrating the difference in adipocyte perimeter (um) between the lateral (L) and medial (M) claws. The cell area was determined by calculating the average cell area for each cushion where >200 adipocytes were measured for that individual site, an average of this was taken for each animal's lateral and medial claw. These samples were dissected from a cohort of 13 animals culled from the Langhill herd based at SRUC's Crichton Royal Farm. This represents pilot data from a more comprehensive dataset.

4.4 Discussion

4.4.1 Summary of Results

This is the first study to describe the collagen content of the digital cushion, in a large sample of adult dairy cattle. The current study identified a substantial range in the collagen composition of the digital cushion present within the lateral claws of animals at cull (23.6 – 83.8%). Animals that had an increased mean lameness score in the four weeks before slaughter were more likely to have a decreased proportion of Type 1 collagen in their digital cushion in the lateral claws at cull. This decreased proportion of Type 1 collagen thereby means that an increased proportion of the collagenous tissue contained within the digital cushion was Type 3 (due to proportion of Type 1 collagen being calculated as a function of the sum of Type 1 and Type 3 collagen). The ranges of adipocyte sizes (cross-sectional area) and perimeters were also identified in the pilot data presented within this Chapter. For the purposes of this discussion, the results calculated from the PSR analysis are discussed in detail will be discussed.

4.4.2 Causality

As described in Chapter 3, the retrospective nature of this study design means that the direction of causality cannot be established from our results. There are two clear mechanisms through which the differences in collagen content may be associated to lameness, namely, 1) The study animals experienced lameness which is underpinned by localised inflammatory mechanisms occurring within the hoof capsule. These inflammatory mechanisms could lead to a turnover of collagen tissue and the remodelling of the adipose tissue supported therein. 2) A non-lameness-based event occurred to cause a change in the content of collagen within the digital cushion which predisposed the animals to lameness, thereby creating the association observed within the current study. Regardless of the direction of causality, however, there is a link between lameness before cull, and the proportion of Type 1 (and from the opposite results Type 3) collagen contained within the lateral claws of the hindlimbs at cull.

4.4.3 Collagen Structures

Type 1 collagen is essential for the organisation and competence of many key structures throughout the body (irrespective of species). It is organised into regular fibril "bundles" which can undergo post-transcriptional changes to optimise functionality in-situ (Charvolin and Sadoc, 2019). Type 3 collagen exists as single fibrils and is essential in the healing of wounds (Volk et al., 2011). Typically Type 3 collagen is deposited first, and the proportion of Type 1 increases as wound healing progresses (Clore et al., 1979). Furthermore, Type 3 collagen is typically deposited by mesenchymal cells (particularly fibroblasts), which means it has a substantial role to play in various inflammatory related conditions (such as Crohn's disease and lung disease in humans) (Ku et al., 2006). It is understood that the MMPs have a specific role to play in the degradation of collagen (Van Doren, 2015), which is typically not affected by proteolytic enzymes and processes. MMPs are expressed as inflammatory mediators (Van Doren, 2015), highlighting a potential inflammatory pathway through which the proportion of collagens may be altered due to pathology.

4.4.4 Collagen and the Digital Cushion

Previous studies have identified how the digital cushions of older animals appeared to contain more densely collagenous tissue than those of heifers (Räber et al., 2004). The authors of that study suggested that changes occurred within the first lactation which led to differences in the digital cushion structure. They went on to elaborate how CHL pathogenesis and calving/transition may have a role to play in the alteration of digital cushion structure.

The proposed inflammatory pathogenic mechanisms underpinning CHL aetiology (Newsome et al., 2016) may directly affect the digital cushion in other means than purely utilisation of fatty acids as mediators in biochemical processes. If the inflammatory processes described by Newsome et al. (2016) are occurring within the hoof capsule, then it is likely that these could have an impact on the proportions of Type 1 and Type 3 collagen contained within the digital cushion. The impacts of this change in proportion of collagen are unknown, however it could be hypothesised that an increase of Type 3 collagen (which is often indicative of scarring), may inhibit the digital cushion's ability to dissipate the concussive forces associated with locomotion and standing. This could be due to two

mechanisms, firstly, the irregularity in structure of Type 3 collagen may mean that it is less structurally "sound" than Type 1 collagen (i.e., it offers less support and structure to the adipocytes contained within the digital cushion) (Clore et al., 1979). Additionally, the deposition of more Type 3 collagen will likely mean that the proportion of collagen to adipocytes is likely to change also. This may, once again, impede the functionality of the digital cushion in its ability to protect the corium from excessive compression. This could occur, even once Type 1 collagen has been deposited as part of the healing process, as this will likely alter the proportion of collagen to adipocytes once again, thereby changing the structure and the ability of the digital cushion to support the pedal bone within the hoof capsule. Newsome et al. (2016) also identified that exostoses developed on the most caudal aspect of the pedal bone, and the extent of these bony proliferations were correlated with the lameness history of the animal. The authors went on in a subsequent publication (Newsome et al., 2021) to explain how the development of this exostoses could deteriorate the structure of the digital cushion as they both exist in the same site and so interaction is highly likely. The authors suggested that the histology of the digital cushion may change due to the trauma exerted on it by the excessive development of new bone, thereby predisposing the animal to future CHLs due to the compromised digital cushion alongside the development of the exostoses. These changes to the structure of the digital cushion could potentially "lower the tolerance" of the functional anatomy of the hoof to compressive and concussive forces. This will mean that the threshold required for a CHL to occur will be lowered considerably, meaning that the animal will be predisposed to future CHLs.

Furthermore, the ability of the digital cushion to act as a metabolically active tissue may change as the differences in structure continue to become vaster. One mechanism through which this could occur is through fatty acids becoming less readily available locally within the hoof capsule due to adipose tissue remodelling as described by Contreras et al. (2017a; b). This could lead to other localised changes to anatomy and metabolic function within the hoof capsule which may predispose the animal to future CHLs.

Another mechanism through which this change in the collagenous makeup of the digital cushion could occur is through the role of relaxin and systemic inflammation around the time of parturition

and transition. Relaxin is produced and circulates systemically around the time of calving to slacken the ligaments around the birth canal to allow easier passage of the neonate (Sherwood, 2004). This, however, is hypothesised to also function on the suspensory apparatus within the hoof capsule, meaning that this structure is weakened, thereby lowering the tolerable force threshold and predisposing animals to CHLs (Tarlton et al., 2002; Knott et al., 2007). The mechanism through which relaxin functions is through MMP activation (Kapila et al., 2009). MMPs are understood to be one of the few groups of enzymes which are able to degrade collagen fibres (Van Doren, 2015), meaning that they may have a potential impact on the collagen content of tissues systemically, including those contained within the digital cushion. This could mean that the structure of the digital cushion post-partum is disrupted, leading to a risk period immediately after parturition in which the corium is susceptible to contusions caused by excessive forces being exerted by the pedal bone. MMP activation also occurs as a part of normal inflammatory processes. Bradford et al. (2015) described how dairy cattle undergo a degree of systemic inflammation during the transition period. The degree to which this inflammation occurs has been linked to the "success" of the animals upcoming lactation, with a predisposition to numerous diseases and conditions being linked to an excessive inflammatory response during transition. If an individual undergoes an excessive inflammatory response, then it would be logical to state that the level of inflammatory mediators circulating and functioning systemically would be increased. An animal that is experiencing a high degree of systemic inflammation at the time of calving may experience a higher degree of collagen degradation due to an increased level of MMPs circulating and functioning locally. If higher levels of MMPs are present within the hoof capsule, then this may degrade the collagen contained within the suspensory apparatus and the digital cushion, meaning that the functional anatomy of the hoof will have a lower tolerance for the concussive and compressive forces associated with locomotion and standing respectively, meaning that contusions within the corium are more likely to occur, leading to the disruption of horn production and CHL onset.

The degradation of collagenous structures driven by MMPs may lead to the permanent compromising of the digital cushions ability to adequately support the pedal bone within the hoof capsule. If animals

experience several calvings and the transition periods (and thereby the systemic inflammation associated with these), then this degradation and compromise of the digital cushions functional structure may accumulate over several lactations. The current study did not isolate age or DIM as a risk factor for a decreased Type 1 (and thereby an increased Type 3) collagen content, however, the cross-sectional nature of this study was not designed to investigate the specific effect of age on the digital cushion. A means of collecting multiple samples from live animals across the duration of a lactation may allow for a more comprehensive investigation of the effect of transition and age on the digital cushion's structure.

4.4.5 Predisposition

The genetic and developmental components associated with digital cushion development have already been described in Chapter 3.4.3. However, it could be hypothesised that the histological structure of the digital cushion may vary between animals due to their genetic potential and the rearing system in which they were managed as youngstock. No research in this field has examined the effects of either of these inputs on the histological structure of the digital cushion, nor on the lameness outcomes potentially generated from such studies. Understanding how the proportions of collagen and adipocytes impact on the functionality of this structure may provide a crucial insight into how the structure of the digital cushion functions across a lactation and the challenges contained therein. Future research is warranted in this area, given the large amount of unexplained variation described in the current study.

4.4.6 Haematoxylin and Eosin Pilot

This pilot study is aiming to expand on the current understanding of adipocyte morphology contained within the digital cushion. Previous work has already comprehensively described the associations between BCS, age, etc. and the histology of the digital cushion (Räber et al., 2004, 2006; Hiss-Pesch et al., 2019; Newsome et al., 2021), however there is currently no published research exploring the associations between lameness and the histology of the digital cushion. The current data has identified that between each individual cushion within each claw there is variation in adipocyte morphology which are similar to those described by Hiss-Pesch et al. (2019). The next stage of this research is to

generate further data to allow for suitably powered statistical analysis to explore associations between adipocyte morphology within the digital cushion and the history of lameness for that respective individual.

4.4.7 Limitations

There are several limitations which should be considered when interpreting the findings of our study. Firstly, and as previously stated, the direction of causality cannot be attributed from a retrospective study such as this. The associations described should be interpreted with care and it is recommended that they are used to inform hypotheses for future prospective intervention studies. Secondly, the study population examined was based on a convenience sample of 54 animals from a single UK dairy research centre. These animals were selected for further analysis as they had a comprehensive lameness history up to the point of cull, which may have created a bias in the population studied. It is not possible to comment on the extent of any potential bias or the impact of that potential bias on the results, however, no factors which would suggest the animals studied are not representative of intensively managed dairy cattle have been identified. The use of cadaver material should also be considered when interpreting our results, with the observations of the current study representing an end-of-life effect which is the culmination of all experiences the animal may have had.

4.4.8 Conclusions

The proportion of Type 1 collagen in the lateral claws of the hind limbs varies substantially (23.6-83.8%) between adult dairy cattle as did the proportion of Type 3 (16.2%-76.4%) respective to each other. The average lameness score in the four weeks before cull explained a small proportion of this variation. A large proportion of the observed variation remains unexplained. This chapter hypothesises that some of this variation can be attributed to the inflammatory pathogenesis associated with CHLs. This inflammatory response may cause the synthesis of “newer” Type 3 collagen, thereby reducing the proportion of Type 1 collagen. The impacts this may have on the digital cushion’s functionality warrant further investigation, however it could be postulated that this deposition of Type 3 collagen may deteriorate the digital cushion’s ability to dissipate the concussive forces of locomotion and standing. It would also be useful to undertake further studies into the overall impact

of collagen content versus adipocyte content within the cushion based on differing factors. Whether not just structure could be affected but other functions such as fatty acid deposition and release are affected.

Future research furthering our understanding of the histology of the digital cushion in relation various inputs (e.g., lameness events) and how these inputs alter the overall structure and function of the digital cushion is required. If the aetiopathogenesis of CHLs is better understood (particularly in relation to digital cushion structure), then better treatment and prevention protocols can be developed and tested to improve the mobility of the global dairy herd.

5 Non-Steroidal Anti-Inflammatory Drugs at Calving Reduce the Lifetime Risk of Lameness: A Randomized Controlled Trial in Cows from First Calving.

5.1 Introduction

Lameness presents a substantial challenge to the sustainability of the dairy industry. It causes financial losses through depression of milk production, reduced reproductive efficiency and increased culling risk (Huxley, 2013), and greatly impacts cow welfare (Whay and Shearer, 2017). Since the current mean prevalence of lameness is estimated at ~30% in the UK (Griffiths et al., 2018; Randall et al., 2019b), between ~22-55% in the USA (Cook, 2003; Espejo et al., 2006; Von Keyserlingk et al., 2012), and ~19% in Australia (Ranjbar et al., 2016) a substantial reduction in lameness remains a key goal for the global dairy industry.

The causes of lameness can broadly be categorised into infectious conditions (such as digital dermatitis and interdigital necrobacillosis) and the CHLs. CHLs are primarily SU, SH and WLD. Claw horn lesions are reported to be the most common cause of lameness in intensive dairy systems (Leach et al., 2012; Solano et al., 2016).

Whilst the aetiopathogenesis and epidemiology of infectious causes of lameness are well described (Blowey and Sharp, 1988; Krull et al., 2016; Kontturi et al., 2019), the underlying pathological mechanisms and risk factors for CHLs are less well understood (Randall et al., 2018b). Mechanisms reported to increase the risk of CHLs include local inflammatory events within the hoof (Newsome et al., 2017a), weakening of the suspensory apparatus attaching the distal phalanx to the claw capsule around parturition (Tarlton et al., 2002; Knott et al., 2007), mobilisation of adipose from the digital cushion in the rise to peak milk yield (Bicalho et al., 2009; Newsome et al., 2016) and excessive pressures on the corium arising from external environmental factors (Nuss et al., 2019). However, none of these pathways has led to a clear understanding of how to prevent lameness derived from CHLs.

Recently, the role of subacute inflammation during the transition period has been highlighted as a possible mechanism underlying the risk of poor health outcomes in dairy cows (Bradford et al., 2015). It is believed that all cows experience some degree of systemic inflammation in the days after parturition and that the extent of such inflammatory processes may dictate the risk of subsequent disease. Subacute systemic inflammation around the time of parturition may affect physiological processes in the hoof, such as lipolysis of the digital cushion and / or weakening of the suspensory apparatus, which could thereby predispose a cow to CHLs. It is possible that the use of NSAIDs at parturition could limit the extent of subacute inflammation and reduce the risk of lameness.

NSAIDs have already been shown to improve cure rates for the treatment of CHLs (Thomas et al. 2015) indicating these products may have a role in treatment as well as prevention of lameness. However, evidence to the contrary also exists (Laven et al., 2008) (both studies contain challenges in their design and implementation which has been previously described in section 1.3.7) Since it has been shown that once cows become lame, they have an increased lifetime risk of lameness (Randall et al., 2016, 2018a), and it has been hypothesised that inflammation may contribute to pathological change in the hoof (Newsome et al., 2016), it is possible that use of NSAIDs at treatment could also reduce the risk of lameness in later life.

To this regard, the prevention of heifers first becoming lame and the early intervention in first lameness cases appears critical in ensuring that these animals are not predisposed to a future lifetime of lameness. If the prevention of lameness or even the intervention early in first lameness is achieved successfully with an appropriate treatment regime, then it may be possible to minimise the predisposing effect that lameness has on the animal's future lifetime risk.

The null hypothesis was that strategic administration of NSAIDs at first and subsequent calvings and when treating animals for lameness would have no impact on the lifetime risk of lameness in dairy cows; this was tested in a randomised controlled trial over three years. A cohort of dairy heifers, enrolled at first parturition, were allocated to one of four intervention groups, for the duration of the study, to evaluate the impact of NSAIDs administered at parturition and as a lameness treatment, on the lifetime risk of lameness and culling.

5.2 Materials and Methods

5.2.1 Study Design

A randomised controlled trial (RCT) was designed and conducted in accordance with the REFLECT guidelines (O'Connor et al., 2010), to investigate the efficacy of NSAIDs in the treatment and prevention of lameness. The study was conducted with permission from The University of Nottingham, School of Veterinary Medicine and Science ethics committee (Reference No: 1913 161208). The study was granted approval by the Veterinary Medicines Directorate under an Animal Test Certificate Type S (Reference No: ATC-S-090).

5.2.2 Study Herd and Herd Management

A description of the study herd is given in Chapter 2.3.

5.2.3 Enrolment

All heifers that calved into the study herd between 8th January 2018 and 22nd June 2020 were eligible for recruitment. Animals were allocated to one of four treatment groups using a block randomisation procedure based on the animal's expected calving date (calculated from insemination and pregnancy diagnosis by a veterinarian) as follows. Animals were listed in chronological order of expected calving date from the earliest to the latest for a four-month period. Animals were chronologically grouped into fours based on the expected calving date and allocated one of the four treatment groups which was unique to that block of four, using an online random block generator (Haahr, 2012). Cows remained in the study until culling, or the study ended. Heifers that aborted or calved greater than two weeks earlier than their expected first calving date were not included in the study. All study animals were intermingled and managed within the herd for the duration of the study and were therefore subjected to all routine day-to-day management procedures.

5.2.4 Treatment Groups

The RCT was designed to evaluate four different approaches to the treatment and prevention of lameness.

Animals were assigned to one of four treatment groups which they remained in for the duration of the study. The treatment groups and their respective regimens were as follows:

- Group 1 animals received a therapeutic trim (as described below) and received an orthopaedic block (wooden block, ergonomically shaped to the claw of the cow; 110mm X 55mm X 25mm – dimensions given at widest/longest points) on the sound claw if deemed appropriate by the operator, every time they were identified and treated for lameness.
- Group 2 animals received a therapeutic trim, an orthopaedic block if deemed appropriate by the operator and a three-day course of the NSAID ketoprofen (Irrespective of the cause of lameness, Dinalgen, 150mg/ml, Bayer PLC, administered by deep intra-muscular injection, dose rate – 14ml/animal/day), every time they were identified and treated for lameness.
- Group 3 animals received a therapeutic trim, an orthopaedic block if deemed appropriate by the operator and a three-day course of the NSAID ketoprofen every time they were identified and treated for lameness (dose as for Group 2). In addition, animals in this group, received a three-day course of ketoprofen every time they calved (first dose administered 24-36 hours after calving, with two further doses 24 and 48 hours administered after the first dose; Dinalgen, 150mg/ml, Bayer PLC, administered by deep intra-muscular injection, dose rate – 14ml/animal/day).
- Group 4 animals received a three-day course of the NSAID ketoprofen every time they were identified with lameness (dose as for Group 2). No therapeutic trim or orthopaedic block was administered unless they were scored as “severely lame” (i.e., Score ≥3a).

The treatments provided for each group are summarised in Table 5-1.

Treatment	Lameness Trim	Lameness NSAID	Calving NSAID
Group 1	✓	×	×
Group 2	✓	✓	×
Group 3	✓	✓	✓
Group 4	× (unless severely lame)	✓	×

Table 5-1 A summary of the four treatment regimens applied in a three year long randomised controlled trial investigating the efficacy of non-steroidal anti-inflammatory drugs in the treatment and prevention of lameness and the subsequent risk of culling.

5.2.5 Treatment Routines

The treatment cycles described were conducted by administrating technicians (JW, DB, NB, JT, HHA) as follows. On a fortnightly basis, the entire milking herd would be lameness scored by JW (with NB providing holiday cover). All lame animals that were not in their refractory period would be listed alongside their lameness score and the limb identified as lame. Treatments would be noted in the form of "Trim", "NSAID" or "Trim and NSAID" for each animal. All animals would be treated within 72 hours of being scored as lame. JW and DB administered most lameness treatments concurrently using two crushes, with NB providing relief cover. Whilst treatments were administered, NB recorded treatments, lesions present, other comments deemed appropriate to make, and carried out other study administration. Treatment recordings were made on a specific data capture form. As animals were treated, their unique ID was added to a list denoting those requiring intra-muscular injection and the relevant NSAID was drawn into syringes for administration by farm staff over the following days. At the end of each treatment day, both the list denoting animals requiring treatment and the list of animals to be treated was cross-checked to ensure no errors occurred and to identify animals that required treatment the following day(s). NB managed all data input in a relational database (Microsoft Access, Microsoft Corp., 2016) which was used to construct lists of animals requiring blocks to be removed as described previously.

Three trained technicians (JW, DB, NB) undertook all lameness treatments; hoof trimming protocols and implementation were reviewed on an annual basis by a specialist external hoof care auditor to ensure consistency and best practice in trimming technique was being maintained (as described in Chapter 2.3.5). Animals were trimmed using the Dutch Five-Step Method (Toussaint Raven, 1971) with the adaptations described by Archer et al. (2015) and Stoddard (2018). Both the lame limb and the contralateral limb were examined in all animals and trimmed as appropriate. Blocks were applied to healthy contralateral claws as required.

Blocks were removed according to the timeframes above. All blocks were removed unless it was deemed to potentially compromise the immediate welfare of the animal. Once the block was removed, a therapeutic trim was administered to restore balance between the two claws to ensure an even weight bearing area was created without compromising claw integrity. This trim took the format of the Dutch Five-Step Method with the two adaptations previously described.

Once the necessary blocks were removed, all animals in the milking herd were scored by one of the two trained outcome scorers (JT or HHA). Animals were scored in the same fashion as for the determination of treatment. Typically, the study involved administering technicians visiting the farm site for four working days in each fortnightly rotation.

5.2.6 Lameness Outcome Scores

Lameness outcomes were collated in the form of a blinded lameness score in which the operating technician had no other involvement in farm management or trial administration. A comprehensive description of the outcome lameness scoring is given in Chapter 2.3.4.

5.2.7 Data Collection and Descriptive Analysis

Data were collated on a fortnightly basis and stored in a relational database (Microsoft Access, Microsoft Corp., 2016). The major outcomes of interest were incidences of lameness (measured through blinded fortnightly lameness scoring) and time to culling (determined by on farm recordings of culling or death). Lameness was defined as a binary variable for each fortnightly score; lame cows were categorised if their score was ≥2a and non-lame if their score as <2a. Severe lameness was

defined in a similar manner but using a threshold for lameness of ≥3a. Scores were amalgamated to allow for a binary lame/non-lame category to be created.

To ensure that there was no influence on the subjectivity of either the "treatment" or the "outcome" scores, the outcome lameness scores were stored and managed separately from the remaining data. Data handling and analysis were carried out using Microsoft Excel 2016 (Microsoft Corp., 2016) and R V4.0.3 (R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). Data collated included calving dates, treatment groups, lameness scores, time on the study and culling date. Initial data handling, and screening was undertaken to identify missing or anomalous data. Anomalous lameness scores were thoroughly examined to determine the origin of any error before inclusion or exclusion from the final dataset, as was the recorded cull data.

5.2.8 Statistical Analysis

To evaluate the study hypotheses, three statistical models were constructed:

1) Two mixed effects logistic regression models were used to evaluate the impact of treatment group on the risk of lameness or severe lameness over time using the R package "lme4" (Bates et al., 2015). Lameness was defined as a single lameness score submitted as equal to or greater than 2a. Severe lameness was defined as a single lameness score submitted as equal to or greater than 3a. The mixed-effects logistic regression model took the form:

$$\text{Lameness}_{ij}\,(1=\text{lame},\ 0=\text{non-lame}) \sim \text{Bernoulli}\,(\mu_{ij})$$

$$logit(\mu_{ij}) = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j + u_j$$

$$u_j \sim N(0, \sigma^2)$$

Where μ_{ij} was the probability of an animal being lame at the ith mobility score of the jth cow, β_0 the model intercept , x_{ij} a matrix of covariates linked to each lameness score (e.g. days in milk or week of study), $\beta_1$the coefficients for x_{ij}, x_j a matrix of covariates linked to each cow (e.g. treatment group),

β_2 the coefficients for x_j, u_j represented a random effect for the j[th] cow and σ^2 the variance of the random effects u_j.

