GENETIC AND METABOLIC ASPECTS OF CLAW HORN LESION AETIOPATHOGENESIS IN HOLSTEIN COWS

MATTHEW BARDEN

A thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

JUNE 2022

Abstract

Genetic and metabolic aspects of claw horn lesion aetiopathogenesis in Holstein cows. *Matthew Barden*

Sole haemorrhage (**SH**), sole ulcers (**SU**), and white line lesions (**WL**), collectively referred to as claw horn lesions (**CHL**), are important causes of lameness in dairy cattle. This thesis aimed to explore the aetiopathogenesis of CHL, with a focus on genetic and metabolic factors. A cohort of 2,352 Holstein cows was enrolled on four herds in the UK and each animal was assessed at four production stages. Data collected included detailed foot lesion records, ultrasound measurements of digital cushion thickness (**DCT**) and, on one herd, blood samples for analysis with proton nuclear magnetic resonance (**^{1}H NMR**) spectroscopy. Pedigree records and genotypes were obtained for all animals.

Single-step genetic analyses indicated sole lesions (SH and SU) had a moderate heritability, while WL had a low heritability. The genetic correlation was strong between SH and SU, moderate between SU and WL, and weak between SH and WL. The heritability estimates of DCT were low-to-moderate depending on the stage of production and location of DCT measurement. The genetic correlation between DCT and sole lesions was generally negative. The recovery of sole lesions between early and late lactation assessments was heritable and appeared only weakly genetically correlated with sole lesion susceptibility.

Genome-wide association analyses of CHL and DCT traits revealed a polygenic background, with candidate genes identified relating to immunity and inflammation, as well as carbohydrate and lipid metabolism. Analysis of serum using ^{1}H NMR spectroscopy indicated the serum metabolome could not accurately differentiate between healthy cows and those affected with sole lesions, but a small number of metabolites appeared to be associated with SU development. Finally, the potential of breeding for improved lameness resistance using national genetic indexes was assessed, and the Lameness Advantage index was significantly associated with SH and SU development.

The additive genetic variance of CHL could be utilised to select for increased resistance to these lesions; novel traits such as DCT and sole lesion recovery may also be useful auxiliary traits to reduce CHL prevalence. The genetic relationships between investigated traits, in addition to the genomic regions and genes associated with CHL and DCT, provide further insights into the genetic background and potential aetiopathogenesis of CHL. The metabolic influence on CHL development is poorly understood with the results of this work suggesting the serum metabolome is not directly associated with sole lesion development, however, further metabolomic studies of CHL would be worthwhile. Breeding for reduced CHL by selecting on the Lameness Advantage index is likely to be effective, although more direct CHL traits would be more efficient. Genetic selection alongside management and environmental changes should be considered as the optimal approach to reducing CHL in dairy cattle.

Acknowledgements

I would like to start by thanking my supervisor, George Oikonomou, for the enthusiasm, opportunities and support over the past few years, as well as my secondary supervisors, Jennifer Duncan and Georgios Banos, for their valuable input and advice. This PhD was funded by the School of Veterinary Science, University of Liverpool with project funding from the Biotechnology and Biosciences Research Council and the Technology Directorate Strategy Group, University of Liverpool; I am very grateful to all parties.

I feel particularly indebted to the unswerving commitment and diligence of Alkis Anagnostopoulos, Beth Griffiths, and Cherry Bedford. I am also grateful to those who gave up their time to help with this project: Aiglyne Chaperone, Theologia Menka, Mollie Rudd, Kerry Long, Amy Russon, Charlotte Smith, Konstantinos Georgakoudis, Konstantina Kasiora, and Ioanna Soumtaki, as well as to the staff on all participating farms who assisted us with data collection.

I would like to thank Bingjie Li and Marie Phelan for sharing their expertise and guiding me through their respective fields of genetics and metabolomics, Bobby Hyde for his advice on data analysis, and Androniki Psifidi and Alex Watson-Lazowski for their input in the preparation of this thesis. I am grateful to the staff at Neogen and EGENES for providing and imputing genotypes, Enrique Sánchez-Molano and Vanessa Kapsona for their contributions to the analysis of genomic data, and the staff at ADHB and NBDC for supplying genetic evaluations.

Finally, I feel extremely fortunate to have had so much support from my friends and family, and particularly from Hannah who I have relied on completely.

Abbreviations

£PLI	Profitable lifetime index
^{1}H NMR	Proton nuclear magnetic resonance
AHDB	Agriculture and Horticulture Development Board
ARI	Absolute risk increase
BCS	Body condition score
BLUP	Best linear unbiased prediction
BTA	*Bos taurus* autosome
CHL	Claw horn (disruption) lesion(s)
CI	Confidence interval
CNV	Copy number variant(s)
CPMG	Carr-Purcell Meiboom-Gill
DCT	Digital cushion thickness
EBV	Estimated breeding value(s)
EGENES	Edinburgh Genetic Evaluation Services
GEBV	Estimated genomic breeding value(s)
GWA	Genome-wide association
HDI	Highest density interval
HR	Hazard ratio
HYS	Herd-Year-Season
ICAR	International Committee for Animal Recording
IGF	Insulin-like growth factor
Lasso	Least absolute shrinkage and selection operator
LD	Linkage disequilibrium
MMP	Matrix metalloproteinase(s)
NEFA	Non-esterified fatty acid(s)
NSAID	Non-steroidal anti-inflammatory drug(s)
MS	Mass spectroscopy
OR	Odds ratio
PCA	Principal component analysis
PLSDA	Partial least squares discriminant analysis
PTA	Predicted transmitting ability
QTL	Quantitative trait loci
SARA	Subacute ruminal acidosis
SD	Standard deviation
SE	Standard error
SH	Sole haemorrhage
SL	Sole lesion (sole haemorrhage or ulcer)
SNP	Single nucleotide polymorphism(s)
SU	Sole ulcer(s)
UK	United Kingdom
USA	United States of America
VIP	Variable importance in projection

Publications

All research chapters have or will be submitted for publication, the structure and content of these chapters reflect this. As of June 2022:

Chapter 2 has been submitted for publication.

Li B*, Barden M*, Kapsona V, Sánchez-Molano E, Anagnostopoulos A, Griffiths BE, Bedford C, Xiaoxia D, Coffey M, Psifidi A, Oikonomou G, Banos G. Single-step genome-wide association analyses of claw horn lesions in Holstein cattle using linear and threshold models *(Under review).* *Shared first authorship

Chapter 3 has been accepted for publication.

Barden M, Li B, Griffiths BE, Anagnostopoulos A, Bedford C, Watson C, Psifidi A, Banos G, Oikonomou G. 2022. Genetic parameters and genome-wide association study of digital cushion thickness in Holstein cows. J. Dairy Sci.

Chapter 4 has been submitted for publication.

Barden M, Anagnostopoulos A, Griffiths BE, Li B, Bedford C, Watson C, Psifidi A, Banos G, Oikonomou G. Genetic parameters of sole lesion recovery in Holstein cows. *(Under review).*

Chapter 5 is being prepared for submission.

Barden M, Phelan M, Hyde R, Anagnostopoulos A, Griffiths BE, Bedford C, Green M, Psifidi A, Banos G, Oikonomou G. ^{1}H NMR-based serum metabolomic analysis of sole lesion development in Holstein cows. *(Not submitted).*

Chapter 6 has been published.

Barden M, Anagnostopoulos A, Griffiths BE, Bedford C, Winters M, Li B, Coffey M, Psifidi A, Banos G, Oikonomou G. 2022. Association between a genetic index for lameness resistance and the incidence of claw horn lesions in Holstein cows. Vet Rec. Apr 25:e1632. doi: 10.1002/vetr.1632.

Contents

Contents

List of figures

List of tables

Chapter 1: Introduction and literature review

1.1 Introduction to lameness in dairy cattle

Against the backdrop of a growing global population, diminishing natural resources and climate change, global food security has never been more important (Tomlinson, 2013; FAO, 2018). At the forefront of this challenge is the sufficient and sustainable production of protein (Semba, 2016; Clark and Lenaghan, 2020), a word derived from the Greek adjective πρώτειος (proteios) meaning "primary" (Vickery, 1950). Bovine milk is an important source of protein in the human diet (Pereira, 2014) and there is an increasing worldwide demand for dairy products (Muehlhoff et al., 2013). But while the nutritional value of milk is clear, there is a widespread drive to promote plant-based alternatives to dairy due to concerns regarding the sustainability of livestock farming (Willett et al., 2019). Therefore, the dairy industry needs to demonstrate that the increased demand for milk production can be met sustainably (Britt et al., 2021). Sustainability in agriculture can be evaluated from economic, environmental, and social aspects (Latruffe et al., 2016); lameness in dairy cattle presents a direct threat to the sustainability of dairy farming from each of these fronts.

1.1.1 Detection of lameness

Lameness is the abnormal locomotion or posture which results from pain or dysfunction in the locomotor system (Bell, 2016). In dairy cattle, lameness is primarily detected by the visual assessment of gait and posture, and a range of locomotion scoring systems have been developed to formalise the detection and severity grading of lameness. Most modern systems are direct or adapted versions of the five-point scale proposed by Sprecher et al. (1997). Despite minor differences, most systems are broadly comparable to each other (Whay, 2002). In the United Kingdom (**UK**), a simplified version of this five-point system, termed "mobility scoring", was promoted by the Agricultural and Horticulture Development Board (**AHDB**) and has become the industry standard in this country (Whay et al., 2003a; AHDB, 2020a).

1.1.2 Frequency of lameness

In the UK, a large number of studies have measured the frequency of lameness in dairy cows. A recent meta-analysis of 68 UK-based studies estimated the national prevalence of lameness in dairy cattle to be 30% (95% confidence interval (**CI**) 27 - 32%) with an incidence rate of 31 cases per 100 cow-years (95% CI 26 - 38) (Afonso et al., 2020). Similar levels of lameness in dairy herds have been reported in other European countries (Dippel et al., 2009; Sárová et al., 2011), as well as in North America (Espejo et al., 2006; von Keyserlingk et al., 2012; Hoffman et al., 2013; Solano et al., 2015), South America (Tadich et al., 2010; Bran et al., 2018), Asia (Chapinal et al., 2014; Sadiq et al., 2017; Ali, 2020), and Australasia (Fabian et al., 2014; Ranjbar et al., 2016). In short, lameness in dairy cattle is a universal problem.

Although the previously referenced studies used different locomotion scoring systems, the thresholds to classify cows as lame were relatively high, such as an immediately detectable lameness (AHDB, 2020a) or an arched back posture whilst standing (Sprecher et al., 1997). Therefore, these prevalence estimates have not been inflated by mild cases and relate to lameness of clinically significant severity. Lameness has been identified as the most pressing problem affecting the modern dairy industry in Europe (European Food Safety Authority (EFSA), 2009; Nalon and Stevenson, 2019), and it is hard to dispute this conclusion when around one in three cows on modern dairy farms are lame. Recent cross-sectional studies from the UK reported that within-herd prevalence ranged from 6% to 65% (Griffiths et al., 2018; Randall et al., 2019), indicating that lameness is much more effectively controlled in some herds compared to others, therefore a low national prevalence of lameness should be achievable.

1.1.3 Aetiology of lameness

Lameness in dairy cattle is a symptom of a wide range of diseases with disorders of the foot considered to be the predominant cause (Huxley, 2013). The importance of foot lesions in lameness is underpinned by observations such as the presence of clinically significant foot lesions in 90% of lame cows (Murray et al., 1996; Somers and O'Grady, 2015); the positive correlation between lameness severity and the likelihood of a painful foot lesion being recorded during foot-trimming (Spearman correlation coefficient 0.48 ± 0.04) (Bicalho et al., 2007a), and the increased odds of lameness in animals with a foot lesion compared to unaffected animals (odds ratio (**OR**) 2.7, 95% CI 1.7 – 4.3) (van Huyssteen et al., 2020).

The International Committee for Animal Recording (**ICAR**), which is a collaborative, international initiative aiming to standardise data recording in agriculture, published an atlas of 16 foot and claw disorders (Egger-Danner et al., 2020). These disorders can broadly be attributed to either infectious or non-infectious causes (**Table 1.1a** and **Table 1.1b**).

The importance of individual foot lesions in dairy cattle can be determined by consideration of the severity of the lesion, which relates to the economic and welfare impact, and the prevalence. Digital dermatitis is highly prevalent in UK dairy herds (Barker et al., 2010) and is consequently considered the most significant infectious cause of lameness in dairy cattle (Refaai et al., 2013). The major non-infectious foot lesions which are associated with lameness in dairy cattle are sole haemorrhage (**SH**), sole ulcers (**SU**), and white line lesions (**WL**). These lesions have a high prevalence in dairy cattle (Murray et al., 1996; Laven and Lawrence, 2006; Somers and O'Grady, 2015) and, relative to other foot lesions, have been associated with the most severe pain responses (Whay et al., 1998; Pastell et al., 2010), economic impacts (Amory et al., 2008; Bruijnis et al., 2010; Dolecheck et al., 2019), and environmental consequences (Mostert et al., 2018).

Table 1.1a. Infectious foot and claw disorders described in the ICAR Claw Health Atlas (Egger-Danner et al., 2020).

Disorder	**Description**
Digital dermatitis	Infection of digital or interdigital skin causing circumscribed superficial ulcerations, and/or chronic hyperkeratosis
Interdigital/superficial dermatitis	Mild superficial dermatitis around the claws or in the interdigital cleft
Heel horn erosion (Slurry heel)	Irregular loss of the horn around the heel bulbs resulting in V-shaped grooves
Interdigital phlegmon (Foul of the foot, Foot rot)	Symmetrical, acute inflammation of the subcutaneous tissues of the foot with a characteristic odour
Swelling of the coronary band and/or heel bulb	Swelling of tissue proximal to the horn capsule in the absence of a penetration injury or concurrent claw horn lesion

Table 1.1b. Non-infectious foot and claw disorders described in the ICAR Claw Health Atlas (Egger-Danner et al., 2020).

Disorder	**Description**
Asymmetric claws	Differences in claw dimensions between medial and lateral claws which cannot be corrected by trimming
Concave dorsal wall	Concave shape of the dorsal wall of the claw
Corkscrew claws	Deviation of the dorsal wall of the claw causing twisting of the claw along the longitudinal axis
Double sole	Multiple layers of underrun sole horn
Horn fissure	Fissures in the claw wall, either horizontal or vertical
Interdigital hyperplasia	Proliferative, fibrous mass in interdigital cleft
Scissor claws	One or both toes deviate axially to cross over each other
Sole haemorrhage	Discolouration of the sole or white line due to blood staining (pink, purple, blue, or yellow discolouration possible); lesions can be circumscribed or diffuse
Ulcers (Sole ulcers, Heel ulcers, Toe ulcers, and Toe necrosis)	Exposure of fresh or necrotic corium through the sole horn; toe necrosis can develop due to non-healing toe ulcers
Thin sole	Sole horn is easily compressed when finger pressure is applied, usually reflecting a sole horn thickness < 5 mm
White line disease	Separation of the junction between the wall and sole (white line) which can progress to abscess development

1.1.4 The threat of lameness to sustainable dairy farming

Economic effects. In a comparison of herd-level economic losses associated with production diseases in dairy cattle, lameness was second only to clinical mastitis (Kossaibati and Esslemont, 1997). In addition to treatment costs, losses due to lameness occur as a result of reduced milk production, involuntary culling, and poorer fertility (Enting et al., 1997). It has been estimated that foot lesions result in annual losses of around $75 per cow (Bruijnis et al., 2010). The costs associated with specific foot lesions have also been estimated, for example, losses of $216 were estimated for each case of SU (Cha et al., 2010). More recent studies have shown that the costs associated with foot lesions depend on the lesion severity, the parity of the affected animal, and the stage of lactation at which the lesion develops (Charfeddine and Pérez-Cabal, 2017; Dolecheck et al., 2019). For example, the total costs associated with mild cases of SU and WL were estimated to be $232 and $221 per cow per year, respectively; whereas severe SU and WL were associated with losses of $622 and $590, respectively (Charfeddine and Pérez-Cabal, 2017). Dolecheck et al. (2019) found the total cost associated with SU and WL was greatest when lesions developed in multiparous animals in the first 60 days after calving ($282 and $249, respectively), with a lower cost associated with lesions which developed in primiparous animals or later in lactation.

Reduced milk yield is a major driver of the economic losses associated with SU and WL (Cha et al., 2010; Charfeddine and Pérez-Cabal, 2017; Dolecheck et al., 2019). Milk yield has been shown to be lower in cows before and after the detection of lameness (Green et al., 2002; Archer et al., 2010b; Reader et al., 2011). Milk yield reductions following lameness are estimated to be in the range of 0.5 kg to 1.55 kg per day (Warnick et al., 2001; Archer et al., 2010b), with a 360 kg reduction (95% CI 163 – 552 kg) in milk yield over a lactation attributable to lameness (Green et al., 2002). Lactation yield reductions of 574 kg (95% CI 307 – 840 kg) were estimated in cows which developed SU and reductions of 369 kg (95% CI 137 – 600 kg) in cows which developed WL, compared to unaffected cows (Amory et al., 2008); this effect on production can persist into subsequent lactations (Oikonomou et al., 2013).

Removal of inefficient animals from a herd promotes profitability but forced culling of diseased animals (known as involuntary culling) has the opposite effect (Rogers et al., 1988; Fetrow et al., 2006). Lame cows were found to be more likely to be involuntarily culled or die compared to non-lame cows (Hazard ratio (**HR**) 1.45, 95% CI 1.12 - 1.78) (Bicalho et al., 2007b). Similarly, cows which developed SU around peak lactation had a higher chance of being culled than unaffected cows (HR 2.7, 95% CI 1.3 – 6.0) (Booth et al., 2004).

Infertility is one of the most frequent reasons for culling dairy cows (Bascom and Young, 1998; Brickell and Wathes, 2011). As lameness negatively affects fertility (Hernandez et al., 2001; Bicalho et al., 2007b), there is an additional indirect relationship between lameness and involuntary culling. It has been shown that lame cows were more likely to be culled for fertility reasons than non-lame cows (HR 8.4, 95% CI 1.2 – 59.6) (Sprecher et al., 1997). Herd productivity is closely related to reproductive efficiency (Britt, 1985) and profitable milk production requires cows to become pregnant relatively early in lactation, the period when the incidence of lameness is highest (Green et al., 2002). Lameness in the first 30 days of lactation has been associated with a lower chance of becoming pregnant compared to non-lame cows (HR 0.43, 95% CI 0.28 - 0.66) (Melendez et al., 2003); in a similar vein, severe lameness during the first 35 days of lactation was associated with an increased chance of delayed ovarian cyclicity compared to mildly lame or sound animals (OR 3.5, 95% CI 1.00 – 12.21) (Garbarino et al., 2004). Therefore, the costs incurred through poorer fertility are another significant source of economic losses due to lameness.

Environmental effects. The environmental impact of livestock farming is under scrutiny, with a particular focus on the emissions of greenhouse gases such as methane (Steinfeld et al., 2006; Rotz, 2020). Enteric methane production from beef and dairy cows contributes a large proportion of the total greenhouse gas emissions from the livestock sector (Caro et al., 2014). Several strategies such as nutritional changes, feed additives, and genetic selection indexes have been explored and may help align dairy production with global goals to reduce greenhouse gases (Carrazco et al., 2020; Dillon et al., 2021; Richardson et al.,

2022). Lameness in dairy cows has been directly linked to increased greenhouse gas emissions, as well as other detrimental environmental impacts (Chen et al., 2016), so improving the health and welfare of dairy cattle is another important step toward reducing the environmental impact of dairy farming (Hristov et al., 2013).

One logical approach to reducing the environmental impact of dairy farming is to maximise productivity so that fewer animals are required to produce the same volume of milk; or to put it another way, by increasing milk production the emissions associated with each animal are effectively diluted (Boadi et al., 2004; Gerber et al., 2011). As previously discussed concerning the economic effects, lameness has a marked effect on milk yield (Green et al., 2002; Archer et al., 2010b; Reader et al., 2011) and therefore lameness needs to be well controlled to achieve maximum productivity.

As well as preventing the negative effects of lameness on milk production, effective fertility management is required to achieve maximum productivity in a dairy herd. Ensuring cattle become pregnant in early lactation is important because it minimises the proportion of time spent in late lactation, where yields are low, and reduces the requirement for prolonged non-productive dry periods (Wall et al., 2012). The negative effect of lameness on fertility (Melendez et al., 2003; Garbarino et al., 2004; Bicalho et al., 2007b) is another reason why high levels of lameness must be addressed to reduce the environmental impact of dairy farming.

Intensification of dairy farming would seem an effective approach to increasing production, and high-input with high-output dairy farming systems are reported to have a lower environmental impact than low-input, pasture-based systems (Capper et al., 2009; Britt et al., 2021). However, increased herd turnover is often overlooked. Increasing the productive lifespan of dairy cattle is a key strategy to offset the environmental costs of rearing replacement heifers (Knaus, 2009; Grandl et al., 2019; De Vries and Marcondes, 2020). High replacement rates are a common feature of intensive dairy farming systems (Overton and Dhuyvetter, 2020); larger herd sizes and greater milk production tend to be

correlated with higher herd turnover and shorter productive lifespans (Weigel et al., 2003; Hadley et al., 2006; Knaus, 2009). As previously discussed, lameness is associated with more involuntary culling which limits the productive lifespan of dairy cattle and increases the number of required replacements (Booth et al., 2004; Bicalho et al., 2007b).

Social effects. Social sustainability encompasses internal and external factors (Lebacq et al., 2013). Internal factors include the well-being of farm staff; a relevant example from a recent study showed that poor mental health of farmers was associated with a higher prevalence of severely lame cows in a herd (King et al., 2021). External factors relate to public opinion of farming practices, principally the perception of animal welfare (Lebacq et al., 2013); lameness is a particular issue in this respect.

Lameness is an intrinsically painful condition which is also associated with hyperalgesia and allodynia (Whay et al., 1997, 1998; Bruijnis et al., 2012). Consequently, lameness is the highest-ranked animal-based measure of welfare in dairy cattle (Whay et al., 2003a). Animal welfare has traditionally been measured using the "Five Freedoms" (FAWC, 1993), and it has been convincingly argued that lameness in dairy cattle undermines every one of these freedoms (Whay and Shearer, 2017). Concerningly, the "Five Freedoms" approach is now considered to be too limited in scope to truly promote "good" welfare (Mellor, 2016), so it would be even harder to reconcile the high prevalence of lameness in dairy cattle with more modern welfare standards. Another approach to assessing animal welfare is to consider three overlapping areas of welfare concern: biological functioning, affective state and natural living (Fraser et al., 1997). Lameness is clearly in opposition to each of these areas, as summarised by von Keyserlingk et al. (2009), "a lame cow is in pain (affective state), has lower milk production and reproduction (poor biological function), and has reduced mobility (natural behaviour)".

To conclude this section, the sustainability of dairy farming is a challenge that the industry must address. Low levels of lameness are a prerequisite of sustainable dairy farming; current levels of lameness on dairy farms undermine

the economic profitability of herds, prevent environmentally efficient milk production, and represent an unquestionable failure of animal welfare. Sustainable dairy farming is achievable, but not without reducing the levels of lameness currently found on the majority of dairy farms.

1.2 Aetiopathogenesis of claw horn lesions

Reducing the number of lame dairy cows requires an understanding of the aetiology and pathogenesis of the condition (Huxley, 2012). This thesis will explore the aetiopathogenesis of the three major non-infectious foot lesions: sole haemorrhage (**SH**), sole ulcers (**SU**), and white line lesions (**WL**). These lesions are collectively referred to as claw horn lesions (**CHL**) (Offer et al., 2000), but have previously been known as "laminitis-associated lesions" (Ossent and Lischer, 1998) or "claw horn disruption lesions" (Hoblet and Weiss, 2001).

The reason SH, SU, and WL are often grouped together stems from the proposed aetiopathogenesis, which is thought to centre around compromised horn production (Hoblet and Weiss, 2001), hence the synonym: claw horn disruption lesion. Horn production is the result of the cornification of epidermal keratinocytes, cells which are dependent on the vascular corium (Hirschberg, Mülling and Budras, 2001). Damage to the corium is viewed as the likely initiating factor in CHL development, however, the aetiology of this damage is still fairly speculative. Broadly, it is thought that pathological changes can develop in the corium because: (1) the suspensory apparatus fails to prevent the downward movement of the distal phalanx within the hoof capsule causing compression of the corium (Lischer et al., 2002b); (2) the digital cushion is ineffective in dissipating forces away from the corium (Bicalho et al., 2009); (3) bone development on the distal phalanx creates focal points of pressure on the corium (Newsome et al., 2016), or (4) mechanical damage occurs due to overgrowth or poor conformation of the hoof, or prolonged standing on hard surfaces (Manske et al., 2002a; Webster, 2002).

Observational studies have identified risk factors for CHL which may be explained by one or more of these underlying pathological pathways. For example, the incidence of CHL peaks around three to four months after calving (Leach et al., 1997; Offer et al., 2000; Barker et al., 2009), and there is a lag of approximately two months between instigating pathology in the corium and the detection of visible lesions (Hoblet and Weiss, 2001). Therefore, changes around calving and early lactation are of particular interest. Consequently, the reduction

in strength of the suspensory apparatus following parturition (Tarlton et al., 2002; Knott et al., 2007) and the nadir in digital cushion thickness the week after calving (Newsome et al., 2017a; Bach et al., 2021) or in early lactation (Bicalho et al., 2009; Griffiths et al., 2020), may contribute to this increased incidence.

Claw horn lesion development has been shown to increase the risk of CHL in future lactations (Hirst et al., 2002; Oikonomou et al., 2013; Randall et al., 2016). Fatty acids from the digital cushion have been hypothesised to be used as inflammatory mediators, causing a reduction in thickness following CHL and an increased risk of future recurrence (Ossent and Lischer, 1998; Lischer et al., 2002b; Räber et al., 2006). Another consequence of chronic inflammation with CHL is bone formation on the distal phalanx which can create sharp spurs and increase the likelihood of future lesion development (Lischer et al., 2002b; Newsome et al., 2016). This same mechanism may also underlie the correlation between age and CHL risk (Lim et al., 2015; Randall et al., 2015; Newsome et al., 2016).

Twice yearly foot-trimming has been associated with reduced CHL (Manske et al., 2002a), and concrete flooring has been linked to more severe SH (Bergsten and Frank, 1996). These results may point to a biomechanical aetiology (Mülling, 2019), but aberrant forces may be exacerbating rather than initiating factors (Cook and Nordlund, 2009).

1.2.1 Description of the claw horn lesions

Sole haemorrhage. Contusions to the corium in the foot cause blood staining of the germinal horn which manifests as areas of haemorrhage in the sole (**Figure 1.1**). Sole haemorrhage is inconsistently associated with lameness (Tadich et al., 2010), but it is considered a direct precursor of more severe SU (Croué et al., 2017; Newsome et al., 2017b). Although this is a logical conclusion, the relationship between SH and SU has not been well characterised. Sole haemorrhage may be a precursor to SU, a milder presentation of the same pathological process, or an unrelated, albeit similar, condition. Previous longitudinal studies have not observed a relationship between SH and SU in a single lactation (Leach et al., 1997; Capion et al., 2009), but these studies focused

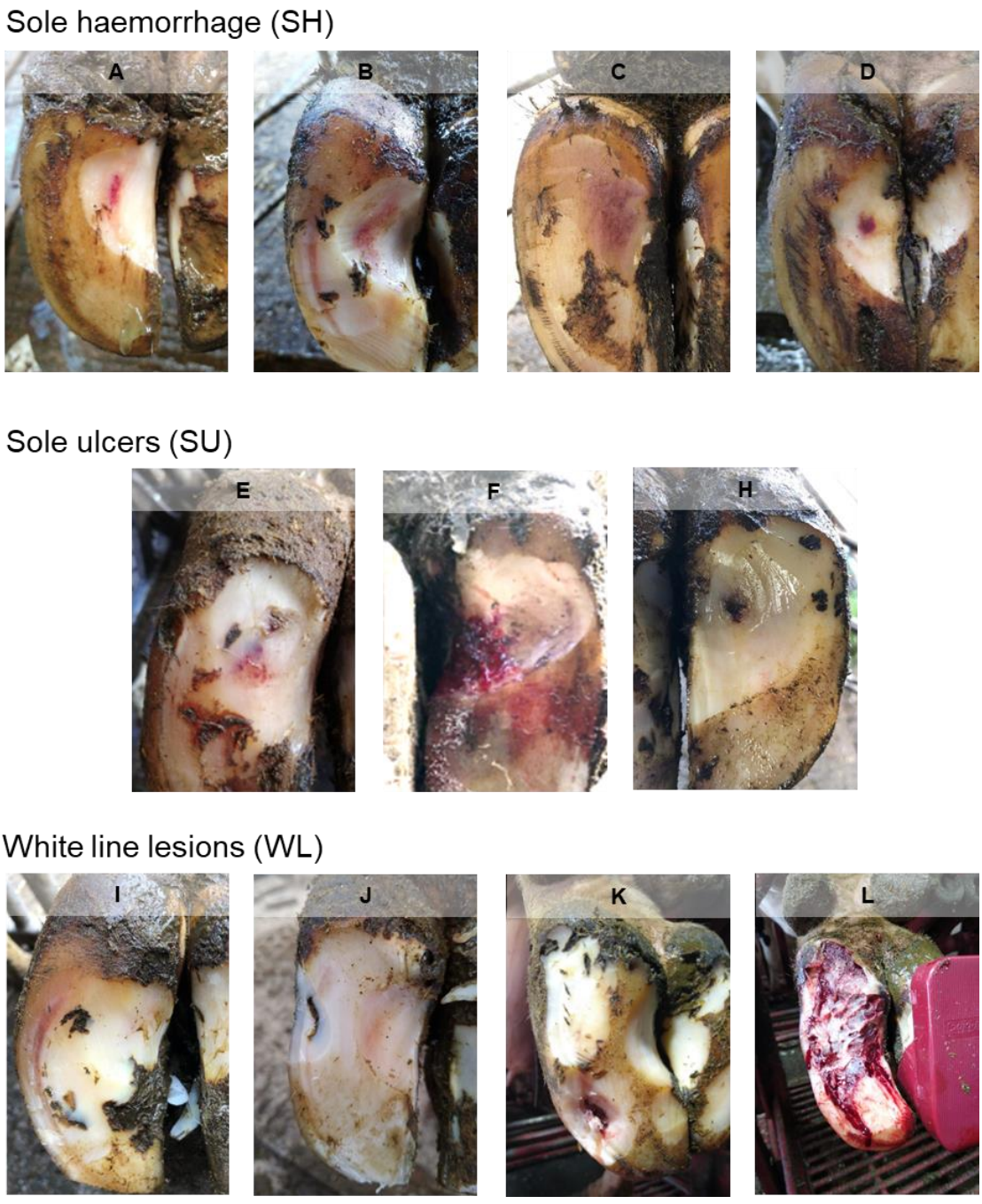
exclusively on first lactation animals which tend to have a lower incidence of SU than older animals (Barker et al., 2009; Somers and O'Grady, 2015), and severe SH in the first lactation may be associated with increased risk of CHL in future lactations (Hirst et al., 2002; Randall et al., 2016).

Sole ulcers. Sole ulcers occur when the corium perforates through the sole horn (**Figure 1.1**). Sole ulcers develop in a typical region of the sole because the flexor tuberosity of the distal phalanx creates a focal area of compression of the corium during walking (van der Tol et al., 2003; van Amstel and Shearer, 2006). Ulcers in the heel or bulb region of the sole can be regarded as distinct lesions to SU (Blowey et al., 2000), but as there are only subtle differences, it is practical to group sole and heel ulcers together for most purposes. Ulcers at the toe region of the sole may arise from similar pathology to typical SU (Kofler, 2017), but toe ulcers are also associated with a generalised thin sole due to over-wear or excessive foot-trimming (Sanders et al., 2009; Kofler, 2017). Therefore, although toe ulcers could be considered a CHL, these lesions can also have a distinct aetiopathogenesis and therefore will not be regarded as CHL in this thesis.

White line lesions. The white line is the junction between the hoof wall and the sole. White line lesions include haemorrhage of the white line, separation along the white line between the hoof wall and sole, and ascending infections or abscess formation between the hoof wall and dermis (**Figure 1.1**). The horn in the white line is softer and structurally weaker than other parts of the foot and the production of inferior claw horn heightens this vulnerability (Kempson and Logue, 1993; Leach et al., 1998; Winkler and Margerison, 2012). This weakness makes the white line vulnerable to excessive forces if animals slip during walking, for example, due to inappropriate herding or inadequate flooring surfaces (Barker et al., 2009). The white line is widest in the abaxial aspect of the hoof which also corresponds to the point of the sole under maximum pressure during standing; consequently, this region is predisposed to separation which can lead to ascending infections (Hoblet and Weiss, 2001; van der Tol et al., 2002). Although WL are considered to arise from the same disease process as SH and SU, the peak incidence of WL is later in lactation than SH and SU in multiparous animals

(Offer et al., 2000), and it has been suggested that WL can also occur with or without prior disturbance of horn production (Mülling, 2002).

Figure 1.1. Examples of claw horn lesions (CHL). Images A to D are sole haemorrhage (SH): discolouration of the sole due to haemorrhage in the corium, colour ranges from light red (A, B) to dark purple (C, D), lesions can be diffuse (C) or circumscribed (D); images E to H are sole ulcers (SU): a defect of the horn exposing the underlying corium which may be fresh (E, F) or necrotic (G); images I to L are white line lesions: haemorrhage localised to the white line (I) or separation of the white line without (J) or with purulent discharge (K, L).



1.2.2 Body condition and lameness history

One of the risk factors for lameness in dairy cows which has received the most attention is body condition. However, it is also one of the least specific in terms of reflecting underlying pathophysiological pathways which may contribute to CHL development. Within a production cycle, the peak incidence of CHL is usually observed shortly after the nadir in body condition, therefore the relationship between body condition and CHL appears pertinent (Bruckmaier et al., 1998; Aeberhard et al., 2001; Amory et al., 2008; Sanders et al., 2009). Body condition can be assessed and assigned a score following visible evaluation and palpation of specific anatomical points; in the UK, the most common body condition scoring system is a one to five scale with quarter-point intervals (Edmonson et al., 1989; Ferguson et al., 1994), however, scoring systems vary by country and are not always well correlated (Roche et al., 2004).

The nature of the association between body condition score (**BCS**) and lameness is not always clear. A single herd study found a quadratic relationship between BCS and lameness, with higher locomotion scores (i.e. more severe lameness) associated with either a low or high BCS (Onyiro et al., 2008). A study of 50 dairy herds found an association between BCS and lameness prevalence, but the greatest and only statistically significant difference existed between cows with BCS ≤ 2.5/5 compared to those with BCS > 2.5/5 (52.6% vs 22.1% $P < 0.01$) (Espejo et al., 2006). The results of these studies indicate that although BCS and lameness are associated, the relationship is not linear.

One of the key questions in terms of CHL aetiopathogenesis is whether poor body condition precedes, or results from lameness. Lame cows appear to spend less time eating (González et al., 2008; Gomez and Cook, 2010) and have a lower dry matter intake (Bach et al., 2007). But whether this translates to a lower BCS following lameness is hard to determine, and is complicated by the association between milk yield and lameness (Archer et al., 2010b; Reader et al., 2011) and milk yield and body condition (Onyiro et al., 2008; Roche et al., 2009), as well as how both milk yield and body condition affect dry matter intake. If CHL result in a loss of body condition, then it becomes difficult to establish if poor body

condition preceding lameness is linked to lameness history, or whether poor body condition is an independent risk factor.

If cows with CHL are not treated promptly and effectively (Pedersen and Wilson, 2021) lameness can persist, particularly if cows are chronically lame when first treated (Thomas et al., 2016). It is thought that inflammation due to CHL may cause new bone development on the distal phalanx which is responsible for a lifelong increased risk of CHL (Lischer et al., 2002b; Newsome et al., 2016). This may be why high rates of recurrence are observed for CHL in consecutive lactations (Enevoldsen et al., 1991; Foditsch et al., 2016; Charfeddine and Pérez-Cabal, 2017).

There is some support for the assertion that poor body condition, or loss of body condition, precedes lameness. Two studies demonstrated that a low BCS at calving was associated with subsequent lameness (Hoedemaker et al., 2009; Lim et al., 2015), but only one of these studies found a statistically significant association with body condition loss in early lactation (Lim et al., 2015). However, neither of these studies controlled for the effect of previous lameness events or milk yield. Another long-term study of a single herd demonstrated loss of ≥ 0.25 BCS during the first four weeks of lactation was associated with a slight increase in odds of visible lameness compared to no change in BCS (OR 1.21 95% CI 1.03 - 1.42); important confounding factors were included as covariates in this statistical model (Randall et al., 2015). This same study also reported an association between cows recorded with BCS <2/5 and visible lameness within the next three weeks, compared to cows with BCS ≥2/5; similarly, the same BCS difference in the previous two weeks was associated with first lifetime lameness events in primiparous animals. Given the presumed time it takes for CHL to develop (Hoblet and Weiss, 2001), if poor body condition is part of the aetiopathogenesis of CHL then poor body condition two or three weeks before lameness cannot convincingly be considered causative as opposed to consequential. First lifetime events of lameness in multiparous were associated with mild and severe lameness after a sixteen-week and eight-week lag, respectively (Randall et al., 2015). This presents a more convincing picture that

low body condition might precede lameness, although the number of animals in this sub-set was small. The relevance of the results from all these studies for CHL aetiopathogenesis is uncertain as the causes of lameness were not described.

Studies which investigate specific lesions rather than non-specific lameness are rare. A single herd study based on foot-trimming records found cows with BCS ≤ 2/5 in the two months before lesion diagnosis, compared to cows with BCS > 2/5, had an increased odds of both SU and WL (OR 1.39 95% CI 1.10 - 1.75) and SH (OR 2.44 95% CI 1.69 - 3.57) (Green et al., 2014). The same comparison between BCS of cows revealed an increased odds of lameness two to four months later due to SU and WL (OR 1.67 95% CI 1.22 - 2.27) and possibly SH (OR 1.43 95% CI 0.85 - 2.44). Analysis adjusted for the important confounding factors of the stage of lactation, parity, milk yield, and previous lameness, and the BCS variable was sufficiently lagged to infer a role in the development of CHL. The same trends were apparent when data were analysed for the first cases of lameness during each lactation, with lameness in previous lactations included as a covariate in the model. Although the reliance on foot-trimming records may limit the data quality for lesions such as SH, which does not consistently cause a visible lameness but can affect future CHL risk (Tadich et al., 2010; Randall et al., 2016), this study provides the most convincing evidence that poor body condition increases the risk of CHL development.

Subsequent analysis of data from two of these previously discussed studies (Green et al., 2014; Randall et al., 2015) demonstrated that around 8% of lameness could be prevented by avoiding the loss of half a BCS, whereas the population attributable risk of lameness due to a historic lameness event (> 16 weeks previous) was approximately 10 - 20% (Randall et al., 2018a). Therefore, the weaker association between poor body condition and the first lifetime lameness event and the lower population attributable risk for body condition compared to historic lameness, highlight the important long-term effects of lameness cases on the risk of recurrence.

Body condition is a reflection of subcutaneous fat and skeletal muscle coverage (Megahed et al., 2019). A lower prepartum back fat thickness was associated with a greater increase in SH severity, but cows with the greatest reductions in back fat thickness had the smallest increase in SH severity (Wilhelm et al., 2017). These results would suggest that the depth of subcutaneous fat may explain part of the association of lameness with poor body condition but possibly not the association with body condition loss; however, these results should be interpreted cautiously as statistical analysis did not extend beyond univariable comparisons. Considering skeletal muscle loss occurs simultaneously with subcutaneous fat (Bruckmaier et al., 1998), it would be useful to also investigate the relationship between lameness and muscle thickness.

Overall, there is reasonable evidence to support the assertion that poor body condition predisposes lameness, and a meta-analysis of the results from two additional epidemiological studies (Solano et al., 2015; King et al., 2017) also reached this conclusion (Oehm et al., 2019). Rather than a linear relationship between body condition and lameness, there appears to be a critical threshold below which lameness risk increases. The loss of body condition also seems to be important independent of the absolute body condition. However, historic lameness cases may be more influential on future lameness risk than body condition.

As mentioned previously, poor body condition, or loss of body condition, can be linked to a wide range of pathophysiological processes, many of which could have a role in the aetiopathogenesis of CHL. Body condition is the biological endpoint of various interacting metabolic pathways, which include physiological processes such as carbohydrate, protein, and lipid metabolism (Tamminga et al., 1997; Roche et al., 2009; Megahed et al., 2019), and potentially pathophysiological processes such as periparturient inflammation and oxidative stress (Sordillo et al., 2009; Abuelo et al., 2015; Bradford et al., 2015; Mann, 2022). The association between body condition and CHL could reflect the metabolic processes which induce, result from, or occur concurrently with fat mobilisation

(Ossent and Lischer, 1998; Lischer et al., 2002b). These changes may cause inflammation or laxity in the suspensory apparatus of the distal phalanx which results in it sinking downwards within the hoof and increasing pressure on the corium. Another hypothesis proposes the most consequential effect of fat mobilisation during early lactation is the depletion of the digital cushion which leaves the bulbar dermis more susceptible to damage during mechanical loading of the hoof (Bicalho et al., 2009). These hypotheses are not mutually exclusive and either mechanism could, theoretically, potentiate the effects of the other within the hoof.

1.2.3 The digital cushion

The digital cushion is composed of three parallel pads of soft fat and loose collagenous tissue which separates the distal phalanx and sole horn (Räber et al., 2004). It is thought the function of the digital cushion is to dissipate forces away from the bulbar dermis and prevent damage to this vulnerable tissue (Budras, 2003; Räber et al., 2004).

The digital cushion could be the link in the association between body condition and lameness. Cadaver studies have reported moderate correlations between body condition and adipocytes in the digital cushion (Newsome, 2016; Hiss-Pesch et al., 2019). However, studies which measure the digital cushion thickness (**DCT**) in vivo with ultrasonography report varying strengths of this association between body condition and digital cushion thickness (Bicalho et al., 2009; Machado et al., 2011; Newsome et al., 2017a; Griffiths et al., 2020). Interestingly, the study which reported the weakest correlation between body condition and DCT was the only study in which DCT was measured blind to body condition and stage of lactation, and the thickest digital cushion coincided with the nadir of the back fat thickness (Newsome et al., 2017a). Therefore, it is possible that the stronger associations reported elsewhere could be inflated by sub-conscious biases during measurement (Griffiths et al., 2020). There is also a lack of consistency between studies in whether the middle or axial fat pad was measured, this inconsistency may explain some of the variations in results, but despite being different sizes (Räber et al., 2004), adipocyte size in both fat pads

are both reported to correlate with body condition (Newsome, 2016; Hiss-Pesch et al., 2019).

If DCT reflects body condition, the dynamics of both throughout a production cycle would be expected to correlate. The first study to address this concluded that "digital cushion thickness decreases steadily after parturition, reaching a nadir four months into the lactation" (Bicalho et al., 2009), however, this assertion is not robustly supported by the presented results. In addition to the problems of inferring progressive change from a cross-sectional study, only lame cows were assessed outside of the routine twice-yearly foot-trimming which occurred in early lactation and before drying off. Therefore, this conclusion regarding DCT changes during lactation may be affected by the time points in lactation when cows were assessed other than routine foot-trimming, i.e., at the treatment of lameness. In agreement with Bicalho et al. (2009), a thinner digital cushion at approximately 70 days postpartum than within the first week after calving was reported by Griffiths et al. (2020). However, Stambuk et al. (2019) reported that during lactation DCT was thinnest at 8 – 34 days postpartum for multiparous animals, although the nadir was at 93 – 118 days postpartum in primiparous heifers. Finally, Newsome et al. (2017a), which included the most frequent measurements from the individual cows during lactation, reported the nadir of DCT occurred approximately seven days postpartum. Overall, there is not a clear picture of how the digital cushion relates to body condition. It should also be noted that despite referring to this ultrasonographic measurement as DCT, it technically refers to the thickness of all soft tissues between the sole and distal phalanx and therefore the thickness of the corium, which is not a constant, may influence the results (Newsome et al., 2017a; b).

Despite the lack of clarity regarding the relationship between digital cushion and body condition, there is an agreement in the literature that a thin digital cushion increases the subsequent risk of a CHL (Machado et al., 2011; Toholj et al., 2014; Newsome et al., 2017b; Stambuk et al., 2019; Griffiths et al., 2020). However, the effect of a concurrent CHL on DCT is less clear with some studies reporting it is associated with an increased DCT (Toholj et al., 2014; Newsome et

al., 2017b) and others the opposite (Bicalho et al., 2009; Griffiths et al., 2020). To date, there is only one study which addresses changes in the digital cushion throughout lactation and its association with CHL development, and a reduction in DCT during early lactation did not increase the risk of CHL development (Newsome et al., 2017b). Therefore, these results would not support the hypothesis that fat mobilisation is associated with the thinning of the digital cushion and subsequent CHL development, however, replication of these results in further longitudinal studies is needed.

The assumed association between DCT and body condition should also be interpreted in the context of how body condition relates to lameness. An increased lameness risk has only been associated with a poor body condition, rather than a linear relationship between body condition and lameness risk (Green et al., 2014; Lim et al., 2015; Randall et al., 2015). The study which reported the strongest association between body condition and DCT observed an average difference of 0.6 mm in the DCT between cows with body condition scores of 2/5 and 2.5/5 (Bicalho et al., 2009). If the digital cushion is the mechanistic link between poor body condition and lameness this highlights the exceptionally fine margins that exist between dairy cows developing a sole ulcer or remaining sound.

The shock-absorbing capacity of the digital cushion may be more dependent on its composition than its dimensions, and this may explain the inconsistencies between studies which have measured the digital cushion with ultrasonography. Cadaver studies indicate the composition of the digital cushion tended to vary with parity (Räber et al., 2004; İzci et al., 2019), although standard errors were either unreported or very large. Another cadaver study reported there was significantly less fat in the digital cushion of primiparous than multiparous animals, but did not account for the stage in lactation or body condition of the animals (Räber et al., 2006). It is plausible that subtle changes in DCT may be indicative of more substantial changes in composition which could have a greater bearing on the biomechanics of the foot, but more research is needed to support this. It is equally possible that changes in DCT are simply a result of the

sinking of the distal phalanx within the hoof causing compression of the tissue below; in healthy cows, this is prevented by a system of collagen fibres called the suspensory apparatus (Mülling, 2012).

1.2.4 The suspensory apparatus

The distal phalanx of the bovine hoof is encased within the hoof capsule and suspended from the hoof wall by collagenous fibres attached to the dermal laminae, this is referred to as the suspensory apparatus (Lischer et al., 2002b; Budras, 2003). The body mass of a cow is primarily transferred from the distal phalanx to the ground via the suspensory laminae and hoof wall. If the suspensory apparatus of the distal phalanx fails to prevent downward movement of the phalanx within the hoof then the corium can be damaged leading to subsequent CHL development (Lischer et al., 2002b).

The calving effect. One widely accepted cause of weakening of the suspensory apparatus is parturition. Two studies, using similar study designs, demonstrated a difference in the strength of the suspensory apparatus of the distal phalanx in heifers on either side of their first calving (Tarlton et al., 2002; Knott et al., 2007). Biomechanical strength in the laminae was decreased in samples collected after parturition and weakest at the final time point twelve weeks postpartum. Although these results are frequently considered to highlight parturition as a risk factor for CHL development, changes in the laminae could relate to either parturition or the onset of lactation, or a combination of both. Furthermore, as the study population was nulliparous heifers, the comparison in these studies is between animals which have never calved and those which have calved once. Therefore, it is possible that observed changes are specific to the first parturition, or the commencement of the first lactation, and may not necessarily repeat at every subsequent parturition.

The significance of the periparturient period on CHL development is supported by observations that increased standing times in the weeks on either side of parturition was associated with future CHL development (Proudfoot et al., 2010; Sepúlveda-Varas et al., 2018). This observation may indicate the soft tissues of the foot are more vulnerable to compressive forces in this

periparturient period which would support the hypothesis of laxity in the suspensory apparatus. Equally, this same behaviour has been associated with other conditions which develop subsequently, such as clinical ketosis, and in this instance, it is considered to reflect a generalised discomfort (Itle et al., 2015). Therefore, both the consequences and causes of increased periparturient standing times should be considered as possible direct or indirect risk factors for CHL development.

Matrix metalloproteinases. The activity of matrix metalloproteinases (**MMP**), and particularly their effects on the suspensory apparatus, are of interest concerning CHL aetiopathogenesis. Matrix metalloproteinases are a family of enzymes which are principally involved in remodelling structural components of the extra-cellular matrix, but also have roles in the regulation of inflammation (Mccawley and Matrisian, 2001; Parks et al., 2004; Vandenbroucke and Libert, 2014). Two MMP, MMP-2 and MMP-9, are specifically associated with collagen degradation (Parks et al., 2004), and MMP-2 has been highlighted as a putative cause of periparturient weakening of the suspensory apparatus of the distal phalanx in cattle (Tarlton et al., 2002; Knott et al., 2007). Cows with SU had increased gene expression for MMP-13, which could indicate MMP-13 is directly associated with SU, as it is associated with inflammation, or indirectly via the activation of MMP-2 and MMP-9 (O'Driscoll et al., 2015). The scope of this study could not differentiate whether expression of MMP-13 occurred before the development of SU or if it was elevated as a response to inflammation caused by the lesion.

Physiologically, MMP are primarily controlled by steroid hormones, such as progesterone, oestrogen, and cortisol (Cury et al., 2007; Takagi et al., 2007; Mishra et al., 2012). Insulin-like hormones also have roles in MMP control, for example, relaxin appears to increase MMP expression and said MMP are associated with the release of insulin-like growth factors (**IGF**) (Mccawley and Matrisian, 2001; Bathgate et al., 2013). Matrix metalloproteinases have been demonstrated to mediate the remodelling of the postpartum endometrium and facilitate the softening of soft tissues before parturition (Samuel et al., 1998;

Walter and Boos, 2001; Schuler et al., 2018). This has led to the endocrinological changes associated with parturition, particularly increases in oestrogen and relaxin, to be considered as possible drivers of MMP activity in the connective tissue of the hoof around calving (Knott et al., 2007), although this link has not been substantiated. It should be noted that multiple hormones which might affect MMP activity, such as progesterone, oestrogen, insulin, and IGF-1, are typically lower in multiparous animals (Sartori et al., 2004; Wiltbank et al., 2006; Wathes et al., 2007; Brown et al., 2012), therefore it may not be accurate to assume MMP activity in the hoof is the same postpartum in primiparous and multiparous animals.

The effects of relaxin in cattle are not well understood and many of the presumed effects are extrapolated from other species; unlike other mammals, cattle do not produce the relaxin-1 peptide and therefore care needs to be taken with these assumptions (Southey et al., 2009). Furthermore, many MMP receptors can be activated by both relaxin and insulin-like peptides (Bathgate et al., 2013) and the role of other hormones in MMP control may also be worth consideration. For example, the upregulation of MMP in the endometrium of cattle has also been associated with postpartum negative energy balance (Wathes et al., 2011). Cows with negative energy balance have a lower concentration of IGF-1 (Wathes et al., 2011), and it has been shown in vitro that IGF-1 has a protective effect on the MMP degradation of collagen in bovine cartilage (Hui et al., 2001). Therefore, it is plausible that MMP within the connective tissue of the hoof may also respond to metabolic changes which are not directly related to parturition but rather the endocrinological background of early lactation.

Systemic inflammation. The expression of MMP increases during endotoxin-induced systemic inflammation (Pagenstecher et al., 2000; Vandenbroucke and Libert, 2014); inflammation is another key area of interest in the aetiopathogenesis of CHL. Theoretically, any systemic inflammation may be associated with localised inflammation within the hoof. Inflammation of the laminae is hypothesised to cause laxity in the suspensory apparatus of the distal

phalanx (Ossent and Lischer, 1998); inflammation of the soft tissues in the foot may also affect the digital cushion or result in bone development on the distal phalanx (Lischer et al., 2002b; Newsome et al., 2016). Any or all of these consequences of inflammation could contribute to CHL development.

Biomarkers of systemic inflammation, such as serum amyloid A and haptoglobin, can be elevated in animals with CHL, but results are inconsistent (Laven et al., 2004; Kujala et al., 2010; Smith et al., 2010; Tothova et al., 2014). Furthermore, as most studies only measure biomarkers of systemic inflammation after CHL diagnosis, they do not necessarily implicate systemic inflammation in the aetiopathogenesis of these lesions. A small study retrospectively matched six lame cows in the first three weeks of lactation, with six healthy controls, and analysed pre and postpartum serum samples for biomarkers of inflammation (Zhang et al., 2015). Cows which were lame had higher concentrations of interleukin-1 and serum amyloid A eight weeks prepartum, both these biomarkers were also elevated four weeks prepartum as well as interleukin-6 and haptoglobin. Although there were limitations to this study design and analysis, including but not limited to the small study population and unknown causes of lameness, these results could support a potential role of inflammation in the aetiopathogenesis of CHL. Another study analysed the biochemical profiles of 48 periparturient Holstein cows on a single Brazilian dairy herd, in which eleven cows were diagnosed as lame in the first week postpartum (Paiano et al., 2019). Albumin was significantly lower in lame cows from 18 days before parturition to 60 days postpartum, and as there were no differences in other markers of protein intake and synthesis, Paiano et al. (2019) suggest this indicated a pro-inflammatory state. While this assertion may be correct, other unrecorded and common inflammatory conditions, such as uterine infections, can reduce also albumin levels, so this association can only be tentatively attributed to lameness (Megahed et al., 2019).