Covariates were deemed significant and remained in the model when P<0.05. Interactions between significant covariates were tested and retained in the model when P<0.05. Investigation of model fit was made by assessing realised discrepancies between cumulated predicted probabilities and observed outcomes (Gelman et al., 1996) and the assessment of normality of random effects. Since random effects were over dispersed, the final model was repeated with outlying cows omitted (>10 incidents of lameness during the study period) to check there were no meaningful changes in model parameters or their interpretation. Predictions from the final model were visualised graphically to illustrate the lameness risk by treatment group for the duration of the study.

2) A Conventional Cox proportional hazards model was constructed using the "survival" (Therneau and Grambsch, 2000) and "survminer" (Kassambara et al., 2020) packages in R to investigate the effect of treatment group on the risk of culling, the model took the form:

$$h(t) = h_0(t) \text{ x } \exp(\beta_1 x_1 \ldots \beta_p x_p)$$

Where t represented the survival time to culling, $h(t)$ the hazard function which was dependent on a baseline hazard $h_0(t)$ and p covariates $(x_1 \ldots x_p)$ with $\beta_1 \ldots \beta_p$ the related coefficients.

Covariates were deemed significant and retained in the model when P<0.05. Evaluation of the proportional hazard's assumption and model fit was undertaken by visual assessment of Schoenfeld residuals, the log-log curves and delta betas (Schoenfeld, 1982; Grambsch and Therneau, 1994). The data were visualised graphically using Kaplan-Meier plots to illustrate time to culling by treatment group.

5.3 Results

5.3.1 Study Denominators

Pre-calving randomisation led to the following recruitment: 132 animals to Group 1, 132 to Group 2, 132 to Group 3 and 132 to Group 4. Of those, 116 in Group 1, 120 in Group 2, 112 in Group 3 and

118 in Group 4 successfully calved within two weeks of their expected calving date and continued in the study. Subsequently 20 cows (5 from Group 1, 3 from Group 2, 6 from Group 3 and 4 from Group 4) were removed because of severe issues at first calving which resulted in them not being presented for lameness scoring and 8 animals (0 from Group 1, 0 from Group 2, 6 from Group 3 and 2 from Group 4) were removed because they posed a danger to farm staff or technicians during handling for lameness treatment. These reasons for exit were considered to have occurred at random, i.e., they were not linked to treatment group. Therefore, a total of 438 animals were included in the final analysis with 111 in Group 1, 117 animals in Group 2 animals, 100 animals in Group 3 and 110 animals in Group 4.

5.3.2 Descriptive Statistics

Study animals received 973 lameness treatments (271 under Group 1 regimens, 254 under Group 2, 151 under Group 3 and 297 under Group 4). From the lameness treatment records, 99 cases of lameness were associated with infectious causes (e.g., digital dermatitis), 675 with CHLs (SH, SU, WLD) and 199 with other sources (e.g., musculoskeletal damage, no visible claw or skin lesion). Outcome lameness scores were collated for the entire study period (146 weeks), yielding 13,886 individual lameness scores. A summary of the treatments administered, and outcome lameness scores are provided in Table 5-2.

During the study period, a total of 106 study animals were culled (38 from Group 1, 24 from Group 2, 17 from Group 3 and 27 from Group 4). Primary reasons for culling as recorded by farm staff included fertility (n = 50), lameness (n = 8), udder health (n = 8), other (n = 22), and no recorded reason (n=18). A description of the culling reasons by treatment group is described in Table 5-3.

	Grp1	Grp2	Grp3	Grp4
Proportion of outcome lameness scores submitted as lame.	892/3605	870/4044	564/2899	859/3338
Proportion of outcome lameness scores submitted as severely lame.	79/3605	55/4044	23/2899	70/3338
Number of lameness treatments administered.	271	254	151	297
Incidence of Treatment (cases of treatment per cow-week)	0.08	0.06	0.05	0.09
Average lameness treatments administered per animal (Min-Max).	2.13 (0-19)	1.90 (0-9)	1.63 (0-13)	2.48 (0-17)
Number of orthopaedic blocks applied.	87	82	68	34
Interim “First aid” treatments required.	4	7	2	19
Veterinary referrals.	2	1	0	1
Cause of lameness – SH.	169	141	101	22
Cause of lameness – SU.	34	33	21	21
Cause of lameness – WLD.	30	21	14	68
Cause of lameness – DD.	28	20	15	36

Table 5-2 Descriptive statistics of the lameness data derived from a three year long randomised controlled trial investigating the efficacy of non-steroidal anti-inflammatory drugs in the treatment and prevention of lameness and the subsequent risk of culling. The dataset this is constructed from contains 13,886 individual lameness scores from 438 animals over a three-year period. Animals were recruited at first calving, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

Treatment Group	Culling Reason				
	Udder Health	Fertility	Lameness	Other	No Recorded Reason
1	4	19	3	5	7
2	1	10	2	6	5
3	1	5	2	5	4
4	2	16	1	6	2

Table 5-3 Descriptive statistics of the culling data derived from a three year long randomised controlled trial investigating the efficacy of non-steroidal anti-inflammatory drugs in the treatment and prevention of lameness and the subsequent risk of culling. The dataset this is constructed from contains 106 cull recordings from 438 animals over a three-year period. Animals were recruited at first calving, and lameness/calving treatments were administered according to the treatment group to which the animal belonged. Culling reasons were determined by farm managers and were not influenced by study administrators.

5.3.3 Statistical Modelling

5.3.3.1 Model 5A. Mixed-effects logistic regression model to evaluate the risk of lameness (score ≥2a).

Final results of Model 5A are provided in Table 5-4. Compared to cows in Group 1, cows in Group 3 were less likely to become lame during the study period (Odds Ratio (OR) 0.66, *P=0.03*). Cows in Group 2 were at a substantially reduced risk of experiencing lameness in their lives, however this difference was not statistically significant. The risk of lameness over time for cows in each treatment group is illustrated in Figure 5-1. Model fit was identified to be good, and omission of outlying cows had no impact on model parameters (<5% alteration in coefficients and no changes in significance Figure 5-2, Figure 5-3).

	Estimate	Odds Ratio	Standard Error	P Value
Intercept	-7.01	<0.01	1.54	5.44E-06
Treatment Group 1	Reference	–	–	–
Treatment Group 2	-0.29	0.75	0.17	0.10
Treatment Group 3	-0.42	0.66	0.19	0.03
Treatment Group 4	0.03	1.04	0.18	0.83

Table 5-4 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of lameness (lameness score ≥2a). The dataset this model is constructed from contains 13,886 individual lameness scores from 438 animals over a three-year period. Animals were recruited at first calving, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

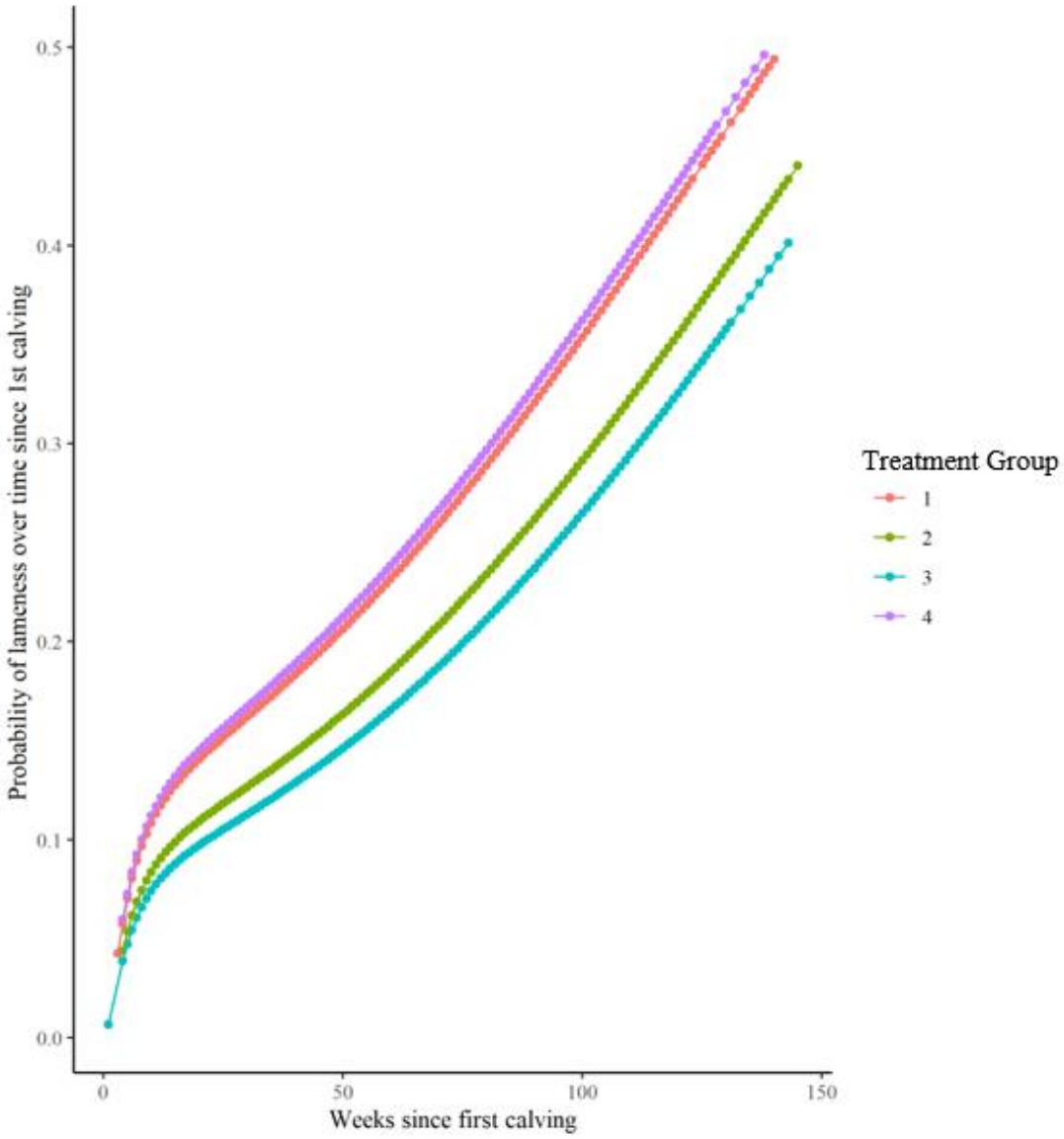


Figure 5-1 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of lameness (lameness score ≥2a) described in Table 5-4. The dataset this model is constructed from contains 13,886 individual lameness scores from 438 animals over a thirty-four-month period. Animals were recruited at first calving, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

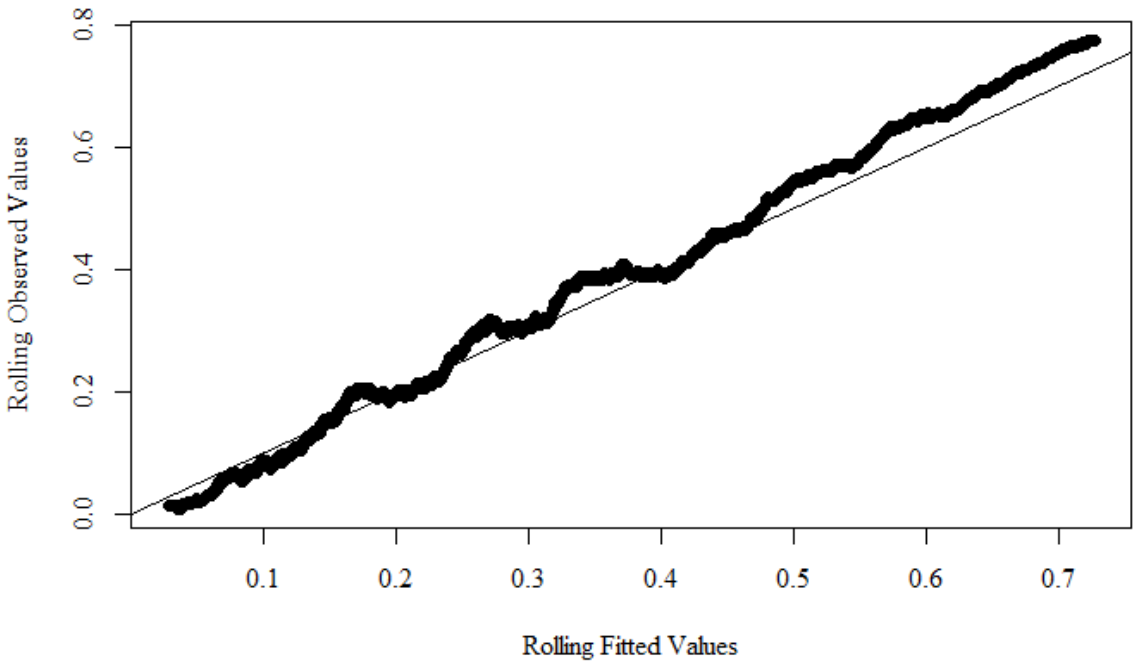


Figure 5-3 A plot of the 800-day rolling fitted and observed values generated from Model 5A which is described in Table 5-4. Model 5A investigated the effect of treatment group on the risk of an animal being scored as lame (≥ score 2a) over time. The fitted and observed values closely aligned and so model fit was deemed good.

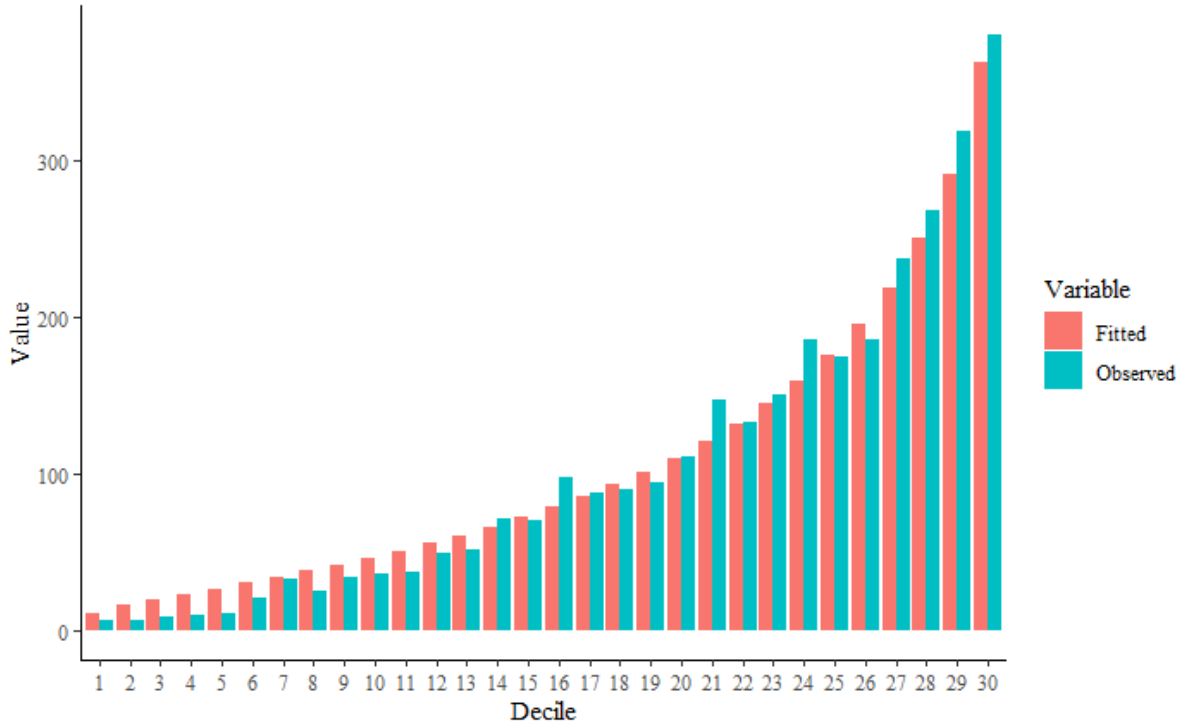


Figure 5-2 A plot of the fitted and observed values for model 5A which is described in Table 5-4. Model 5A investigated the effect of treatment group on the risk of an animal being scored as lame (≥ score 2a) over time. The fitted and observed values are consistently similar and so model fit was deemed as good.

5.3.3.2 Model 5B. Mixed-effects logistic regression model to evaluate the risk of severe lameness (score ≥3a).

Final results of Model 5B are provided in Table 5-5. Compared to cows in Group 1, cows in Group 3 were less likely to become severely lame during the study period (OR=0.28, *P=0.04*). The risk of severe lameness over time for cows in each treatment group is illustrated in Figure 5-4. As for Model 5A, model fit was good (Figure 5-5, Figure 5-6).

	Estimate	Odds Ratio	Standard Error	P Value
Intercept	-23.70	5.08E-11	6.69	3.93E-04
Treatment Group 1	Reference	–	–	–
Treatment Group 2	-0.50	0.61	0.51	0.32
Treatment Group 3	-1.27	0.28	0.60	0.04
Treatment Group 4	-0.30	0.74	0.53	0.57

Table 5-5 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of severe lameness (lameness score ≥3a). The dataset this model is constructed from contains 13,886 individual lameness scores from 438 animals over a thirty-four-month period. Animals were recruited at first calving, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

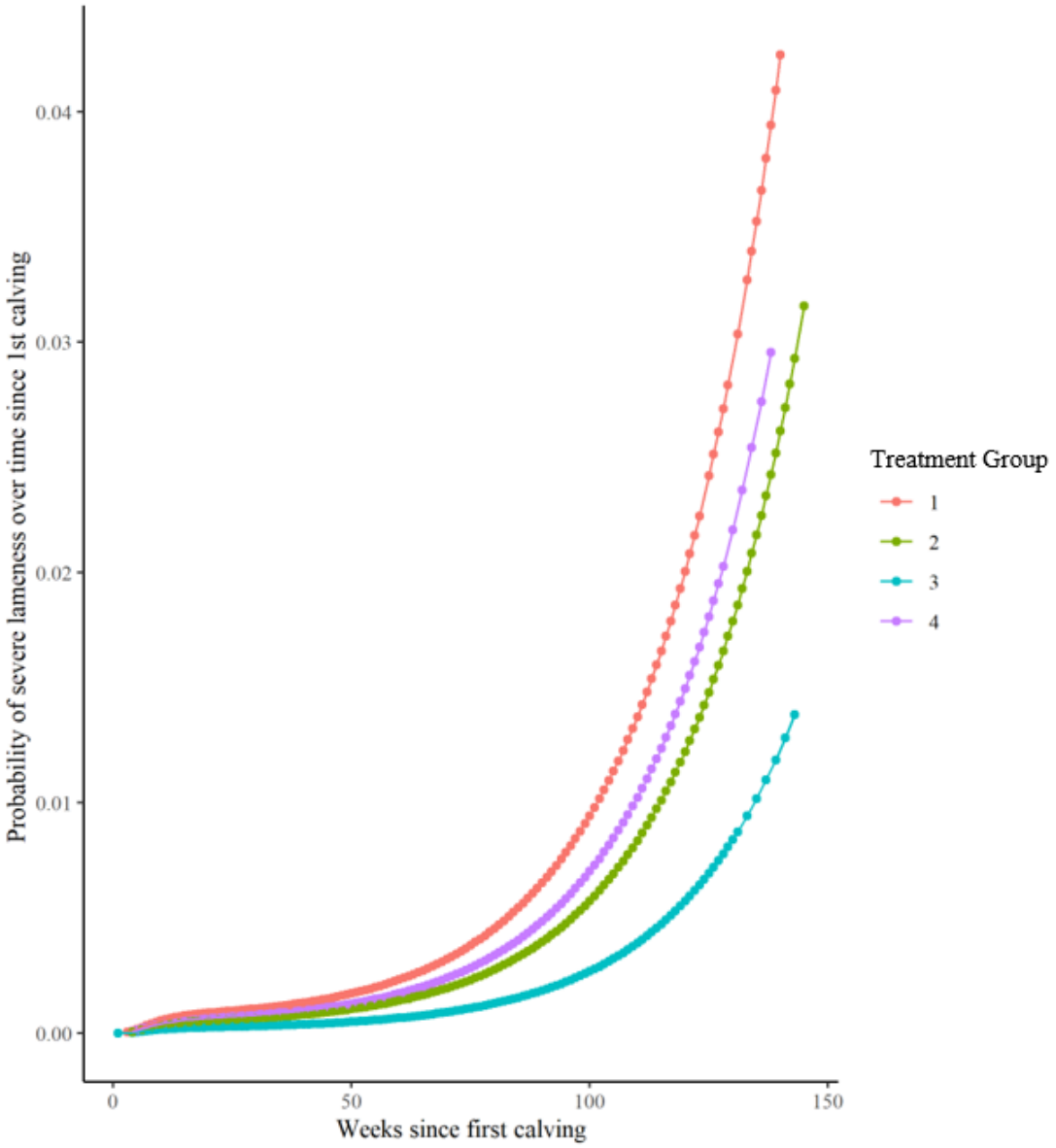


Figure 5-4 A graphical illustration of predictions from the mixed effects logistic regression model described in Table 5-5 investigating the impact of treatment group on the risk of lameness (lameness score ≥3a). The dataset contained 13,886 individual lameness scores from 438 animals over a thirty-four-month period.

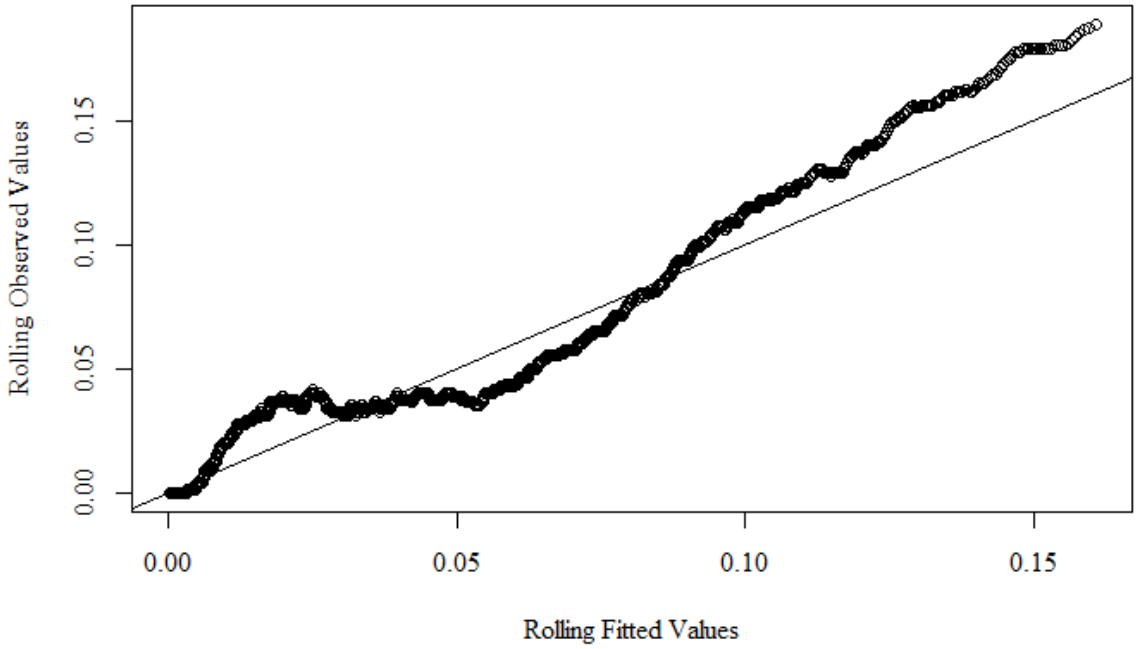


Figure 5-5 A plot of the 800-day rolling fitted and observed values generated from Model 5B which is described in Table 5-5. Model 5A investigated the effect of treatment group on the risk of an animal being scored as lame (≥ score 2a) over time. The fitted and observed values closely aligned and so model fit was deemed good.

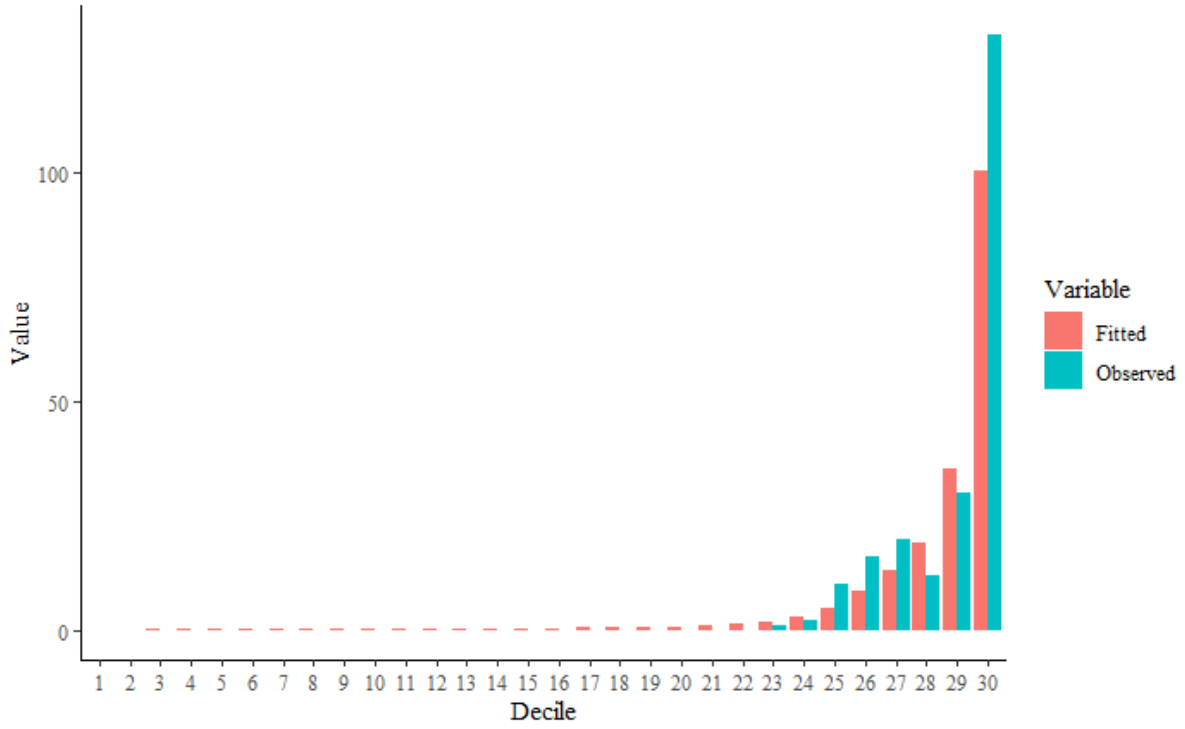


Figure 5-6 A plot of the fitted and observed values for model 5B which is described in Table 5-5. Model 5B investigated the effect of treatment group on the risk of an animal being scored as lame (≥ score 2a) over time. The fitted and observed values are similar and so model fit was deemed as good.

5.3.3.3 Model 5C. Cox proportional hazards model for survival to culling.

Final results of Model 5C are provided in Table 5-6. Compared to cows in Group 1, cows in Groups 2 and 3 were at a reduced risk of culling during the study period (Hazard Ratio=0.55 (*P=0.02*) and 0.56 (*P<0.05*) respectively). A Kaplan-Meier plot to illustrate survival time to culling by treatment group is presented in Figure 5-7. Investigations of model fit indicated that the model assumption of proportionality of hazards was met and residual analysis indicated fit was good (Figure 5-8).

Model term	Coefficient	Hazard Ratio	P Value
Treatment Group 1	Baseline	–	–
Treatment Group 2	-0.61	0.55 (95% CI: 0.33 – 0.91)	0.02
Treatment Group 3	-0.58	0.56 (95% CI: 0.32 – 0.99)	<0.05
Treatment Group 4	-0.29	0.75 (95% CI: 0.46 – 1.2)	0.25

Table 5-6 Results of the final Cox proportional hazards model for time to culling. The dataset on which this model is based consists of 438 animals within a single commercial herd, of which 106 were culled during the study period. Animals were recruited at first calving, and 'Treatment Group' represents four different types of treatment administered at lameness and calving.

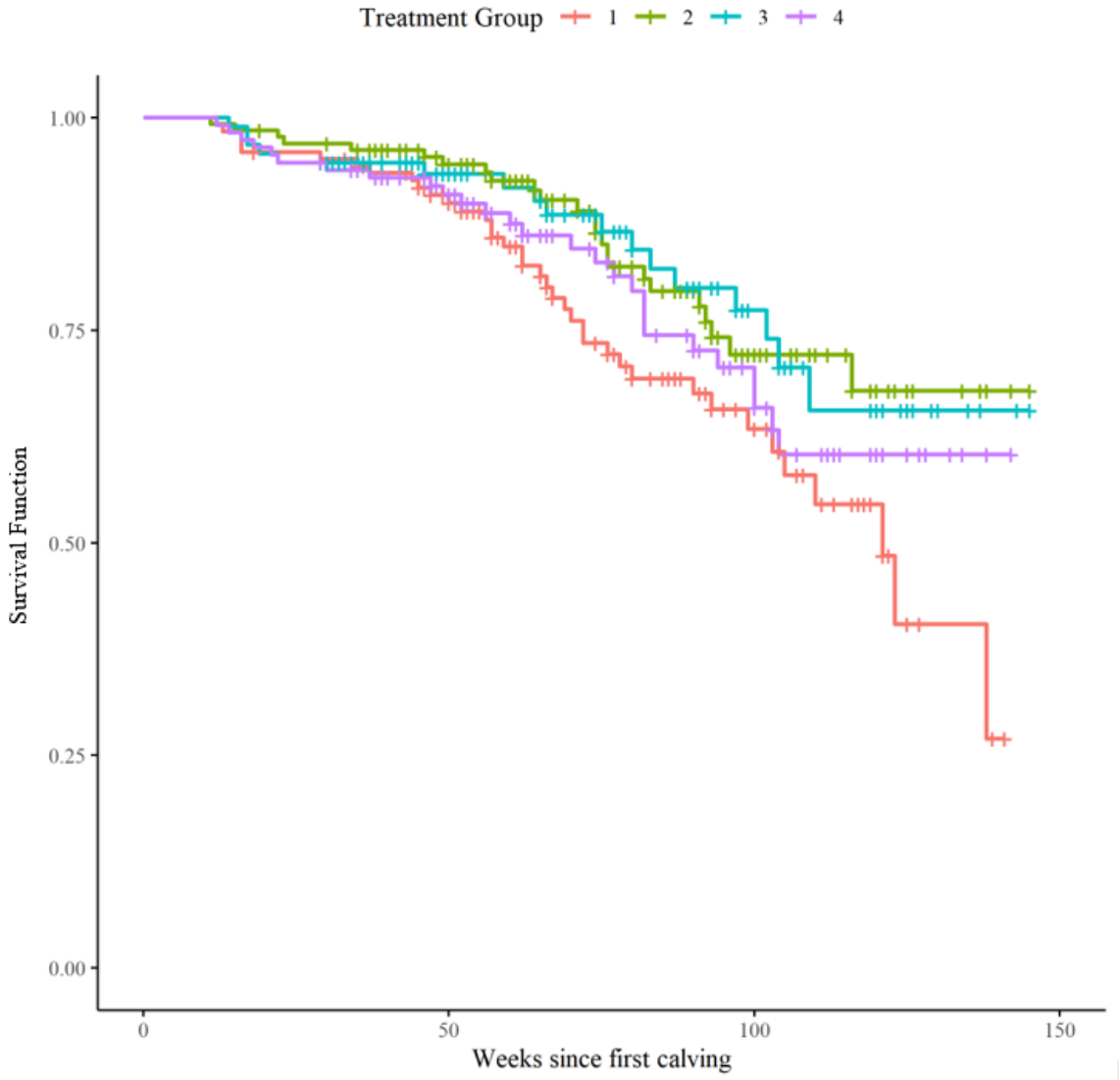


Figure 5-7 A post-model Kaplan-Meier Plot for the survival analysis presented in Table 5-6 of heifers subjected to one of four lameness treatment regimens over a thirty-four-month long randomised controlled trial. The trial investigated the efficacy of non-steroidal anti-inflammatory drugs in the treatment and prevention of lameness.

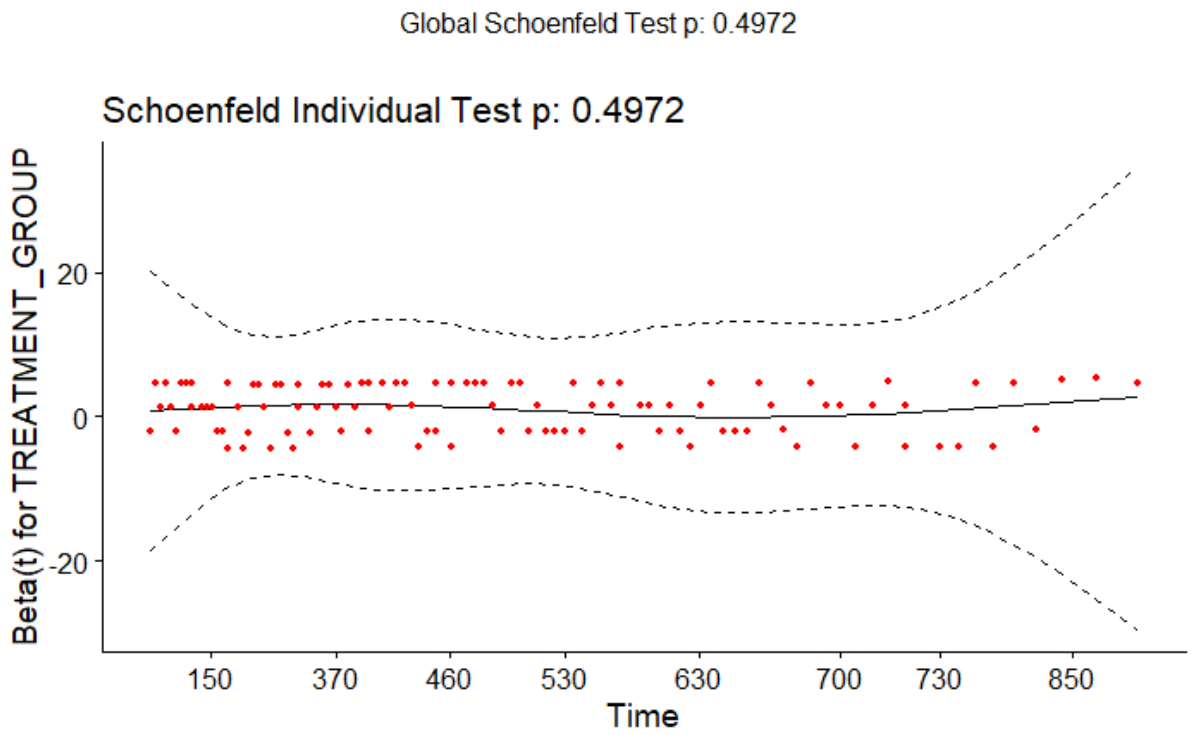


Figure 5-8 A plot of the Schoenfeld residuals for Model 5C which is presented in Table 5-6. Model 5C was a Cox proportional hazards model investigating the effect of lameness treatment group on the risk of culling through the thirty-four-month study period. In the current plot, there is no systemic change in Beta (t) as time progresses, and so the model fit is deemed to be good.

5.4 Discussion

5.4.1 Summary of Results

To the authors knowledge, this RCT is the first to investigate the effect of an intervention on the risk of lameness over a period longer than one year (Thomas et al., 2015; Groenevelt et al., 2016; Mahendran et al., 2017; Warner et al., 2020) and is in fact the longest treatment RCT in cattle which can be identified in the literature. Like many of the endemic production diseases of cattle, lameness is known to have cumulative and long-lasting repercussions (Newsome et al., 2016; Randall et al., 2016, 2018a), hence long term studies are vital if the industry wishes to further elucidate treatment and prevention strategies which lead to long term benefits on-farm. Importantly, our results indicated a particularly large effect size (for both lameness and severe lameness) when NSAIDs are routinely administered at first and subsequent calvings and every time animals are treated for lameness. It was identified that a cohort of animals receiving the Group 3 treatment protocol tested, had an absolute reduction in lameness risk of approximately 10% compared to animals treated with the conventional best practice of a corrective trim and appropriate application of orthopaedic blocks (Figure 5-1) i.e.

the administration of NSAID at first and subsequent calvings alongside lameness events (in conjunction to therapeutic trimming and appropriate application of orthopaedic blocks) results in 1 in ten fewer cows being lame. Furthermore, the same intervention reduced the risk of these animals being culled. If the results of this study are generalisable to the wider population, this strategic use of NSAIDs has the potential to substantially reduce the overall prevalence of lameness in a dairy herd by an absolute value of 10% (i.e., 10 in 100 fewer cows would be lame on any day of assessment), and the prevalence of severe lameness by 3% (i.e., 3 in 100 fewer cows would be severely lame on any day of assessment) (Figure 5-4). Given the considerable welfare implications of lameness (Whay and Shearer, 2017) and the high prevalence of lameness in most dairy nations (Von Keyserlingk et al., 2012; Ranjbar et al., 2016; Randall et al., 2019a), the potential benefits of this intervention for the dairy industry are extraordinary.

Importantly, the design of this study, a randomised controlled trial, means that results were unlikely to be affected by bias or confounding; RCT are considered to provide strong evidence of a causal effect (Evans, 2003; Backmann, 2017). No negative effects were noted with the intervention implemented as part of the Group 3 protocol.

5.4.2 The Role of Inflammation in Claw Horn Lesion Pathogenesis

The primary outcome measure in this study was lameness score assessed on a fortnightly basis, no other metrics of NSAID intervention on distal limb anatomy were recorded, meaning that the mechanism behind the causal effect of the NSAID intervention tested, cannot be elucidated from this study. This Chapter hypothesises several mechanisms through which its benefits could occur. The extent of systemic inflammation during transition is considered to be associated with the risk of an individual experiencing disease during lactation (Bradford et al., 2015). The reduction of systemic and local inflammation in the hoof capsule associated with NSAID administration post-partum could be a key driving factor behind the reduction in lameness observed. It is possible that systemic inflammation has a detrimental impact on the functional anatomy of the hoof leading to increased susceptibility to lameness. With increased susceptibility, additional management factors may

exacerbate the pressures exerted with the hoof leading to an increased risk of lameness (Knott et al., 2007).

Systemic inflammation derived from the transition period may predispose an animal to future lameness by driving pathological change to the anatomy of the hoof This chapter hypothesises that this inflammation can lead to a predisposition to lameness in the animals' immediate and distant future, through immediate changes to anatomic structures and long-term deterioration to the functionality of the anatomy, respectively. The digital cushion is composed of a range of fatty acids (Newsome et al., 2021). The lameness risk generated from this inflammation could be derived from the utilisation of fatty acids as inflammatory mediators (Calder, 2006; Bradford et al., 2015; Contreras et al., 2017a). The metabolism of digital cushion fatty acids could present a mechanism through which these fatty acids are mobilised as inflammatory mediators (as described in Chapter 3). The metabolism of digital cushion fatty acids presents two risks; firstly, this release of fatty acids could, in turn, drive the further the long-term deterioration of the digital cushion structure through acting as mediators to the inflammatory processes locally (Calder, 2006). These inflammatory processes could lead to the deposition of collagenous scar tissue which may have a negative impact on the digital cushion's ability to function. Secondly, the immediate functional anatomy of the digital cushion may be impeded through the thinning of the digital cushion on a short-term basis (Newsome et al., 2016), limiting its capacity to dissipate force during limb loading. Studies using trans-sole ultrasonography have noted that the thickness of the sole soft tissues appears to be reduced in the period immediately post-partum (Bicalho et al., 2009; Newsome et al., 2017a; b) which has been identified as a risk factor for CHLs (Newsome et al., 2017a; b). This could be due to fatty acid metabolism thinning the digital cushion which in turn impedes its functionality leading to increased pressures on the corium, and thereby CHLs. The metabolism of fatty acids and exposure to inflammation is also understood to cause a remodelling of tissue structure (Contreras et al., 2017a). This remodelling could impede the ability of the digital cushion to function over and across lactations, leading to a longer-term predisposition to lameness also.

Secondly this Chapter hypothesises that systemic inflammation around the time of calving could drive a long-term predisposition to lameness through the stimulation of exostosis on the caudal palmer / plantar aspect of the distal phalanx. Newsome et al. (2016) postulated that the development of exostosis was a direct result of the inflammation associated with CHLs. Indeed, this could be true for most development observed within that study, however this hypothesis needs to be added to by postulating that systemic inflammation associated with transition could also be in part responsible for the development of exostosis on the weight bearing surface of the distal phalanx. Within the study reported by Newsome et al. (2016) they reported that exostosis on the distal phalanx were positively correlation with age and a large proportion of the variation in extent of new bone development was unexplained. It is possible that systemic inflammation associated with transition is another driver of this pathological change to distal limb anatomy. Systemic inflammation could be sourced from various origins (e.g., mastitis, endometritis, etc) all of which may have a detrimental impact indirectly on the functional anatomy of the hoof. If systemic inflammation, derived from any source, has impacts on the functional anatomy within the hoof capsule (i.e., stimulates exostosis development on the distal phalanx or metabolism of the digital cushion), then these present potential timepoints when intervention with NSAID may further reduce the risk of an animal being scored as lame in the future. This relationship may exist due to the NSAIDs ability to “protect” the functional anatomy of the hoof capsule from the detrimental effects of systemic inflammation.

The same changes to distal limb anatomy as described above have been suggested to occur at the time of lameness also (Newsome et al., 2016, Chapter 3). The inflammation associated with CHLs has been postulated to stimulate exostosis development (Newsome et al., 2016) and also lead to digital cushion adipose metabolism (as described in Chapter 3). Within the current study, the EDPET principle was implemented. This has already been shown to improve 35d cure rates (Thomas et al., 2015, 2016) and improve lameness prevalence generally (Groenevelt et al., 2014), however its relevance to the management of pathological change is not understood. This Chapter postulates that through early intervention in lameness cases within the current study, that any changes to the distal

phalanx or the digital cushion through the use of NSAIDs not only at calving, but also at the earliest point of CHL onset, have effectively been minimised.

5.4.3 The Management of Pain Derived from Claw Horn Lesions

The management of acute and chronic pain in the dairy cow is an important consideration when assessing the efficacy of a treatment protocol including NSAIDs. Dairy cattle suffering from CHLs have been shown to exist in a state of hyperalgesia (Whay et al., 1998), and it has been demonstrated that NSAIDs are important in the management of this state of pain (Whay et al., 2005). Animals experiencing a repeated noxious stimulus can undergo pathophysiological changes to the nervous system which causes a transition from acute to chronic pain (Devor, 2006; Greene, 2010; Voscopoulos and Lema, 2010). The transition from acute to chronic pain, does not necessarily require nerve damage to take place, and actually repeated exposure to inflammation can create the same effect (Devor, 2006; Voscopoulos and Lema, 2010). This transition causes the region affected to become more sensitive to noxious stimuli, meaning that a heightened pain response will be experienced (Bartfai, 2001). In the case of the CHLs, the pathogenesis of severe lesions takes place over a longer time duration which could lead to this heightened experience of pain (Whay et al., 2005)through the remodelling of neural processing networks local to the hoof (Apkarian et al., 2009; Denk et al., 2014). This may result in animals having a lower nociceptive threshold, even when free of disease (Apkarian et al., 2009; Denk et al., 2014). NSAIDs have been shown to prevent the transition from acute to chronic pain (Bartfai, 2001; Samad et al., 2001; Telleria-Diaz et al., 2010), meaning that the benefit observed in Group 3 may be derived from pain management supplementary to inhibiting the pathogenic pathways associated with CHLs.

Furthermore, it is possible that intervention with NSAIDs as a prophylactic treatment for lameness may alter the animal's perception of pain. It is recognised that the average heifer calving into the herd at ~24 months is still immature in terms of development. It has been suggested that animals exposed to pain when young are more susceptible pain in their later lives, and that this can be modulated by the administration of pain relief (Apkarian et al., 2009; Denk et al., 2014). It is possible that the nervous system of these animals is not fully developed and that through intervening with NSAIDs at

first calving may have a positive outcome on the pain experienced by these individuals for the remainder of their lives.

5.4.4 A Cost-Effective Solution?

The findings from this study support the adoption of routine NSAID administration at calving and lameness as a cost-effective solution to lameness management within a dairy enterprise. As a part of the Group 1 (n=111) treatment regimen 271 lameness treatments were administered i.e. 2.44 treatments per animal. Based on a cost of £20 per treatment for a therapeutic trim and hoof block, this equated to £49 per Group 1 animal. Similarly Group 3 (n=100) received 151 treatments at an average cost of £30 per animal. In Group 3, the NSAIDs at first and subsequent calving (152 treatments) and when animals were lame (156 treatments) resulted in 308 three-day courses of NSAID being administered, at a total cost of £9,240 (based on £30 per 3-day treatment) or £92 per animal. Consequently, total treatment costs were £49 for Group 1 animals and £122 for Group 3 animals, a differential of £73 per animal between groups. A total of 38 animals were culled from Group 1. Assuming an average, mid-market price in the UK of £650 for cull cows and £1,350 for dairy cows in milk over the course of the study (Anon, 2021), this equates to an average cost of culling of £240 per animal in the group ((£1,350-£650) x 38 / 111). Similarly, 17 were culled from Group 3, at an average cost of £119 per animal, a differential of £121 per animal between groups. Overall, it cost an additional £73 per animal to treat Group 3 to gain a saving of £121 per animal, a return on investment of 1.66:1. This estimate is likely conservative as it takes no account of other likely benefits, for example milk yield and fertility, and despite its rudimentary nature, 529 can be very confident that the intervention is better than cost neutral under UK conditions.

5.4.5 Limitations

The main limitation of this study was that it was conducted on a single commercial dairy unit and so generalisability to other herds and situations is unknown. Although the herd was representative of housed dairy systems in the UK, additional research would be useful to evaluate the effects of the Group 3 treatment protocol on lameness.

5.4.6 Conclusions

In conclusion, our results indicate that the Group 3 treatment protocol reduced the risk of lameness, severely lameness and culling during the three-year RCT. This Chapter hypothesises that systemic inflammation at the time of calving and localised inflammation at lameness predisposes cows to future lameness and that this is limited by treatment with NSAIDs. Furthermore, an improved pain management programme associated with the repeated administration of NSAIDs at painful events (such as calving and lameness) will likely lead to a substantial improvement to animal welfare. The administration of NSAIDs at calving and lameness appears to set animals on a trajectory to experience less lameness in future life.

6 Non-Steroidal Anti-Inflammatory Drugs at Calving have no Effect on the Subsequent Risk of Lameness: A Randomized Controlled Trial in Adult Cattle.