Conditions such as mastitis are associated with a systemic inflammatory response, even in the absence of systemic clinical signs (Eckersall et al., 2001). Early lactation clinical mastitis was observed to increase the odds of subsequent

SU development (Griffiths et al., 2020; Watson et al., 2022). This would support a link between systemic inflammation and claw health, although results may also be affected by changes in animal behaviour such as decreased lying time due to painful mammary glands (Siivonen et al., 2011), or common predisposing factors underlying both conditions. One mechanism through which laminae may be affected by systemic inflammation is via the action of toxic compounds such as lipopolysaccharides. An in vitro study demonstrated lipopolysaccharides caused inflammation of the dermal cells in the hoof (Tian et al., 2019), and systemic administration of lipopolysaccharides in vivo has been reported to induce histological changes in the laminae (Boosman et al., 1991). The translocation of lipopolysaccharides into systemic circulation has been demonstrated from naturally and experimentally induced mastitis and metritis (Eckel and Ametaj, 2016), therefore, it is possible that systemic inflammatory conditions could affect the laminae in the hoof, although further work is needed to develop this theory.

One way to directly evaluate the importance of inflammation in CHL aetiopathogenesis is to assess the effect of pharmacologically reducing inflammation. A randomised control trial demonstrated that the most successful treatment of CHL included the administration of three days of a non-steroidal anti-inflammatory drug (**NSAID**) (Thomas et al., 2015), and consequently administration of an NSAID has been advocated as the best practice for CHL treatment, in addition to therapeutic foot-trimming and the application of a foot block to the unaffected claw (Pedersen and Wilson, 2021). Although the follow-up in this study was not long enough to record the recurrence of lesions in subsequent lactations (Thomas et al., 2015), the results suggest that inflammation due to CHL may prevent successful recovery. The same study design applied to chronic cases indicated that recovery rates were poor regardless of treatment (Thomas et al., 2016), implying any pathological changes due to inflammation may be prevented with NSAID, but not reversed.

Another randomised controlled trial of primiparous heifers on a single herd, which ran over consecutive lactations, compared study groups which either included or omitted an NSAID as part of lameness treatment, and one group in

which all animals also received three days of an NSAID following each calving (Wilson et al., 2022). The group which received NSAID following calving, as well as during treatment for lameness, had a reduced risk of visible lameness or culling when compared to those that received no NSAID at calving or during lameness treatment (Wilson et al., 2022). The results of this study suggest periparturient inflammation may have an important role in lameness development, however, although around 70% of lameness cases were attributed to CHL, no analysis was reported to indicate how the results related specifically to cases of CHL. Additionally, results were reported for all cases of lameness so it was not possible to examine the effects on first cases in lactation compared to repeated cases; this would be of particular relevance to differentiate the effects of postpartum inflammation from inflammation caused by lameness. Interestingly, there was no effect of NSAID following parturition on lameness in multiparous animals enrolled in the same study (Wilson, 2021). This is possibly a reflection of the unknown lameness history of multiparous animals in this study population, which may include previous cases of CHL. It may also suggest that the effects of inflammation around parturition are only consequential the first time an animal calves, or that there are other important risk factors for lameness in older animals, such as increased milk production and metabolic changes, which mask any benefit of reduced periparturient inflammation.

A randomised control trial of Holstein dairy cows reported that prepartum administration of pegbovigastrim, which elevates circulating neutrophils, was associated with a significantly greater incidence of lameness in the first 30 days after calving (OR 1.79, 95% CI 1.16 - 2.76) (Zinicola et al., 2018). Most of these lame cows did not have detectable foot lesions at the time, but lesion incidence after the first 30 days of lactation was not reported. These results could support a causal link between immune response, or at least leucocytosis (McDougall et al., 2017), and subsequent lameness, but should be interpreted cautiously as investigating this hypothesis was not the primary aim of this study. Conversely, the administration of the pro-inflammatory cytokine interleukin-8 to prepartum dairy cows reduced the incidence of lameness within the first 30 days of lactation by approximately 50% (lameness recorded in 11/70 untreated controls, 6/70

treated with intrauterine interleukin-8, 5/70 treated with intravenous interleukin-8) (Zinicola et al., 2019b). This result would weaken the case for inflammation being implicated in lameness, but this study was also designed to test a different hypothesis and this result was not statistically significant (P = 0.16). Experimentally inducing systemic inflammation in randomly controlled trials may be the most robust approach to determine the role of systemic inflammation in the aetiopathogenesis of CHL, and this area would benefit from further research.

Laminitis. Inflammation of the laminar corium is called laminitis, although it is contested whether inflammation causes degeneration of the laminae, or degeneration of laminae causes secondary inflammation due to tissue damage (Shearer and van Amstel, 2017). Either way, degeneration of the laminae would prevent the suspensory apparatus from functioning effectively.

Acute ruminal acidosis is associated with acute laminitis (Thoefner et al., 2004; Danscher et al., 2009), but this was not found to be to be associated with a reduction in the tensile strength of the laminae (Danscher et al., 2010), undermining the importance of laminitis in the aetiopathogenesis of CHL. Sub-acute ruminal acidosis (**SARA**) is common in early lactation due to the change onto a high-starch ration, and CHL development may be a consequence of subclinical laminitis due to this nutritional change (Vermunt and Greenough, 1994; Ossent and Lischer, 1998; Cook et al., 2004; Offer et al., 2004; Vermunt, 2007). Two studies observed no differences in CHL development during early lactation in cows fed a high concentrate-to-forage ratio diet compared to cows fed a ration with a low concentrate-to-forage ratio (Livesey et al., 2003; Offer et al., 2004). Other studies have demonstrated that a low dry matter diet was associated with an increased CHL incidence in primiparous heifers (Webster, 2001; Offer et al., 2003). The results of these studies should be interpreted with caution because analyses were limited to either exclusively univariable comparisons or multivariable analyses without important confounding factors such as body condition and milk production.

Despite equivocal results, the association between rumen health and claw health should not be disregarded. The relationship between SARA and CHL

development is hindered, in part, by the limited understanding and characterisation of SARA as a condition, which is difficult to diagnose in individual animals (Grove-White, 2004). An association between SARA and CHL is supported by the results of a randomised control trial which found that cows which were changed onto a high-energy diet after calving developed more severe CHL than cows which were already on the high-energy diet prepartum (Donovan et al., 2004), although this study did not find an association between rumen pH and CHL development.

The specific association between rumen pH and CHL development has been an obstacle in attempts to link rumen health and claw health. This has led some authors to dismiss this proposed component of CHL aetiopathogenesis (Randall et al., 2018b), however, rumen health cannot be surmised exclusively by its pH which correlates poorly with other indicators of rumen health and function, such as concentrations of volatile fatty acids and ammonia (Bramley et al., 2008; Lean and Golder, 2018). Moreover, the diurnal fluctuations in rumen pH render point measurements of pH less predictive of SARA than the rumen pH kinetics (Sato, 2016; Villot et al., 2018). Therefore, further research which attempted to capture more of the complexity of rumen dynamics would be useful to investigate the possible role of SARA in CHL aetiopathogenesis.

The proposed mechanism by which SARA affects claw health is through the translocation of toxic compounds from the rumen which can subsequently damage the connective tissues in the hoof (Lean et al., 2013; Tian et al., 2019). Although toxic compounds generated during SARA have been measured in the rumen, there is some debate as to how consistently these translocate across the rumen epithelium (Plaizier et al., 2018). Experimentally induced SARA caused increased systemic levels of lipopolysaccharides, in addition to other systemic inflammatory markers (Khafipour et al., 2009; Guo et al., 2017). Additionally, there appears to be cross-talk between the regions of the gastrointestinal tract, and hindgut fermentation is also affected by the amount of dietary starch (Steele et al., 2016). The epithelium of the distal intestines is more susceptible to acidic damage than the rumen and translocation of toxic compounds occurs more

frequently, therefore SARA may not exclusively reflect rumen health but could indicate changes across the entirety of the gastrointestinal tract (Plaizier et al., 2018). In horses, hindgut fermentation of excessive carbohydrates is associated with systemic inflammation and clinical laminitis (Katz and Bailey, 2012), although insulin resistance is now considered to be the predominant aetiopathogenesis in most cases (de Laat et al., 2010; Patterson-Kane et al., 2018; Durham et al., 2019).

Metabolic pathophysiology. In dairy cows, insulin resistance in the periparturient period is considered a necessary homeorhetic adaption to supply glucose to the reproductive tract and mammary gland (De Koster and Opsomer, 2013), and insulin resistance is linked to increased milk production (Chagas et al., 2009). There is a well-recognised correlation between milk production and lameness (Archer et al., 2010b; Reader et al., 2011) and therefore the relationship between insulin resistance and lameness may be pertinent (Newsome, 2016). This relationship may include systemic inflammation: the administration of the pro-inflammatory cytokine interleukin-8 induced insulin resistance in a small number of male calves (Zinicola et al., 2019b), and the administration of interleukin-8 to prepartum dairy cows increased subsequent milk production (Zinicola et al., 2019a).

In early lactation dairy cows, the combined effect of reduced dry matter intake and accelerating milk production is a metabolic state which reaches, or at least approaches, a state of negative energy balance (Collard et al., 2000). This deficit is mitigated by extensive lipolysis which manifests as a loss of body condition (Sordillo and Raphael, 2013). Lipolysis releases non-esterified fatty acids (**NEFA**) from adipocytes and if NEFA production exceeds the metabolic capacity of the liver, hyperketonaemia can develop (Raboisson et al., 2014).

Over-conditioned cows at parturition are recognised to be at increased risk for hyperketonaemia (Vanholder et al., 2015), whereas under-conditioned cows are at the highest risk of lameness (Green et al., 2014; Lim et al., 2015; Randall et al., 2015). Therefore the observation that cows which developed claw lesions in mid-lactation had elevated NEFA and hyperketonaemia around parturition is not

intuitive (Sepúlveda-Varas et al., 2018). This paradox highlights the complexity of the periparturient metabolic environment which cannot be determined by the concentration of one or two hormones in isolation. Lipid metabolism involves interactions between hormones such as cortisol, growth hormone, insulin and leptin (Roche et al., 2009), and adipose tissue itself expresses mRNA for many other hormones and cytokines (De Koster and Opsomer, 2013). Additionally, changes in muscle metabolism, which occur concurrent with lipid metabolism in early lactation (Bruckmaier et al., 1998; Megahed et al., 2019), have yet to be thoroughly investigated for an association with lameness. Therefore, although the metabolic changes in early lactation may contribute to changes within the connective tissues of the hoof, the specific links can be difficult to unravel. The utilisation of -omics technologies offers the greatest potential to capture the full complexity of these relationships.

Studies which attempt to characterise the entirety of biological molecules within a biological system have the suffix "-omics", for example, studies which describe all proteins within a tissue (the proteome) are termed proteomic studies. A proteomic case-control study using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (**MS**) determined that the expression profiles of proteins in the plasma were different between lame cows and sound cows (Dong et al., 2015). There were multiple differences between cases and controls including increased expression of proteins associated with cholesterol synthesis, proteins implicated in lipopolysaccharide-induced inflammation, and acute phase inflammatory proteins such as haptoglobin. Unfortunately, this study had limitations such as not controlling for the stage of lactation and the limited number of controls compared to the number of cases, nevertheless, a proteomic approach to investigating CHL development would be worth pursuing.

Metabolomic techniques have also been employed to investigate lameness in dairy cows. Metabolomics uses high-throughput platforms to detect large numbers of low-molecular-weight metabolites within a biological sample (Wishart, 2008). The primary technologies used in metabolomics are chromatographic separation coupled with MS, and proton nuclear magnetic

resonance (**^{1}H NMR**) spectroscopy (Emwas et al., 2019). To date, all research in this area comes from a single case-control study of six lame cows and 20 non-lame controls which has generated multiple ^{1}H NMR- and MS-based metabolic analyses of serum, milk, and urine (Dervishi et al., 2019; Eckel et al., 2020; Zhang et al., 2020b; a; Zwierzchowski et al., 2020). The study period spanned eight weeks on either side of parturition and highlighted large numbers of metabolites which may be associated with lameness, both several weeks before and after calving and the onset of clinical signs. The results indicated metabolic pathways associated with inflammation, muscle catabolism and immunosuppression were up-regulated before parturition in lame cows. Other metabolite differences suggested the presence of prepartum inflammation, in addition to oxidative stress and acid-base imbalance. Results also implied metabolic markers could be used to discriminate between lame and non-lame cows with almost perfect accuracy in all evaluated biofluids; although the reported predictive accuracy may be over-optimistic due to the risk of feature selection bias with the analytical approach described (Xia et al., 2013; Kuhn and Johnson, 2018). There were other limitations to these studies (Dervishi et al., 2019; Eckel et al., 2020; Zhang et al., 2020b; a; Zwierzchowski et al., 2020) which limit the interpretation and application of the results. In addition to the small sample size, the lameness history of these cows was not reported so it is unclear whether lameness developed for the first time in early lactation or if the cows were also lame prepartum. This latter point is crucial because it affects whether the results indicate changes which occurred before clinical lameness or changes which occurred as a result of a pre-existing lameness. Furthermore, no details were provided about the cause of the severe lameness, which is implied to have an acute onset. If lameness cases had an infectious aetiology, such as interdigital phlegmon, then evidence of preceding or concurrent inflammation may not be unexpected (Zheng et al., 2016), but if lameness cases were due to CHL then this would be a valuable insight into the aetiopathogenesis. Despite these limitations, these results suggest that metabolomic studies have the potential to further the understanding of metabolic mechanisms in CHL aetiopathogenesis, and even

that it may be possible to identify animals at risk of future lameness using metabolite profiles.

1.2.5 Conclusions

Despite a large number of known risk factors, the aetiopathogenesis of CHL remains poorly understood. It appears that CHL aetiopathogenesis is multifactorial, but it is not clear whether CHL development is dependent on the cumulative effect of multiple factors or if there are key mechanisms which are essential for lesion development and other factors which exacerbate the pathogenesis.

There are many metabolic and inflammatory changes associated with parturition and lactation in dairy cows, making it difficult to isolate specific changes that influence CHL development. It appears that body condition is related to CHL risk, but it is unclear whether this is because it reflects concurrent metabolic processes or the functionality of the digital cushion. Prior lameness due to CHL also appears to be an important risk factor for future CHL, and this could be through bone development on the distal phalanx, permanent changes within the soft tissue of the hoof, or via another causal risk factor.

From a practical perspective, some of the risk factors for CHL development can be managed on farms, such as reducing damaging forces in the hoof by ensuring optimal foot shape and avoiding unnecessary standing on hard surfaces. Other risk factors, such as the effect of calving, cannot be avoided. One aspect of CHL control programmes which has yet to receive much attention is the use of breeding strategies to increase the resistance to CHL.

1.3 Genetics of claw horn lesions

Sole ulcers are also referred to as Rusterholz ulcers after Anton Rusterholz who published the first detailed description of the condition in 1920 (Rusterholz, 1920; Mülling, 2019). When discussing the potential pathogenesis of SU, Rusterholz stated that due to the relationship between the anatomy of the claw and SU development, the lesion "genetischem Interesse sein dürften" [may be of genetic interest] (Rusterholz, 1920). A century or so later, there is still interest in the genetics of SU, and lameness more generally.

Development of the modern dairy cow. Around 90% of Holsteins in the UK are descended from North American Holsteins (Oltenacu and Algers, 2005). Between 1920 and 2020, there was a fivefold increase in the lactation milk yield of the average North American Holstein; approximately half of this increase can be attributed to genetic changes (Miglior et al., 2017; Cole and VanRaden, 2018). While this illustrates the remarkable genetic progress which can be achieved with intense selection pressure, unfavourable genetic correlations between production and fitness traits have contributed to a marked decline in the health and welfare of dairy cows (Jones et al., 1994; Oltenacu and Algers, 2005). So, although the development of the North American Holstein dairy cow is an example of the power of genetic selection, it is also a cautionary tale.

The *Bos taurus* species emerged in present-day Syria from the domestication of the wild ox (*Bos primigenius*) around 10,000 years ago (Magee et al., 2014). Domesticated cattle spread across Europe along agricultural trade routes with the Holstein-Friesian breed first recognised about 8,000 years later in North Holland and West Friesland (McGuffey and Shirley, 2011; Elischer, 2014). Holstein-Friesians were bred for high milk production so they were an attractive breed to import into the United States of America (**USA**) to supply the growing milk market in the mid-19th century (Elischer, 2014). Over the next 100 years or so, three key developments were instrumental in creating the modern Holstein dairy cow.

The first was the integration of phenotypes with pedigree information. In the USA, the Dairy Herd Improvement Association was formed in 1908 and collated

the results from regular testing of milk yields and constituents so that accurate and clearly defined production phenotypes of progeny could be linked back to individual bulls (Weigel et al., 2017). The second was the emergence of the field of quantitative genetics which led to the development of estimated breeding values for individual animals (McGuffey and Shirley, 2011; Weigel et al., 2017). At this point, the science allowed the identification and selection of bulls with the best genetic potential for production, but it was not until the development of artificial insemination that the science could be efficiently applied. Building on a large body of previous research, artificial insemination techniques were refined and upscaled in the 1940s so that semen from the bulls with the highest genetic merit for milk production could be used on a wider distribution and greater number of female cows than would be possible with natural mating (Foote, 2002). Effective freezing techniques followed and enabled the transport of bull semen all over the world (Oltenacu and Algers, 2005), resulting in the predominance of North American Holstein genetics in the UK dairy herd.

1.3.1 Genetic selection for reduced lameness

Breeding programmes aim to improve the average phenotype for a given trait within a population. The phenotype of an animal is partly dictated by the genes it inherits, a proportion of this genetic component is additive which means it can be measured and influenced by selection (Simm, 2000). The additive genetic value of an animal is termed the estimated breeding value (**EBV**). An equivalent expression of an animal's genetic potential is predicted transmitting ability (**PTA**), which is half the EBV and relates directly to the genetic merit passed on to offspring.

Selection for profitability. In general, genetic selection aims to improve the lifetime profitability of animals. To achieve this, individual trait indexes (either EBV or PTA) are often combined into a composite index where each trait is weighted according to their relationship to each other and their economic value (Cole and VanRaden, 2018). A selection index for production, which includes milk constituents and yield, has been used in the UK since the 1980s (Hill and Swanson, 1983). Traditionally, breed societies focussed on certain physical

attributes to define breeds and inform breeding decisions. These physical attributes were the basis of linear type scores, which were introduced as breeding values in the UK in 1983 (Veerkamp et al., 1995). In the early 1990s, most countries used profitability selection indexes which prioritised production alongside linear type traits (Leitch, 1994). This was partly due to the large number of accessible phenotype records required to evaluate national breeding values. Regular milk recording allowed production phenotypes to be recorded and collated with relative ease, similarly, breed society classifying results were collected as part of the registration of pedigree animals.

In the latter part of the 20th century, it became clear that while increased production had increased profitability, future progress would be offset by the costs associated with the genetic decline in health and fertility (Pryce et al., 2000b). Effective selection indexes to reverse this trend were not readily available. Longevity was the first functional trait to receive much attention, and in the UK, it was included in an "index for total economic merit" (ITEM) using the linear type traits of Angularity, Foot Angle, Udder Depth, and Teat Length as indicator traits for Longevity (Veerkamp et al., 1995). Shortly after this, the number of lactations was also incorporated into the index as a direct longevity trait (Brotherstone et al., 1998), and the index was renamed the "profitable lifetime index" (**£PLI**). However, selection on £PLI was not enough to mitigate the negative consequences of selection for production (Pryce et al., 2000b), and £PLI was broadened further to include specific health and fertility traits. A locomotion assessment had been included in breed society classifying since 1998, and this was added to £PLI, along with other functional traits, bringing the total proportion of non-production traits in £PLI to 24% (Stott et al., 2005). Despite these changes, this was still only expected to slow down the deterioration of health conditions such as lameness and mastitis, rather than drive an improvement. As the urgency to increase genetic health and function became more widely accepted, and more direct functional traits were evaluated, the weighting of non-production traits in £PLI increased further; 66% of the current £PLI is made up of non-production traits (AHDB, 2020b). The equivalent selection index in the USA, Lifetime Net Merit Index (NM$), has followed a similar trend over the past decades (Cole and

VanRaden, 2018). However, due to the low heritability of lameness traits, genetic progress is likely to be slow unless lameness traits are heavily weighted within selection indexes (Neuenschwander et al., 2012).

Selection for reduced lameness. There is a high level of motivation in dairy farmers to improve claw health because it is a major driver of welfare and efficiency in the herd (Dutton-Regester et al., 2019). One such avenue is through genetic selection, and farmers prioritise the genetic progress of functional traits to breed robust cows (Egger-Danner et al., 2014). Lameness traits include linear type traits which are indirectly associated with lameness, such as locomotion assessed during classifying and conformation of limbs and feet, and traits which directly relate to foot lesions or clinical lameness (van der Werf and Pryce, 2019).

Historically, farmers wishing to reduce CHL in their herd through improved genetics could only select on indirect traits (McDaniel, 1998). Selection on indicator traits can be used in place of direct selection providing three criteria are met: (1) there is a strong genetic correlation between the indicator and target trait, (2) the indicator trait can be recorded more cheaply, more easily, and earlier in life than the target trait, and (3) the heritability of the indicator trait is greater than the target trait (Shook, 1989). Although measurement of lameness-associated type traits, such as foot and leg conformation is cheaper, easier and possible in younger animals (McDaniel, 1998), the heritability of most type traits is low or moderate, and crucially, the genetic correlation between indicator and direct lameness traits is variable (Pryce et al., 2000a; Berry et al., 2004; Oliveira Junior et al., 2021). Lameness-associated type traits are not genetically correlated with CHL strongly enough to be used as effective indicator traits to increase resistance to CHL, but they have been shown to increase the reliability of breeding values (Chapinal et al., 2013; Häggman and Juga, 2013) and the expected response to selection (Koenig et al., 2005; van der Linde et al., 2010).

Selection on direct health traits is thought to be the most effective approach to achieving genetic progress (Egger-Danner et al., 2014). It has been advocated that to be included in a breeding programme traits should meet three criteria: (1) have a demonstrable economic value, (2) have a reasonable degree of additive

genetic variance (and heritability), and (3) be measurable at a low cost and be clearly defined so that recording is consistent (Shook, 1989). For direct CHL traits, the first of these three points is not contested due to the recognised costs of these lesions, the second point is less clear with mixed reports about the magnitude of CHL heritability, but the third point is the major obstacle to the selection on direct CHL traits because repeated foot lesion records are required for each animal, and these records cannot be obtained passively or automatically.

Some countries have developed the infrastructure to collect foot-trimming records so that these can be directly incorporated into selection indexes (van der Linde et al., 2010; Ødegård et al., 2015). In the Netherlands, the incorporation of foot lesions into a composite selection index has been predicted to have the capacity to reduce lameness prevalence by 0.1 - 0.7% per year (van der Linde et al., 2010). A sub-index for hoof health, based on lesions recorded by hoof trimmers, is now available in Canada (Malchiodi et al., 2020), with the mean reliability of CHL breeding values ranging from 73 - 76%. In recent years, private companies have also identified the value of providing genetic predictions of health traits to dairy farms (Eastham, 2019), including schemes such as Clarifide Plus (www2.zoetis.co.uk/clarifide-plus/, accessed 13 April 2020) and Evolution International (www.evolution-int.com/en/our-innovations, accessed 13 April 2020).

Where the infrastructure to record foot-trimming lesions on a large scale has not been established, countries have instead utilised farm records of lameness for genetic evaluations (Zwald et al., 2004; Pritchard et al., 2013; Parker Gaddis et al., 2014). In the UK, Pritchard et al. (2013) demonstrated that farm records could be used as lameness phenotypes. In 2018, a genetic selection index to reduce lameness, termed “Lameness Advantage”, was published by the AHDB with a reported heritability of 0.04 (AHDB, 2018a).

The Lameness Advantage index is calculated from farm records of lameness and digital dermatitis (collected via milk recording organisations) in combination with traits from type classification. The individual traits and their

heritability (in parentheses) in the Lameness Advantage index are the number of lameness events from farm records (0.03), digital dermatitis events (0.01), Bone Quality (0.16), Locomotion (0.08), and Feet and Legs (0.10) (M Winters, 2021, personal communication, 16 September). Feet and Legs is a composite index from the overall assessment by the classifier incorporating Foot Angle, Rear Legs Side View, Locomotion, and Bone Quality. The Lameness Advantage index is expressed as a percentage ranging from -5% (bad) to +5% (excellent), with every 1% change in a bull's Lameness Advantage PTA expected to translate to an equivalent change in the percentage of daughters becoming lame in each lactation (AHDB, 2018a).

1.3.2 Genomics

The bovine genome has been mapped using markers selected to provide coverage of the whole genome with alleles which are common in the population (Zimin et al., 2009). There are approximately 2.89 billion base pairs (2.89 Gb) in the bovine genome, and up to 12.1 million variants have been identified in female Holstein cows (Das et al., 2015). The genotype of an individual can be determined using genomic markers, often single nucleotide polymorphisms (**SNP**). In the USA, the first commercial SNP microarray chip for Holstein cows was released in 2007 and included 54,000 SNP (Wiggans et al., 2017).

Estimated breeding values are calculated with best linear unbiased prediction (**BLUP**). Traditionally, EBV were based on pedigree relationships. Estimated genomic breeding values (**GEBV**) can be calculated using the same method but based on genomic relationships derived from SNP, or with a combination of both pedigree and genomic information, referred to as a single-step approach (Hayes et al., 2009; Weigel et al., 2017; Aguilar et al., 2019). The correlation between estimated and true breeding values is termed the accuracy, the squared accuracy is called the reliability (Simm, 2000). The reliability of GEBV are improved by the incorporation of both genomic and pedigree information (Hayes et al., 2009). For example, the reliability of a non-infectious foot lesion PTA was increased by 0.18 (0.42 to 0.66) by using genomic information in addition to pedigree information (Dhakal et al., 2015). The genetic homogeneity

of Holsteins in different countries, due to the ubiquity of artificial insemination and the global dissemination of semen and embryos (Zenger et al., 2007), has resulted in vast numbers of offspring records per sire, this has dramatically improved the accuracy of GEBV in the Holstein breed (Taylor et al., 2016; Weigel et al., 2017).