6.1 Introduction

Lameness derived from CHLs is a chronic condition (Newsome et al., 2016; Randall et al., 2018a), affecting dairy cattle worldwide (Cook, 2003; Von Keyserlingk et al., 2012; Ranjbar et al., 2016; Randall et al., 2019a). Research evidence suggests that lameness begets future lameness with a substantial proportion of the risk of disease being attributed to previous occurrence, demonstrating that the occurrence of this condition has long-lasting effects on the health and welfare of the dairy cow (Randall et al., 2016, 2018a). It is currently thought that this predisposition may be driven by changes to the anatomy of the hoof driven by inflammatory processes associated with CHL pathogenesis. Furthermore, calving appears to be an important time in the development of CHL.

In a study conducted by Newsome et al. (2016) the development of exostosis on the caudal aspect of the pedal bone of cull cattle was strongly associated to the age and lameness/CHL history of the animal in question. In Chapter 3 it was identified that the digital cushion contained within the hind lateral claws of cull cows with a history of CHLs/lameness was significantly lower in volume when compared to their sound counterparts. Supplementary to this, in Chapter 3, the proportion of Type 3 collagen (indicative of inflammatory disease) was increased in those animals that experienced an increased lameness score in the four weeks before cull, suggesting that lameness has a subsequent impact on the structure of the digital cushion. The ability of the digital cushion and distal phalanx to function normally is likely to be impeded by these anatomical changes which are driven by lameness and CHL pathogenesis. These changes are considered to predispose animals to a lifetime of CHLs through an increased force being exerted on the horn producing germinal epithelium which sits beneath these two structures and/or decreased functionality of the digital cushion. This is hypothesised to be caused by the digital cushion being less able to dissipate the forces associated with locomotion and standing away from this sensitive tissue, and the distal phalanx exerting a greater

overall or point pressure through bony proliferations. It is likely that the anatomical changes described by Newsome et al. (2016) and in Chapters 3 and 4 exist permanently, thereby predisposing the animal to lameness for the duration of their lives. This is supported by the reported increase in lameness risk with occurs with increasing parity (Mason, 2017).

Evidence has demonstrated that NSAID administration (as part of a treatment regimen) in newly lame animals has a role to play in improving short-term lameness cure rates (Thomas et al., 2015), however their use in the management of chronically lame animals is likely not to expand further than pain relief (Laven et al., 2008; Thomas et al., 2016). The use of NSAID in the long-term management of lameness has never been investigated other than in Chapter 5 where the effect of NSAID at calving and lameness were investigated in parity one animals. Further evidence is required to understand the implementation of lameness control programmes in adult dairy cattle where there is the potential for chronic pathological changes to be driving future lameness risk. The effectiveness of NSAID administration on reducing the risk of lameness (both as a treatment and prevention) in the adult dairy cow may be reduced in comparison to their heifer counterparts. This could be driven by the pre-existing pathogenic exostosis and a diminished digital cushion due to previous lameness. These alterations to distal limb anatomy could mean that an animal has a strong disposition towards lameness due to existing pathological change to hoof anatomy which exert excessive pressures on the corium. If this were the case, the effects of preventive NSAID administration at calving or at the time of lameness as a treatment may not be as beneficial in the adult dairy cow compared with heifers.

Within the study presented in Chapter 5, heifers were recruited pre-first calving and treated at lameness and at first and subsequent calvings according to the treatment group they were randomised into. Within that Chapter, it was demonstrated that the Group 3 treatment protocol (NSAID at first calving and subsequent calvings, and at each lameness treatment) led to animals being at a significantly lower risk of being scored as lame and being culled. This has highlighted the potential importance of systemic inflammation associated with the transition period on the structures contained within the hoof capsule. In Chapter 5 it was hypothesised that the administration of NSAID post-partum functioned to minimise the effects of transition period inflammation on the functional anatomy

of the hoof and improve the animal's future experiences of pain. This was hypothesised to be through a reduction in digital cushion metabolism and osteoma development on the distal phalanx alongside the protection of the suspensory apparatus from the actions of relaxin through MMP inhibition. Pain inhibition was postulated to be driven by the prevention of nerve remodelling which reduces the risk of animals living in a state of chronic pain. For the heifers recruited to the study presented in Chapter 5, it was assumed that these animals were naïve to the challenges of lameness and transition meaning that the effects observed are directly associated with our intervention. The effects of the intervention would not be "masked" by a predisposition to lameness due to previous pathological change having occurred within the hoof capsule (i.e., no osteoma would have developed, no depletion of the digital cushion, or weakening of the suspensory apparatus would have occurred which would increase pressures on the germinal epithelium, thereby predisposing an animal to CHLs), prior to their inclusion in the study.

If, however, NSAID administration can reduce the future risk of lameness in the adult cow, then this would allow for "blanket" management strategies to be implemented in a cost-effective manner to reduce the prevalence of lameness worldwide. The current RCT was designed to investigate the efficacy of NSAID administration in the management of lameness in a cohort of adult dairy cattle, this would allow for comparisons to be made between interventions in adult cattle and in heifers calving into the herd (i.e., treatments from first calving and first lameness as a guarantee). This was investigated by dividing the dataset into two parts – 1) All animals recruited who had calved at least once (these animals had no treatments at first calving, and an unknown lameness history); 2) Animals in their first lactation at study onset (these animals had no treatments at first calving, and an unknown lameness history, however, this would have been over a shorter duration than the remainder of the adult herd). The null hypothesis was that NSAID administration at calvings, and when treating animals for lameness would have no impact on the lifetime risk of lameness in a cohort of adult dairy cows who may not be naïve to lameness.

6.2 Materials and Methods

6.2.1 Study Design

Methods for the current Chapter can be taken to be the same as in Chapter 5, unless expressly described otherwise.

6.2.2 Study Herd and Herd Management

A description of the continuously housed, high input/output study herd is given in Chapter 2.3.1.

6.2.3 Enrolment

All lactating or dry adult dairy cattle (that had calved at least once) were eligible for recruitment unless they had already been denoted for cull in the six-month period immediately following study initiation. Animals were allocated to one of four treatment groups using a block randomisation procedure which was stratified based on lactation number (1, 2, 3, 4+), days in milk (DIM) at study initiation, and on the previous eight-week history of lameness (based on fortnightly scores, classified into animals presenting ≥50% lame scores and those presenting <50% lame scores). Animals were categorised into lists of ≥50% lameness and <50% lameness for their respective lactations. The animal ID numbers were then listed in order of their respective DIM at study onset (from lowest to highest). Animals were grouped into fours sequentially and randomly allocated to one of the four treatment groups which was unique to that block of four, using an online random block generator (Haahr, 2012). Cows remained in the study until culling, or the study ended. All study animals were managed in the same manner within the herd for the duration of the study and were therefore subjected to the same routine day-to-day management procedures.

6.2.4 Treatment Groups

The randomised controlled trial was designed as described in Chapter 5.2.

Cows were assigned to one of four treatment groups, prior to study onset, and were subjected to different treatment protocols at the time of lameness and calving. The treatments provided for each group are described in Chapter 5.2.4. The treatments provided for each group are summarised in Table 6-1.

Treatment	Lameness Trim	Lameness NSAID	Calving NSAID
Group 1	✓	×	×
Group 2	✓	✓	×
Group 3	✓	✓	✓
Group 4	×	✓	×

(Unless severely lame)

Table 6-1 A summary of the four treatment regimens applied in a three year long randomised controlled trial investigating the efficacy of non-steroidal anti-inflammatory drugs in the treatment and prevention of lameness and the subsequent risk of culling.

6.2.5 Treatment Routines

The treatment routines within the current Chapter are exactly the same as those described in Chapter 5.2.5. That is to say, the heifers and adult cattle within the study herd were lameness scored and treated at the same time.

6.2.6 Lameness Outcome Scores

Lameness outcomes were collated in the form of a blinded lameness score in which the operating technician had no other involvement in farm management or trial administration. A comprehensive description of the outcome lameness scoring is given in Chapter 2.3.4.

6.2.7 Data Collection and Descriptive Analysis

Data were collated on a fortnightly basis and stored in a relational database (Microsoft Access, Microsoft Corp., 2016). A full description of data collection, management and handling is given in Chapter 5.2.7.

6.2.8 Statistical Modelling

To evaluate the study hypotheses, two cohorts of animals were examined; namely all adult cattle that had calved at least once (herein described as all adult cattle), and those animals in their first lactation

at study onset (herein described as first lactation animals). To evaluate the study hypotheses, two statistical models were constructed for each cohort of animals:

1) A mixed effects logistic regression model was used to evaluate the impact of treatment group on the risk of lameness over time using the "lme4" (Bates et al., 2015) package in R. Lameness was defined as a single lameness score submitted as equal to or greater than 2a. Severe lameness was defined as a single lameness score submitted as equal to or greater than 3a. The mixed-effects logistic regression model took the form:

$$\text{Lameness}_{ij}\,(1=\text{lame},\ 0=\text{non-lame}) \sim \text{Bernoulli}\ (\mu_{ij})$$

$$logit(\mu_{ij}) = \beta_0 + \beta_1 x_{ij} + \beta_2 x_j + u_j$$

$$u_j \sim N(0, \sigma^2)$$

Where μ_{ij} was the probability of an animal being lame at the ith mobility score of the jth cow, β_0 the model intercept , x_{ij} a matrix of covariates linked to each lameness score (e.g. days in milk or week of study), $\beta_1$the coefficients for x_{ij}, x_j a matrix of covariates linked to each cow (e.g. treatment group), β_2 the coefficients for x_j, u_j represented a random effect for the jth cow and σ^2 the variance of the random effects u_j.

Covariates were deemed significant and remained in the model when P<0.05. Interactions between significant covariates were tested and retained in the model when P<0.05. Investigation of model fit was made by assessing realised discrepancies between cumulated predicted probabilities and observed outcomes (Gelman et al., 1996) and the assessment of normality of random effects. Since random effects were over dispersed, the final model was repeated with outlying cows omitted (>10 incidents of lameness during the study period) to check there were no meaningful changes in model parameters or their interpretation. Predictions from the final model were visualised graphically to illustrate the lameness risk by treatment group for the duration of the study.

2) A Conventional Cox proportional hazards model was constructed using the "survival" (Therneau and Grambsch, 2000) and "survminer" (Kassambara et al., 2020) packages in R to investigate the effect of treatment group on the risk of culling, the model took the form:

$$h(t) = h_0(t) \text{ x } \exp(\beta_1 x_1 \ldots \beta_p x_p)$$

Where t represented the survival time to culling, $h(t)$ the hazard function which was dependent on a baseline hazard $h_0(t)$ and p covariates $(x_1 \ldots x_p)$ with $\beta_1 \ldots \beta_p$ the related coefficients.

Covariates were deemed significant and retained in the model when P<0.05. Evaluation of the proportional hazard's assumption and model fit was undertaken by visual assessment of Schoenfeld residuals, the log-log curves and delta betas (Schoenfeld, 1982; Grambsch and Therneau, 1994). The data were visualised graphically using Kaplan-Meier plots to illustrate time to culling by treatment group.

6.3 Results

6.3.1 Study Denominators – All Adult Cattle

Initial randomisation led to 107 animals being randomised into each of the four treatment groups, totalling 428 animals randomised in the study herd. Of those, 105 in Group 1, 107 in Group 2, 107 in Group 3 and 106 in Group 4 were presented for lameness scoring (i.e., they were not culled between randomisation and being eligible to be scored). Subsequently 13 cows (3 from Group 1, 5 from Group 2, 1 from Group 3 and 4 from Group 4) were removed because they posed a danger to farm staff or technicians during handling for lameness treatment. These reasons for exit were considered to have occurred at random, i.e., they were not linked to treatment group. Therefore, a total of 412 animals were included in the final analysis with 102 in Group 1, 102 animals in Group 2 animals, 106 animals in Group 3 and 102 animals in Group 4.

6.3.2 Descriptive Statistics – All Adult Cattle

Study animals received 2,028 lameness treatments (506 under Group 1 regimens, 417 under Group 2, 529 under Group 3 and 576 under Group 4). From the lameness treatment records, 238 cases of lameness were associated with infectious causes (e.g., digital dermatitis), 1,422 with CHLs (SH, SU, WLD) and 368 with other sources (e.g., musculoskeletal injury not associated with the hoof, no visible claw or skin lesion). Outcome lameness scores were collated for the entire study period (146 weeks), yielding 14,021 individual lameness scores. A summary of the treatments administered, and outcome lameness scores are provided in Table 6-2.

During the study period, a total of 345 study animals were culled (84 from Group 1, 84 from Group 2, 89 from Group 3 and 88 from Group 4). Primary reasons for culling as recorded by farm staff included fertility (n = 94), lameness (n = 31), udder health (n = 155) and other (n = 65)

	Grp1	Grp2	Grp3	Grp4
Proportion of outcome lameness scores recorded as lame.	1633/3587	1500/3421	1586/3546	1726/3467
Proportion of outcome lameness scores recorded as severely lame.	138/3587	99/3421	154/3546	147/3467
Number of lameness treatments administered.	506	417	529	576
Incidence of Treatment (cases of treatment per cow-week)	0.14	0.12	0.15	0.16
Average lameness treatments administered per animal (Min-Max).	4.52 (0-18)	4.01 (0-20)	4.56 (0-19)	5.38 (0-20)
Number of orthopaedic blocks applied.	197	174	233	46
Interim "First aid" treatments required.	15	8	12	13
Veterinary referrals.	10	8	4	15
Cause of lameness - SH	221	212	231	127
Cause of lameness - SU	62	70	54	86
Cause of lameness – WLD	78	84	90	107
Cause of lameness – Infectious Causes	63	52	53	70

Table 6-2 Descriptive statistics of the lameness data derived from a three year long randomised controlled trial investigating the efficacy of non-steroidal anti-inflammatory drugs in the treatment and prevention of lameness and the subsequent risk of culling. The dataset this is constructed from contains 14,021 individual lameness scores from 412 animals over a 34-month period. Animals were recruited as of the 8th January 2018, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

6.3.3 Statistical Modelling – All Adult Cattle

6.3.3.1 Model 6A. Mixed-effects logistic regression model to evaluate the risk of lameness (score ≥2a).

Final results of Model 6A are provided in Table 6-3. Compared to cows in Group 1, cows in Groups 4 more likely to become lame during the study period (OR = 1.68, *P=0.*01). The risk of lameness over time for cows in each treatment group is illustrated in Figure 6-1. Model fit was identified to be good, and omission of outlying cows had no impact on model parameters (<5% alteration in coefficients and no changes in significance, Figure 6-2, Figure 6-3).

	Estimate	Odds Ratio	Standard Error	P Value
Intercept	-1.26	0.28	0.49	0.01
Treatment Group 1	Reference	–	–	–
Treatment Group 2	0.05	1.05	0.20	0.81
Treatment Group 3	0.03	1.03	0.20	0.89
Treatment Group 4	0.52	1.68	0.20	0.01

Table 6-3 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of lameness (lameness score ≥2a). The dataset this model is constructed from contains 14,021 individual lameness scores from 412 animals over a 34-month period. Animals were recruited on the 8th January 2018, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

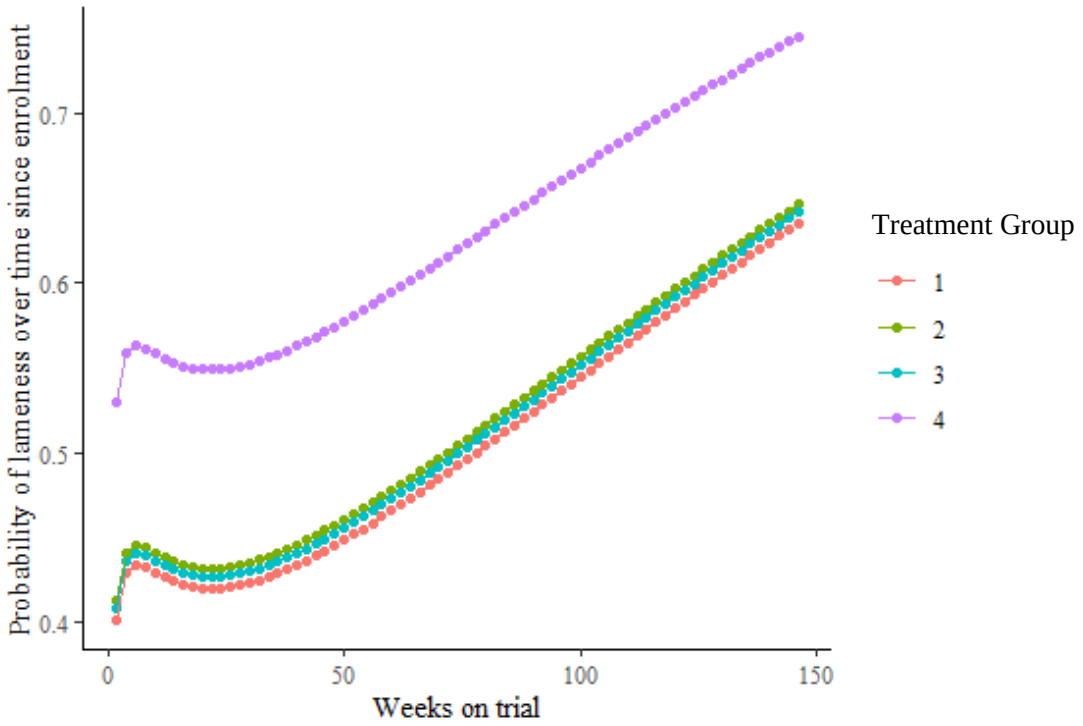


Figure 6-1 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of lameness (lameness score ≥2a) described in Table 6-3. The dataset this model is constructed from contains 13,886 individual lameness scores from 412 animals over a thirty-four-month period. All adult animals that had calved at least once were recruited on 8th January 2018 and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

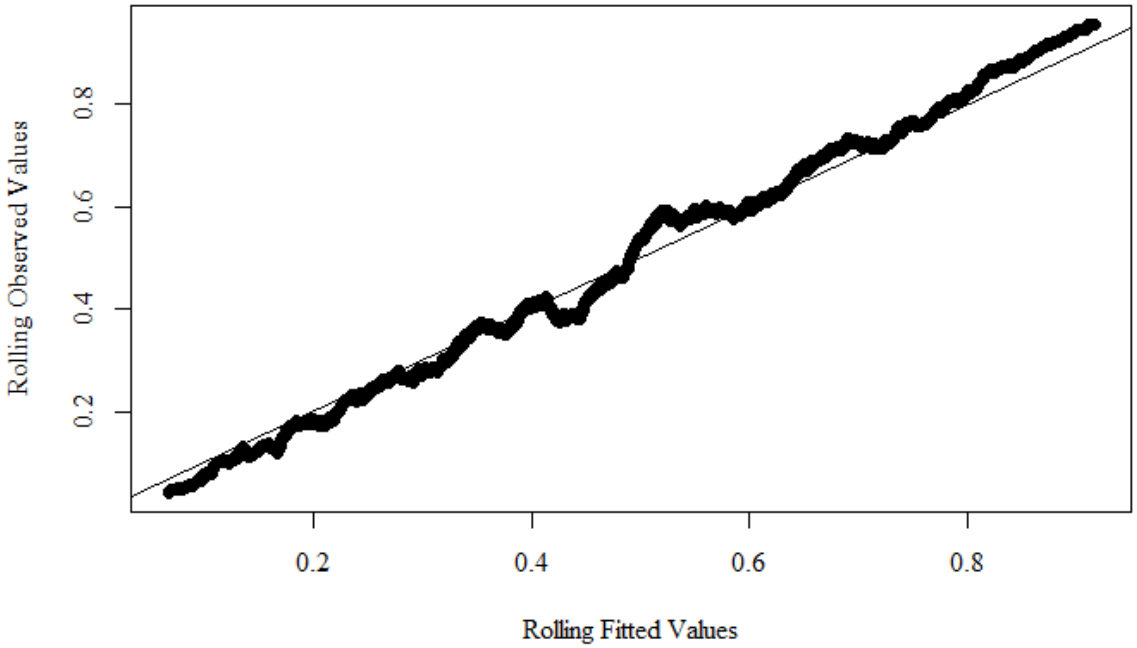


Figure 6-2 A plot of the 800-day rolling fitted and observed values generated from Model 6A which is described in Table 6-3. Model 6A investigated the effect of treatment group on the risk of an animal being scored as lame (≥ score 2a) over time. The fitted and observed values closely aligned and so model fit was deemed good.

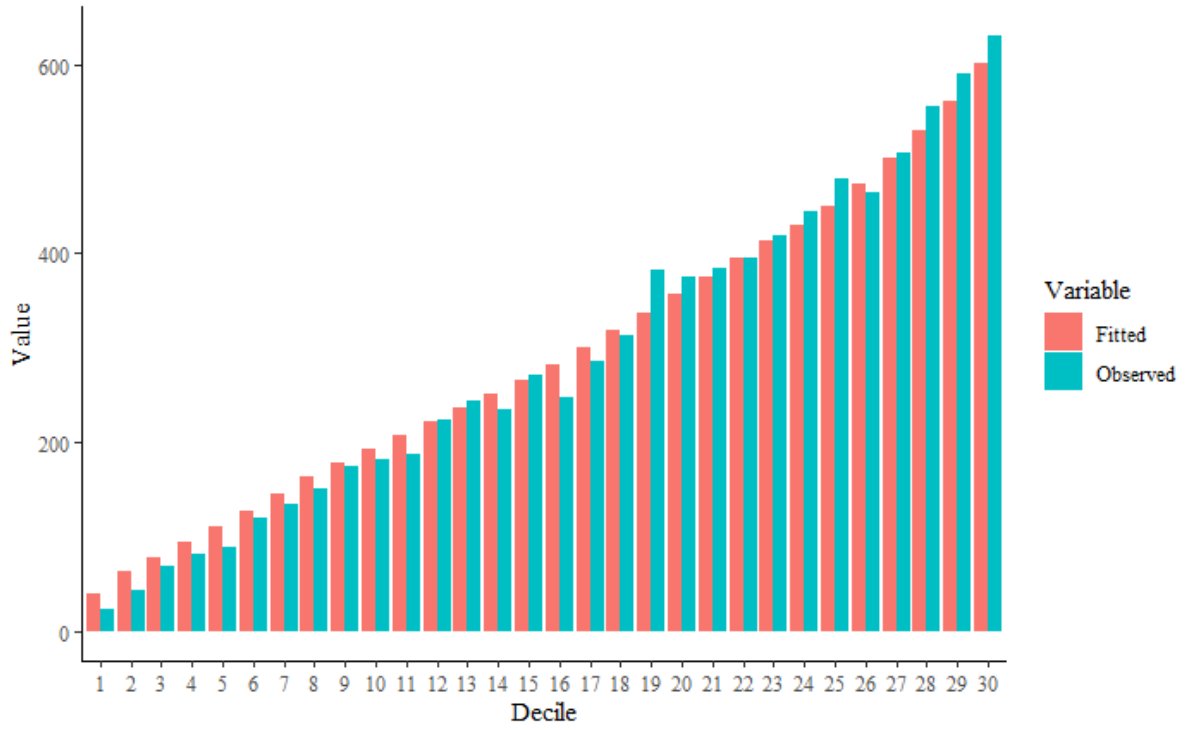


Figure 6-3 A plot of the fitted and observed values for model 6A which is described in Table 6-3. Model 6A investigated the effect of treatment group on the risk of an animal being scored as lame (≥ score 2a) over time. The fitted and observed values are consistently similar and so model fit was deemed as good.

6.3.3.2 Model 6B. Mixed-effects logistic regression model to evaluate the risk of severe lameness (score ≥3a).

Final results of Model 6B are provided in Table 6-4 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of severe lameness (lameness score ≥3a). The dataset this model is constructed from contains 14,021 individual lameness scores from 412 animals over a 34 month. Animals were recruited on the 8th January 2018, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.. No significant effect was observed between the treatment groups on the risk of an animal being scored as severely lame. The risk of severe lameness over time for cows in each treatment group is illustrated in Figure 6-4. As for Model 6A, model fit was good (Figure 6-5, Figure 6-6).

	Estimate	Odds Ratio	Standard Error	P Value
Intercept	-1.93	0.15	1.15	0.10
Treatment Group 1	Reference	-	-	-
Treatment Group 2	-0.25	1.28	0.37	0.49
Treatment Group 3	0.16	1.17	0.36	0.65
Treatment Group 4	0.28	1.32	0.36	0.43

Table 6-4 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of severe lameness (lameness score ≥3a). The dataset this model is constructed from contains 14,021 individual lameness scores from 412 animals over a 34 month. Animals were recruited on the 8th January 2018, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

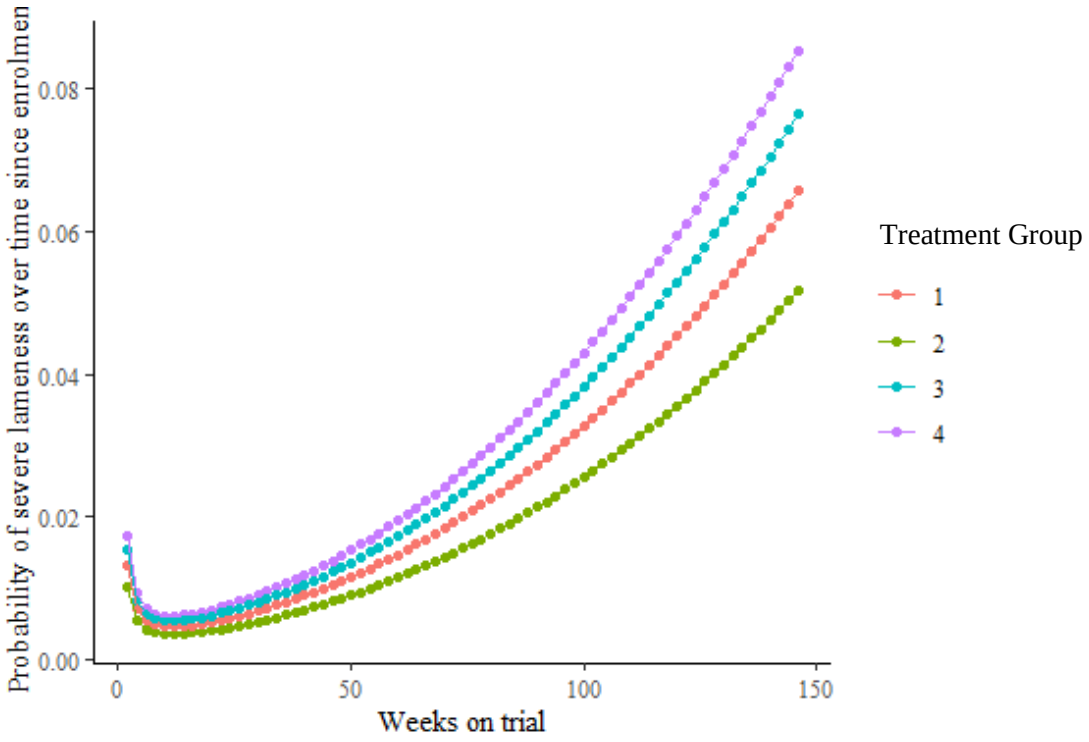


Figure 6-4 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of severe lameness (lameness score ≥3a) described in Table 6-4. The dataset this model is constructed from contains 13,886 individual lameness scores from 412 animals over a thirty-four-month period. All adult animals that had calved at least once were recruited on 8th January 2018 and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

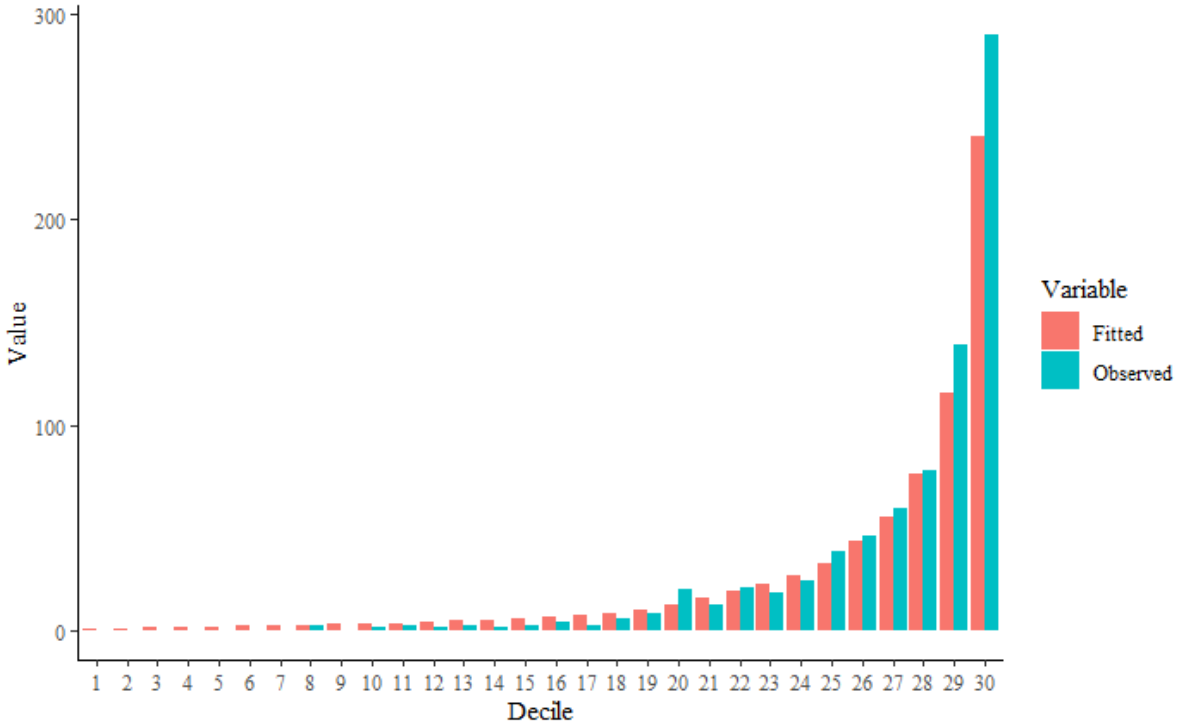


Figure 6-5 A plot of the fitted and observed values for model 6A which is described in Table 6-3. Model 6A investigated the effect of treatment group on the risk of an animal being scored as severely lame (≥ score 3a) over time. The fitted and observed values are consistently similar and so model fit was deemed as good.

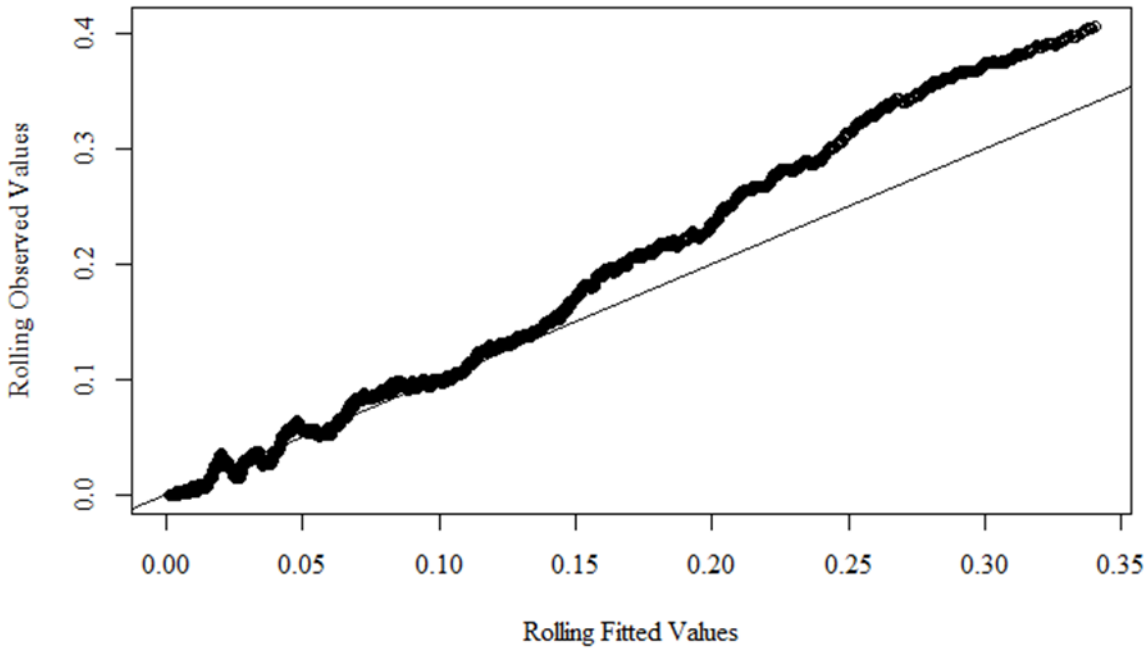


Figure 6-6 A plot of the 800-day rolling fitted and observed values generated from Model 6B which is described in Table 6 4. Model 6B investigated the effect of treatment group on the risk of an animal being scored as severely lame (≥ score 3a) over time. The fitted and observed values closely aligned and so model fit was deemed good.

6.3.3.3 Model 6C. Cox proportional hazards model for survival to culling.

Final results of Model 6C are provided in Table 6-5. No significant effect was observed between the treatment groups on the risk of an animal being culled at any point of the study period. A Kaplan-Meier plot to illustrate survival time to culling by treatment group is presented in Figure 6-7. Investigations of model fit indicated that the model assumption of proportionality of hazards was met and residual analysis indicated fit was good (Figure 6-8).

Model term	Coefficient	Hazard Ratio	P Value
Treatment Group 1	Baseline	–	–
Treatment Group 2	-0.08	0.92 (95% CI: 0.68 – 1.23)	0.63
Treatment Group 3	0.17	0.85 (95% CI: 0.87 – 1.61)	0.28
Treatment Group 4	0.08	0.92 (95% CI: 0.80 – 1.48)	0.61

Table 6-5 Results of the final Cox proportional hazards model for time to culling. The dataset on which this model is based consists of 412 animals within a single commercial herd, of which 169 were culled during the study period. All adult animals that had calved at least once were recruited on 8th January 2018, and 'Treatment Group' represents four different types of treatment administered at lameness and calving.

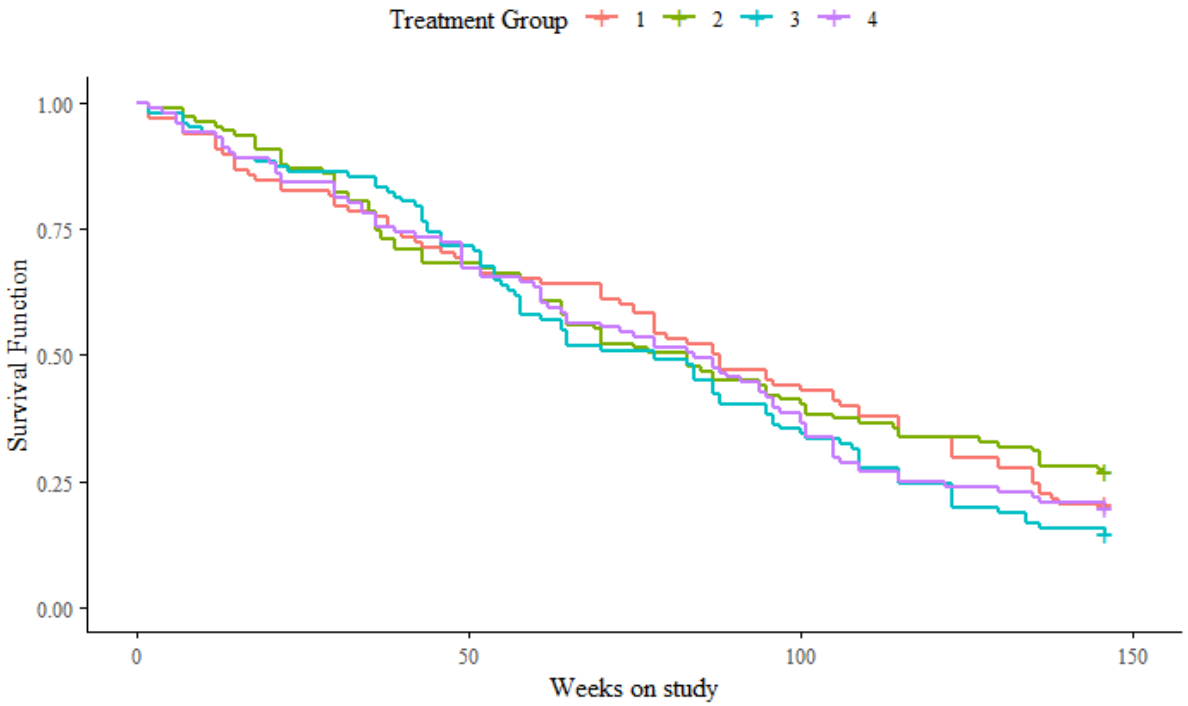


Figure 6-7 A Kaplan-Meier Plot for the survival analysis presented in Table 6-5 of adult cows subjected to one of four lameness treatment regimens over a thirty-four month long randomised controlled trial. The trial investigated the efficacy of non-steroidal anti-inflammatory drugs in the treatment and prevention of lameness.

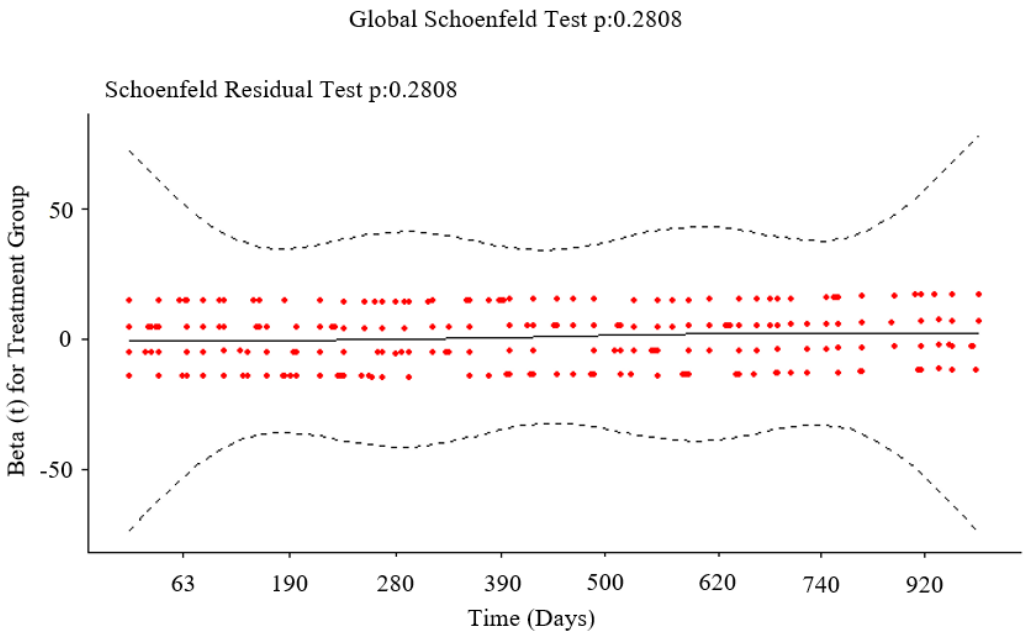


Figure 6-8 A plot of the Schoenfeld residuals derive from Model 6C (described in Table 6-5) against time. Model 6C was a Cox proportional hazards model investigating the effect of lameness treatment group on the risk of culling through the thirty-four-month study period. As time progressed, biased shift in beta was not detected, meaning model fit was good.

6.3.4 Study Denominators – First Lactation Animals

Initial randomisation led to the randomisation of 98 animals who were in their first lactation at study onset. Of these, 25 were in Group 1, 24 were in Group 2, 24 were in Group 3 and 25 were in Group 4. All animals randomised into this cohort were presented for lameness scoring (i.e., they were not culled between randomisation and being eligible to be scored). Subsequently 2 cows (0 from Group 1, 1 from Group 2, 1 from Group 3 and 0 from Group 4) were removed because they posed a danger to farm staff or technicians during handling for lameness treatment. These reasons for exit were considered to have occurred at random, i.e., they were not linked to treatment group. Therefore, a total of 96 animals were included in the final analysis with 25 in Group 1, 23 animals in Group 2 animals, 23 animals in Group 3 and 25 animals in Group 4.

6.3.5 Descriptive Statistics – First Lactation Animals

Study animals received 413 lameness treatments (104 under Group 1 regimens, 99 under Group 2, 69 under Group 3 and 141 under Group 4). From the lameness treatment records, 42 cases of lameness were associated with infectious causes (e.g., digital dermatitis), 276 with CHLs (SH, SU, WLD) and 95 with other sources (e.g., musculoskeletal injury not associated with the hoof, no visible claw or skin lesion). Outcome lameness scores were collated for the entire study period (146 weeks), yielding 4,880 individual lameness scores. A summary of the treatments administered, and outcome lameness scores are provided in Table 6-6.

During the study period, a total of 31 animals were culled (7 from Group 1, 8 from Group 2, 7 from Group 3 and 9 from Group 4). Primary reasons for culling as recorded by farm staff included fertility (n = 11), lameness (n = 4), udder health (n = 7) and other (n = 9).

	Grp1	Grp2	Grp3	Grp4
Proportion of outcome lameness scores recorded as lame.	351/1189	407/1199	274/1070	520/1422
Proportion of outcome lameness scores recorded as severely lame.	14/1189	19/1199	18/1070	20/1422
Number of lameness treatments administered.	104	99	69	141
Incidence of Treatment (cases of treatment per cow-week)	0.09	0.08	0.07	0.10
Average lameness treatments administered per animal (Min-Max).	4.16 (0-14)	4.13 (0-13)	2.88 (0-10)	5.64 (0-16)
Number of orthopaedic blocks applied.	49	41	29	13
Interim “First aid” treatments required.	6	4	2	3
Veterinary referrals.	2	1	2	5
Cause of lameness – SH	49	43	36	17
Cause of lameness – SU	19	16	8	23
Cause of lameness – WLD	18	15	6	28
Cause of lameness - DD	10	10	8	14

Table 6-6 Descriptive statistics of the lameness data derived from a three year long randomised controlled trial investigating the efficacy of non-steroidal anti-inflammatory drugs in the treatment and prevention of lameness and the subsequent risk of culling. The dataset this is constructed from contains 4,880 individual lameness scores from 96 animals over a three-year period. Animals were in their first lactation as of the 8th January 2018, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

6.3.6 Statistical Modelling – First Lactation Animals

6.3.6.1 Model 6D. Mixed-effects logistic regression model to evaluate the risk of lameness (score ≥2a).

Final results of Model 6D are provided in Table 6-7. Compared to cows in Group 1, cows in Group 4 were more likely to be scored as lame during the study period (OR = 1.70, *P=0.08*). The risk of lameness over time for cows in each treatment group is illustrated in Figure 6 9. Model fit was identified to be good, and omission of outlying cows had no impact on model parameters (<5% alteration in coefficients and no changes in significance, Figure 6-10, Figure 6-11).

	Estimate	Odds Ratio	Standard Error	P Value
Intercept	-3.35	0.04	1.03	<0.01
Treatment Group 1	Reference	–	–	–
Treatment Group 2	0.29	1.34	0.94	0.35
Treatment Group 3	-0.19	0.83	-0.57	0.57
Treatment Group 4	0.53	1.70	1.75	0.08

Table 6-7 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of lameness (lameness score ≥2a). The dataset this model is constructed from contains 4,880 individual lameness scores from 96 animals over a thirty-four-month period. The Animals recruited to this analysis were in their first lactation as of the 8th January 2018, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

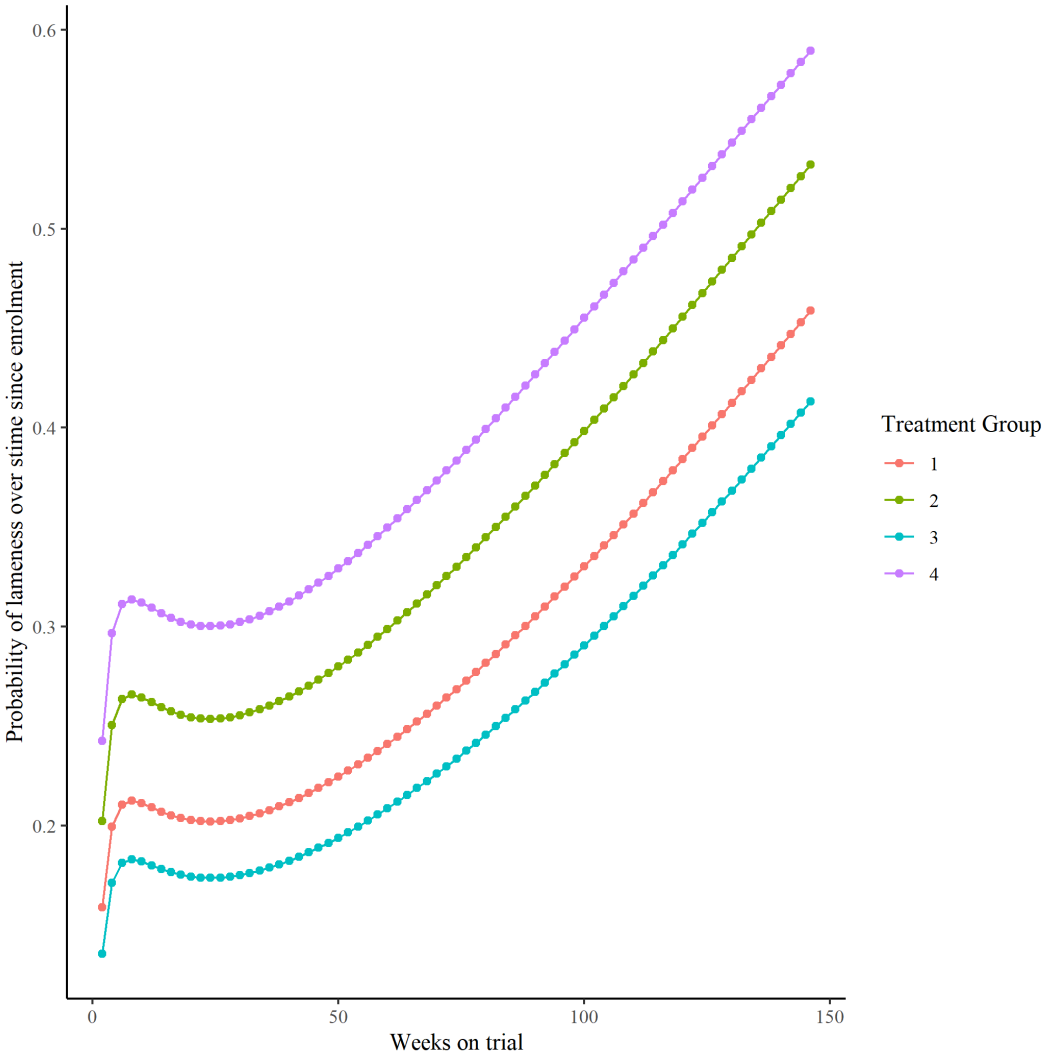


Figure 6-9 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of lameness (lameness score ≥2a) described in Table 6-7. The dataset this model is constructed from contains 4,880 individual lameness scores from 96 animals over a thirty-four-month period. The Animals recruited to this analysis were in their first lactation as of the 8th January 2018, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

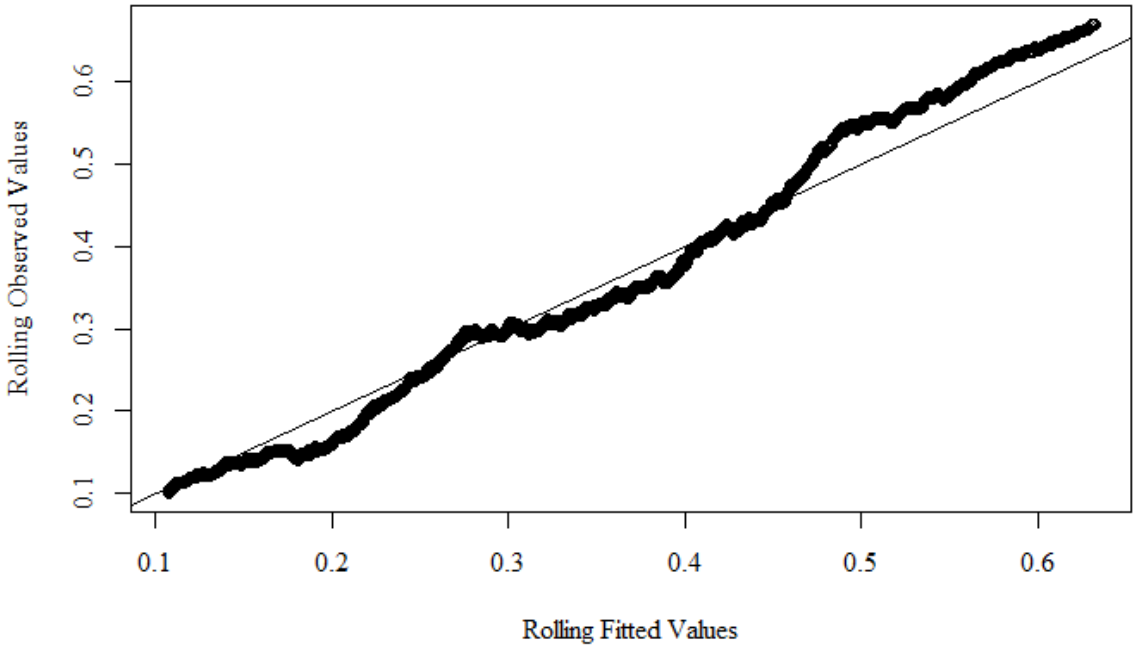


Figure 6-11 A plot of the 800-day rolling fitted and observed values generated from Model 6D which is described in Table 6-7. Model 6D investigated the effect of treatment group on the risk of an animal being scored as lame (≥ score 2a) over time. The fitted and observed values closely aligned and so model fit was deemed good.