Another reason for the increased reliability of GEBV is that relationships between animals are defined from actual allele, or haplotype block, sharing rather than probabilities of shared alleles (Speed and Balding, 2015). For example, although full siblings share half of their additive genetic variance, genomic analysis has determined that the range of shared alleles can range from 0.37 to 0.62 (Visscher et al., 2006). Therefore, unproven full siblings can have different and more accurate GEBV, whereas their EBV would initially be identical (Hayes et al., 2009). Consequently, there has been widespread uptake of genomic testing from breeding companies because GEBV are available for young bulls, at a time when the breeding values could only be estimated from parental averages with limited accuracy (Calus et al., 2013a; Wiggans et al., 2017). This has reduced the generation interval and accelerated the rate of genetic progress (García-Ruiz et al., 2016).

The accuracy of GEBV is closely related to the level of linkage disequilibrium (**LD**) between genomic markers and causative alleles (Meuwissen et al., 2001). There is a relatively high level of LD in Holstein cows (Qanbari et al., 2010). The inclusion of dam GEBV further increases the accuracy of genomic selection (Kaniyamattam et al., 2016). If genomic testing of females is augmented by the strategic use of sexed-semen, which facilitates greater selection intensity, then even greater genetic gains can be made compared to bull selection alone (Schaeffer, 2006). Currently, only a relatively small proportion of heifers in the UK have been genotyped, although the uptake in North American countries has been higher, and this is likely to increase in the future (Eastham, 2019).

The accuracy of GEBV for claw health and lameness is impeded by the paucity of historical data (Heringstad et al., 2018). Claw horn lesions are labour-intensive to record due to the need for feet to be inspected in a foot crush,

therefore alternative phenotype sources have been used. Producer-recorded lameness cases may correlate poorly to independently assessed lameness prevalence (Whay et al., 2003a), therefore this source of phenotypic data may not be reliable, and in any case, is likely to be skewed towards painful lesions which require intervention. Foot-trimming records are a valuable resource for genetic evaluations, but they also have limitations due to variability between individual recorders and differences in terminology between countries (Christen et al., 2015). Furthermore, less clinically important lesions, which often only require minimal intervention, may be under-recorded when the primary purpose of handling cattle is to perform preventative or therapeutic foot-trimming (Archer et al., 2010a). Nevertheless, genomic predictions based on foot-trimmer records still differentiated between the best and worst 10% of cows, despite the poor accuracies reported (Croué et al., 2019). A genomic heritability estimate of 0.25 was reported for hoof health based on foot-trimmer recorded lesions, and this indicates that genomic selection for improved claw health could be based on this source of phenotypic data (Suchocki et al., 2020).

1.3.3 Heritability of claw horn lesions and associated traits

Heritability is the proportion of phenotypic variance in a population that can be explained by genetic variance. The evaluation of different breeding strategies requires accurate prediction of selection responses, calculated as a function of heritability (Falconer and Mackay, 1996). Therefore, effective breeding for a reduced incidence of CHL is dependent on understanding the additive genetic variance, and heritability, of these lesions.

The heritability of CHL has been studied extensively in dairy cows. Lesion records from professional foot-trimmers are the most common source of these data for genetic studies because they can be collated for a large number of cows, but there are some inherent biases in this source of data (Heringstad et al., 2018). A study of foot-trimmer records, which used professional foot-trimmer records from two years, reported considerable variation in the proportion of animals assessed in each herd, ranging from less than 10% to greater than 90% (Croué et al., 2017). In the absence of screening an entire population, a sample of the

population can be assessed which, if randomly selected, can be representative of the entire population (Dohoo et al., 2012). The subsets of individual herds in foot-trimmer records are not randomly selected and are likely to over-represent lame cows which will affect the estimated phenotypic and genetic variance (Köck et al., 2019). Studies have tried to minimise this problem by excluding herds if records were only available for a small proportion of animals or assuming non-trimmed cows are healthy, but both of these approaches appear to introduce other sources of bias and inaccuracy to estimates of genetic parameters (van der Spek et al., 2013; Croué et al., 2017; Malchiodi et al., 2017).

Heritability estimates of CHL vary between studies (**Table 1.2**), but in general, all CHL are reported to have a low to moderate heritability, with SU generally reported to have the highest heritability of the three lesions. A study of 52 seasonally calving dairy herds evaluated the differences in genetic parameters using different trait definitions of SH and WL and found heritability estimates were higher if SH and WL were graded by severity, rather than dichotomised (Ring et al., 2018). Ring et al. (2018) concluded the average lesion severity across all feet would be the most effective trait to select for reduced SH and WL. Heritability was also estimated for severe SH and WL, with mild cases classed as healthy, which caused a marked reduction in heritability of SH but not WL. This result may imply the genetic variance for WL susceptibility is driven by animals which develop severe lesions, with only minimal genetic variance underlying mild WL.

Although the presence or absence of a CHL is a binary trait, they can be modelled as linear variables to estimate heritability (Pérez-Cabal and Charfeddine, 2015; Malchiodi et al., 2017). However, there are limitations to this approach, and another method is to assume the underlying disease risk is continuous; heritability can be estimated on this continuous underlying liability scale using a threshold model, with a threshold which relates to disease prevalence (Gianola, 1982; Lynch and Walsh, 1998). These two approaches can produce different heritability estimates, particularly when the trait prevalence is less than 0.25 (Tenesa and Haley, 2013), which is frequently the case for CHL.

Heritability estimates with threshold models are usually higher than heritability estimates from linear models (**Table 1.2**).

Table 1.2. Heritability estimates for sole haemorrhage (SH), sole ulcers (SU) and white line lesions (WL) on either the observed or underlying scale.

Reference	Study population (N)	Breed	Country	Heritability (observed)			Heritability (underlying)		
				SH	SU	WL	SH	SU	WL
(Huang and Shanks, 1995)	1,239	Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey	United States of America	-	0.03	0.08	-	0.02	0.15
(Koenig et al., 2005)	5,634	Holstein	Germany	-	0.09 (0.006)	0.10 (0.001)	-	-	-
(van der Waaij et al., 2005)	21,611	Holstein-Friesian	The Netherlands	0.08 (0.02)	0.01 (0.01)	0.02 (0.01)	-	-	-
(König et al., 2008)	5,360	Holstein	Germany	-	0.10 (0.07)	0.10 (0.05)	-	0.14 (0.07)	0.14 (0.05)
(Swalve et al., 2008)	16,681	Holstein	Germany	-	0.06 (0.01)	0.05 (0.01)	-	0.17 (0.02)	0.10 (0.02)
(van der Linde et al., 2010)	62,187	Holstein-Friesian	The Netherlands	0.06	0.12	0.03	-	-	-
(Buch et al., 2011)	63,962	Swedish Red	Sweden	0.05 (0.007)	0.03 (0.006)	-	0.09a	0.17a	-

Reference	Study population (N)	Breed	Country	Heritability (observed)			Heritability (underlying)		
				SH	SU	WL	SH	SU	WL
(Johansson et al., 2011)	171,000	Holstein, Swedish Red	Denmark, Finland Sweden	0.04 – 0.05	0.02 – 0.04	0.01	-	-	-
(Gernand et al., 2012)	19,870	Holstein	Germany	-	-	-	-	0.07 (0.02)	0.09 (0.02)
(Häggman et al., 2013)	52,598	Ayrshire	Finland	-	-	-	0.03 (0.01)	0.15 (0.03)	0.11 (0.01)
(Häggman and Juga, 2013)	24,685	Holstein	Finland	-	-	-	0.02 (0.01)	0.08 (0.03)	0.04 (0.02)
(Oberbauer et al., 2013)	5,043	-	United States of America					0.30 (0.08-0.63)	0.24 (0.10 - 0.42)
(Ødegård et al., 2013)	178,452	Norwegian Red	Norway	-	-	-	0.07 (0.01)	0.18 (0.02)	0.06 (0.02)
(van der Spek et al., 2013)	20,474	Holstein-Friesian	France	0.02 (0.01)	0.03 (0.01)	0.04 (0.01)	0.05 (0.1)[a]	0.11 (0.01)[a]	0.09 (0.01)[a]
(Pérez-Cabal and Charfeddine, 2015)	35,337	Holstein	Spain	-	0.04 (0.004)	0.02 (0.003)	-	0.15 (0.024)	0.09 (0.021)
(Croué et al., 2017)	25,511	Holstein	France	0.02 (0.01)	0.05 (0.01)	0.06 (0.01)	-	-	-

Reference	Study population (N)	Breed	Country	Heritability (observed)			Heritability (underlying)		
				SH	SU	WL	SH	SU	WL
(Malchiodi et al., 2017)	53,654	Holstein	Canada	0.02 (0.003)	0.04 (0.006)	0.02 (0.004)	0.09 (0.02)	0.14 (0.02)	0.06 (0.01)
(Ring et al., 2018)	6,814	Holstein-Friesian	Ireland	0.03 (0.008) – 0.27 (0.03)[b]	-	0.02 (0.004) – 0.21 (0.03)[b]	-	-	-
(Sánchez-Molano et al., 2019)	554	Holstein-Friesian	United Kingdom	0.14 (0.08)	0.35 (0.10)	0.13 (0.08)	-	-	-
(Shabalina et al., 2020)	90,215	Holstein	Germany	-	0.06 – 0.12[c]	0.09 – 0.19[c]	-	-	-
(Malchiodi et al., 2020)	127,729	Holstein	Canada	0.03 (0.003)	0.05 (0.003)	0.04 (0.003)	-	-	-
(Oliveira Junior et al., 2021)	500,000	Holstein	Canada	0.03 (0.002)	0.05 (0.003)	0.02 (0.002)	-	-	-

a Transformed to underlying scale after estimation on the observed scale

b Range denotes heritability estimates using different trait definitions for each lesion

c Range denotes heritability estimates at different stages of lactation for different parities

Estimates of heritability can be affected by study design because they are dependent on the phenotypic variance recorded, which is calculated as the sum of the genetic and environmental (residual) variance estimates. Genetic variance within a population, environmental factors which affect phenotypic variance, and the correlation between genes and the environment can all change over time (Lynch and Walsh, 1998). Heritability estimates of CHL have been reported to vary by parity (van der Linde et al., 2010) and stage of lactation (Gernand et al., 2013), therefore characteristics of the study population may produce different results in differently designed studies. Studies based on the cows which producers have presented to foot-trimmers may over-represent painful foot lesions and therefore may not capture the full genetic variance within a population (Köck et al., 2019).

The majority of the studies included in **Table 1.2** used pedigree information to estimate heritability. Pedigree-based heritability estimates rely on the probability of relatedness between individuals which may not always be accurate (Speed and Balding, 2015). A pedigree error rate of approximately 10% has been found in UK dairy farms (Visscher et al., 2002), which will further limit the accuracy of pedigree-based heritability estimates. Genomic relationships may not explain all of the genetic variance captured by pedigree data (Hayes et al., 2009); although, it may be that genomic estimates are closer to the true values (Speed and Balding, 2015). This explains why genetic variance estimated using pedigree and genomic relationships can vary. For example in one study, the genomic heritability estimate of visible lameness in early lactation was estimated to be 0.14 to 0.21, which was substantially lower than the pedigree-based heritability estimate of 0.30 (Lopes et al., 2020). On the other hand, the genomic heritability estimate of non-infectious lesions in first-lactation animals was 0.12, which was higher than the pedigree-based heritability estimate of 0.08 (Dhakal et al., 2015).

Pedigree-based heritability estimates are calculated under assumptions of random mating, linkage equilibrium, no additive-epistatic genetic interactions and no universal environmental influence (Lynch and Walsh, 1998). Some of

these assumptions are unlikely to hold in modern dairy herds. For example, as many dairy herds use artificial insemination and breeding decisions based on breeding values, the assumption of random mating may not be met. Additionally, the small effective population size of Holstein cows and the high rate of inbreeding creates a greater degree of LD than found in wild populations, violating the assumption regarding linkage equilibrium (Rodríguez-Ramilo et al., 2015). Finally, female calves are born into the same environment as their mothers and siblings and therefore there can be a familial correlation between environmental effects. Other explanations for differences in genomic and pedigree-based calculations relate to how genomic data is analysed, including assumptions about SNP effects, minor allele frequency and levels of LD (Speed et al., 2017). All of these factors could influence estimates of additive genetic variance, and therefore heritability.

1.3.4 Genetic correlation between claw horn lesions and associated traits

Genetic correlation refers to the correlation between the additive genetic variance of two traits. Genetically correlated traits imply either the same genes are associated with both traits, attributable to either pleiotropy or a direct causative relationship between traits, or it can indicate genes which control both traits exist in LD within a population (van Rheenen et al., 2019). Estimates of genetic correlation often have large standard errors and can be markedly different between populations, therefore the results of different studies should be compared cautiously (Falconer and Mackay, 1996).

The results of multiple studies which have estimated the genetic correlation between CHL are shown in **Table 1.3**. In general, the genetic correlation between SH and SU is strongly positive. Interestingly, Croué et al. (2017) estimated that SU was strongly genetically correlated with circumscribed SH (0.79), but the genetic correlation between SU and diffuse SH was weak (0.22) and not statistically different from zero. The genetic correlation between SU and WL is reported to be positive, but variable in strength. In some studies, the genetic correlation between SH and SU and between SU and WL are of comparable

strength, but other studies estimate the genetic correlation between SU and WL to be weaker. The genetic correlation between SH and WL is reported to be positive but generally weak or moderate, it is relatively consistently estimated to be the weakest genetic correlation between individual CHL.

Table 1.3. Genetic correlation estimates between claw horn disruption lesions: sole haemorrhage (SH), sole ulcer (SU) and white line lesions (WL).

Reference	Genetic correlation		
	SH - SU	**SH - WL**	**SU - WL**
(Koenig et al., 2005)	-	-	0.44 (0.12)
(van der Waaij et al., 2005)	0.81 (0.26)	0.30 (0.21)	0.95 (0.15)
(Swalve et al., 2008)	-	-	0.01
(van der Linde et al., 2010)	0.58 – 0.79[a]	0.06 – 0.51[a]	0.41 – 0.60[a]
(Buch et al., 2011)	0.04 (0.01)	-	-
(Johansson et al., 2011)	0.68 - 0.74[b]	0.62 – 0.73[b]	0.74 – 0.78[b]
(Häggman and Juga, 2013)	0.38 (0.15)	0.39 (0.12)	0.31 (0.13)
(Ødegård et al., 2013)	-	-	0.79 (0.08)
(van der Spek et al., 2013)	0.90 (0.10)	0.10 (0.17)	0.49 (0.13)
(Pérez-Cabal and Charfeddine, 2015)	-	-	0.80 (0.05) - 0.98 (0.05)[c]
(Croué et al., 2017)	SHD: 0.22 (0.17) SHC: 0.79 (0.12)	SHD: 0.07 (0.17) SHC: 0.44 (0.15)	0.58 (0.11)
(Malchiodi et al., 2017)	0.80 (0.08)	0.52 (0.13)	0.75 (0.08)
(Malchiodi et al., 2020)	0.56 (0.03)	0.21 (0.05)	0.17 (0.05)
(Oliveira Junior et al., 2021)	0.83 (0.02)	0.46 (0.04)	0.60 (0.04)
(Lai et al., 2021a)	-	-	0.92 (0.46)

a Range denotes the genetic correlation between lesions across parities

b Range denotes the genetic correlation between lesions in different breeds

c Range denotes the genetic correlation between lesions using linear and threshold models

SHD: diffuse sole haemorrhage, SHC: circumscribed sole haemorrhage

The genetic correlation between DCT and CHL was estimated to be negative (-0.60 ±0.29) in a study of 972 Holstein cows in a single herd (Oikonomou et al., 2014). The genetic correlation between CHL and conformation traits has been estimated in several studies. Koenig et al. (2005) reported correlations between breeding values for SU and various conformation traits. Although the correlation between breeding values can reflect genetic correlation, this is only an approximation, furthermore, no error terms are reported, so the uncertainty of these estimates is unknown. With these caveats in mind, there appeared to be a genetic correlation between SU and all conformation traits, with the largest correlation coefficients reported for Feet & Legs (composite index), Rear Leg Side View, Rear Leg Rear View, Strength, and Foot Angle. More recently, a statistically significant genetic correlation was reported between SU and Feet & Legs (composite index), Rear Leg Side View, and Locomotion, but no genetic correlations with linear type traits were significant for either SH or WL (van der Linde et al., 2010). A statistically significant genetic correlation was found between SU and Rear Leg Rear View and Foot Angle; between SH and Rear Leg Side View, and between WL and Foot Angle (Häggman and Juga, 2013). In summary, the estimated genetic correlations between CHL and conformation traits are inconsistent, but it appears that a weak correlation may exist in many instances.

One limitation of estimating the genetic correlation between CHL and conformation traits is that the relationship may not be linear (Heringstad et al., 2018). For example, intermediate values of linear type trait phenotypes are associated with a reduced risk of SU and WL (Pérez-Cabal and Charfeddine, 2016), and a similar trend has been reported between sire PTA for type traits and SU and WL (Oikonomou et al., 2013). Therefore, if both extremes of conformation traits are genetically associated with increased CHL susceptibility, the non-linearity of this relationship may deflate estimates of genetic correlation.

1.3.5 Genome-wide association studies of claw horn lesions and associated traits

Genome-wide association (**GWA**) studies aim to detect genomic variants which influence a trait. With complex traits, genetic variance is usually spread over a large number of quantitative trait loci (**QTL**) across the genome, therefore finding variants with observable effects can be challenging (Kemper and Goddard, 2012). There is also a risk of stochastic noise creating an apparent association with the trait, or indirect association, due to LD or population structure, which can be mistakenly inferred as causal (Platt et al., 2010). Therefore, GWA studies of CHL are challenging to design, analyse, and interpret.

In Denmark, health traits have been recorded and used for national genetic evaluations of dairy cattle since 1990 (Nielsen et al., 2000). One of these health traits, "feet and leg diseases", includes 11 lameness-related conditions (including infectious and non-infectious foot lesions, proximal limb disorders, and lameness of unknown aetiology). This lameness phenotype was analysed in the first GWA study of a direct lameness trait (Buitenhuis et al., 2007). Since then, several GWA studies have been published using a range of CHL phenotypes from farm records (Naderi et al., 2018; Sánchez-Molano et al., 2019), foot-trimmer records (van der Spek et al., 2015; Croué et al., 2019; Suchocki et al., 2020; Lai et al., 2021a; b), veterinary records (Suchocki et al., 2020), researcher recorded records (Swalve et al., 2014; Sánchez-Molano et al., 2019), and deregressed breeding values (Wu et al., 2016; Butty et al., 2021).

There is agreement from all GWA studies that lameness traits are highly polygenic with very few, if any, QTL with large effects. A summary of QTL identified in GWA studies of CHL or CHL-related phenotypes is shown in **Table 1.4**. There is limited agreement from all GWA studies regarding specific QTL, however, there are a few instances of genomic regions which have been highlighted by more than one study. For example, two QTL on *Bos taurus* autosome 8 (**BTA-8**) around 74.0 Mb were associated with SU in two separate studies (van der Spek et al., 2015; Lai et al., 2021b), and similarly, the region around 12.0 Mb on BTA-12 was independently detected for SU in another two studies (Croué et al., 2019; Sánchez-Molano et al., 2019). Two regions on BTA-20

have been linked to CHL. Adjacent QTL around 47.0 - 49.0 Mb were identified for SU and a composite CHL trait in two separate GWA studies (van der Spek et al., 2015; Croué et al., 2019), and a copy number variant (**CNV**) was also recently detected for SH at 71.0 Mb (Butty et al., 2021) which was close to a locus previously linked to a composite CHL trait (van der Spek et al., 2015). Replication of QTL in independent GWA studies increases the confidence that an observed effect may be genuine, and these regions would warrant further exploration.

Three GWA studies of DCT have been published. One study of 360 cows did not highlight any QTL associated with DCT in the week after calving (Sánchez-Molano et al., 2019). Two other GWA studies, both of a similar population of approximately 600 Holstein and Jersey cows, characterised DCT as a complex trait and identified candidate genes related to inflammation, fat tissue deposition, bone growth, and keratinocyte function (Stambuk et al., 2020a; b).

Table 1.4 The chromosome (BTA) and position of quantitative trait loci (QTL) reported in genome-wide association studies of phenotypes which directly relate to (or could include) claw horn lesions in dairy cattle. Results should be compared cautiously due to differences in study design, study population, analysis, trait definition, and reference genome assembly from which QTL positions are drawn.

BTA	Position (Mb)	Trait	Reference
1	18.2	SU	(van der Spek et al., 2015)
	38.6 - 38.8	WL	(Croué et al., 2019)
	39.6	WL	(Croué et al., 2019)
	93.6	CHL (+ DS)	(van der Spek et al., 2015)
	109.7 - 111.0	SU	(Croué et al., 2019)
	119.3 - 119.4	SU	(Croué et al., 2019)
	125.5 - 125.8	SU & DD*	(Lai et al., 2021a)
	128.3	WL	(Croué et al., 2019)
	135.7	FD - trim.	(Suchocki et al., 2020)
	136.8	SHC	(Croué et al., 2019)
	158.1	SHC	(Croué et al., 2019)
2	3.1 - 3.6	SHC	(Croué et al., 2019)
	4.5 - 5.6	SH	(Sánchez-Molano et al., 2019)
	4.9	SH	(Sánchez-Molano et al., 2019)
	57.4 - 57.7	WL	(Croué et al., 2019)
	64.0	FLD	(Wu et al., 2016)
	95.0 - 95.2	WL	(Croué et al., 2019)
	102.9 - 103.2	WL	(Croué et al., 2019)
3	15.7 - 16.2	SU	(Croué et al., 2019)
	98.3 - 98.5	SU	(Croué et al., 2019)
4	28.1 - 28.6	SU	(Croué et al., 2019)
	33.9	LAM	(Naderi et al., 2018)
	67.6 - 67.7	WL	(Croué et al., 2019)
	118.9 - 120.5	SU	(Croué et al., 2019)
5	8.5 - 8.6	SHC	(Croué et al., 2019)
	44.2	FLD	(Buitenhuis et al., 2007)
	71.7	SU	(van der Spek et al., 2015)
	79.7	LAM	(Naderi et al., 2018)
	94.4	WL	(Sánchez-Molano et al., 2019)
	105 - 109	FLD	(Wu et al., 2016)
6	22.0 - 63.0	FLD	(Wu et al., 2016)
	53.9 - 54.1	SHD	(Croué et al., 2019)
	87.0 - 88.1	WL	(Croué et al., 2019)
	88.4 - 89.0	SU	(Croué et al., 2019)

7	0.8 - 1.6	WL	(Croué et al., 2019)
	10.4	WL	(Butty et al., 2021)
	36.5	SU	(Croué et al., 2019)
	56.3	WL	(Croué et al., 2019)
	61.6	LAM	(Naderi et al., 2018)
	63.0 - 65.0	FLD	(Wu et al., 2016)
	63.2	LAM	(Naderi et al., 2018)
	68.2	SU	(van der Spek et al., 2015)
	75.1	WL	(Sánchez-Molano et al., 2019)
	96.6 - 97.9	FD - no.	(Suchocki et al., 2020)
	109.5 - 109.6	SHC	(Croué et al., 2019)
8	5.4	SU	(van der Spek et al., 2015)
	6.3	WL	(Croué et al., 2019)
	36.4	WL	(Croué et al., 2019)
	42.9 - 44.6	SU & WL* SU & DD* SU & MET*	(Lai et al., 2021a)
	46.0	FLD	(Wu et al., 2016)
	67.8	SU	(van der Spek et al., 2015)
	74.0	SU	(van der Spek et al., 2015)
	74.3 - 77.5	SU	(Lai et al., 2021b)
	78.5	SU	(van der Spek et al., 2015)
	84.0	FLD	(Wu et al., 2016)
	100.8	SU	(van der Spek et al., 2015)
	100.5	SU	(van der Spek et al., 2015)
	101.0	SU	(van der Spek et al., 2015)
	102.8 - 103.6	SHD	(Croué et al., 2019)
	106.6	SU	(van der Spek et al., 2015)
9	1.3	SU	(van der Spek et al., 2015)
	23.7	SU	(Croué et al., 2019)
	79.2	SU	(van der Spek et al., 2015)
10	6.8 - 7.0	SU	(Croué et al., 2019)
	26.0	FLD	(Wu et al., 2016)
	31.0 - 41.0	FLD	(Wu et al., 2016)
	31.1	SU	(van der Spek et al., 2015)
	44.0	SU	(van der Spek et al., 2015)
	47 - 58	FLD	(Wu et al., 2016)
	51.0 - 51.1	SU	(Croué et al., 2019)
	54.0	WL	(Croué et al., 2019)
	78.5 - 78.8	SH	(Butty et al., 2021)

11	6.7.	SHD	(Croué et al., 2019)
	21.0	FLD	(Wu et al., 2016)
	23.3	LAM	(Naderi et al., 2018)
	48.1	SU	(van der Spek et al., 2015)
	64.8 - 64.9	SHC	(Croué et al., 2019)
	76.0 - 79.0	FLD	(Wu et al., 2016)
12	11.6 - 12.1	SU	(Croué et al., 2019)
	12.6	SU	(Sánchez-Molano et al., 2019)
	54.1 - 54.3	SU	(Croué et al., 2019)
	62.3	CHL (+ DS)	(van der Spek et al., 2015)
	62.6	CHL (+ DS)	(van der Spek et al., 2015)
	86.1 - 86.3	SU	(Butty et al., 2021)
13	29.6	CHL (+ DS)	(van der Spek et al., 2015)
	31.3	SU	(van der Spek et al., 2015)
	45.2 - 47.6	SU/WL	(Lai et al., 2021b)
	45.2 - 47.6	NICL	(Lai et al., 2021b)
	46.3 - 47.5	WL	(Lai et al., 2021b)
	51.7 - 51.9	FD - trim.	(Suchocki et al., 2020)
	53.1	SU	(Croué et al., 2019)
	82.1 - 82.6	WL	(Croué et al., 2019)
14	2.6 - 4.1	WL	(Croué et al., 2019)
	5.8	WL	(Sánchez-Molano et al., 2019)
	6.2	SU	(van der Spek et al., 2015)
	6.8 - 7.7	WL	(Sánchez-Molano et al., 2019)
	20.0 - 29.0	FLD	(Wu et al., 2016)
	44.9 - 45.0	SU	(Croué et al., 2019)
	62.1 - 62.5	FD - vet.	(Suchocki et al., 2020)
	65.3 - 65.7	FD - no.	(Suchocki et al., 2020)
	66.3	SHD	(Croué et al., 2019)
	81.6	SU & DD*	(Lai et al., 2021a)
15	2.3	SU	(Croué et al., 2019)
	53.0 - 53.1	FD - vet.	(Suchocki et al., 2020)
	56.5 - 57.1	SHD	(Croué et al., 2019)
	67.1	SU	(van der Spek et al., 2015)
	67.0	WL	(Croué et al., 2019)
	70.6 - 70.8	WL	(Croué et al., 2019)
16	57.8	WL	(Croué et al., 2019)
	62.0	FLD	(Wu et al., 2016)
	63.9 - 65.0	WL	(Croué et al., 2019)

17	10.2 - 10.4	SU	(Croué et al., 2019)
	23.3	WL	(Croué et al., 2019)
	41.3	SU & WL*	(Lai et al., 2021a)
	46.2	SU	(van der Spek et al., 2015)
	61.6 - 61.7	SU	(Croué et al., 2019)
	74.3	SHC	(Croué et al., 2019)
18	23.8	SU	(van der Spek et al., 2015)
	25.0	SU	(van der Spek et al., 2015)
	34.5 - 34.6	WL	(Croué et al., 2019)
	35.8 - 36.2	WL	(Croué et al., 2019)
	42.7 - 43.3	WL	(Croué et al., 2019)
	58.1 - 58.6	SU	(Croué et al., 2019)
19	12.1	FLD	(Buitenhuis et al., 2007)
	30.4	WL	(Croué et al., 2019)
	50.2 - 50.5	SU	(Croué et al., 2019)
20	5.6	WL	(Croué et al., 2019)
	37.7	CHL (+ DS)	(van der Spek et al., 2015)
	47.5 - 48.5	SU	(Croué et al., 2019)
	48.9	CHL (+ DS)	(van der Spek et al., 2015)
	70.8 - 71.1	SH	(Butty et al., 2021)
	71.4	CHL (+ DS)	(van der Spek et al., 2015)
21	7.5	SU	(van der Spek et al., 2015)
	22.5	SH	(Swalve et al., 2014)
	46.0	SH	(Sánchez-Molano et al., 2019)
22	8.1	FLD	(Buitenhuis et al., 2007)
	14.3	SU	(van der Spek et al., 2015)
	15.2	SU	(van der Spek et al., 2015)
	29.0 - 35.0	FLD	(Wu et al., 2016)
	32.2 - 32.3	WL	(Croué et al., 2019)
	32.6	SU	(Croué et al., 2019)
	38.0 - 39.0	FLD	(Wu et al., 2016)
	39.3 - 39.5	FD - trim.	(Suchocki et al., 2020)
	46.5 - 46.8	SHD	(Croué et al., 2019)
	49.0 - 54.0	FLD	(Wu et al., 2016)
	56.0	WL	(Croué et al., 2019)
23	15.7	SU	(van der Spek et al., 2015)
	25.9 - 26.1	WL	(Butty et al., 2021)
	40.9 - 41.4	WL	(Croué et al., 2019)
	44.5 - 45.3	WL	(Croué et al., 2019)
	45.5	LAM	(Naderi et al., 2018)

24	19.6 - 20.0	SU	(Croué et al., 2019)
	31.8 - 32.1	SU	(Croué et al., 2019)
	58.6	WL	(Croué et al., 2019)
25	3.1 - 4.3	SU	(Sánchez-Molano et al., 2019)
	22.1 - 22.9	SU & MET*	(Lai et al., 2021a)
	31.0 - 31.1	SHC	(Croué et al., 2019)
	34.7 - 34.9	WL	(Croué et al., 2019)
	36.2 - 36.6	SHC	(Croué et al., 2019)
26	45.3 - 46.0	WL	(Croué et al., 2019)
	56.1	FLD	(Buitenhuis et al., 2007)
27	31.6 - 32.2	WL	(Croué et al., 2019)
	37.5 - 38.9	SU & WL*	(Lai et al., 2021a)
28	15.1 - 16.1	WL	(Croué et al., 2019)
	25.0 - 25.1	SU	(Croué et al., 2019)
	25.7 - 26.0	SU	(Croué et al., 2019)
	26.7 - 27.0	SU	(Croué et al., 2019)
	36.0 - 36.4	WL	(Croué et al., 2019)
	37.7	SHD	(Croué et al., 2019)
29	49.6	SU	(Butty et al., 2021)

CHL: claw horn lesions; DD: digital dermatitis; DS: double sole; FD - no.: number of foot disorders, FD - trim.: foot disorder recorded during foot-trimming; FD - vet.: foot disorder detected by a veterinary surgeon; FLD: foot and leg disorder; LAM: laminitis; MET: metritis, NICL: non-infectious claw lesion; SH: sole haemorrhage; SHD: diffuse sole haemorrhage; SHC: circumscribed sole haemorrhage; SU: sole ulcer; WL: white line lesion

**Two-trait genome-wide association analysis*

Interpreting and comparing GWA studies is hindered by several factors. Some of these factors are intrinsic and difficult to circumvent, such as differences between populations. The majority of GWA studies of CHL have been with Holstein cows, but some have included other breeds such as Braunvieh and Fleckvieh (Suchocki et al., 2020); a GWA study of multiple breeds found no consistency in QTL between breeds (Wu et al., 2016).

As genotyping cost and availability have improved, more recent GWA studies have genotyped female cows so that the same animals have phenotypes and genotypes (Sánchez-Molano et al., 2019; Lai et al., 2021b). Compared to GWA studies using sire genotypes and daughter phenotypes, this may allow the detection of QTL with smaller or weaker effects. Another consequence of improved technology and the reduced genotyping cost is the use of higher-density genotypes. Earlier GWA studies were based on relatively sparse arrays of 300 - 400 markers (Buitenhuis et al., 2007; Swalve et al., 2014), whereas more recent studies have used higher density arrays of 777,000 SNP (Lai et al., 2021b; a) or analysed structural variants in the genome such as CNV (Butty et al., 2021). If a genetic variant confers fitness, then over time genetic selection will reduce the allele which is detrimental to fitness, such as an allele associated with disease susceptibility. As lower density SNP arrays are designed to include variants which occur at a high frequency within a population, the effects of rare alleles associated with disease susceptibility may not always be captured (Wray et al., 2013).

The genomic variants most frequently identified during GWA analyses are markers in LD with causative alleles, therefore the number of genomic markers is arguably less important than the LD between genomic markers and causative alleles (VanRaden et al., 2013). However, as the linkage phase varies between families these results may generalise poorly to other populations (Dekkers, 2004). With high-density genotypes, the genome can be analysed in small windows to identify markers in short-range LD with causative alleles (Dekkers, 2004), this may improve the replicability of results from GWA analyses in the future.

The source of phenotypes varies between studies. As discussed previously, there is typically a trade-off between the size of the study population and phenotype accuracy. However, one difficulty in comparing GWA studies is differences in how lesions are defined. For example, the QTL reported for laminitis (Naderi et al., 2018) are difficult to compare to other GWA studies because laminitis is not officially recognised as a foot lesion (Egger-Danner et al., 2020). Although laminitis is often a synonym for SH (Swalve et al., 2014), it is not clear whether this was the case in this instance, nor how the case definition provided ("aseptic infection of the corium") could realistically be determined through inspection of the foot.

A major challenge with GWA studies of complex traits is that few, if any, markers are associated with large effects. The power of GWA studies depends on the number of animals in the study and the number of SNP analysed (Goddard and Hayes, 2012), as well as the genetic background of the trait in question (Loh et al., 2022) and its heritability (Shin and Lee, 2015).

To increase study power, many GWA studies analyse composite traits. For example, Lai et al. (2021b) identified a locus on BTA-13 for WL but not for SU. However, this locus had stronger evidence of an effect (i.e., a smaller *P*-value) when SU was included with WL, and stronger still when other non-infectious claw lesions were included (SH, sole fracture, sole abscess, wall abscess, heel abscess, and laminitis). Therefore, the genomic differences at this locus appear most pronounced in cows with WL but still occur in cows with SU and other non-infectious claw lesions. Further investigation of this region could justify the analysis of all non-infectious foot lesions together to improve study power.

Multivariate GWA analyses of correlated traits have to been shown to increase study power (Zhou and Stephens, 2014), and two-trait GWA analyses have recently been applied to CHL (Lai et al., 2021a). When SU was analysed in combination with either WL, digital dermatitis or metritis, the same genomic region (BTA-8, 42.9 - 44.6 Mb) was detected. This result may reflect "horizontal pleiotropy", where the same variant influences both phenotypes, "vertical pleiotropy", due to a causal relationship between the two phenotypes, or

"spurious pleiotropy", where two variants exist in LD in this region, and each independently affects one of the two traits (van Rheenen et al., 2019). The latter is less likely due to the moderate-to-strong genetic correlation between each trait pair (Lai et al., 2021a), therefore, these results suggest there is a genetic or biological relationship between SU and WL, digital dermatitis and metritis.

Due to the large number of genomic markers tested in GWA analysis, statistical significance needs to be adjusted for type one error, but this needs to be balanced against the power of the study, and there is little point in minimising type one error if the increase in type two error eliminates the chances of detecting any QTL from an experiment (Wakefield, 2008). Type one error, although potentially misleading, can be highlighted by similar studies or subsequent fine mapping of candidate regions, which is the natural follow-up to GWA studies (Schaid et al., 2018). On the other hand, type two error could only be addressed by conducting higher-powered studies, which would be even more demanding on resources and funds. Therefore, the consequences of applying stringent corrections for multiple testing may be more detrimental than the consequences of reporting some false positive results (Perneger, 1998). There are inconsistencies between GWA studies in approaches to correct for multiple testing and therefore the strength of evidence for reported QTL varies between studies.

One of the goals of GWA is to identify candidate genes which may improve understanding of the biological background of the trait. Candidate genes are usually selected based on their proximity to detected QTL, however, LD varies across the genome and between populations (Qanbari, 2020). In humans, a locus identified for obesity in multiple GWA studies, but without a clear biological link to the phenotype, has since been found to be functionally connected to a distant regulatory gene (Smemo et al., 2014), illustrating a limitation to positional gene mapping in GWA studies. Furthermore, scrutiny of plausible candidate genes is inherently difficult in GWA studies of non-specific phenotypes which can include a range of lameness diseases which do not have a common aetiopathogenesis. Finally, speculation about biologically plausible candidate genes is vulnerable to

availability and confirmation biases because speculation about specific genes focuses on genes with recognised biological roles which can be easily linked to the accepted pathophysiology.

1.3.6 Conclusions

Compared to other health conditions of comparable importance, genetic selection to improve resistance to CHL has been limited. One reason for this may be that the heritability of CHL is generally low, and therefore it is often easier and more immediately rewarding to focus on environmental and management factors to reduce CHL (Cole and VanRaden, 2018). But genetic variance for CHL has been demonstrated and therefore there is the opportunity to utilise this in breeding programmes. One reason the heritability estimates of CHL are typically low may be due to the inaccuracy of phenotypes sources from farm or foot-trimming records, which inflate the residual variance. It would be useful to estimate the genetic parameters of CHL using accurate phenotypes to understand the extent of this problem.

The polygenic nature of lameness traits is advantageous for selective breeding because non-additive genetic effects are diluted by a large number of QTL, and therefore the genetic variance is mainly additive (Hill et al., 2008). However, each allele is responsible for only a small proportion of the overall genetic variance, and this can make it challenging to identify specific genomic regions. Therefore, the lack of agreement between GWA studies is not surprising, but there would be a benefit to additional GWA studies if they are based on accurate phenotypic data.

1.4 Thesis aims and objectives

This thesis aims to investigate the aetiopathogenesis of CHL with a particular focus on genetic and metabolic factors.

The first three chapters (**Chapter 2**, **Chapter 3**, and **Chapter 4**) describe genetic analyses relating to CHL. The objectives of these chapters are to estimate the genetic parameters of traits which include SH, SU, WL, DCT, and the recovery from SH and SU. A further objective in **Chapter 2** and **Chapter 3** is to characterise the genomic background of SH, SU, WL, and DCT; the identification of candidate genes associated with these traits could further the understanding of the aetiopathogenesis of CHL. Many of the current theories concerning the aetiopathogenesis of sole lesions relate to metabolic pathways, this is addressed in **Chapter 5** using ^{1}H NMR spectroscopy to explore the serum metabolome before and after the development of SH and SU in early lactation. Finally, the effectiveness of genetic selection to reduce the incidence of CHL is assessed in **Chapter 6** by quantifying the association between the current selection index for lameness resistance in the UK, and the development of CHL.

Chapter 2: Genetic parameters and genome-wide association study of claw horn lesions in Holstein cows

2.1 Introduction

Lameness affects approximately one in three dairy cows in the United Kingdom (**UK**) (Afonso et al., 2020) and reducing this prevalence is a key priority for the UK dairy industry (GB Cattle Health & Welfare Group, 2020; Rioja-Lang et al., 2020). Lameness in dairy cattle is primarily caused by foot lesions (Murray et al., 1996; Bicalho et al., 2007a; van Huyssteen et al., 2020). Three of the most important foot lesions are sole haemorrhage (**SH**), sole ulcers (**SU**), and white line lesions (**WL**) (Somers et al., 2003; Solano et al., 2015; O'Connor et al., 2019; Arango-Sabogal et al., 2020), collectively referred to as claw horn (disruption) lesions (**CHL**) (Offer et al., 2000). Relative to other foot lesions, CHL have been associated with the most severe pain responses (Whay et al., 1998; Pastell et al., 2010), economic consequences (Amory et al., 2008; Bruijnis et al., 2010; Dolecheck et al., 2019), and environmental impacts (Mostert et al., 2018).

In the UK, initiatives have been developed to reduce lameness and the incidence of CHL in dairy cattle through management and environmental improvements (AHDB, 2018b). A large number of studies have estimated the heritability of CHL to be between 0.01 – 0.20 for SH; 0.01 – 0.35 for SU, and 0.01 – 0.19 for WL (van der Waaij et al., 2005; Johansson et al., 2011; Croué et al., 2017; Malchiodi et al., 2017; Heringstad et al., 2018; Sánchez-Molano et al., 2019; Shabalina et al., 2020). Despite the variation in these results, there is a general agreement that CHL are heritable, which means current CHL prevention programmes could be more effective if husbandry-based approaches are complemented with genetic selection.

Genetic analysis of CHL traits requires a large volume of foot lesion records. Given the time and effort involved to lift cows' feet, the majority of previous studies on CHL have utilised foot lesion data which had been recorded by farmers or foot-trimmers (Koenig et al., 2005; van der Linde et al., 2010; van der Spek et al., 2013; Pérez-Cabal and Charfeddine, 2015; Malchiodi et al., 2017). Although

foot-trimming records are a valuable resource for genetic evaluations, they have limitations as a phenotype due to variability between individual recorders and differences in terminology between countries (Christen et al., 2015). Furthermore, less clinically important lesions, which often only require minimal intervention, may be under-recorded when the primary purpose of handling cattle is to perform preventative or therapeutic foot-trimming (Archer et al., 2010a). Ring et al. (2018) reported lower heritability estimates for SH when only severe lesions were considered as cases, as opposed to all severities of SH; consequently, if foot-trimming records under-report mild lesions this may influence the estimation of genetic parameters.

In addition to possible inconsistencies or insensitivities in foot-trimming records, the study population may also be skewed in these records as only a proportion of herds are assessed at each visit. In datasets of foot-trimming records, the proportion of individual herds represented can range from less than 10% to greater than 90% of each herd (Croué et al., 2017). These sub-populations may over-represent animals requiring intervention due to painful foot lesions (van der Spek et al., 2013; Croué et al., 2017), or those with poorer genetic merit for foot health (Köck et al., 2019). Previous studies have addressed this by excluding herds unless a minimum proportion of each herd was reflected in foot-trimming records (van der Waaij et al., 2005; van der Linde et al., 2010), but heritability estimates appear dependent on this proportion, suggesting that this approach could still introduce biases (van der Spek et al., 2013; Croué et al., 2017).

Genome-wide association (**GWA**) studies have progressed understanding of the genetic background of CHL (Swalve et al., 2014; van der Spek et al., 2015; Croué et al., 2019; Suchocki et al., 2020; Butty et al., 2021; Lai et al., 2021b; a). Results from these studies indicate that CHL are polygenic traits, although results generally differ between studies concerning the presence and location of possible quantitative trait loci (**QTL**). Given the complex genetic background, limited consistency between studies regarding QTL is expected (Kemper and Goddard, 2012; Robinson et al., 2014); however, other factors may contribute to this lack of replicability, such as differences in study design and experimental

population, trait definition, data handling and analysis, and study power (McCarthy et al., 2008; Wang et al., 2019; Crouch and Bodmer, 2020).

2.1.1 Objectives

The objectives of this study were to use a dataset of accurate and detailed claw horn lesion records from a cohort of prospectively enrolled Holstein cows to (i) estimate the genetic parameters of sole haemorrhage, sole ulcers, and white line lesions; and (ii) characterise the genetic background of these lesions with genome-wide association analyses.

2.2 Materials and methods

2.2.1 Study design and population

The study was conducted following ethical approval by the University of Liverpool Research Ethics Committee (VREC269a, VREC466ab) and procedures regulated by the Animals (Scientific Procedures) Act were conducted under a UK Home Office License (P191F589B).

A prospective cohort study was designed to record foot lesions at four time points during a production cycle. Data collection was conducted on four dairy herds (A - D) in the northwest of the UK which were selected for convenience based on the practicalities of frequent visits and assessments. Herds A to C housed lactating cows all-year-round, milked cows three times daily and recorded 305-day milk yields of approximately 11,000 - 11,500 L. Herd D housed lactating cows all-year-round except for lower-yielding cows which were grazed during the summer; cows were milked twice daily and the 305-day milk yield was approximately 9,000 L. Parous cows on all herds were routinely foot-trimmed twice a year before drying off and 60 - 120 days after calving. On all herds, lactating cows were regularly footbathed after milking. Herd A footbathed cows three times a week with either copper sulphate or formalin; herd B footbathed cows twice daily with formalin, herd C footbathed cows daily with either copper sulphate or formalin and herd D footbathed three times a week with formalin.

All animals which were registered as Holsteins and expected to calve between April and December 2019 were prospectively enrolled with no additional inclusion or exclusion criteria applied. A total of 2,352 animals were enrolled. Data were collected by qualified veterinary surgeons during weekly or twice weekly visits to each herd from February 2019 to July 2020 (with a break from March to June 2020 due to COVID-19 restrictions). Animals were assessed for foot lesions at four time points: before parturition (**T1-Precalving**), immediately after parturition (**T2-Calving**), in early lactation around the time of peak milk yield (**T3-Early**), and in late lactation (**T4-Late**). The sample size was determined by resource constraints. All eligible animals were enrolled until the final

assessments (T4-Late) began, at which point further enrolments ended because simultaneous data collection at four time points was not feasible.

2.2.2 Data collection

Animals were restrained in a foot-trimming crush and lesions on each claw were recorded by qualified veterinary surgeons using case definitions as described in the ICAR claw health atlas (Egger-Danner et al., 2020). Foot lesions were recorded either during routine foot-trimming or following light trimming of the sole horn to allow visualisation of lesions. If lesions were visible initially but disappeared following the removal of the claw horn these were still recorded as present. Lesions were graded according to severity (**Table 2.1**), broadly comparable to absent (score 0), mild (score 1), moderate (score 2), and severe (score 3). All foot lesions were examined and recorded by qualified veterinary surgeons; over 90% by a single researcher, and the remainder by three other researchers. Data collection was the same at all time points except for T2-Calving on herd C where only the hind feet were assessed to reduce the handling time of recently calved cows, this was only required on this herd due to the large numbers of cows calving each week.

Table 2.1. Case definitions and severity scoring system for assessing sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL).

Lesion	Case definition	Severity grading
Sole haemorrhage (SH)	Discolouration of the sole horn	Grade 1: light pink lesion < 2 cm diameter or diffuse discoloration of sole Grade 2: light pink lesion ≥ 2 cm diameter or dark pink/purple lesion < 2 cm diameter Grade 3: dark pink/purple lesion ≥ 2 cm diameter or discolouration with a blue tinge
Sole ulcer (SU)	Exposure of fresh or necrotic corium	Grade 1: < 2 cm diameter lesion covered by a thin layer of horn before modelling Grade 2: ≥ 2 cm diameter lesion with < 1.5 cm granulation tissue protruding through the horn Grade 3: ≥ 1.5 cm granulation tissue protruding through the horn or secondary bacterial infection
White line lesion (WL)	Lesion localised to the white line region	Grade 1: haemorrhage of the white line or discolouration or separation of the white line which disappears after limited trimming Grade 2: deeper separation or discolouration of the white, lesion is still present after limited trimming Grade 3: separation of the white line which extends to the corium, purulent exudate or necrotic tissue may be present

2.2.3 Trait definitions

Foot lesion records from all time points were used to define two traits for each CHL (SH, SU, and WL): a binary trait to represent overall susceptibility, and a continuous trait to reflect lesion severity.

Susceptibility. A binary trait classified animals as either "susceptible" or "resistant" to each CHL over the whole study period. Animals were classified as susceptible if a lesion was present on any claw (i.e., lesion severity = 1 - 3) at any assessment, regardless of lesion severity, the number of claws affected, the number of time points the lesion was present, or the total number of records for that animal. Animals were classified as resistant if the lesion was absent (i.e., lesion severity = 0) from all claws in a complete set of records from all four time points. Therefore, animals were unclassified by this trait if they were unaffected by a lesion but did not have records from all four time points. This resulted in a slight reduction in study power for this trait, due to a small proportion of incomplete lesion records for animals which had otherwise always been unaffected, but provided the highest confidence in the classification of animals as resistant to each lesion.

Severity. A continuous trait which reflected both the severity and distribution of each CHL was calculated by taking the maximum severity of each lesion from the medial and lateral claw of each foot and then averaging this across all feet. This approach was intended to try and capture the severity and distribution of CHL, whilst minimising the diluting effect of healthy claws in animals which were affected with CHL. This trait was calculated for each time point, so each animal had repeated records of lesion severity at each time point.

2.2.4 Pedigrees and genotypes

Pedigree details for the study population were extracted from the national database of dairy cattle by tracing back seven generations for each animal. Blood samples were collected from the coccygeal vein of each animal into EDTA vacutainers and used to genotype each animal with the Illumina BovineSNP50 BeadChip (Illumina Inc., USA). Genotypes were subsequently imputed to 80K single nucleotide polymorphism (**SNP**) genotypes by Edinburgh Genetic

Evaluation Services (EGENES) using an in-house procedure which has been developed for all national genomic evaluations of dairy cattle in the UK. Briefly, this imputation process uses the Illumina BovineSNP50 BeadChip and Illumina BovineHD BeadChip (Illumina Inc., USA), in addition to other commercial genotyping arrays, extra gene tests, and large-effect sequence variants. Following imputation, genotypes included 79,051 SNP spanning the entire genome. Chromosomal locations of the imputed 80K SNP panel were drawn from the latest assembly of the Bos taurus genome (ARS-UCD 1.2)(Rosen et al., 2020).

Imputed genotypes were available for 2,250 animals. Genotype quality control was implemented using PREGSF90 (Aguilar et al., 2014) within the BLUPF90 software suite (Misztal et al., 2018). Quality control included the removal of SNP with a call rate < 0.90 (N = 10,977), SNP with a minor allele frequency < 0.05 (N = 3,008), monomorphic SNP (N = 36), or SNP showing a strong deviation (> 0.15) from Hardy-Weinberg equilibrium (N = 14) (Wiggans et al., 2009). Additionally, animals were removed if sample call rate < 0.90 (N = 63) or there were parent-progeny Mendelian conflicts (N = 20). Quality control procedures resulted in a final dataset of 2,167 animals with genotypes of 65,211 SNP.

2.2.5 Population structure

2.2.6 Genetic parameter estimation

Before genetic analyses, fixed effects were evaluated via mixed-effect linear (or generalised linear) regression of repeated observations of each trait, with the animal as a random effect in the model. This analysis was conducted in R (R Core Team, 2021) with the *lmerTest* package (Kuznetsova et al., 2017). The following fixed effects were tested: herd, parity, the season of calving, the season of assessment, days since calving at the assessment, and the researcher who recorded CHL. The importance of each fixed effect was determined by finding the multivariable model with the lowest Akaike information criterion. The effect of which researcher examined and recorded CHL increased Akaike information criterion, so this term was not included in subsequent genetic analyses.

Additionally, the genetic structure of the study population was explored with a principal component analysis of animal genotypes using GEMMA (Zhou and Stephens, 2012); this revealed no distinct clusters in the genotypes which could be attributed to a known population characteristics (**Figure 2.1**).

Susceptibility. Binary traits relating to SH, SU, and WL susceptibility were analysed with threshold models to transform the phenotype to a latent liability scale (Gianola, 1982). A Markov chain Monte Carlo approach was used to obtain marginal posterior distributions for model parameters via the Gibbs sampling algorithm in THRGIBBS1F90 (Tsuruta and Misztal, 2006). Convergence of Gibbs sampling was assessed using the *coda* package in R (Plummer et al., 2006); a chain length of 500,000 samples with a 50,000 sample burn-in produced consistent results and was used for all models. Lag correlation between consecutive samples was reduced with a thinning interval of 50, therefore genetic parameters were estimated from the posterior distribution of 9,000 Gibbs samples.

The animal threshold model used for each lesion was:

$$\boldsymbol{\lambda} = \mathbf{Xb} + \boldsymbol{Z}\mathbf{a} + \mathbf{e} \quad (1)$$

where $\boldsymbol{\lambda}$ is a vector of unobserved liabilities for susceptibility to either SH, SU, or WL; **b** is a vector of the fixed effect of parity (8 levels: 1st parity to ≥8th parity) and herd-year-season of calving (12 levels); $\mathbf{a}$ is a vector of random additive genetic effects for each animal; $\mathbf{e}$ is a vector of random residual effects, and $\mathbf{X}$, and $\boldsymbol{Z}$ are incidence matrices for $\mathbf{b}$ and $\mathbf{a}$, respectively. Random effects were assumed to be normally distributed with a mean of zero and covariance structure of:

$$var\begin{bmatrix}\boldsymbol{a}\\ \boldsymbol{e}\end{bmatrix} = \begin{bmatrix}\boldsymbol{H}\sigma_a^2 & 0\\ 0 & \boldsymbol{I}\sigma_e^2\end{bmatrix} \quad (2)$$

where σ_a^2 is the additive genetic variance; σ_e^2 is the residual variance; $\boldsymbol{I}$ is an identity matrix, and $\boldsymbol{H}$ is the relationship matrix incorporating pedigree and genomic information in a single-step genomic analyses framework as defined by Legarra et al. (2009). The inverse of $\boldsymbol{H}$ is defined as (Aguilar et al., 2010; Christensen and Lund, 2010):

$$\boldsymbol{H}^{-1} = \boldsymbol{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & (\boldsymbol{G}^{-1} - \boldsymbol{A}_{22}^{-1}) \end{bmatrix} \quad (3)$$

where $\boldsymbol{A}$ is the pedigree relationship matrix; $\boldsymbol{G}$ is the genomic relationship matrix, and $\boldsymbol{A}_{22}$ is the pedigree relationship matrix for genotyped animals. The $\boldsymbol{A}$ matrix includes inbreeding coefficients calculated from pedigree relationships (Meuwissen and Luo, 1992). The genomic relationship matrix was constructed as $0.95\boldsymbol{G}^{*} + 0.05\boldsymbol{A}_{22}$; $\boldsymbol{G}^{*}$ is defined according to VanRaden (2008) as:

$$\boldsymbol{G}^{*} = \frac{\boldsymbol{Z}\boldsymbol{Z}'}{2\sum_{i=1}^{M} p_i(1 - p_i)} \quad (4)$$

where $\boldsymbol{Z}$ is a centred matrix of genotype at each locus (*aa* = 0, *Aa* = 1, and *AA* = 2); M is the number of SNP, and p_i is the minor allele frequency at locus i.