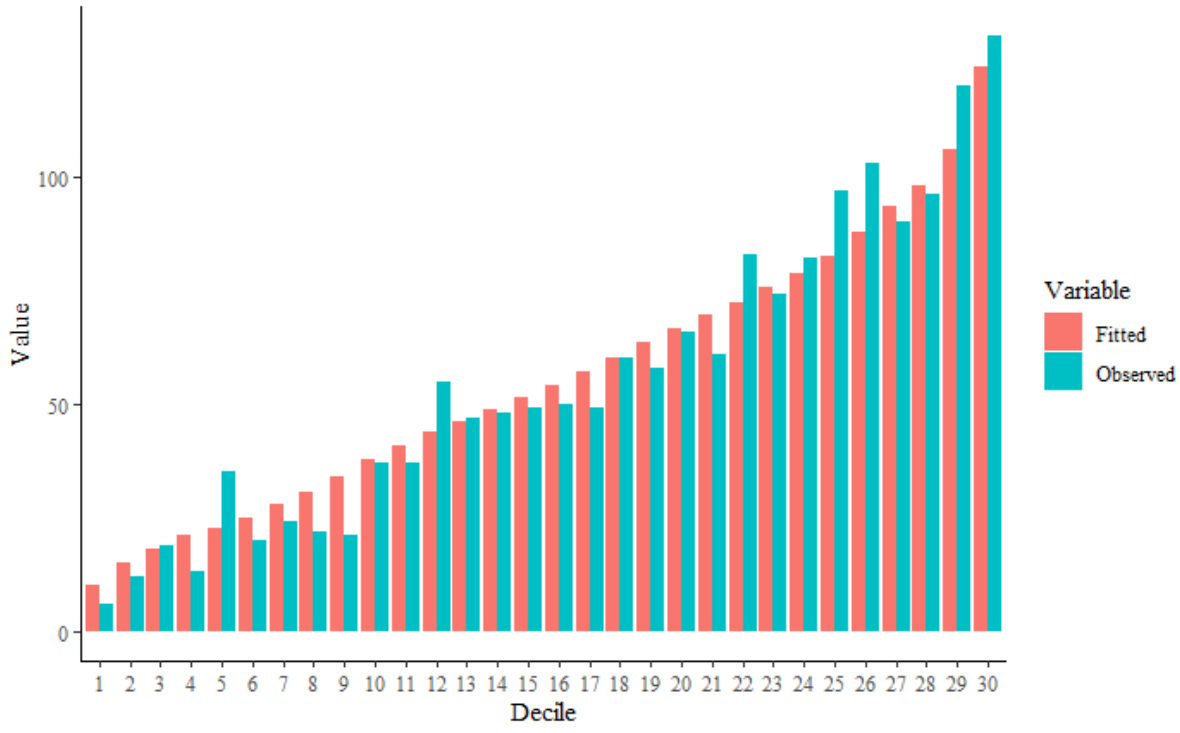


Figure 6-10 A plot of the fitted and observed values for model 6D which is described in Table 6-7. Model 6D investigated the effect of treatment group on the risk of an animal being scored as lame (≥ score 2a) over time. The fitted and observed values are consistently similar and so model fit was deemed as good.

6.3.6.2 Model 6E. Mixed-effects logistic regression model to evaluate the risk of severe lameness (score ≥3a).

Final results of Model 6E are provided in Table 6-8. No significant effect was observed between the treatment groups on the risk of an animal being scored as severely lame. The risk of severe lameness over time for cows in each treatment group is illustrated in Figure 6-12. As for Model 6D, model fit was good (Figure 6-13, Figure 6-14).

	Estimate	Odds Ratio	Standard Error	P Value
Intercept	-100.49	2.27902E-44	74.22	0.18
Treatment Group 1	Reference	–	–	–
Treatment Group 2	0.27	1.30	0.77	0.72
Treatment Group 3	0.17	1.19	0.82	0.84
Treatment Group 4	0.08	1.08	0.75	0.92

Table 6-8 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of severe lameness (lameness score ≥3a). The dataset this model is constructed from contains 4,880 individual lameness scores from 96 animals over a three-year period. Animals were in their first lactation as of the 8th January 2018, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

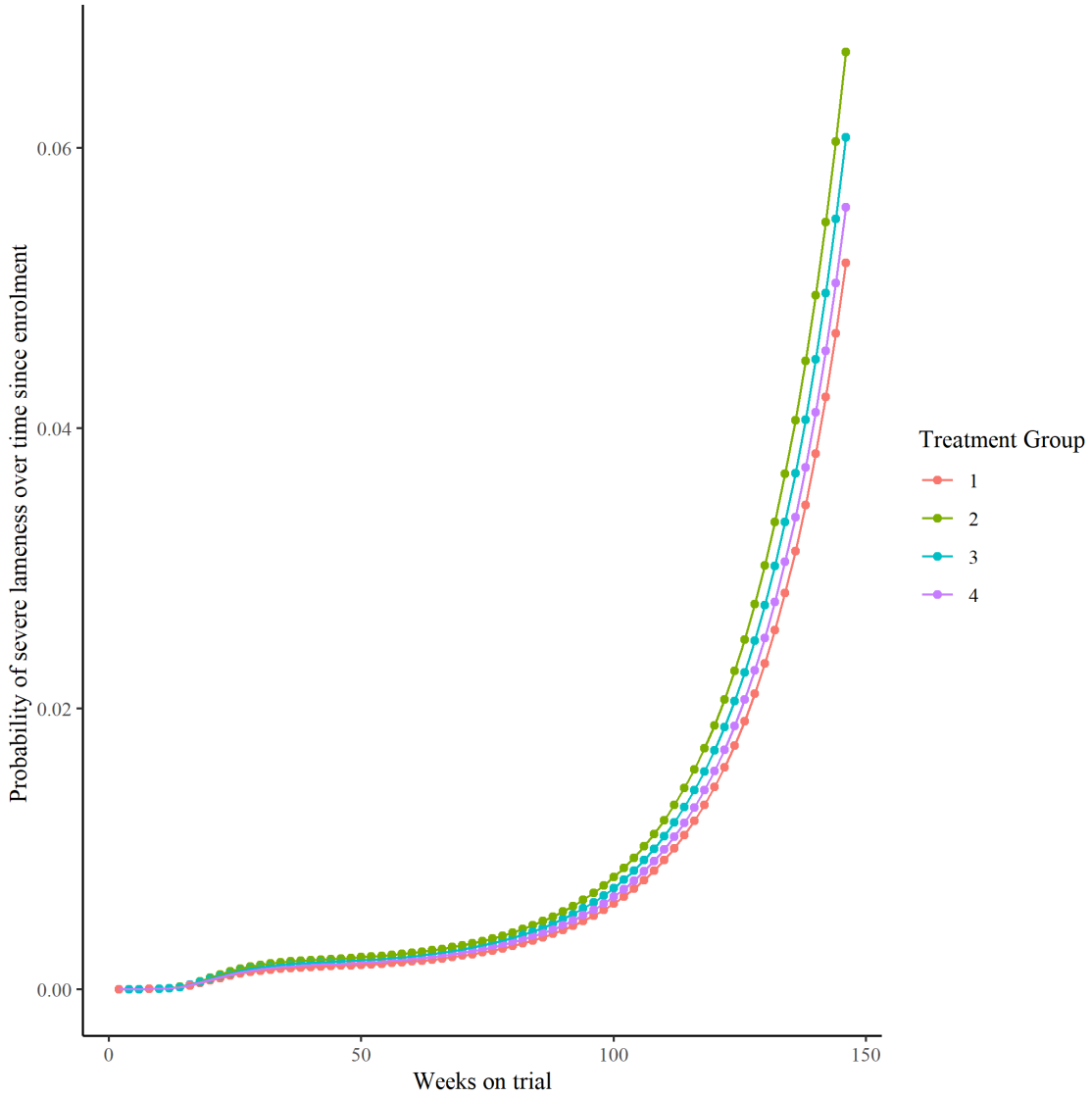


Figure 6-12 The output from a mixed effects logistic regression model investigating the impact of treatment group on the on-going risk of severe lameness (lameness score ≥3a) described in Table 6-8. The dataset this model is constructed from contains 4,880 individual lameness scores from 96 animals over a thirty-four-month period. The Animals recruited to this analysis were in their first lactation as of the 8th January 2018, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

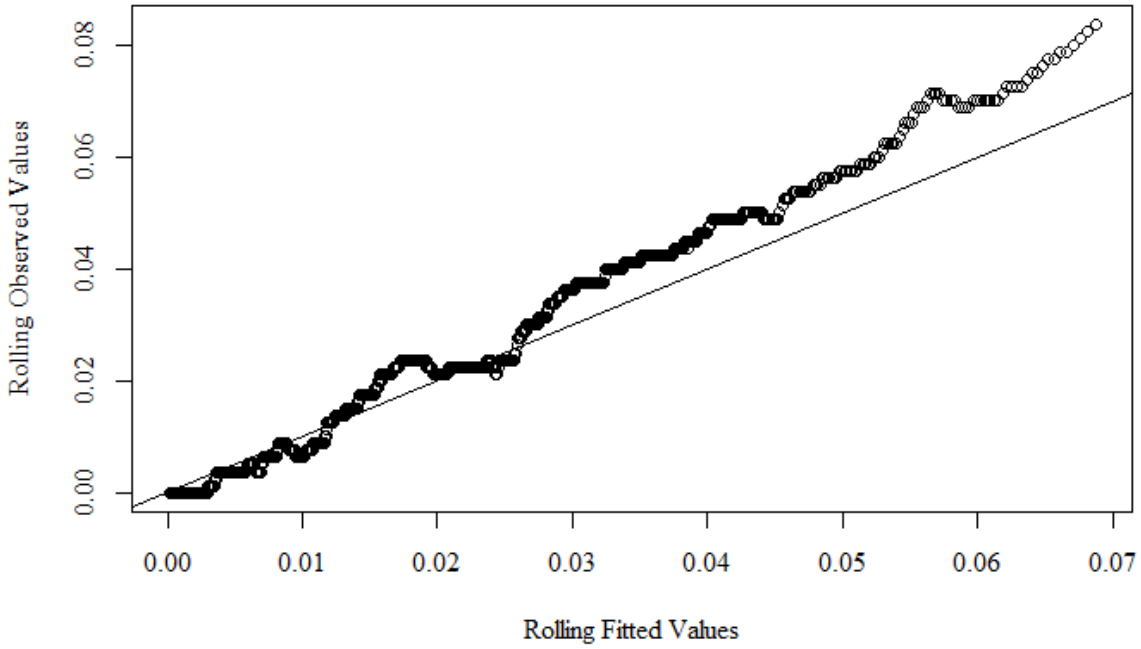


Figure 6-13 A plot of the 800-day rolling fitted and observed values generated from Model 6E which is described in Table 6-8. Model 6E investigated the effect of treatment group on the risk of an animal being scored as severely lame (≥ score 3a) over time. The fitted and observed values aligned and so model fit was deemed moderate.

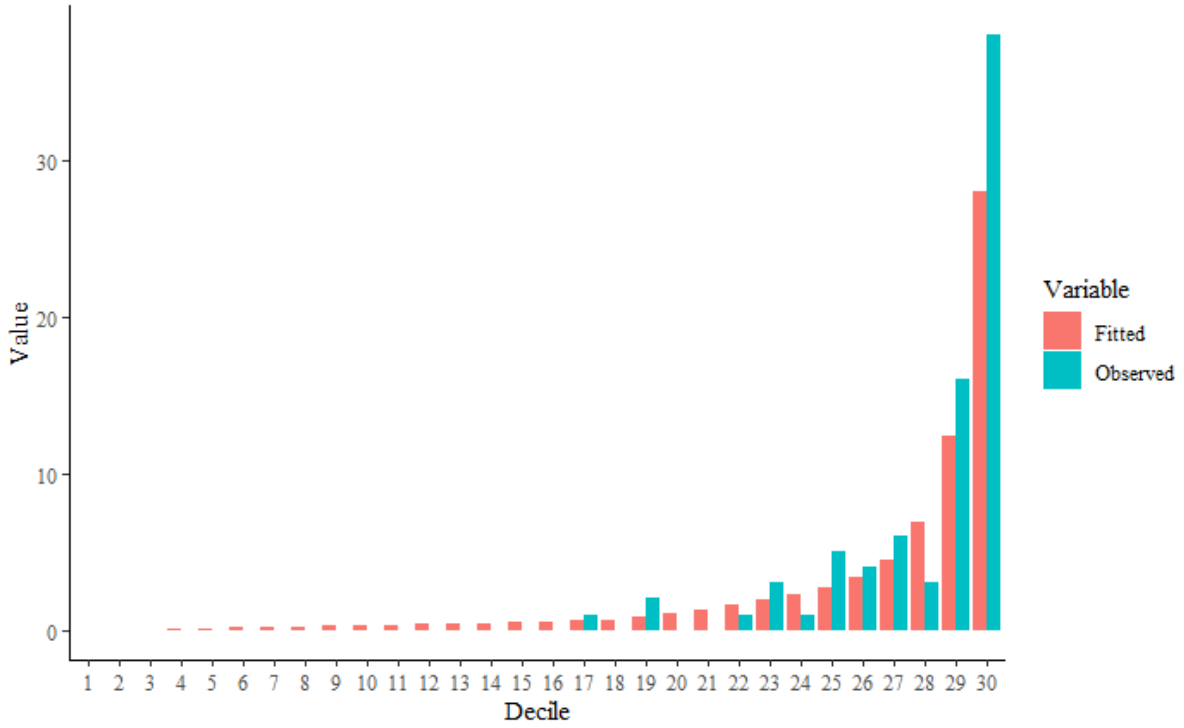


Figure 6-14 A plot of the fitted and observed values for model 6E which is described in Table 6-8. Model 6E investigated the effect of treatment group on the risk of an animal being scored as severely lame (≥ score 3a) over time. The fitted and observed values are similar and so model fit was deemed as moderate.

6.3.6.3 Model 6F. Cox proportional hazards model for survival to culling.

Final results of Model 6F are provided in Table 6-9. No significant effect was observed between the treatment groups on the risk of an animal being culled at any point of the study period. A Kaplan-Meier plot to illustrate survival time to culling by treatment group is presented in Figure 6-1615. Investigations of model fit indicated that the model assumption of proportionality of hazards was met and residual analysis indicated fit was good (Figure 6-16).

Model term	Coefficient	Hazard Ratio	P Value
Treatment Group 1	Baseline	–	–
Treatment Group 2	0.33	1.39 (95% CI: 0.51 – 3.84)	0.52
Treatment Group 3	0.24	1.27 (95% CI: 0.45 – 3.62)	0.66
Treatment Group 4	0.25	1.29 (95% CI: 0.48 – 3.46)	0.61

Table 6-9 Results of the final Cox proportional hazards model for time to culling. The dataset on which this model is based consists of 96 animals within a single commercial herd, of which 32 were culled during the study period. Animals were in their first lactation as of the 8th January 2018, and lameness/calving treatments were administered according to the treatment group to which the animal belonged.

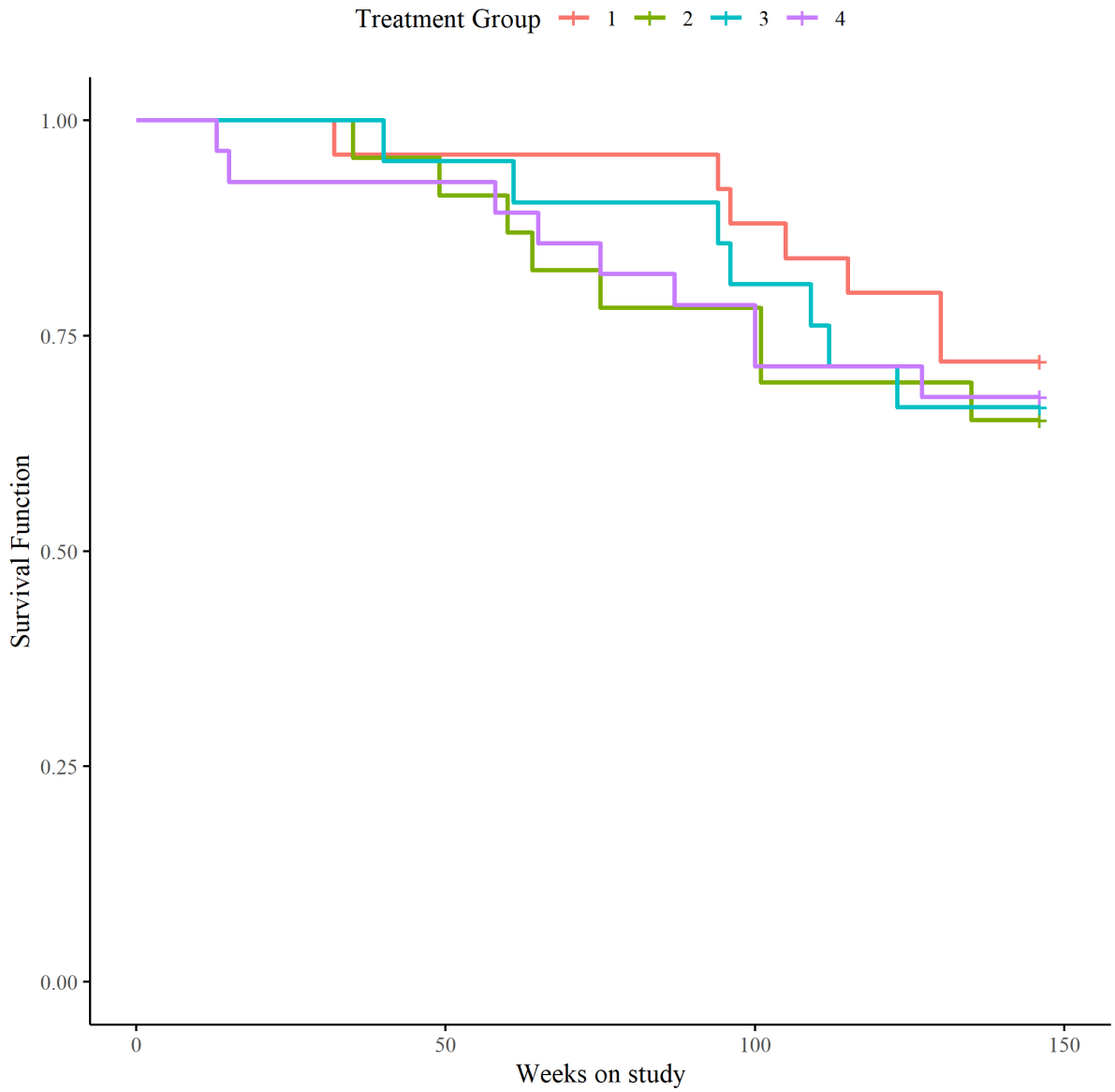


Figure 6-15 A Kaplan-Meier Plot for the survival analysis of cattle in their first lactation at study initiation who were subjected to one of four lameness treatment regimens over a three year long randomised controlled trial. The trial investigated the efficacy of non-steroidal anti-inflammatory drugs in the treatment and prevention of lameness.