Severity. Continuous traits relating to the severity of SH, SU, and WL were analysed with linear mixed models using the average information-restricted maximum likelihood algorithm, implemented in AIREMLF90 (Misztal et al., 2018). The following animal repeatability model was used to take account of the repeated lesion severities from each time point:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wpe} + \mathbf{e} \quad (5)$$

where $\mathbf{y}$ is a vector of the severity of either SH, SU, or WL; $\mathbf{b}$ is a vector of the fixed effect of parity (8 levels), HYS of each time point (12 levels), and time point (i.e. stage of production cycle, 4 levels); $\mathbf{a}$ is a vector of random additive genetic effects for each animal; $\mathbf{pe}$ is a vector of random permanent environmental effects to account for repeated measurements from each animal, $\mathbf{e}$ is a vector of random residual effects; $\mathbf{X}$, $\mathbf{Z}$ and $\mathbf{W}$ are incidence matrices for $\mathbf{b}$, $\mathbf{a}$ and $\mathbf{pe}$ respectively. Random effects were assumed to be normally distributed with a mean of zero and covariance structure of:

$$var\begin{bmatrix} \boldsymbol{a} \\ \boldsymbol{pe} \\ \boldsymbol{e} \end{bmatrix} = \begin{bmatrix} \boldsymbol{H}\sigma_a^2 & 0 & 0 \\ 0 & \boldsymbol{I}\sigma_{pe}^2 & 0 \\ 0 & 0 & \boldsymbol{I}\sigma_e^2 \end{bmatrix} \quad (6)$$

where σ_a^2 is the additive genetic variance; σ_{pe}^2 is the permanent environmental variance; σ_e^2 is the residual variance; $\boldsymbol{I}$ is an identity matrix, and $\boldsymbol{H}$ is the relationship matrix as before (Equation 3).

2.2.7 Heritability

The heritability of the susceptibility to each lesion was estimated on the underlying liability scale (h_l^2) using variance components from the threshold model (Equation 1), as:

$$h_l^2 = \sigma_a^2/(\sigma_a^2 + \sigma_e^2) \quad (7a)$$

with each variance component defined as before in Equation 2. Similarly, the heritability of the severity of each lesion was estimated on the observed scale (h_o^2) using variance components from the linear repeatability model (Equation 5), as:

$$h_o^2 = \sigma_a^2/(\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2) \quad (7b)$$

with each variance component defined as before in Equation 6.

To allow comparison of the heritability of lesion susceptibility and severity which were estimated on different scales, the heritability of lesion susceptibility was transformed to the observed scale, adjusting for the over-representation of cases compared to the study population prevalence (Lee et al., 2011) as:

$$h_o^2 = \frac{h_l^2 z^2 P(1-P)}{K^2(1-K)^2} \quad (8)$$

where h_l^2 is the heritability on the liability scale (Equation 7a), K is the prevalence of the trait in the full study population, P is the proportion of cases in the case-control sub-population, and z is the height of the standard normal probability density function at the truncation threshold given by K.

2.2.8 Genetic correlation

For both the susceptibility and severity traits, additive genetic covariance between different lesions was estimated with bivariate animal models based on the same parameters as the corresponding univariate models (Equation 1 for lesion susceptibility and Equation 5 for lesion severity). The genetic correlation (r_g) between lesions was calculated using (co)variance component estimates from bivariate models as:

$$r_g = cov(t_1, t_2) / \sqrt{var(t_1) var(t_2)} \quad (9)$$

where $cov(t_1, t_2)$ is the additive genetic covariance between trait 1 (t_1) and trait 2 (t_1) and $var(t_1)$ and $var(t_2)$ are the additive genetic variance for trait 1 and trait 2, respectively.

2.2.9 Estimated genomic breeding values

The estimated genomic breeding value (**GEBV**) of each animal for each trait was calculated. The GEBV for lesion susceptibility was estimated during Gibbs sampling; the GEBV for lesion severity was estimated by single-step genomic best linear unbiased prediction implemented in BLUPF90 (Misztal et al., 2018).

2.2.10 Genome-wide association analyses

For both traits, individual marker effects were calculated in POSTGSF90 (Aguilar et al., 2014) following the approach described by Wang et al., (2012) in which SNP effects ($\hat{u}$) are defined as (Strandén and Garrick, 2009):

$$\hat{u} = \boldsymbol{DZ}'[\boldsymbol{ZDZ}']^{-1}\hat{a}_g \quad (10)$$

where $\boldsymbol{D}$ is the diagonal matrix of weights for the SNP effects, $\boldsymbol{Z}$ is a matrix relating genotypes to each locus, and $\hat{a}_g$ is the GEBV of genotyped animals.

Standardised SNP effects were calculated for the susceptibility to each lesion by dividing the absolute SNP effects by the standard deviation of SNP variance. For the lesion severity, *P*-values of marker effects were calculated (Aguilar et al., 2019) after the test statistic had been adjusted for genomic inflation, assuming constant inflation across the genome (Amin et al., 2007).

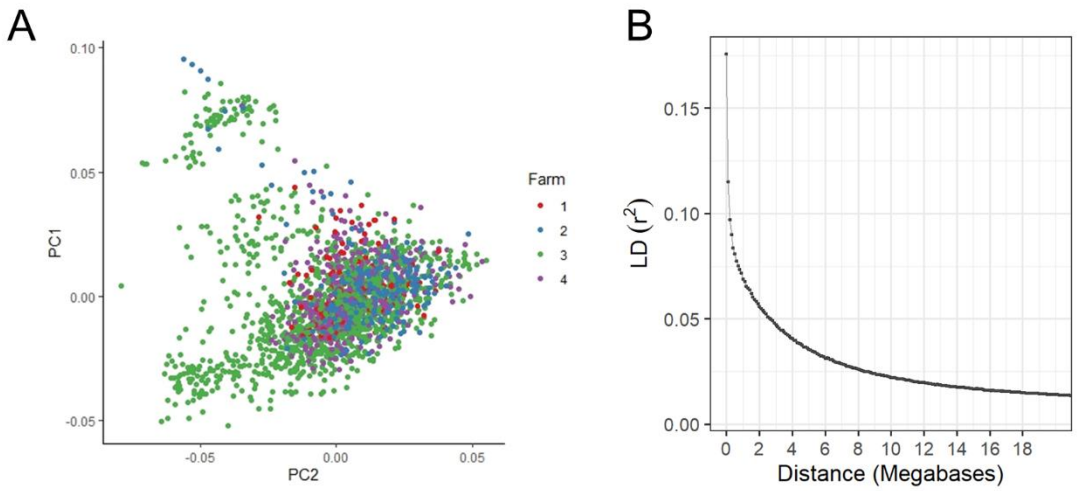
Significant SNP were defined using a statistical significance threshold of *P* ≤ 0.05, which was corrected for multiple testing to 7.67E-07 (*P* ≤ 0.05/number of tested markers). Suggestive SNP were defined using a genome-wide threshold equivalent to one false positive result per genome scan (Lander and Kruglyak, 1995), the suggestive threshold was 1.53E-05 (*P* ≤ 1/number of tested markers).

A window-based GWA approach was used to further explore the association between genomic regions and the susceptibility and severity traits for each lesion. The window size was determined by linkage disequilibrium (**LD**) in the study population (Silva et al., 2020). The magnitude and decay of LD between SNP was evaluated using PLINK (Purcell et al., 2007). On average, LD was found to decay by 50% every 0.65 Mb (**Figure 2.1**) and therefore sliding windows of 0.65 Mb were used for window-based analyses. The proportion of genetic variance explained by each sliding window of 0.65 Mb was calculated using POSTGSF90, as described by Wang et al. (2014):

$$\frac{Var(a_i)}{\sigma_a^2} \text{x } 100\% = \frac{Var(\sum_{0.65Mb} \boldsymbol{Z}_j \hat{u}_j)}{\sigma_a^2} \text{ x } 100\% \quad (11)$$

where a_i is the genetic value of the *i*-th 0.65 Mb window, σ_a^2 is the total genetic variance, $\boldsymbol{Z}_j$ is a vector of the gene content of the *j*-th SNP for all individuals, and $\hat{u}_j$ is marker effect of the *j*-th SNP within the *i*-th region.

Figure 2.1. (A) Principal component analysis of genotypes. (B) Average linkage disequilibrium (LD) decay between markers in the genotyped population.



2.2.11 Quantitative trait loci and functional analysis

Positional candidate genes were identified using the latest assembly of the *Bos taurus* genome (ARS-UCD 1.2) downloaded from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/assembly/GCF_002263795.1/). In each GWA analysis, the closest gene to each significant or suggestive SNP were identified, up to a maximum of 0.2 Mb upstream or downstream from the marker. Additionally, genes were explored if they were contained, or partially contained, within the top genomic windows. The UniProt database (The UniProt Consortium, 2021) was used for functional annotation of positional candidate genes. Enrichment analysis of the candidate genes from single-marker and window-based GWA analyses was conducted using the DAVID bioinformatics resource (Huang et al., 2009a; b)

2.3 Results

2.3.1 Population and dataset description

A total of 2,352 animals were enrolled in this study. In some cases (N = 38), animals were enrolled before parturition but did not subsequently calve because they aborted, died or were euthanised for health reasons. These animals were excluded from further analysis due to the absence of a calving date, despite having phenotypes recorded at T1-Precalving.

Additionally, to ensure environmental factors were broadly consistent at each time point, an acceptable spread of sampling times was defined; T1-Precalving: 0 - 120 days pre-calving; T2-Calving: 0 - 21 days post-calving; T3-Early: 50 - 120 days post-calving, and T4-Late: more than 170 days post-calving. Records were excluded from further analysis if they fell outside of these ranges (T1-Precalving: N = 26, T2-Calving: N = 1, T3-Early: N = 8, T4-Late: N = 6). Most records which fell outside the planned sampling time frame were at T1-Precalving because animals were enrolled based on farm records of expected calving dates which were occasionally inaccurate. A higher number of animals were lost to follow-up between T3-Early and T4-Late due to a break in data collection due to COVID-19 restrictions. There were 2,305 cows with lesion records in the final dataset used for statistical analysis (**Table 2.2**); lesions records were available from 2,277 cows at T1-Precalving, 2,185 at T2-Calving, 2,124 at T3-Early, and 1,931 at T4-Late.

Table 2.2. Number of cows in the final study population by herd and lactation number.

Lactation	**Number of cows**				
	Herd A	Herd B	Herd C	Herd D	Total
1	36	70	445	51	602
2	37	161	462	63	723
3	23	75	237	48	383
4	24	57	191	32	304
5	7	34	86	17	144
6 and greater	5	19	108	17	149
Total	132	416	1,529	228	2,305

There were sporadic instances where all four feet were not assessed for lesions at a time point due to the behaviour of the animal in the foot-trimming crush which risked the safety of the animal or researchers. In the final dataset, 99.5% (2,266/2,277), 99.2% (2,108/2,224), and 96.7% (1,868/1,931) of animals had lesion records from all four feet at T1-Precalving, T3-Early, and T4-Late, respectively. At T2-Calving, although 99.0% (2,164/2,185) of animals had lesion records from both hind feet, only 33.6% (734/2,185) had lesion records from all four feet, due to the change in data collection procedure on herd C at this time point.

The prevalence of SH, SU, and WL at each of the four time points is provided in **Table 2.3**. The peak prevalence of SH (58%), and SU (6%) was at T3-Early, and the peak of WL (59%) was at T4-Late. Details of the susceptibility and severity traits are provided in **Table 2.4**. For the susceptibility trait, 2,139 cows were classified as either susceptible or resistant for SH, 1,925 for SU, and 2,121 for WL.

Table 2.3. Proportion (frequency) of animals with sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL) at each time point.

	T1-Precalving	**T2-Calving**	**T3-Early**	**T4-Late**
SH	0.33 (741)	0.33 (720)	0.58 (1,222)	0.53 (1,022)
SU	0.04 (88)	0.03 (55)	0.06 (129)	0.06 (115)
WL	0.31 (705)	0.30 (661)	0.38 (802)	0.59 (1,143)
Total	2,277	2,185	2,214	1,931

Table 2.4. Summary of the susceptibility and severity traits for sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL). The susceptibility trait classified cows as susceptible if the lesion was present on any claw at any time point, and resistant if the lesion was absent from all claws at all time points, presented as the frequency of animals in each class. The severity trait is average lesion severity at each time point, presented as the mean (standard deviation) of this trait.

	Susceptibility		**Severity**			
	Susceptible	Resistant	T1-Precalving	T2-Calving	T3-Early	T4-Late
SH	1775	364	0.14 (0.25)	0.19 (0.33)	0.33 (0.39)	0.27 (0.34)
SU	268	1657	0.02 (0.09)	0.02 (0.12)	0.02 (0.10)	0.03 (0.14)
WL	1885	236	0.13 (0.24)	0.18 (0.33)	0.16 (0.26)	0.29 (0.32)

2.3.2 Genetic parameters

The variance component and heritability estimates are provided in **Table 2.5** and the genetic correlations between lesions in **Table 2.6**. For both the susceptibility and severity traits, the heritability estimates for SH and SU were higher than for WL. The genetic correlations between lesions were high and positive for SH and SU with both traits. For both traits, the genetic correlation between SU and WL was moderate and positive, however, the standard errors of these estimates were relatively large. The genetic correlation between SH and WL was not statistically different from zero for either trait.

Table 2.5. Additive genetic variance (σ_a^2), permanent environmental variance (σ_{pe}^2) residual variance (σ_e^2), and narrow-sense heritability (h^2) estimates (standard error) for susceptibility and severity of sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL). Heritability was calculated on the liability scale for the susceptibility trait (h_l^2) and also transformed to the observed scale (h_o^2) post hoc, heritability was calculated on the observed scale for the severity trait (h_o^2).

	Susceptibility			**Severity**		
	SH	SU	WL	SH	SU	WL
$\boldsymbol{\sigma_a^2}$	0.42 (0.14)	0.56 (0.20)	0.12 (0.08)	1.2E-02 (1.8E-03)	7.0E-03 (1.7E-04)	3.2E-03 (8.2E-04)
$\boldsymbol{\sigma_{pe}^2}$	-	-	-	9.8E-03 (1.6E-03)	3.3E-03 (2.2E-04)	2.6E-03 (9.6E-04)
$\boldsymbol{\sigma_e^2}$	1.01 (0.04)	1.01 (0.05)	1.01 (0.04)	7.7E-02 (1.4E-03)	8.2E-03 (1.5E-04)	6.9E-02 (1.2E-03)
$\boldsymbol{h_l^2}$	0.29 (0.07)	0.35 (0.08)	0.10 (0.06)	-	-	-
$\boldsymbol{h_o^2}$	0.12 (0.03)	0.15 (0.03)	0.03 (0.02)	0.12 (0.02)	0.06 (0.01)	0.04 (0.01)

Table 2.6. Genetic correlations (standard error) between sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL). Results for lesion severity are above the diagonal; results for lesion susceptibility are below the diagonal.

	SH	**SU**	**WL**
SH	-	0.59 (0.17)	0.03 (0.27)
SU	0.98 (0.04)	-	0.67 (0.33)
WL	0.20 (0.27)	0.70 (0.20)	-

2.3.3 Quantitative trait loci and functional analysis

There was a polygenic background to the susceptibility and severity of SH, SU, and WL. There were no distinct peaks in plots of the standardised SNP effects of lesion susceptibility (**Figure 2.2**). The ten markers with the largest standardised SNP effects for susceptibility to each lesion are provided in **Table 2.7**, but due to the lack of any clear peaks, these markers were not mapped to nearby genes. No markers had a significant effect on lesion severity; markers with a suggestive effect were identified on *Bos taurus* autosome 8 (**BTA-8**) for SU and BTA-23 for WL (**Figure 2.3**). Positional candidate genes located closest to the top markers for the severity of each lesion, and within 0.2 Mb upstream or downstream of the marker, are provided in **Table 2.8**.

Window-based GWA analyses revealed a similarly complex genetic background to the lesion susceptibility traits, although some genomic regions explained relatively greater proportions of the total genetic variance (**Figure 2.4**). Details of the genes contained, or partially contained, within these genomic regions are provided in **Table 2.9**.

The positional candidate genes identified from GWA analyses of all traits were enriched in Gene Ontology (**GO**) biological processes of complement activation, classical pathway (GO:0006957) and complement activation, alternative pathway (GO:0006958). Additionally, genes were enriched in the *Bos taurus* Reactome pathway of sphingolipid metabolism (R-BTA-428157).

Figure 2.2. Standardised single nucleotide polymorphism (SNP) effects for susceptibility to sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL).

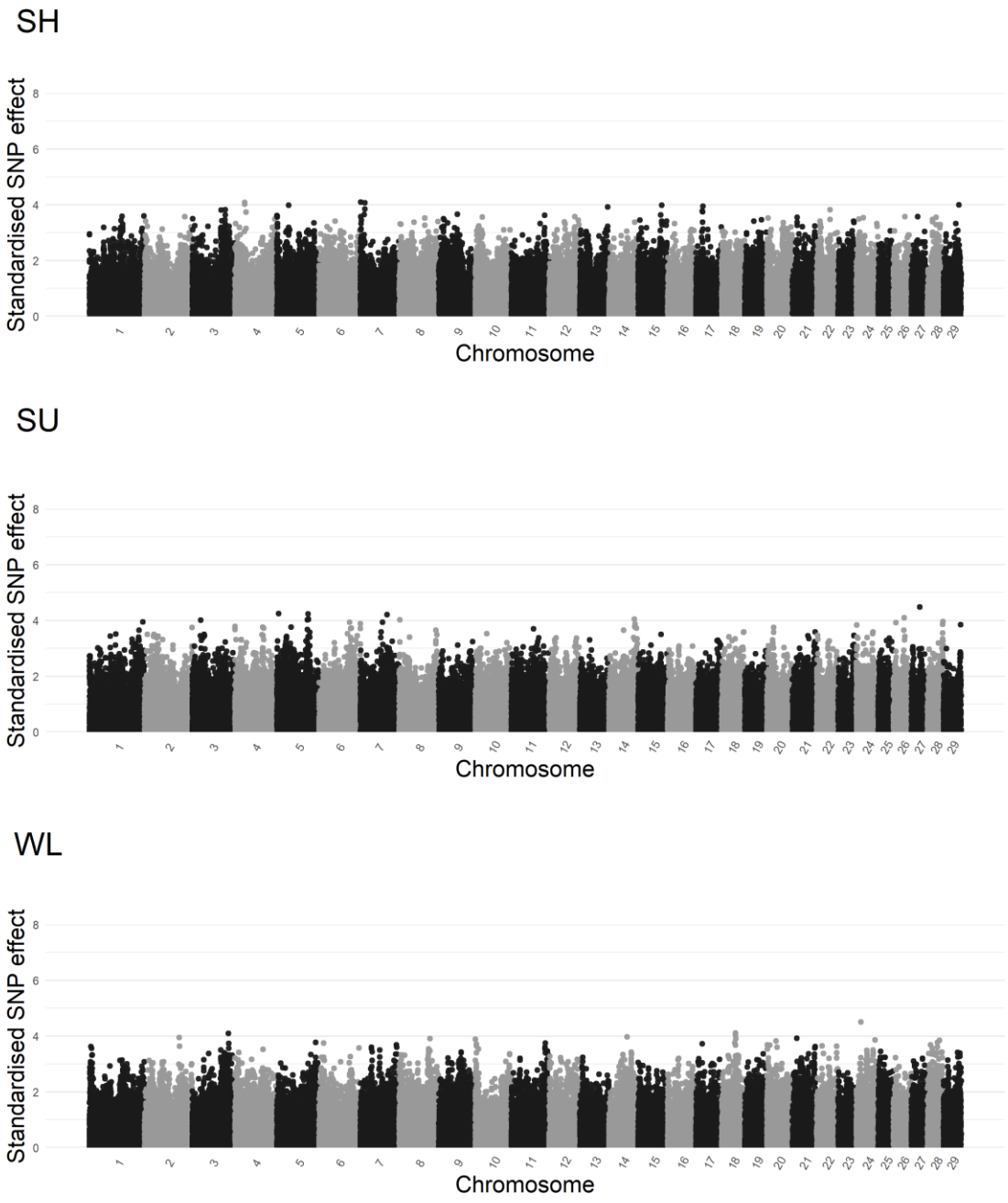


Figure 2.3. Manhattan and quantile-quantile plots of the severity of sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL); -log10 *P*-value of marker effects against marker position on the chromosome. The solid line represents the genome-wide significance threshold ($P \leq$ 7.67E-07, 0.05/number of tested markers) and the dashed line represents the suggestive threshold ($P \leq$ 1.53E-05, 1/number of tested markers).

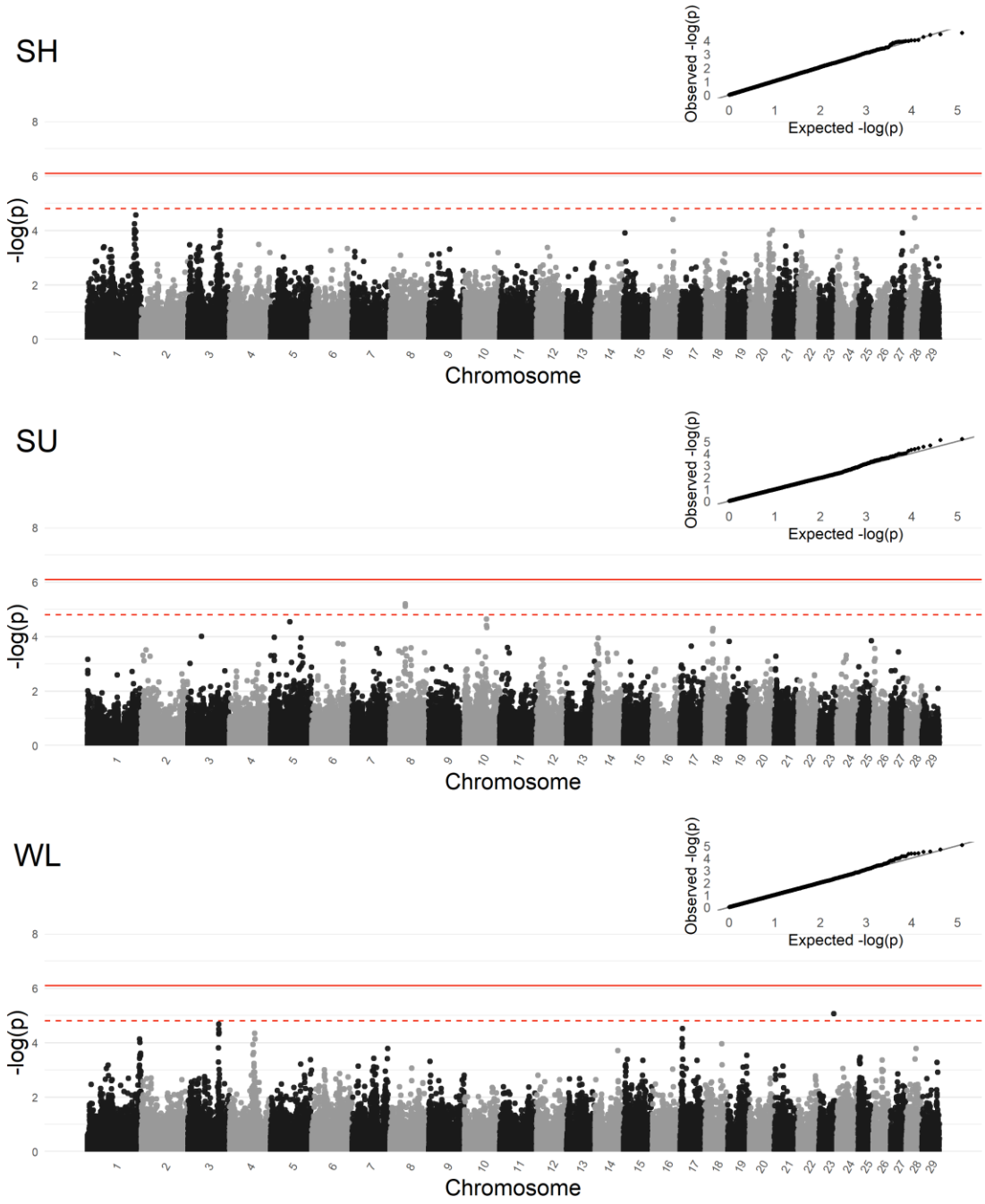


Table 2.7. The ten single nucleotide polymorphisms (SNP) with the largest standardised effects for sole haemorrhage (SH), sole ulcer (SU), and white line disease (WL) susceptibility, including chromosome (BTA), position, and minor allele frequency (MAF). The standardised SNP effects were calculated by dividing the estimated SNP effects by their empirical standard deviation and are presented in descending order for each trait.

Lesion	BTA	Position (bp)	MAF	SNP effect
SH	7	1,796,400	0.28	4.09
	4	28,253,982	0.35	4.08
	7	12,965,356	0.48	4.07
	4	27,890,577	0.40	4.01
	29	44,577,341	0.40	4.00
	15	68,358,382	0.34	3.99
	5	33,560,114	0.48	3.98
	17	19,924,694	0.49	3.95
	13	79,875,689	0.40	3.92
	7	12,994,583	0.46	3.85
SU	27	24,424,298	0.45	4.47
	5	4,889,808	0.33	4.25
	5	89,051,206	0.41	4.23
	7	76,625,461	0.38	4.21
	26	31,562,060	0.36	4.09
	5	90,375,279	0.40	4.05
	14	72,785,677	0.45	4.05
	5	90,364,435	0.29	4.03
	8	1,782,066	0.49	4.02
	5	87,262,266	0.36	4.02
WL	24	12,855,197	0.36	4.50
	18	39,385,561	0.41	4.11
	3	103,194,072	0.44	4.09
	18	39,488,321	0.41	4.08
	18	39,440,689	0.41	4.05
	14	52,361,610	0.48	3.97
	2	99,164,443	0.48	3.95
	21	13,283,849	0.33	3.92
	18	40,219,814	0.21	3.91
	8	88,091,837	0.49	3.91

Table 2.8. The top markers for the severity of sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL), with the respective chromosome (BTA) and position (bp), minor allele frequency (MAF), *P*-value of marker effect, and name and location of the closest gene. Markers with a significant ($P \leq$ 7.67E-07, 0.05/number of tested markers) or suggestive effect ($P \leq$ 1.53E-05, 1/number of tested markers) are denoted with *, markers located inside candidate genes are denoted with **.

Lesion	BTA	Position (bp)	MAF	*P*-value	Gene	Gene location
SH	1	141,365,527	0.21	2.73E-05	*BACE2*	141,381,597 - 141,487,147
	16	60,525,984	0.19	3.93E-05	*ABL2***	60,462,811 - 60,558,443
	28	24,747,891	0.42	3.45E-05	*HNRNPH3*	24,751,331 - 24,762,284
SU	5	56,420,099	0.06	2.86E-05	*TAC3*	56,411,332 - 56,418,012
	8	44,652,431	0.08	6.31E-06*	*PGM5*	44,403,368 - 44,612,837
	10	65,020,001	0.11	2.25E-05	*C10H15orf48*	65,015,661 - 65,019,322
WL	3	89,815,776	0.22	2.10E-05	*PLPP3*	89,607,569 - 89,695,225
	17	7,269,721	0.35	3.07E-05	*RPS3A*	6,717,403 - 6,722,090
	23	43,909,068	0.13	8.73E-06*	*PHACTR1*	43,276,513 - 43,796,605

Figure 2.4. Proportion of the total additive genetic variance explained by sliding windows of 0.65 Mb for the susceptibility and severity traits of sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL).

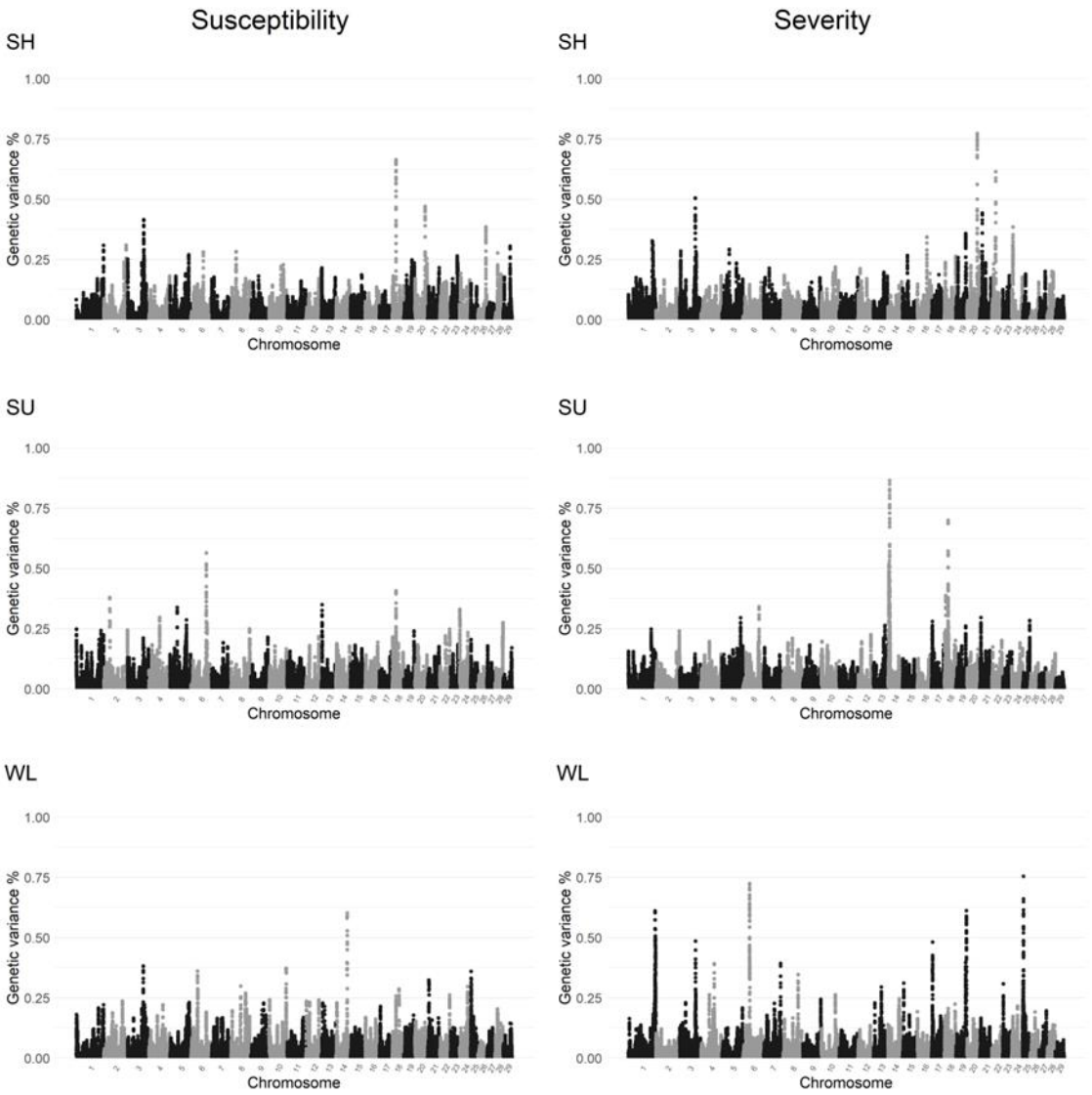


Table 2.9. Genomic regions which explained the greatest proportion of genetic variance for the susceptibility and severity traits of sole haemorrhage (SH), sole ulcer (SU), and white line disease (WL); including chromosome (BTA), window position (bp), the proportion of total genetic variance explained, and the name of genes contained (or partially contained) within these windows.

Lesion	Trait	BTA	Window position (bp)	Variance (%)	Gene(s)
SH	Susceptibility	3	90,725,628 – 91,356,392	0.47	*BSND, TMEM61*
		18	21,574,466 – 22,223,573	0.66	*CHD9, RBL2, AKTIP, FTO*
		20	58,161,594 – 58,795,440	0.41	*ANKH, OTULIN, OTULINL, TRIO*
	Severity	3	88,750,416 – 89,386,272	0.50	*C8B, C8A*
		20	58,161,594 – 58,795,440	0.77	*ANKH, OTULIN, OTULINL, TRIO*
		22	21,284,764 – 21,911,533	0.61	*BHLHE40, ITPR1, SUMF1*
SU	Susceptibility	6	86,762,457 – 87,405,290	0.56	*SLC4A4, GC*
		18	21,422,708– 22,070,855	0.41	*CHD9, RBL2, AKTIP, FTO*
	Severity	14	5,922,777 – 6,526,644	0.52	*KHDRBS3*
		14	8,954,477 – 9,597,429	0.86	*KCNQ3, EFR3A*
		18	21,454,669 – 22,081,129	0.70	*CHD9, RBL2, AKTIP, FTO*

Lesion	Trait	BTA	Window position (bp)	Variance (%)	Gene(s)
WL	Susceptibility	3	88,750,416 - 89,386,272	0.38	*C8B, C8A*
		6	37,796,921 - 38,426,291	0.36	-
		10	97,152,695 - 97,799,000	0.37	
		14	64,061,208 - 64,708,627	0.60	*RNF19A, POLR2K, MGC148714*
	Severity	1	153,405,778 - 154,054,353	0.61	*DAZL, PLCL2*
		1	155,049,738 - 155,696,507	0.49	*SATB1*
		3	89,466,489 - 90,090,412	0.49	*PRKAA2, PLPP3*
		6	37,412,062 - 38,055,681	0.72	*LCORL*
		17	5,843,870 - 6,493,219	0.48	*GATB, MIR2404-1*
		19	59,814,966 - 60,461,025	0.61	-
		25	4,231,415 - 4,872,654	0.75	-

2.4 Discussion

We have corroborated the results of previous studies which showed SH, SU, and WL have a low to moderate heritability (van der Waaij et al., 2005; van der Linde et al., 2010; Johansson et al., 2011; Häggman and Juga, 2013; Croué et al., 2017; Malchiodi et al., 2017; Heringstad et al., 2018; Ring et al., 2018; Shabalina et al., 2020; Oliveira Junior et al., 2021). The heritability estimates for the susceptibility trait were higher than estimates for lesion severity. These differences are predominantly a reflection of the differences in estimating heritability on the liability scale as opposed to the observed scale (Visscher et al., 2008; Tenesa and Haley, 2013). To allow comparison of the susceptibility and severity traits, we transformed the heritability estimates for lesion susceptibility to the observed scale. This transformation adjusted for the over-representation of cases compared to the study population prevalence (Lee et al., 2011), although this was only a minor problem in this dataset as case and control groups were not designed to be balanced. Taking into account the uncertainty of the estimates, the heritability on the observed scale was comparable for both the susceptibility and severity traits, although the greatest difference was apparent for SU which had a higher heritability for susceptibility than severity. This could suggest that the case definition for SU is clearer than SH and WL, and therefore assessment as a binary lesion is more consistent. It has previously been shown that using the average lesion severity from all claws produces larger heritability estimates for SH and WL than a binary lesion trait (Ring et al., 2018), but we did not observe a similar trend between the heritability of SH and WL susceptibility and severity.

2.4.1 Heritability of sole haemorrhage and sole ulcers

The heritability estimates of SH and SU susceptibility on the liability scale were larger than those previously reported from studies that either directly estimated variance components on the liability scale or transformed the heritability to the liability scale post hoc (Buch et al., 2011; Häggman and Juga, 2013; van der Spek et al., 2013; Malchiodi et al., 2017). Although heritability estimates from different populations should be compared cautiously (Visscher

et al., 2008; Tenesa and Haley, 2013), there were some features of our study which may contribute to larger heritability estimates; these relate to phenotype recording, trait definitions, genetic relationships, and study population.

Phenotype recording. The previously referenced studies (Buch et al., 2011; Häggman and Juga, 2013; van der Spek et al., 2013; Malchiodi et al., 2017), included study populations much larger than ours and collated records from multiple foot-trimmers. Harmonising foot lesion recording is challenging due to variability between individuals and differences in terminology (Christen et al., 2015), therefore there may have been unavoidable discrepancies in how lesions were recorded during these studies. Additionally, the animals which are presented to a foot-trimmer during a visit may over-represent those with painful foot lesions and result in a skewed study population; this could affect heritability estimates (van der Spek et al., 2013; Croué et al., 2017). In our study, all foot lesions were recorded by qualified veterinary surgeons, predominantly a single researcher; cows were prospectively enrolled and assessed specifically for research purposes. Therefore, we believe we have minimised these common sources of bias.

Trait definition. We defined the lesion susceptibility trait to differentiate between "susceptible" and "resistant" animals, using a definition which was as robust as possible within the constraints of our study. We used repeated records from a single production cycle so that cows were only considered unaffected (or "resistant") if the lesion was absent on all four occasions. We intended this approach to reduce misclassification bias, however, it did result in a slight reduction in study power due to a small proportion of animals which were unaffected by a lesion but did not have complete records. We were also careful to record even very mild cases of SH, and consequently, 77% (1,775/2,305) of the study population were recorded as being affected with SH (or "susceptible"). This may have influenced our results because the heritability of SH has been shown to be larger when mild cases of SH were classed as affected as opposed to being grouped with unaffected animals (Ring et al., 2018).

Genetic relationships. The majority of animals with recorded phenotypes were genotyped (94%, 2,167/2,305), which was achievable because the study population was comparatively smaller than the previously referenced studies (Buch et al., 2011; Häggman and Juga, 2013; van der Spek et al., 2013; Malchiodi et al., 2017). We were, therefore, able to define relationships between animals using pedigree and genomic information; which minimises the effect of pedigree recording errors, is less affected by violated assumptions such as that of random mating, and describes relatedness by actual allele sharing rather than the probability of related individuals having the same alleles (Visscher et al., 2002, 2006; Speed and Balding, 2015). This may have avoided deflation of the heritability estimates from increased residual variance in the analysis, compared to studies which relied on the accuracy of pedigree information.

Study population. As previously mentioned, we had a smaller study population than previous genetic studies of foot lesions, however, the main limitation of our study was that animals were only from four herds, and three of the four study herds were operating similar systems of zero-grazing and three-times-a-day milking. This homogeneity may have reduced the environmental variance, or the influence of a genotype x environment interaction, and resulted in higher heritability estimates.

2.4.2 Heritability of white line lesions

The heritability estimates of WL were low for both the susceptibility and severity traits: 0.10 and 0.04, respectively. Although the heritability estimates of WL are comparable to previous studies (van der Waaij et al., 2005; van der Linde et al., 2010; Johansson et al., 2011; Häggman and Juga, 2013; Croué et al., 2017; Malchiodi et al., 2017; Heringstad et al., 2018; Shabalina et al., 2020), they were notably smaller than the heritability of SH and SU.

A study of grazing dairy herds in Ireland, which was methodologically comparable to ours in many ways, estimated the heritability of WL to be 0.21 using a trait analogous to the WL severity (Ring et al., 2018). The higher heritability of WL in grazed herds, compared to the heritability we estimated in predominantly housed animals (0.04), would be worth further exploration as WL

is regarded as a more important cause of lameness in grazed cattle compared to other CHL (Chesterton et al., 2008; Navarro et al., 2013; Somers and O'Grady, 2015).

The low heritability of WL suggests there is a more limited capacity for genetic selection to reduce WL frequency in dairy herds compared to SH and SU, although it does not preclude breeding for WL resistance. A Dutch study which evaluated the value of foot lesion records in a composite claw health index reported that the genetic response was minimal for WL compared to other lesions, including SH and SU; additionally, the inclusion of WL in the index did not improve the reliability of the index (van der Linde et al., 2010). Malchiodi et al. (2020) ranked cows by a composite claw health genetic index of direct lesion traits which weighted SH, SU, and WL at 3%, 20%, and 8% in the index, respectively, and estimated the heritability of SH and WL to be similar. The difference in lesion prevalence between the best and worst cows for this index was similar for SH and WL (5 - 6%), despite the proportionally greater weighting of WL in the index. The results of these two studies also suggest genetic selection for WL resistance will be slower than for SH and SU unless selection intensity is proportionally higher. However, another explanation for the differences in SH and WL prevalence reported by Malchiodi et al. (2020) is a positive genetic correlation between SH and SU, because SU was one of the most heavily weighted lesions in the index, and therefore the reduction in SH prevalence may have been a correlated selection response.

2.4.3 Genetic correlations between lesions

Genetic correlations between SH and SU were high for both lesion susceptibility (0.98) and severity (0.59), indicating the possibility of genetic improvement in one trait through selection on the other. Previous studies have reported similar genetic correlations between SH and SU ranging from 0.38 to 0.90 (van der Waaij et al., 2005; Buch et al., 2011; Johansson et al., 2011; Häggman and Juga, 2013; van der Spek et al., 2013; Malchiodi et al., 2017; Heringstad et al., 2018). It has also been shown that the partial genetic correlation between SH and SU is much smaller than the genetic correlation

(Buch et al., 2011); in this case, the partial correlation was calculated with other traits held constant (infectious foot lesions and production, health and fertility traits). This result implies the shared genetic background between SH and SU is more limited when the effects of other traits are considered. We only assessed genetic correlation with bivariate models, so we can only speculate as to whether this same result would have been apparent in our study population. Interestingly, Croué et al. (2017) showed the strength of the genetic correlation between SH and SU was dependent on the distribution pattern of SH; if SH was circumscribed, the genetic correlation with SU was 0.72, but it was only 0.22 if SH was diffuse. We did not differentiate between the distribution patterns of SH, but the severity grading we used reflected this to some extent. The trait definition of susceptibility to SH and SU, which did not take the severity or the distribution of the lesions into account, indicated a very strong genetic correlation between SH and SU; this has an important practical implication. In general, SH is associated with a less severe lameness than SU (Tadich et al., 2010; Blackie et al., 2013), and therefore may not be as reliably recorded in foot-trimming records (Archer et al., 2010a). Furthermore, there can be ambiguity as to what severity threshold should be used to denote lesion presence, resulting in the misclassification of animals with mild SH as unaffected (Ring et al., 2018); the case definition for SU is much clearer in this regard. Therefore, as SH and SU are strongly and positively genetically correlated based on a simple present or absent classification (i.e., lesion susceptibility trait), selection for reduced SU would be expected to result in a reduction in SH, overcoming potential challenges with SH recording.

The genetic correlation between SU and WL was positive for both lesion susceptibility (0.70) and severity (0.67), but with relatively large standard errors. The genetic correlation between SU and WL has most frequently been reported to be between 0.4 and 0.8 (Koenig et al., 2005; van der Linde et al., 2010; Johansson et al., 2011; van der Spek et al., 2013; Croué et al., 2017; Malchiodi et al., 2017, 2020), but ranges from 0.01 to 0.98 (Swalve et al., 2008; Pérez-Cabal and Charfeddine, 2015). Our results, in combination with previous studies, would suggest that although the genetic variance of SU and WL may correlate to an extent, there is a substantial proportion of the genetic background which is

distinct to each lesion. The genetic correlations between SH and WL were not statistically different from zero in our study. The genetic correlation between SH and WL is generally reported to be in the range of 0.2 to 0.55 (van der Waaij et al., 2005; van der Linde et al., 2010; Malchiodi et al., 2017, 2020), and, similar to SU, circumscribed SH has been reported to have a stronger genetic correlation with WL than diffuse SH (Croué et al., 2017).

Taken together, our results suggest that WL may have a different genetic background to SH and a lesser extent, SU. Although CHL are often regarded as pathophysiologically similar lesions, it is also thought that WL can also develop as primary lesions without prior disruption of claw horn production (Mülling, 2002). Epidemiological studies lend some support to this distinction because different predisposing environmental factors have been identified for SH and SU compared to WL (Sogstad et al., 2005; Barker et al., 2009; Cramer et al., 2009; Sanders et al., 2009; Moreira et al., 2019). Further work which specifically focuses on WL separately from SH and SU would be beneficial; but it is perhaps also worth reconsidering the assumption that all CHL have a completely shared aetiopathogenesis, at least from a genetic perspective.

2.4.4 Quantitative trait loci

The SH, SU, and WL susceptibility and severity traits all had a complex genetic background, in agreement with previous research (van der Spek et al., 2015; Croué et al., 2019; Sánchez-Molano et al., 2019; Lai et al., 2021b). As these traits are highly polygenic and our study population was relatively small for a GWA study, there is a high risk of stochastic noise being misinterpreted as meaningful (Lander and Kruglyak, 1995; Platt et al., 2010; Wray et al., 2013; Visscher et al., 2017). Therefore, we interpret the GWA results cautiously and focus on QTL which were either associated with multiple traits or lesions, or those which have also been highlighted in previous studies.

Inference of the genomic region on BTA-14 (5.9 – 6.5 Mb), which explained 0.52% of the total genetic variance for SU severity, is strengthened because this region includes a locus which has previously also been linked to SU in a population of Holstein-Friesian cattle (van der Spek et al., 2015). The candidate

gene in this region was *KHDRBS3* (KH RNA Binding Domain Containing, Signal Transduction Associated 3), which was also highlighted during GWA analyses of digital cushion thickness using the same dataset (Chapter 3). Digital cushion thickness is linked, both phenotypically and genetically, to CHL development (Machado et al., 2011; Oikonomou et al., 2014; Toholj et al., 2014; Newsome et al., 2017b; Stambuk et al., 2019; Griffiths et al., 2020). In cattle, the *KHDRBS3* gene is expressed in adipose and muscle tissue (Fang et al., 2020), has been associated with average daily gain (Seabury et al., 2017), and is in LD with a neighbouring gene associated with intramuscular fat deposition (Barendse et al., 2004; Gibbs et al., 2009). The digital cushion is primarily composed of adipose tissue and its fat content is correlated with CHL incidence (Lischer and Ossent, 2002; Räber et al., 2006; Wilson et al., 2021). Therefore, the *KHDRBS3* gene may play a role in SU development via mechanisms which relate to the digital cushion. Furthermore, enrichment of the sphingolipid metabolism pathway also suggests genetic control of lipid metabolism may have a role in CHL development, this is a mechanism which could underlie the observations that subcutaneous fat thickness is correlated with CHL risk (Newsome et al., 2017b).

A pleiotropic locus on BTA-8 (42.9 - 44.6 Mb) was associated with increased susceptibility to SU, WL, digital dermatitis, and metritis in a GWA study of Holstein cattle (Lai et al., 2021a). The marker which had the strongest effect on SU severity in our study was adjacent to this locus (44.6 Mb), lending support to the results reported by Lai et al. (2021a). Inflammation in early lactation has been posited as a component of SU pathogenesis; early lactation clinical mastitis is associated with an increased risk of SU development (Griffiths et al., 2020; Watson et al., 2022), and metritis has been tentatively linked to increased SU incidence (Enevoldsen et al., 1991); therefore a locus with pleiotropic effects on SU and metritis has interesting implications. The translocation of lipopolysaccharides into systemic circulation has been demonstrated from naturally and experimentally induced mastitis and metritis (Eckel and Ametaj, 2016); lipopolysaccharides are thought to affect the soft tissues of the suspensory apparatus of the distal phalanx (Boosman et al., 1991; Tian et al., 2019), which is considered a key mechanism in SU development (Lischer et al., 2002b). The

closest gene to the marker associated with SU severity was *PGM5* (phosphoglucomutase 5) which codes for a key enzyme in glucose metabolism and is related to adipogenesis, early gestation, and bovine tuberculosis susceptibility in cattle (Yu et al., 2009; Cerri et al., 2012; Richardson et al., 2016); as well as to muscle mass in sheep (Zlobin et al., 2021), and tissue healing in lower limb injuries in mice (Aguilar et al., 2015). Therefore, although there is not an obvious biological pathway which links the *PGM5* gene with SU, WL, digital dermatitis, and metritis; this region warrants further consideration.

For both SH susceptibility and SH severity, one of the greatest proportions of genetic variance was explained by the same genomic region on BTA-20 (58.1 – 58.7 Mb). Genes within this candidate region are linked to inflammation, innate immune signalling, and leukocyte migration (van Rijssel et al., 2012; Fiil et al., 2013; Damgaard et al., 2016), implying genetic control of inflammatory processes may influence SH development, a conclusion which is also supported by the enrichment of two complement activation pathways.

The genomic region on BTA-25 (4.2 – 4.8 Mb) which explained 0.75% of the total genetic variance associated with WL severity overlaps a genomic region which has previously been associated with SU in Holstein cows (Sánchez-Molano et al., 2019). Genes in this region may contribute to the genetic correlation we observed between SU and WL. Similarly, a genomic region on BTA-6 (86.7 – 87.4 Mb), which explained 0.56% of the total genetic variance associated with SU susceptibility, overlapped with a genomic region previously linked to WL (Croué et al., 2019). A marker located within this region on BTA-6 was associated with Rump Width in dairy cattle in a previous GWA study (Hiendleder et al., 2003). Rump Width is a linear type trait which is positively genetically correlated with sole overgrowth and WL (Ring et al., 2018), and SU incidence was reported to be twice as high in animals whose sire's breeding value for Rump Width was in the top tercile (positive value) compared to the bottom tercile (negative value) (Oikonomou et al., 2013). One of the candidate genes in this region was *GC* (GC vitamin D binding protein), a causal variant within this gene has been established to relate to clinical mastitis resistance in dairy cattle (Freebern et al., 2020; Lee

et al., 2021). Phenotypically there is a relationship between early lactation mastitis and subsequent SU development (Griffiths et al., 2020; Watson et al., 2022), and SU is positively genetically correlated with subclinical and clinical mastitis (Koenig et al., 2005; Buch et al., 2011); the *GC* gene may contribute these observations.

The same genomic window on BTA-3 (88.7 – 89.3 Mb) was one of the top windows, based on the proportion of the total genetic variance explained, for both SH severity and WL susceptibility. This region contains the genes *C8A* (complement C8 alpha chain) and *C8B* (complement C8 beta chain) which are related to the complement system and have previously been associated with reproductive function and temperament in cattle (Chan, 2012; Tenghe et al., 2016). A nearby marker on BTA-3 was also associated with WL severity (89.8 Mb) and mapped to *PLPP3* (phospholipid phosphatase 3), which has been reported as a candidate gene related to mastitis resistance in Holstein cattle (Cai et al., 2018). Both this genomic region and marker were located slightly upstream of a marker previously associated with digital dermatitis (90.4 Mb) (Sánchez-Molano et al., 2019). The genetic correlation between SH and digital dermatitis has been estimated to be very low or zero, whereas the genetic correlation between WL and digital dermatitis is often negative with estimates typically between -0.1 and -0.3 (Swalve et al., 2008; van der Linde et al., 2010; Croué et al., 2017; Malchiodi et al., 2017, 2020).

Overlapping windows on BTA-18 were associated with SH susceptibility and SU susceptibility and severity (21.5 – 22.2 Mb and 21.4 – 22.0 Mb, respectively). Genes within this region are related to cell division and apoptosis, regulation of lipid metabolism, regulation of fat mass, adipogenesis and energy homeostasis (Jia et al., 2011; Zhou et al., 2015). These regions include, or neighbour, a marker which was previously been associated with heel horn erosion in cattle (Croué et al., 2019). Although heel horn erosion has historically been considered a CHL (Hoblet and Weiss, 2001), it is now generally regarded to have an infectious aetiology (Bergsten and Herlin, 1996; Greenough, 2007). The genetic correlation between SH or SU and heel horn erosion has been reported to range from -0.07

to 0.50 (Heringstad et al., 2018), although partial correlations were higher, when other traits including infectious foot lesions and production, health and fertility traits, were held constant (Buch et al., 2011).

A marker on BTA-23 (43.9 Mb) had the strongest evidence of an effect on WL severity, although the lack of deviation from the baseline in adjacent SNP means this result should be interpreted cautiously. This marker was located within *PHACTR1* (phosphatase and actin regulator 1) which is involved in the regulation of actin cytoskeleton dynamics, tubule formation and endothelial cell survival (Jarray et al., 2011; Allain et al., 2012). This marker is also within a genomic region which was previously associated with interdigital hyperplasia in Holstein cattle (Sánchez-Molano et al., 2019). Additionally, another genomic region on BTA-14 (64.0 – 64.7 Mb), which explained 0.60% of the total genetic variance associated with WL susceptibility, is close to a marker (64.0 Mb) which has also been previously linked with interdigital hyperplasia (van der Spek et al., 2015). Previous estimates of the genetic correlation between WL and interdigital hyperplasia generally report minimal or no genetic correlation (Heringstad et al., 2018), although there have been reports of a moderate and positive genetic correlation between these two lesions (Koenig et al., 2005).