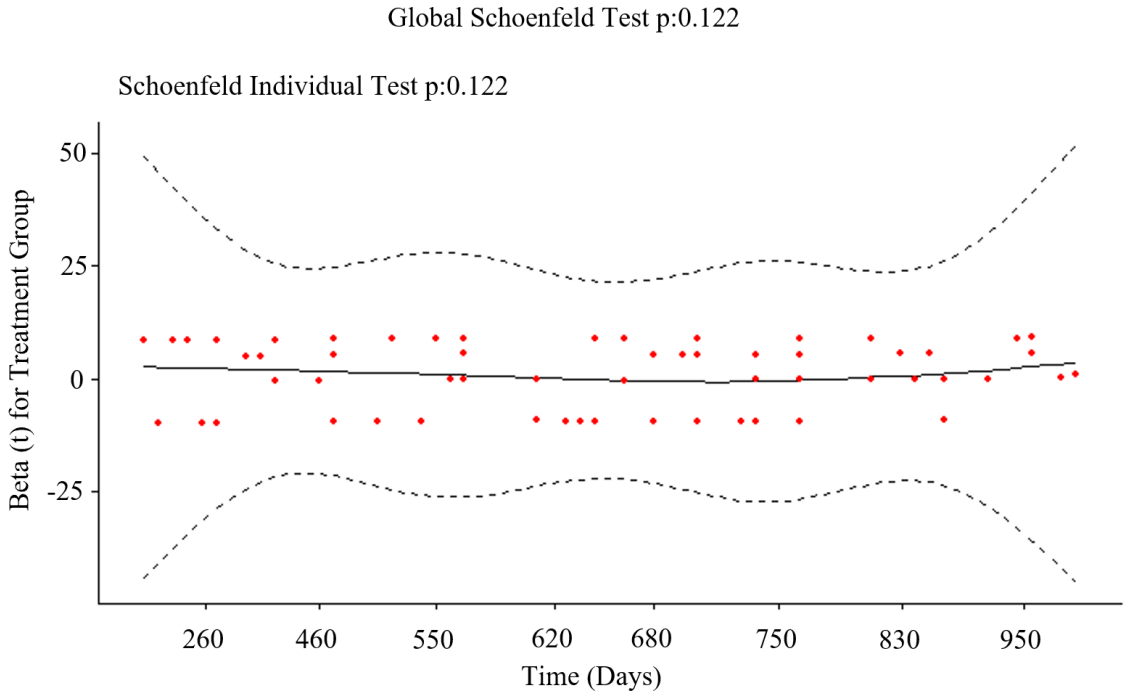


Figure 6-16 A plot of the Schoenfeld residuals for Model 6F which is presented in Table 6-9. Model 6F was a Cox proportional hazards model investigating the effect of lameness treatment group on the risk of culling through the thirty-four-month study period. In the current plot, there is no systemic change in Beta (t) as time progresses, and so the model fit is deemed to be good.

6.4 Discussion

6.4.1 Summary of Results

The only detectable significant effect within the current Chapter that was identified was that all adult cattle in Group 4 were more likely to be scored as lame throughout their time on the study, highlighting the importance of therapeutic hoof trimming and blocking in adult dairy cattle. There was no effect on the risk of culling identified in this study between the four treatment groups. Heifers in Group 3 that were lactating at study onset displayed trends similar to those presented in Chapter 5, in that they appeared to benefit substantially from NSAID administration at calvings, and at lameness. However, the number of animals available in this sub-section of the study meant that the study lacked the statistical power required to detect significance. The trends, however, suggest that there may be value to NSAID administration in these animals to reduce the risk of lameness across the animal's future life. If generalisable, the effect size and positive association observed within Chapter 5, when taken with the current results from the same study design, can be used to inform commercial lameness intervention strategies alongside future study design. Importantly, the design of this study, a

randomised controlled trial, means that results were unlikely to be affected by bias or confounding; RCTs are considered to provide strong evidence of a causal effect (Evans, 2003; Backmann, 2017). No negative effects were noted with this intervention however, and the production outcomes associated with lameness (such as milk yield reduction and reduced reproductive performance) still warrant further investigation.

6.4.2 Pain Management

Furthermore, the analgesic properties of NSAID administration on the pain experienced by lame cows should not be de-valued, even if their administration does not reduce future lameness risk. The pain associated with CHLs is already well understood and the role of NSAIDs in the modulation of this has been previously reported (Whay et al., 1998, 2005). Whay et al. (2005) demonstrated that NSAID administration had a positive impact on the short-term (28 day) nociceptive threshold of animals with CHLs, demonstrating their ability to improve the welfare of lame animals rapidly. This study identified that animals had a higher nociceptive threshold 28 days after treatment if NSAIDs were a part of the treatment regimen. When taken in conjunction with the current study, this could be taken to explain that the short-term lameness prospects of the animal improve, however there is no long-term benefit to their administration as part of a lameness management programme in adult dairy cattle with regards to future lameness risk. This avenue of investigation warrants further investment, as understanding the long-term pain management of lame cattle may become vital in aiding those cows who cannot have a positive long-term lameness outcome. However, their importance in managing long-term lameness in adult cattle must be examined to ensure that these animals have positive, long-term lameness prospects.

6.4.3 Inflammatory Pathogenesis

Systemic inflammation derived from the transition period and inflammation occurring locally within the hoof (derived from disease) may predispose an animal to future lameness by driving pathological change to the anatomy of the hoof as described in Chapter 5, i.e., weakening of the suspensory apparatus and the thinning of digital cushion and development of new bone on the caudal aspect of the distal phalanx, respectively.

It is possible that many of the adult animals in the current study have experienced lameness in some form prior to study initiation. This means that inflammation could have already occurred locally in the hoof to drive pathological change (metabolism/remodelling of the digital cushion the development of exostosis on the distal phalanx), thereby creating a predisposition to future lameness through a permanently increased pressure on the germinal epithelium. This predisposition through pathological change to the internal anatomy of the hoof may also be driven by systemic inflammation associated with the transition period.

It has been demonstrated that the extent of systemic inflammation around the transition period has an important role to play in the establishment of a successful and healthy future lactation (Bradford et al., 2015), however future research is required to understand the mechanisms through which this could influence lameness occurrence. This systemic inflammation could drive pathological change to distal limb anatomy through the mobilisation of fatty acids from the digital cushion (as inflammatory mediators), the weakening of the suspensory apparatus through MMP activation and through the deposition of new bone on the caudal aspect of the distal phalanx (a more comprehensive description of these is given in Chapter 5). These changes are likely to increase pressures on the germinal epithelium of the corium and predispose an animal to CHLs. This existing predisposition to lameness may have reduced the observed longer-term prophylactic or therapeutic effect of NSAIDs administered as part of this study. The anti-inflammatory effects of NSAIDs are likely unable to alleviate the physical pressures exerted on these cells which are caused by pathological changes to anatomy. Indeed, it was observed in the current study, that the risk of animals being scored as lame was markedly increased in Group 4 when compared to Groups 1 and 2. These animals received no therapeutic trim or block as part of their treatment protocol (unless identified as being severely lame), suggesting that the animals in Groups 1, 2 and 3 benefitted from a therapeutic trim and the potential application of an orthopaedic block. It could be taken that the physical alleviation of weight from diseased regions of the claws in these animals is particularly important due to the potentially compromised internal anatomy within the hoof capsule. If animals have an excessive baseline pressure exerted on the germinal epithelium, then it is unlikely that NSAIDs would have any benefit

in the alleviation of this trauma when not used in conjunction with a therapeutic trim and orthopaedic block. The implementation of a therapeutic trim and orthopaedic block would mean that the physical weight and pressure exerted on the germinal epithelium is alleviated, despite the presence of pathological change. This study could be taken to show the importance of trimming and blocking these animals that are predisposed to lameness to at least ensure that the chronicity cycle is reduced in its impacts. The ability of the contralateral claw to cope with the extra pressures associated with orthopaedic block application warrant further investigation to understand potential prophylactic use. This could be investigated using a multitude of outcomes and means; however, this thesis would propose that understanding the length of time an orthopaedic block could be retained without causing trauma to a healthy claw could warrant investigation. Also, some form of survival time analysis in the prophylactic application of orthopaedic blocks in late-stage disease lame animals may also prove useful in understanding how to manage end-stage disease to improve animal welfare.

In a previous randomised controlled it was found that animals that had been lame for longer than two weeks before treatment had a significantly reduced cure rate irrespective of treatment regimen implemented (Thomas et al., 2016), when compared to their acutely lame counterparts (Thomas et al., 2015). This delay in treatment could prolong the extent to which localised inflammation occurs inside the hoof capsule, meaning that the longer the delay, the greater the development of pathological change within the hoof capsule. Within the current study, the lameness history of these animals was largely unknown, meaning that predisposition to CHLs derived from previous CHLs is likely to be present. Anatomical change that has occurred previously due to transition and CHLs/lameness could potentially drive disease and thereby future anatomical change. These challenges to hoof anatomy are unlikely to be reversed using NSAIDs as either a treatment or prevention regimen once they have occurred.

6.4.4 Pain Modulation

These animals may have also undergone remodelling of neural signalling pathways (described in Chapter 5.4.3) which has led to their nociceptive threshold being lowered (alongside pathological change to distal limb anatomy). The potential lack of pain management delivered before study onset

could mean that these animals have experienced a history of pain associated with CHLs which has led to them entering a state of "chronic pain". Chronic pain is different to having a lower nociceptive threshold, however, regardless of the mechanism in place driving a predisposition to pain, both would result in animals experiencing more pain, even in the absence of pathology. This would mean that regardless of treatment or management protocol in place to minimise the risk of CHLs that these animals are likely to have an increased lameness score purely due to the historic remodelling that has occurred to the neural signalling pathways associated with nociception. These animals require careful management as this presents a substantial welfare challenge, at this stage, it is important to emphasise the potential short-term benefits imparted onto the animal with routine NSAID administration which may allow the animal to exist with a degree of comfort and allow for the "maintenance" rather than deterioration of the individuals nociceptive threshold.

6.4.5 First Lactation Animals

First lactation animals already within the herd at study onset may not have experienced a lameness event, however, these animals will have already experienced transition around first calving. This could mean that these animals have a depleted digital cushion (associated with a loss in BCS post-calving), a slightly weakened suspensory apparatus, and a small degree of exostosis development on the most caudal aspect of the pedal bone. In any degree, these pathological changes to distal limb anatomy may predispose an animal to a lifetime of future lameness, meaning that any intervention strategies implemented after first transition may be ineffective at managing the future of the animal's lameness status. The findings of this Chapter, when read in conjunction with the results of Chapter 5 suggest that the first calving an animal experiences is a critical event in the animal's lameness story and that NSAID administration at this event appears to present a substantial lameness mitigation strategy.

6.4.6 Welfare

It is important to note that this Chapter is not de-valuing the routine use of NSAID in the treatment of CHLs in adult cattle. Indeed, the CHLs are painful lesions which can cause animals to live in a state of hyperalgesia (Whay et al., 1998). It has previously been demonstrated that NSAIDs have an

important role to play in the modulation of pain associated with CHLs (Whay et al., 2005), meaning that they are important in improving the welfare of lame dairy cattle worldwide. This pain management may go further than the initial modulation of the responsiveness of dorsal horn neurons and may have an important role in the prevention of pain "wind-up". It should be reiterated that NSAID administration in adult cattle is vital not only in pain modulation, but also in offering potential economic paybacks. Animals experiencing pain derived from lameness are likely to display abnormal behaviours (Bach et al., 2007; Whay and Shearer, 2017) which are likely to lead to production losses (Huxley, 2013). The importance of NSAID use in minimising this production loss is still to be investigated to allow for an appropriate cost-benefit analysis to be presented to producers and the benefits of using NSAID in the treatment and prevention of CHLs in heifers entering the milking herd has already been demonstrated.

6.4.7 Limitations and Future Research

The main limitation of this study was that it was conducted on a single commercial dairy unit and so generalisability to other herds and situations is unknown. The likelihood of the study herd not being representative of the wider UK population of intensively managed dairy cattle is low, this was a well-managed herd which had a lameness control programme in line with what is common on many commercial units prior to study initiation. Factors that could influence the effect observed in the current study could include genetics, transition management, disease management and the system through which the animals are reared as youngstock. Within the commercial unit recruited to the study, there is no abnormal management regimen that could be identified as a confounder for the current study results. Despite this, additional research on multiple farms would be useful to evaluate the effects of administration of NSAIDs at calving and lameness on future lameness risk. The nature of the current study must be considered when replicate studies are designed and implemented across other farming systems. The body of work involved in the management of a study such as this is substantial. Organising fortnightly farm visits to lameness score and treat the lame animals (typically over two days) alongside coordinating the ordering of equipment, the removal of orthopaedic blocks, first aid intervention, management of treatments administered, and outcome scoring was a time-

consuming process. Furthermore, the financial cost in implementing a study such as this is considerable. This was a resource intensive study to manage and run, which is likely to be a barrier to multiple-farm studies of this magnitude and should be considered when designing replicates or supplementary studies in the future. With this in mind, it is unlikely that repeats of a trial such as this would occur purely due to the investment and timescale required to enact it. If repeats were to take place, it would be recommended that these focus on the pre-calving heifer to continue to set this animal on a trajectory to succeed with respect to lameness. Supplementary to this, trials investigating the generalisability of this study may prove important in furthering our understanding of the pathogenesis of CHLs across different dairy management systems. The present farm was an intensive, continuously housed, all year-round calving system wherein cows were milked three times a day. Comparisons between the management of lameness in a system such as this and an extensive once a day milking system in New Zealand for example where the cows are walking vast distances should be assessed. However, the CHLs are commonly experienced amongst both systems and indeed different production structures globally. The assumption with many lameness control programmes worldwide is that the pathogenesis of these lesions is similar regardless of the system, however, this is a substantial assumption to make, given the prevalence of the CHLs. Further research investigating the risk factors in different systems would allow for specific interventions to be designed and tested for each system to ensure appropriate, cost-effective solutions are offered to farmers irrespective of geographical location or cattle management structure in place.

6.4.8 Applications

This study, in conjunction with Chapter 5, has highlighted the importance of first calving and lameness in the risk of future lameness. This must be considered not only in the implementation of lameness control programmes in a commercial setting, but also in the development of future research studies. Previous lameness and calving should be observed as a potential confounder when testing the true impact of lameness prevention and treatment protocols. From this work, it is clear that any form of intervention programme must enrol pre-calving heifers to assess the true efficacy of an intervention to minimise confounding effects associated with previous calving and lameness history. That being

said, future research on management strategies for adult dairy cattle need to be optimised to manage the pain experienced by these animals and to endeavour to minimise the risks of future lameness. When introducing a lameness control programme such as this, the time delay in observing any kind of benefit must be considered. Chronically lame cows will stay lame and the only way that they will not support the prevalence of lameness on farm appears to be through them being culled from the herd. With this in mind, any reductions in lameness may take months or years to be observable, as the time required to rear replacement heifers and cull chronically lame adult cattle creates a "lag period" in which the benefit may not be substantial across the herd (purely caused by the presence of adult, chronically lame animals that cannot be cured, but require appropriate management).

6.5 Conclusions

In conclusion, the results presented in this chapter indicate that administration of NSAID at second calving onwards and at the time of all lameness events, had no effect on the risk of lameness and severe lameness during the three-year study period. From this research, it can be hypothesised that the animals within the current study had already experienced systemic inflammation through the transition period and pre-study lameness events, which reduced the beneficial effects of NSAID administration at these times compared to when given to heifers from their first calving. This inflammation would mean that pathological changes may have occurred in these animals that has put them on a trajectory towards future lameness that cannot be prevented or reduced by subsequent administration of NSAIDs.

7 Discussion and Conclusions

7.1 Introduction

The lack of tangible, long term evidence to support the design and implementation of lameness control programmes is a result of several factors. At the forefront of this is the lack of recognition that lameness has historically received regarding industry attention. This is despite it being one of the most substantial challenges to the welfare of the future dairy herd globally. This lack of recognition, in line with a lack of pharmaceutical investment into lameness research (due to a perception that the treatment of lameness will not drive pharmaceutical sales substantially) has resulted in a substantial shortfall in our understanding of optimal long-term management protocols. As a result, the condition is highly prevalent across the global dairy herd. Indeed, over the last ~15 years, the prevalence of lameness in the UK has increased.

Furthering our understanding of the pathogenesis of lesions that cause lameness is of vital importance to curtail the growth in prevalence observed in the last 10 years globally. The development of cost-effective, high impact management strategies to limit this disease are required to improve the health of the global dairy herd, the productivity of the individual farm business, and improve consumer confidence in the welfare of the animals producing their foodstuffs.

7.2 Summary of Findings

Chapter 3 identified that a lifetime history of lameness and claw horn lesions is associated with a reduced digital cushion volume at slaughter. It was postulated that genetic and developmental foundations in digital cushion structure may predispose an animal to lameness through a reduced digital cushion volume. This predisposition could then lead to the utilisation of fatty acids as inflammatory mediators associated with CHL pathogenesis. This results in the potential deterioration in the functionality of the digital cushion as part of the disease process associated with CHLs, thereby predisposing animals to future CHLs. Chapter 4 identified that in line with an increased lameness score in the four-weeks before slaughter, animals would have an increased proportion of Type 3 collagen in comparison to Type 1 contained within the digital cushions of their lateral claws. Again,

this could impair the functionality of the digital cushion, making it less able to dissipate the concussive forces of hoof-strike in the future. It was hypothesised that this was due to the presence of MMPs which act as key mediators in the inflammatory process and can degrade the structure of collagen, leading to turnover and deposition of "newer" Type 3 collagen. However, it is also possible that external factors changed the collagenous structure of the digital cushion which predisposed the animal to lameness. Both Chapters 3 and 4 postulate that lameness has a long-term negative impact on the structure and function of the digital cushion, which would in turn predispose the animal to future CHL onset, and future degradation of these key structures, leading to a cyclical pathway resulting in a chronically lame animal.

A 34-month long RCT was implemented on a single farm to produce the results described in Chapters 5 and 6. This research has identified the importance of NSAID administration post-partum and as a lameness treatment. Chapter 5 identified that NSAID administration at first and subsequent calvings and at lameness events reduced the risk of an animal being scored as lame in the future or being culled from the herd. It was postulated that the intervention with NSAIDs minimised the pathological change occurring within the hoof capsule which is understood to be driven by inflammation associated with the transition period. Parallel to this, was the hypothesis that the use of NSAIDs at calving and lameness allowed for more appropriate management of the pain experienced by the animal, this would block the remodelling of neural networking mechanisms which (if left unmanaged) would result in the animal having a permanently lower nociceptive threshold. This study also identified that the intervention that appeared most effective in the management of lameness in heifers that have not experienced the challenges of calving and lameness previously (i.e., the animals described in Chapter 5). This resulted in animals being at a significantly reduced risk of culling (whether this was due to a reduced risk of lameness or due to another effect of NSAID administration at calving is still to be investigated). Cattle that were already in the milking herd appeared to receive little benefit of routine NSAID administration at calving and lameness with respect to the risk of an animal being scored as lame or being culled. A subset of data was analysed which was pertinent to first lactation animals (those animals that had calved once before study onset) within the milking herd. These individuals

that received NSAID at calving and lameness appeared to have a trend towards a reduced lameness risk, however within that dataset lacked the statistical power to infer significance. The results of this study have demonstrated that routine NSAID administration at first and subsequent calvings, and at lameness events reduces the risk of an animal being scored as lame or culled, and that animals that may have experienced lameness previously, have little hope of a long-term positive lameness state.

The results of this thesis should be utilised to inform future research, the on-farm management of lameness and the development of policy, to improve global dairy cow welfare.

7.3 Aetiology Review

The research presented within this thesis allows for the current hypothesised aetiopathogenesis of CHLs to be updated. The evidence provided continues to place emphasis on the importance of inflammation in the predisposition and onset of these lesions. The study described in Chapter 5 has highlighted the importance of NSAID administration post-partum in the long-term management of lameness, suggesting that the inflammation experienced during this time is an important contributory factor in the aetiology of CHLs. Below the potential mechanisms through which inflammation (sourced from lameness or other factors) can lead to a predisposition towards CHLs are described.

Systemic and localised inflammation could act as a precursor to CHLs by manipulating the functional anatomy of the hoof, thereby increasing the pressures exerted on the horn-producing germinal epithelium. Systemic inflammation around the transition period and the extent to which it is present has been previously described as a significant mechanism influencing the health status of the animal for the remainder of her lactation (Bradford et al., 2015). In the study described in Chapter 5, the routine administration of NSAID post-partum reduced the risk of an animal being scored as lame, suggesting that inflammation post-partum has a role to play in the development of lameness. In that Chapter, it was postulated that routine NSAID administration at calving protects the functional anatomy of the hoof through:

- Inhibiting the function of MMPs on the suspensory apparatus. The systemic secretion of relaxin has been postulated to influence the structure of the suspensory apparatus around the

time of parturition (Tarlton et al., 2002; Knott et al., 2007). Relaxin partially functions through the action of MMPs and their respective function. Numerous studies exist demonstrating that MMP production and action are inhibited by the administration of NSAIDs (Sadowski and Steinmeyer, 2001; Pan and Hung, 2002). If this is the case within the current study, then what is being observed is in part a reduction of "the calving effect", the extent to which this individual factor predisposes the dairy cow to CHLs is unknown, however it is commonly accepted as being a significant contributory factor to the high risk period for CHLs that occurs post-partum. Tarlton et al. (2002) identified the weaking of the suspensory apparatus around the time of calving, however the duration of this weakening was not identified. This weakening would mean that the pedal bone is less well suspended in place, leading to additional pressures on the germinal epithelium. This thesis postulates that the suspensory apparatus may never recover from this "calving effect", meaning that through repeated calving events over time, that the suspensory apparatus only becomes weaker and weaker as the parity of the animal increases.

- Protecting the fatty acids contained within the digital cushion from being utilised as part of an inflammatory process. The inflammation associated with the transition period could require the utilisation of fatty acids systemically as mediators (Calder, 2006; Bradford et al., 2015). Their utilisation as mediators could lead to the systemic metabolism of adipose tissue to meet the demand of the inflammatory process. This systemic adipose metabolism could influence the digital cushion, leading to a reduction in volume, meaning that the digital cushion is less able to support the distal phalanx which will exert excessive pressures on the germinal epithelium. Contreras et al. (2017a) identified that adipose tissue can effectively "remodel" during periods of lipolysis and inflammation. The effects that this remodelling could have on the functionality of the digital cushion is unknown, as is the ability of the digital cushion to recover from this process. When the fatty acid content of the digital cushion in heifers is compared to adult cattle, the potential role of inflammation becomes pertinent. The digital cushion of heifers contains a significantly larger volume of arachidonic acid than that of adult cattle (Räber et al., 2006). The metabolism of arachidonic acid is associated with

inflammatory events and the release of inflammatory mediators. With more of this fatty acid being present in the heifer digital cushions, it is possible that arachidonic acid is being utilised as an inflammatory mediator more regularly in the digital cushions of adult cattle as they are more likely to experience lameness/CHLs (Mason, 2017).

- Protection of the collagen contained within the digital cushion from the actions of MMPs associated with relaxin activity. Relaxin functions through the degradation of collagenous fibres via the actions of MMPs (Kapila et al., 2009; Van Doren, 2015). A large proportion of the digital cushion is primarily connective, collagenous tissue which may be susceptible to MMP activity. This degradation of collagen in the digital cushion may impede its ability to dissipate the concussive forces of hoof strike, thereby predisposing the animal to CHLs. It could be postulated that this could take place due to systemic inflammation around the transition period, localised inflammation during CHL occurrence, or through the actions of relaxin around parturition. The pharmacological mechanisms through which NSAIDs function inhibit MMP activity (Sadowski and Steinmeyer, 2001; Pan and Hung, 2002), which may in part explain the effects observed in Chapter 5.
- Inhibiting the development of exostosis on the most caudal aspect of the distal phalanx. Newsome et al. (2016) identified that the development of new bone growth was associated with age and a history of lameness. The authors of that paper postulated that inflammation associated with CHLs was the driving force behind the development of this new bone formation. They also suggested that this new bone formation would likely predispose the animal to future CHLs. It could be suggested that the association between age and extent of growth could be caused by systemic inflammation arising during the transition period. Typically, the older the cow, the more calvings she will have experienced, meaning that alongside to an increased exposure to lameness risk, there is also an increased exposure to systemic inflammation during the transition period. The more transition periods an animal experiences, the more systemic inflammation they are likely to experience and the additional new bone that could be developed on the caudal aspect of the pedal bone. This is where

targeted NSAID administration may inhibit the development of this new bone, through reducing the impact of transition sourced inflammation as well as at the time of lameness.

Our understanding of the changes to anatomy described above are still in their infancy. However, it can be postulated that with each transition period an animal experiences, the more degradation occurs to the functional distal limb anatomy. The suspensory apparatus, the digital cushion and the distal phalanx may never truly recover from the pathological challenges associated with transition and CHLs. This is still to be understood fully; however, it is logical to suggest that once the structure of the suspensory apparatus and the digital cushion have been initially compromised, that there is very little scope for their recovery. The same applies to the distal phalanx, unless the exostosis was somehow internally metabolised or degraded, there is little scope for this bone development to reduce or disappear over time. What may then be observed over several lactations is a diminishment of the ability of the anatomy of the hoof to be able to protect itself, even without the challenge of claw horn disease.

The extent to which these pathological changes occur also needs to be examined. Within our current evidence base for their contribution to the aetiopathogenesis of CHLs, there is a reliance on cadaver studies which whilst convenient, present challenges in interpreting the results. Firstly, in Chapter 3, the differences observed in digital cushion volume are of an unknown origin. Although they are associated with lameness during life, no causal mechanisms could be derived from that study and so it becomes a question of “chicken and egg”. What came first, the loss of digital cushion or the CHLs? Obtaining longitudinal measures of the structure of the digital cushion through the animals young as well as adult life may provide a useful insight as to how the digital cushion may be pre-conditioned to optimise the hoof health of the animal in the future. Furthermore, the volume of digital cushion could be tracked along a lactation in a robust fashion to identify risks for CHLs and examine the response to treatment once disease arises. The study reported by Newsome et al. (2016) relied on material sourced from cadavers and so inferring causality does present some issues. However, the primary challenge with interpreting the results of this study is in the cross-sectional nature of the study, and the method of image analysis used. The challenges of cross-sectional study design have already been described,

but briefly it is difficult to understand the nature of pathological change without longitudinal measures capturing its progression. The nature of image analysis relied on a standardised measure of bone, which for the investigations taking place, was appropriate. This measurement of bone relied on the use of landmarks on the distal phalanx to establish measurements of the exostosis. However, what may not have been measured using the methods described is small, uniform developments of bone on the plantar aspect of the distal phalanx. This would mean that low-grade pathological change to the anatomy of the distal limb may go un-recorded, therein presenting a bias to under-report pathological change to anatomy. Developing imaging modalities capable of capturing accurate measurements from live animals (i.e., not trans-sole ultrasonography) are likely to prove crucial in developing our understanding of the nature of pathological influences on distal limb anatomy. If live-animal recordings are possible, it would allow for longitudinal study designs to be constructed to observe the impact of interventions on the anatomy of the distal limb to deliver high impact research which will empower farmers to reduce lameness prevalence worldwide.

Additionally, understanding the structural importance of each cushion may prove important in understanding the predisposition to CHLs. To this end, more robust means of measuring the digital cushion in situ in live animals need to be established to understand the variables that drive the degradation of this structure. Within Chapter 4 it was identified that different individual cushions can have different adipocyte sizes, perimeters and collagen content. Gaining a better understanding of how each cushion functions in its own individual right may allow for a better understanding of the pathogenesis of WLD and SH/U as separate lesions, to better place emphasis on risk factors associated with these conditions.

What appears to be vital in the management of lameness with respect to its pathogenesis is the first calving and management of the first CHL occurrence. In Chapter 5 it was identified that the Group 3 treatment protocol appeared to set animals onto a trajectory whereby they were less likely to be scored as lame or culled from the herd. This contrasts with the animals presented in Chapter 6 (both adult cattle and milking heifers presented in that Chapter). This would suggest that in order for this treatment regimen to succeed, it needs to be implemented from the first calving event. Most intensive

production systems set a target for their maiden heifers to join the milking herd at 24 months age. At this stage in life, these animals are still relatively immature anatomically. If the functional anatomy of the hoof is compromised by calving or lameness at this crucial timepoint, then it is likely to be impeded for the remainder of the animal's lifetime as the development of key structures such as the suspensory apparatus and digital cushion may be impeded and the growth of the distal phalanx in line with the rest of the animal may be altered. These alterations and degradation may occur purely due to systemic inflammation that occurs around the time of transition, meaning that even without lameness, these animals may be predisposed to lameness. This highlights the importance of first calving and first lameness event as a time when the functional anatomy of the hoof capsule in the maiden heifer needs to be protected by any means necessary.

The anatomical changes may present on farm in a similar fashion to the results reported within Chapter 6 wherein adult dairy cattle received no benefit in terms of lameness and culling risk. The complete lameness history of these animals was unknown, however, due to the nature of the randomisation process, systematic bias was unlikely to have occurred (meaning that animals with a history of lameness were evenly allocated to the treatment groups at random). Any effects observed were expected to be solely caused by the interventions implemented. Within that Chapter, no benefit of the Group 3 intervention was observed, meaning that this intervention was only effective in those animals naïve to lameness and transition (i.e., the heifer cohort reported in Chapter 5). This presents a challenge in the management of lameness on farm in the future as will be described below. The true reasons for this lack of effect are unknown, however, this thesis postulates that the animals that have previously experienced transition or lameness have already begun this downwards spiral which creates the chronically lame individual. If just one transition period (and the systemic inflammation associated) has been experienced, this may be enough to begin the downwards spiral and degradation of functional hoof anatomy. Within the older cohort of animals who may have experienced multiple transition periods and lameness events without NSAID intervention, this becomes particularly important. Some of the animals recorded in the adult herd described in Chapter 6 underwent 20 treatments for lameness (whether derived from CHLs or not) which involved a therapeutic trim,

orthopaedic block and NSAID administration. Certain animals appear to be “incurable” in the long-term with regards to being prone to repeat cases of lameness. From this the question arises as to when it is appropriate to cull an animal that is predisposed to repeated cases of lameness, not only in terms of resources, but also in the ethical implications of sustaining this animal. If bone growth, digital cushion remodelling and degradation of the suspensory apparatus cannot be undone, then it appears that there is little hope for these animals walking without pain throughout their lives. It is important to note that the study presented in Chapter 6 described purely lameness-based outcomes, and although this is a metric used to assess animal welfare, other metrics need to be examined before NSAID administration is removed from the recommended lameness treatment protocol. Understanding the pain experienced by these animals on a more detailed level and the production outcomes associated may provide a strong incentive to routinely administer NSAID to animals at calving and lameness to at least maximise the productivity of that individual whilst also improving welfare through the short-term alleviation of pain.

7.4 Pain Management Associated with Claw Horn Lesions

The effect of NSAIDs on the short term nociceptive thresholds of lame animals has been reported previously (Whay et al., 2005). However, what is not understood, is the role of NSAIDs in the long-term management of the nociceptive threshold of lame and non-lame animals. Animals that experience pain derived from more severe CHLs are understood to have a lower nociceptive threshold than those animals that have not experienced a CHL even once pathology has resolved/cured (Whay et al., 1998, 2005). This is understood to be due to the individual entering a state of hyperalgesia, wherein the nociceptive threshold is so low that these animals are experiencing pain at the slightest stimulus to the affected region of the hoof. This process involves the remodelling of neural signalling networks which are responsible for nociception (Devor, 2006; Voscopoulos and Lema, 2010). This remodelling may lead to the animal being predisposed to experiencing pain for the remainder of their lives.

Many intensive dairy production systems aim for first lactation heifers to calve at 24 months of age, a point at which these animals may not have fully matured. This exposes these animals to the painful

events of calving and potentially lameness when neural processing networks may still be developing. Exposure to these events without an appropriate pain management plan in place may result in the remodelling of these networks, predisposing these animals to having a lowered nociceptive threshold. This lowered nociceptive threshold could predispose cattle to lameness from mild insults which occur as part of locomotion and standing. Further investigation into the physiology of pain in the dairy cattle is warranted, especially with reference to the long-term management of pain derived from CHLs and how more robust animals can be produced using appropriate pain management plans throughout their young and adult lives.

7.5 Pain or Pathology?

In the RCT presented in Chapter 5, it could be argued that the Group 3 intervention has either impeded the development of hoof pathology (i.e., disrupted the inflammatory pathogenic mechanisms associated with CHLs) or it has enabled animals to cope with the pain associated with CHLs more readily. If the latter were to be the case, it could be suggested that these animals are displaying a normal gait, whilst a CHL is present. The ethical question surrounding the management of pain versus the management of pathology arises, the importance of either can only be understood after further robust intervention studies have been conducted to examine production effects of "pain free" lesions, and most importantly, the impact that these pathologies may have on the animal's experience within the herd (i.e., how they may impact on animal welfare).

It could be hypothesised that both the inhibition of pathology and the appropriate management of pain are taking place concurrently to explain the results presented within Chapter 5. Previous research has highlighted the importance of appropriate pain management in the prevention of animals experiencing hyperalgesia or chronic pain (Greene, 2010; Voscopoulos and Lema, 2010). Furthermore, the role of NSAIDs has been suggested by several studies to interfere with the inflammatory aetiology associated with CHLs, suggesting that the pathogenic and pain related cycles associated with CHLs are inhibited.

Regardless of the mechanism through which the Group 3 intervention functioned, this intervention resulted in the reduction of lameness prevalence by an absolute percentage of 10%. One in ten fewer

animals experiencing pain upon locomotion is a substantial reduction and one which warrants stringent future investigation.

7.6 On Farm Implementation

The research conducted by Thomas et al. (2015, 2016) conclusively demonstrated that the routine use of a therapeutic trim, orthopaedic block and three day course of NSAID improved cure rates in newly lame animals. Despite robust evidence to this effect, it is common for lame cows to be left un-treated unless severely lame. Even when identified as lame, it is common for these animals to receive a sub-optimal treatment plan with NSAID administration being a common missing element from the treatment plan. This presents a two-part challenge; firstly, these are animals that are experiencing pain upon locomotion, and the ethical implications of a farmer withholding pain relief from that individual are substantial. Secondly, the opportunity to improve the cure rates associated with the treatments of CHLs is one which should not be missed in terms of the cost of the disease and the understanding surrounding the chronicity of these conditions. Understanding the barriers to farmers using NSAID in the treatment and prevention of lameness may help improve the rate at which appropriate lameness management strategies are implemented globally, thereby improving cow welfare globally. It is well reported that farmers regularly underestimate their lameness prevalence (Leach et al., 2010; Ranjbar et al., 2020), meaning that less importance is placed on lameness control on farm as it is not perceived to be a problem by the primary producer. If farmers are unable to identify lame animals, then their perception is that they may have a healthy herd, meaning that a degree of pride can be taken in the condition of their animals which has been shown to be a substantial driver for behaviour change previously (Leach et al., 2010; Bennett et al., 2014; Ranjbar et al., 2020). Supplementary to this is the hidden losses associated with lameness; farmers do not dispose of milk as they do with a condition such as mastitis, which has been treated with antibiotics. The losses surrounding lameness are hidden/indirect, meaning that quantifying the cost of that lameness event for that individual animal is challenging. Both of the aforementioned factors are substantial barriers to routine NSAID usage in the prevention and treatment of lameness as they remove an emphasis on the welfare and economic importance of the condition.

For the routine treatment of lameness, firstly the condition needs to be recognised. Farmers are typically able to identify severely lame animals, however it is the identification of early stage, mild disease which is understood to deliver the maximum return on investment. For farmers to be able to intervene at the early stages of disease as described in Chapters 5 and 6 and other RCTs (Thomas et al., 2015, 2016), the detection of lameness in a commercial setting needs to be improved from the current standards. Many health and welfare auditing schemes are now encouraging the use of an independent lameness scorer to assess lameness on farm. However, what is commonplace is for the technician scoring to submit a list of lame animals to the farmer, and for this to be passed onto the auditing scheme without direct action on farm being implemented as a result of this data generation. A shift needs to take place to empower farmers to use this mobility data to firstly isolate and treat lame animals, but also to monitor animals on a longitudinal basis to isolate risk factors for management change to occur to improve herd mobility. The farmer needs to have the understanding of how to use these scores in order to benefit as much as possible from this compulsory data capture.

Equally critical to the success of a lameness control programme, is the rapid treatment of the disease-causing pain. Previous research has demonstrated that a delay in CHL treatment leads to a reduced cure rate (Groenevelt et al., 2014; Thomas et al., 2016). A delay in treatment is inevitable if no regular lameness scoring is being implemented or even if quarterly or monthly lameness scores are in place. The requirement of this protocol to succeed relies firstly on the at least fortnightly occurrence of a lameness score (meaning that an animal could become lame the day after and be in that condition for no more than 13 days), and also the rapid and effective treatment of the cause of the condition making the animal lame (Leach et al., 2012; Groenevelt et al., 2014; Bell, 2015). As soon as lameness is identified it needs to be addressed (ideally no longer than 48 hours after identification) to prevent any further compromise in animal welfare and ensure optimum cure rates can be achieved.

The method of treatment is also a key feature in the success of this intervention programme. Hoof trimming is a task that requires skill and knowledge and is commonly compromised in many on farm lameness control programmes. The role of the hoof trimmer is vital in this respect, and the skill that they bring (whether as an internal member of the farm team, or an external contractor) to the

management programme needs to be critiqued as well as appraised. This is particularly where the monitoring of lameness scores over time can be a useful exercise to monitor the performance of the trimmer and the cost-effectiveness of a lameness control plan (e.g., by looking at cure rates or lameness after routine mid-lactation trimming). The use of a contracted trimmer has several benefits such as the specific knowledge in the area of hoof care, larger numbers of animals can be checked in a smaller amount of time and also the reduction in investment in on farm staff training/equipment maintenance (please note that this list is not exhaustive). However, the use of external contractors presents challenges such as irregularity of hoof trimming visits (meaning lame cows may remain untreated between visits) and an over-reliance on external contractors for hoof care (which may result in under-investment in facilities and the lack of skills required to trim a cow's hoof appropriately amongst the farm team).

The final barrier to the implementation of this protocol is the use of NSAIDs routinely in the treatment of mild lameness cases, and routine administration at first and subsequent calvings. The routine administration of NSAIDs as part of a lameness treatment regimen has (as previously stated) been slow in terms of uptake by producers. The evidence provided in Chapter 5 suggests that the potential paybacks regarding the routine NSAID administration are vast regarding both animal welfare and capital return on investment. The costs of NSAIDs licensed for treating lameness in dairy cattle have fallen considerably in the last ten years, however the cost of use is commonly cited as a reason not to administer them routinely. When NSAIDs are administered, it is commonplace that these go to the most severely lame animals who for the purposes of welfare absolutely require some form of pain relief. However, it is the mildly lame animals who are more likely to receive the full benefit of NSAID administration early in the disease cycle. These animals are likely to have minimal pathological change occurring within the anatomy of the hoof (as described by Newsome et al. (2016) and in Chapter 3), and so halting any potential pathological change is likely to prevent these cows from having a disposition towards being lame (i.e. through minimising the changes occurring within the hoof capsule with NSAID administration, the animal is less likely to experience future CHLs than an animal that does not receive NSAID).

The evidence suggests that only in combination will all these factors work to reduce lameness prevalence and improve cow welfare. The omission of a single factor within this treatment regimen will substantially reduce its effectiveness (Groenevelt et al., 2014; Bell, 2015; Thomas et al., 2015, 2016). The appropriate dissemination of this research and optimal treatment regimens to farmers will be vital in the success of the adoption of this programme. Furthermore, there is scope for this research to inform contractual agreements regarding milk purchasing. As mentioned previously, milk purchasers are now auditing the health and welfare status of the dairy cattle producing the milk purchased. This is done primarily to improve the transparency regarding animal welfare on farm, with a view to improve the public perception of the dairy industry. The incentives for implementing the Group 3 treatment protocol as described in Chapter 5 are substantial, with an absolute reduction of 10% in lameness prevalence expected in the heifers to which this regime is applied, the economic benefits for the farm business are vast. However, what is also considerable, is the potential improvement in public perception of the dairy industry. If animals are routinely given NSAID at calving (which is understood to be a painful and challenging process either way) and lameness (which by definition is a painful condition) it can work to improve the consumer perception of farmer empathy towards animal pain. However, what is more worrying, is the potential impact that not implementing this programme may have on the perception of the dairy industry (regardless of the economic paybacks). What has been described in this thesis is a means of potentially reducing the global lameness prevalence by an absolute of 10%. If the dairy industry chooses to ignore or not fully adopt this protocol, then the risks are vast. It would appear highly damaging to identify such a simple intervention through the most robust means of testing said intervention (a RCT), for it to be ignored and not taken forward either in a research perspective (to further investigate generalisability) or for the adoption of it throughout the dairy industry. This is where national welfare auditing schemes and milk purchasers may have a substantial impact on the prevalence of lameness globally, through using research such as this to dictate management interventions which would work to improve animal welfare and improve the consumer base's opinions around the ethics of the dairy industry.

What also needs to be provided is an adequate support mechanism to allow farmers to adopt this intervention regimen with relative ease. Mechanisms need to be employed to reduce the costs and remove barriers associated with routine lameness treatment (regarding both hoof trimming and NSAID administration). What needs to be made more readily available are members of staff or contractors with the skill to identify and treat lame animals appropriate to the cause of pain, and veterinarians and consultants who are willing to analyse data, audit lameness management protocols and deliver high impact advice, pertinent to the treatment regime already in use. With regards to this, the changes in the UK RCVS legislation are perfectly placed to ensure that veterinarians and hoof trimmers are both following the evidence base for lameness management protocols and may allow for the better communication between farmer, vet, and hoof trimmer with the end result of a more cohesive lameness management plan.

The reason why the facilitation of this management protocol is so important is because the implementation of lameness control programmes, particularly with respect to identification and treatment of lame animals, is highly demanding on staff time and morale. Within the farm utilised in the studies described in Chapters 5 and 6, as part of implementing the RCT at times ~100 cows every other week required treatment for the first 8 months of study administration. This was at times challenging for those implementing the study and should bear consideration when a new management protocol is being implemented on farm. When independent scorers attend a farm for the first time, there may be proportionally a lot more lame cows than the producer first realised. This can be demoralising and raise challenging conversations between those scoring, treating the cows and managing the herd. However, from the observations made within Chapter 5, it is clear that the routine treatment of lameness and the proactive approach to treating mildly lame individuals will deliver high impact results on farm (albeit through a large amount of challenging, physical work). Regarding investment and morale, it is also important to note that the positive effect observed within the Group 3 intervention (described in Chapters 5 and 6) only had a positive effect on the heifers that calved into the herd after study onset (i.e., those only described in Chapter 5). On a commercial unit, this would likely mean that any substantial, herd-wide reduction in lameness prevalence is likely to take years to

achieve. This “lag-effect” is due to the proportion of adult cattle respective to those that have calved in as heifers under the new management regime. As more heifers calve into the new management regime, the proportion of animals that are set on a trajectory to live longer and be less lame will increase. Furthermore, as the adult cattle that did not calve into the management protocol as heifers are culled from the herd, the proportion of heifers set to succeed will continue to increase. This will result in the observable benefit of this protocol across the herd taking years to be substantial, however, what can be achieved in that time is remarkable in terms of reduction in risk of lameness.

7.7 Future Research

The research described in this thesis has allowed for our understanding of the aetiopathogenic mechanisms that underpin CHL onset to be better understood. However, several further hypotheses have also been raised as a part of this process which warrant further investigation.

Within Chapter 3 it was described how the reduced digital cushion volume in the lateral claws could have existed before the lameness and could have acted as a predisposing factor to this condition. Further studies examining the digital cushion in live animals using MRI would allow for the assessment of genetic and developmental inputs into its structure, alongside observing how the digital cushion responds to CHLs and differing treatment protocols involving NSAIDs. Furthermore, understanding how the 3D structure of the digital cushion changes throughout lactation and indeed across parities would allow for a better understanding of the effects of sequential transition periods and lameness events on this tissue. Furthermore, perfusing limbs with contrast pre-MRI scan, may allow for a better understanding of the inflammatory effects CHLs have on this structure.

Within Chapter 4 a large variation in collagen content was described, however, only a small proportion of this variation could be explained by the model presented. Better understanding the histology of the “healthy” digital cushion, with a particular focus on optimising functionality may allow for more robust animals to be bred, or for better treatment regimens to be developed. The better understanding of the range of adipocytes, the potential impact of disease on this aspect of the digital cushion’s histology and how the differences in adipocytes impact on the structures function would

also allow for better farm level decision making and the isolation of risk factors predisposing animals to lameness.

The studies presented in Chapters 5 and 6 can both inform on farm decision making regarding lameness in an impactful fashion. However, better understanding the generalisability of these studies may prove critical in their adoption globally. The implementation of these studies across multiple production systems may prove challenging. The nature of these studies meant that they were costly to implement and required a high level of staff input from both the commercial farm and the research team involved. Gaining further financial investment and finding commercial sites where studies such as this can be completed in the future may prove difficult, but the payback in terms of producer investment could be vast. Furthermore, investigating the questions postulated in these studies regarding the management of pain or the management of pathology with NSAIDs may prove crucial in future on-farm decision making. Also examining the other health and production outcomes from these studies may provide further incentive for the routine administration of NSAID at calving and lameness.

The studies in this thesis can also be used to identify the challenges surrounding study designs investigating lameness and CHLs. From the data retrieved, it is clear that previous lameness and CHLs act as a predisposing factor for future lameness and CHLs through the depletion of the digital cushion and altering the histological function alongside the changes described by Newsome et al. (2016). This, when taken with the contrasting observations surrounding risk of lameness made in Chapters 5 and 6, lead to the suggestion that future lameness research should utilise animals naïve to transition, lameness and CHLs as study units when investigating the effect of an intervention. If this is not practical, then animals with a comprehensive lameness history should be utilised and the randomisation taking place should stratify the animals based on their previous lameness history to ensure no systemic bias takes place. Furthermore, the study presented in Chapter 3 has clearly explained how trans-sole ultrasonography is not an appropriate tool for the measurement of digital cushion thickness due to its inability to distinguish between digital cushion and corium. With several animals missing individual digital cushions or indeed, having no digital cushion in their lateral claws

whatsoever, it is clear how the use of trans-sole ultrasonography could lead to the over-reporting of digital cushion thickness.

7.8 Conclusions

This thesis has identified that animals with a history of lameness and CHLs throughout their lifetimes are more likely to have less digital cushion in their lateral claws at slaughter and what digital cushion is present is likely to consist of less Type 1 collagen and more Type 3. It is postulated that part of the differences observed are due to genetic and developmental inputs to digital cushion structure, however these predispose the individual to CHLs in its adult life. The subsequent occurrence of CHLs, could lead to the utilisation of fatty acids from the digital cushion as inflammatory mediators and cause the reduction in digital cushion volume and the remodelling of adipose tissue which could lead to animals being predisposed to future CHLs, creating a cycle of CHL chronicity.

In Chapter 5, maiden heifers that received NSAID at their first and subsequent calving and all lameness events were less likely to be scored as lame or culled throughout a three-year period. It was suggested that this administration of NSAID at first and subsequent calving, protected the functional anatomy of the hoof from the degrading impacts of inflammation and inflammatory mediators which are expressed systemically during the transition period. These mediators could impact on the suspensory apparatus, digital cushion, and distal phalanx in tandem.

In contrast, the routine administration of NSAID in the same protocol in adult cattle had no impact on the risk of lameness or culling. These studies lead to the suggestion, when taken in combination, that lameness is a condition which leads to animals entering a “downwards spiral” wherein the inflammatory pathogenic mechanisms and the remodelling of the neural processing networks associated with nociception are continually changing to predispose the animal to future pathology and pain, respectively. It is hypothesised that the event of first calving and first lameness are critical intervention points where the opportunity presents itself to set an animal on a trajectory to succeed with respect to a reduced risk of lameness and culling.

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