2.5 Conclusions

We followed a prospective cohort study design to carefully record SH, SU, and WL at multiple time points during a production cycle. With this dataset, we estimated the heritability of SH, SU, and WL using two trait definitions relating to the susceptibility and severity of each lesion. Our results corroborate those of previous studies which showed that SH, SU, and WL have a low to moderate heritability. The magnitude of the heritability estimates for SH and SU susceptibility indicates the possibility to improve resistance to these lesions through selective breeding; the heritability estimates for WL were low, and we would be more conservative in our expectations regarding the potential to breed resistance to this lesion. We observed a strong genetic correlation between SH and SU implying that a correlated response would be expected through selection on one of these lesions; additionally, it would suggest the genetic background of these two lesions is similar, in support of the prevailing hypotheses regarding pathogenesis. On the other hand, the genetic correlation between SH and SU with WL was weaker and more variable, implying there is a distinct genetic component to WL not shared by SH and SU. We characterised the genetic background of these lesions as highly polygenic, in agreement with previous studies. We highlighted QTL which may be associated with these lesions, in particular, genes relating to lipid metabolism, inflammation and digital cushion thickness; as well as QTL which have been detected in GWA analysis for other foot lesions and health conditions in dairy cattle.

Chapter 3: Genetic parameters and genome-wide association study of digital cushion thickness in Holstein cows

3.1 Introduction

The digital cushion of the bovine claw is composed of three parallel pads of soft fat and loose connective tissue which are thought to play an important role in protecting the corium from mechanical forces in the foot (Lischer and Ossent, 2002; Räber et al., 2004, 2006). New horn develops via cornification of keratinocytes, cells that are nutritionally dependent on the corium (Hirschberg et al., 2001); consequently, damage to the corium leads to the production of poor-quality horn (Hoblet and Weiss, 2001). The term "claw horn (disruption) lesion" (**CHL**) refers to foot lesions considered to arise from damaged corium; these lesions are sole haemorrhage (**SH**), sole ulcers (**SU**), and white line lesions (**WL**).

In 1999, Kofler and others described an ultrasonographic approach to visualise the digital cushion which could be employed in live animals (Kofler et al., 1999). The digital cushion thickness (**DCT**) has since been measured in a succession of studies, with a general agreement that a thin DCT represents an increased risk of subsequent CHL development (Machado et al., 2011; Toholj et al., 2014; Newsome et al., 2017b; Stambuk et al., 2019; Griffiths et al., 2020). Studies that have focused on CHL as individual lesions have, to date, only demonstrated an association between DCT and SH or SU (Newsome et al., 2017b; Griffiths et al., 2020). Therefore, the contribution of WL to the previously reported associations between DCT and CHL development is unclear.

Variation in DCT has been summarised as being conditional on a combination of genetic, developmental, and pathological factors (Wilson et al., 2021); the genetic background of DCT is the focus of our study. The heritability of DCT, measured using ultrasonography, has been estimated to be 0.33 (± 0.09) using pedigree relationships (Oikonomou et al., 2014), and 0.31 (± 0.13) from multiple genomic analyses (Stambuk et al., 2020b). One other study, which enrolled cows specifically for research purposes, reported the heritability of DCT

in recently calved cows to be 0.23 (± 0.12) (Sánchez-Molano et al., 2019). A negative genetic correlation has been reported between DCT and CHL incidence (Oikonomou et al., 2014), however, the separate genetic relationships between DCT and sole lesions (SH and SU) and between DCT and WL are unknown.

The thickness of the digital cushion has been observed to change around calving and throughout lactation, but with inconsistencies regarding the exact changes which occur (Newsome et al., 2017a; Stambuk et al., 2019; Griffiths et al., 2020; Bach et al., 2021). In some cases, the DCT has been reported to be thinnest during early lactation; suggested to be a consequence of fat tissue mobilisation and a reflection of the concurrent nadir in body condition (Bicalho et al., 2009; Griffiths et al., 2020). Alternatively, other studies have observed the thinnest DCT immediately after calving (Newsome et al., 2017a; Bach et al., 2021), this is thought to be the result of compression due to periparturient laxity in the suspensory apparatus of the distal phalanx (Tarlton et al., 2002; Knott et al., 2007). Therefore, it would be useful to define the genetic parameters of DCT at different periods during lactation, particularly as the stage of lactation has been shown to affect genetic parameter estimates for other foot lesion traits (Gernand et al., 2013).

Genome-wide association (**GWA**) studies have utilised genomic markers to map quantitative trait loci (**QTL**) which may be associated with the genetic variance of DCT. Two recent GWA studies of around 600 Holstein and Jersey cows characterised DCT as a complex trait and identified candidate genes related to inflammation, fat tissue deposition, bone growth, and keratinocyte function (Stambuk et al., 2020a; b). Further studies are needed to corroborate previously highlighted QTL as well as to explore QTL for DCT in different populations.

3.1.1 Objectives

The objectives of our study were to (i) estimate genetic parameters for digital cushion thickness at different stages during a production cycle, (ii) estimate the genetic correlation between digital cushion thickness and claw horn lesions, evaluating sole lesions (sole haemorrhage and sole ulcers) and white line

lesions separately (iii) identify quantitative trait loci associated with digital cushion thickness.

3.2 Materials and methods

3.2.1 Study design and population

The study was conducted following ethical approval by the University of Liverpool Research Ethics Committee (VREC269a, VREC466ab) and procedures regulated by the Animals (Scientific Procedures) Act were conducted under a United Kingdom (**UK**) Home Office License (P191F589B).

A prospective, cohort study was designed to measure the DCT in dairy cows and record SH and SU at four time points during a production cycle. Data collection was conducted on four dairy herds (A - D) in the northwest of the UK which were selected for convenience based on the practicalities of frequent visits and assessments. Herds A to C housed lactating cows all-year-round, milked cows three times daily and recorded 305-day milk yields of approximately 11,000 - 11,500 L. Herd D housed lactating cows all-year-round except for lower-yielding cows which were grazed during the summer; cows were milked twice daily and the 305-day milk yield was approximately 9,000 L. Parous cows on all herds were routinely foot-trimmed twice a year before drying off and 60 - 120 days after calving. On all herds, lactating cows were regularly footbathed after milking. Herd A footbathed cows three times a week with either copper sulphate or formalin; herd B footbathed cows twice daily with formalin, herd C footbathed cows daily with either copper sulphate or formalin and herd D footbathed three times a week with formalin.

All animals which were registered as Holsteins and expected to calve between April and December 2019 were prospectively enrolled with no additional inclusion or exclusion criteria applied. A total of 2,352 animals were enrolled. Data were collected by qualified veterinary surgeons during weekly or twice weekly visits to each herd from February 2019 to July 2020 (with a break from March - to June 2020 due to COVID-19 restrictions). Animals were assessed for foot lesions at four time points: before parturition (**T1-Precalving**), immediately after parturition (**T2-Calving**), in early lactation around the time of peak milk yield (**T3-Early**), and in late lactation (**T4-Late**). The sample size was determined by resource constraints; all eligible animals were enrolled until the final

assessments (T4-Late) began, at which point further enrollments ended as data collection at four time points simultaneously was not feasible.

3.2.2 Data collection

At each assessment time point, animals were restrained in a foot-trimming crush and, if foot-trimming had not been conducted during the visit, the claw horn on the sole of each foot was lightly trimmed to allow inspection of foot lesions. On each claw, CHL were recorded using case definitions as described in the International Committee for Animal Recording (ICAR) claw health atlas (Egger-Danner et al., 2020). All CHL were graded according to severity (**Table 3.1**), broadly comparable to absent (score 0), mild (score 1), moderate (score 2), and severe (score 3). All foot lesions were examined and recorded by qualified veterinary surgeons; over 90% by a single researcher, and the remainder by three other researchers.

Table 3.1. Case definitions and severity grading system for sole haemorrhage (SH), sole ulcers (SU), and white line lesions (WL).

Lesion	Case definition	Severity grading
Sole haemorrhage (SH)	Discolouration of the sole horn	Grade 1: light pink lesion < 2 cm diameter or diffuse discoloration of sole Grade 2: light pink lesion ≥ 2 cm diameter or dark pink/purple lesion < 2 cm diameter Grade 3: dark pink/purple lesion ≥ 2 cm diameter or discolouration with a blue tinge
Sole ulcer (SU)	Exposure of fresh or necrotic corium	Grade 1: < 2 cm diameter lesion covered by a thin layer of horn before modelling Grade 2: ≥ 2 cm diameter lesion with < 1.5 cm granulation tissue protruding through the horn Grade 3: ≥ 1.5 cm granulation tissue protruding through the horn or secondary bacterial infection
White line lesion (WL)	Lesion localised to the white line region	Grade 1: haemorrhage of the white line or discolouration or separation of the white line which disappears after limited trimming Grade 2: deeper separation or discolouration of the white, lesion is still present after limited trimming Grade 3: separation of the white line which extends to the corium, purulent exudate or necrotic tissue may be present

After SH and SU had been recorded, the digital cushion was imaged in the lateral claw of the left-hind digit using B-mode ultrasonography with the foot still lifted off the ground (i.e. non-weight-bearing), as previously described (Kofler et al., 1999). This site was chosen because the lateral claw of the hindlimb digit is the most common site of CHL development (Murray et al., 1996). Time constraints did not allow us to scan more than one claw per cow; the left-hind digit was arbitrarily chosen over the right-hind digit. A 5 cm linear probe inside a gel standoff was used with a DRAMIŃSKI Vet 4 Mini ultrasound machine (DRAMIŃSKI S. A., Olsztyb, Poland); frequency was set to 6 MHz and image depth to 4 cm. The probe was placed on the midline of the sole and the ultrasound image was stored when the digital cushion, arch of the distal phalanx, and flexor tuberosity of the distal phalanx were visible.

The data collection procedure was the same at all time points with two exceptions. At T2-Calving on herd C, only hind feet were assessed to reduce the handling time of cows which had recently calved; this was only required on this herd due to the large numbers of cows calving each week. The other exception was at the T4-Late assessments which resumed in June 2020 following a break due to COVID-19 restrictions; data collection from this point onwards was more limited due to social distancing protocols and digital cushion images were not collected.

Following completion of data collection, DCT was measured on stored images by a single researcher using ImageJ (Schneider et al., 2012). Images were first re-labelled so measurements were blinded to details regarding image collection such as time point, farm, or concurrent lesions. Two measurements were recorded, in both cases between the internal aspect of the sole horn and the distal aspect of the distal phalanx. The first measurement was at the most proximal point in the arch of the distal phalanx, representing the greatest thickness of the digital cushion (**DCT-MAX**); this point corresponds to the interconnecting abaxial and axial pads of the digital cushion (primarily the axial pad) in the midline of the claw (Räber et al., 2004). The second measurement was immediately distal to the flexor tuberosity of the distal phalanx (**DCT-FT**); at this

point, the specific part of the digital cushion measured was assumed to be the middle pad in a typical cow (Räber et al., 2004). (For further details, readers are referred to Figure 1 in Newsome et al. (2017b) which shows a midline sagittal section of the bovine digit with a corresponding ultrasound image; in this figure, site 2 corresponds to DCT-MAX and site 3 to DCT-FT). We only recorded measurements if landmarks were clearly identifiable on each image; necessary landmarks included the interfaces between sole horn and soft tissue and the interface between soft tissue and distal phalanx. Additionally, the DCT-FT measurement was only taken if the point of the flexor tuberosity of the distal phalanx could be clearly identified.

It is important to note that these ultrasonographic DCT measurements do not exclusively relate to the digital cushion and include all soft tissues between the sole horn and distal phalanx, which include connective tissue and the corium (Kofler et al., 1999; Räber et al., 2004). For this reason, DCT measured using ultrasonography is sometimes, more correctly, referred to as sole soft tissue thickness (Newsome et al., 2017a; Griffiths et al., 2020); however, for consistency with the majority of published research in this area, the term digital cushion thickness (DCT) will be used throughout this manuscript.

3.2.3 Trait definitions

The two DCT measurements (DCT-MAX and DCT-FT) were analysed as continuous traits. As per our research objectives, sole lesions (SH and SU) were analysed separately to WL. It is thought that SH and SU represent different stages of the same disease process (Offer et al., 2000; Lischer and Ossent, 2002). Therefore, at each time point, the severity of SH and SU on each claw were combined so that the severity ranged from 0 to 6, with grades 1 to 3 directly corresponding to SH severity, and grades 4 to 6 corresponding to SU severity. The maximum severity of sole lesions from the medial and lateral claw of each foot was taken and then averaged across all feet to create a variable called “sole lesion severity” (**SL-Severity**). This approach was intended to try and capture the severity and distribution of sole lesions whilst minimising the diluting effect of healthy claws in animals which were affected with sole lesions. We followed the

same process for WL (calculating the average of the most severe white line lesion from each foot) to create a variable called “white line lesion severity” (**WL-Severity**). Both SL-Severity and WL-Severity were analysed as continuous traits.

3.2.4 Pedigrees and genotypes

Pedigree details for the study population were extracted from the national database of dairy cattle by tracing back seven generations for each animal. Blood samples were collected from the coccygeal vein of each animal into EDTA vacutainers and used to genotype each animal with the Illumina BovineSNP50 BeadChip (Illumina Inc., USA). Genotypes were subsequently imputed to 80K single nucleotide polymorphism (SNP) genotypes by Edinburgh Genetic Evaluation Services (EGENES) using an in-house procedure which has been developed for all national genomic evaluations of dairy cattle in the UK. Briefly, this imputation process uses the Illumina BovineSNP50 BeadChip and Illumina BovineHD BeadChip (Illumina Inc., USA), in addition to other commercial genotyping arrays, extra gene tests, and large-effect sequence variants. Following imputation, genotypes included 79,051 SNP spanning the entire genome. Chromosomal locations of the imputed 80K SNP panel were drawn from the latest assembly of the Bos taurus genome (ARS-UCD 1.2) (Rosen et al., 2020).

Imputed genotypes were available for 2,250 animals. Genotype quality control was implemented using PREGSF90 (Aguilar et al., 2014) within the BLUPF90 software suite (Misztal et al., 2018). Quality control included the removal of SNP with a call rate < 0.90 (N = 10,977), SNP with a minor allele frequency < 0.05 (N = 3,008), monomorphic SNP (N = 36), or SNP showing a strong deviation (> 0.15) from Hardy-Weinberg equilibrium (N = 14) (Wiggans et al., 2009). Additionally, animals were removed if sample call rate < 0.90 (N = 63) or there were parent-progeny Mendelian conflicts (N = 20). Quality control procedures resulted in a final dataset of 2,167 animals with genotypes of 65,211 SNP.

3.2.5 Genetic parameter estimation

Before genetic analyses, fixed effects were evaluated via mixed-effect linear regression of repeated observations of each trait, with the animal as a random effect in the model. This analysis was conducted in R (R Core Team, 2021) with the *lmerTest* package (Kuznetsova et al., 2017). The following fixed effects were tested: herd, parity, the season of calving, the season of assessment, days since calving at the assessment, and the researcher who recorded CHL. The importance of each fixed effect was determined by finding the multivariable model with the lowest Akaike information criterion. Season of calving had similar effects on model fit as the season of assessment; the effect of which researcher examined and recorded CHL increased Akaike information criterion, so this term was not included in subsequent genetic analyses.

Genetic parameters of each trait (DCT-MAX, DCT-FT, SL-Severity, WL-Severity) were estimated at each time point separately (T1-Precalving, T2-Calving, T3-Early, T4-Late) with single-trait linear mixed models, resulting in a total of 16 univariate models. Models were fit using the average information restricted maximum likelihood algorithm, implemented in AIREMLF90 (Misztal et al., 2018). The genetic parameters of each trait at each of the four time points were estimated with the following univariate animal model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} \quad (1)$$

where $\mathbf{y}$ is a vector of one of the four traits (DCT-MAX, DCT-FT, SL-Severity, or WL-Severity); $\mathbf{b}$ is a vector of the fixed effects including herd (4 levels), parity (5 levels, 5th level = 5th parity and greater), the season of calving (2 levels, April - September or October – March), and days relative to parturition (continuous variable); $\mathbf{a}$ is a vector of random additive genetic effects for each animal; $\mathbf{e}$ is a vector of random residual effects; $\mathbf{X}$, and $\mathbf{Z}$ are incidence matrices for $\mathbf{b}$ and $\mathbf{a}$, respectively. Random effects were assumed to be normally distributed with a mean of zero and covariance structure of:

$$var\begin{bmatrix}\mathbf{a}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{H}\sigma_a^2 & 0\\ 0 & \mathbf{I}\sigma_e^2\end{bmatrix} \quad (2)$$

where σ_a^2 is the additive genetic variance; σ_e^2 is the residual variance; $\mathbf{I}$ is an identity matrix, and $\mathbf{H}$ is the relationship matrix incorporating pedigree and genomic information in a single-step genomic analyses framework (Legarra et al., 2009). The inverse of $\mathbf{H}$ is defined as (Aguilar et al., 2010; Christensen and Lund, 2010):

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & (\mathbf{G}^{-1} - \mathbf{A}_{22}^{-1}) \end{bmatrix} \quad (3)$$

where $\mathbf{A}$ is the pedigree relationship matrix; $\mathbf{G}$ is the genomic relationship matrix, and $\mathbf{A}_{22}$ is the pedigree relationship matrix for genotyped animals. The $\mathbf{A}$ matrix includes inbreeding coefficients calculated from pedigree relationships (Meuwissen and Luo, 1992). The genomic relationship matrix, $\mathbf{G}$, was constructed as $0.95\mathbf{G}^* + 0.05\mathbf{A}_{22}$; $\mathbf{G}^*$ is defined according to VanRaden (2008) as:

$$\mathbf{G}^* = \frac{\mathbf{ZZ}'}{2\sum_{i=1}^{M} p_i(1 - p_i)} \quad (4)$$

where $\boldsymbol{Z}$ is a centred matrix of genotype at each locus (*aa* = 0, *Aa* = 1, and *AA* = 2); M is the number of SNP, and p_i is the minor allele frequency at locus i.

To explore the genetic relationship between the two DCT traits (DCT-MAX and DCT-FT), bivariate models were fit for DCT-MAX and DCT-FT at each of the four time points. To estimate the genetic correlation between stages of production for each DCT trait, bivariate models were fit with each pairwise combination of time points (i.e., DCT-MAX at T1-Precalving and DCT-MAX at T2-Calving; DCT-FT at T1-Precalving and DCT-FT at T2-Calving etc.), resulting in six bivariate models for DCT-MAX and six bivariate models for DCT-FT.

One of our objectives was to evaluate the genetic relationship between DCT and CHL. Therefore, we fit bivariate models for combinations of DCT and CHL traits, both within the same time point and between time points. Specifically, we fit bivariate models for DCT-MAX and SL-Severity at each of the four time points and for every pairwise combination of time points, such as DCT-MAX at T2-Calving and SL-Severity at T3-Early, and so on. This was repeated for DCT-FT and

SL-Severity, DCT-MAX and WL-Severity, and finally for DCT-FT and WL-Severity, resulting in an additional 40 bivariate models.

Bivariate models had the same parameters as the univariate models (Equation 1), random effects were assumed to be normally distributed with a mean of zero and covariance structure of:

$$var\begin{bmatrix}\mathbf{a}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{G}_0 \otimes \mathbf{H} & 0\\ 0 & \mathbf{R}_0 \otimes \mathbf{I}\end{bmatrix} \quad (5)$$

where $\mathbf{G}_0$ is the genetic covariance matrix between traits due to animal additive genetic effects; $\mathbf{R}_0$ is the residual covariance matrix between traits; $\otimes$ is the Kronecker product; $\mathbf{H}$ is the relationship matrix, and $\mathbf{I}$ is an identity matrix.

3.2.6 Estimated genomic breeding values and genome-wide association analyses

To address our objective of identifying QTL associated with DCT, we followed a single-step GWA study approach for each DCT trait (DCT-MAX and DCT-FT) at each of the four time points (Wang et al., 2012). The genetic background of CHL was beyond the scope of this study. First, single-step genomic best linear unbiased prediction was implemented in BLUPF90 (Misztal et al., 2018) to calculate estimated genomic breeding values for DCT-MAX and DCT-FT at each time point. Second, the genomic breeding values for DCT-MAX and DCT-FT were back-solved to estimate individual marker effects and *P*-values, using POSTGSF90 (Aguilar et al., 2014, 2019).

In each GWA analysis, genomic inflation was assessed by calculating the inflation factor of the test statistic (Amin et al., 2007). We adjusted for multiple testing with a Bonferroni correction which was considered appropriate for this study given the sample size, genotype density, correlation structure between markers, and the reported polygenic background of DCT (Loh et al., 2022). Significant SNP were defined using a statistical significance threshold of $P \leq 0.05$, which was corrected for multiple testing to 7.67E-07 ($P \leq 0.05$/number of tested markers). Suggestive SNP were defined using a genome-wide threshold

equivalent to one false positive result per genome scan (Lander and Kruglyak, 1995), the suggestive threshold was 1.53E-05 ($P \leq 1$/number of tested markers).

A window-based GWA approach was used to further explore the association between genomic regions and DCT traits (DCT-MAX and DCT-FT). The window size was determined by linkage disequilibrium (**LD**) in our study population (Silva et al., 2020). The magnitude and decay of LD between SNP was evaluated using PLINK (Purcell et al., 2007). On average, LD was found to decay by 50% every 0.65 Mb and therefore sliding windows of 0.65 Mb were used for window-based analyses. The proportion of genetic variance explained by each sliding window of 0.65 Mb was calculated using POSTGSF90, as described by Wang et al. (2014).

3.2.7 Quantitative trait loci and functional analysis

Positional candidate genes were identified using the latest assembly of the *Bos taurus* genome (ARS-UCD 1.2) downloaded from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/assembly/GCF_002263795.1/). In each GWA analysis, the closest gene to each significant or suggestive SNP were identified, up to a maximum of 0.2 Mb upstream or downstream from the marker. Additionally, genes were explored if they were contained, or partially contained, within genomic windows which explained > 0.5% of the total genetic variance. The UniProt database (The UniProt Consortium, 2021) was used for functional annotation of positional candidate genes. Enrichment analysis of the candidate genes from single-marker and window-based GWA analyses was conducted using the DAVID bioinformatic resource (Huang et al., 2009a; b)

3.3 Results

3.3.1 Population and dataset description

A total of 2,352 animals were enrolled in this study: 132 animals from herd A, 432 animals from herd B, 1,549 animals from herd C, and 239 animals from herd D. Details of the final number of animals with phenotype records at each assessment time point, and the timing of each assessment relative to parturition, are provided in **Table 3.2**. In some cases (N = 38), animals were enrolled before parturition but did not subsequently calve because they aborted, died or were euthanised for health reasons. These animals were excluded from further analysis due to the absence of a calving date, despite having phenotypes recorded at T1-Precalving. Additionally, to ensure environmental factors were broadly consistent at each time point, records were excluded from each time point if they fell outside of the planned sampling time frame (see ranges in **Table 3.2,** number of excluded records: T1-Precalving: N = 26, T2-Calving: N = 1, T3-Early: N = 8, T4-Late: N = 6). Most records which fell outside the planned sampling time frame were at T1-Precalving because animals were enrolled based on farm records of expected calving dates which were occasionally inaccurate. A higher number of animals were lost to follow-up between T3-Early and T4-Late due to a break in data collection due to COVID-19 restrictions. In sporadic instances, all four feet were not assessed for lesions at a time point due to the behaviour of the animal in the foot-trimming crush which risked the safety of the animal or researchers. In the final dataset, 99.5% (2,266/2,277), 99.2% (2,108/2,224), and 96.7% (1,868/1,931) of animals had lesion records from all four feet at T1-Precalving, T3-Early, and T4-Late, respectively. At T2-Calving, although 99.0% (2,164/2,185) of animals had lesion records from both hind feet, only 33.6% (734/2,185) had lesion records from all four feet, due to the change in data collection procedure on herd C at this time point. Measurement of DCT at the two pre-determined anatomical locations (DCT-MAX and DCT-FT) was not always possible with high precision and confidence due to the absence or ambiguity of necessary landmarks, in these cases no measurement was recorded to ensure the DCT phenotypes were as accurate as possible. The DCT-FT measurement was missing more frequently than the DCT-MAX measurement due to difficulties

in clearly identifying the point of the flexor tuberosity of the distal phalanx in the stored images.

Table 3.2. Details of data collection at each time point including the timing of each assessment relative to the calving date, the number of feet assessed, digital cushion images collected, and digital cushion thickness measurements from each animal (maximum digital cushion thickness (DCT-MAX) and digital cushion thickness distal to the flexor tuberosity of the distal phalanx (DCT-FT)).

		Assessment time point			
		T1-Precalving	T2-Calving	T3-Early	T4-Late
Timing of assessment relative to parturition (days)	Mean (SD)	-55.2 (18.9)	+5.4 (2.8)	+84.0 (13.6)	+200.0 (31.0)
	Range	-119 to -1	0 to 21	50 to 120	170 to 307
Total number of feet assessed from each animal	One foot only	1	21	1	2
	Two feet only	4	1,427	2	22
	Three feet only	6	3	13	39
	All four feet	2,266	734	2,108	1,868
	Total	2,277	2,185	2,124	1,931
Digital cushion image collected		2,194	2,139	2,061	1,419
Measurement of DCT-MAX		2,091	2,066	1,995	1,380
Measurement of DCT-FT		1,059	1,157	1,020	670

Descriptive details of the phenotypes are provided in **Table 3.3**. Both DCT measurements (DCT-MAX and DCT-FT) followed a similar trend during the production cycle in both primiparous and multiparous animals: the thinnest DCT was recorded at T2-Calving and the thickest at T4-Late. At each time point, the Pearson correlation coefficient between DCT-MAX and DCT-FT ranged from 0.63 to 0.74. In primiparous animals, the prevalence of SH (all severity grades) was highest at T3-Early (67.4%, 392/582); in multiparous animals, it was highest and similar at both T3-Early (53.8%, 830/1,542) and T4-Late (55.1%, 758/1,375). The prevalence of SU followed a similar pattern, the highest prevalence of SU (all severity grades) in primiparous animals was at T3-Early (3.8%, 22/582) and in multiparous animals, it was highest, and similar, at T3-Early (6.9%, 107/1,542) and T4-Late (7.7%, 106/1,375). The prevalence of WL was highest at T4-Late for both primiparous (54.7%, 304/556) and multiparous animals (61.0%, 839/1,375).

Table 3.3. Mean (standard deviation) of each trait at each time point: the maximum digital cushion thickness (DCT-MAX), the digital cushion thickness distal to the flexor tuberosity of the distal phalanx (DCT-FT), the mean sole lesion severity across all feet (SL-Severity), and the mean white line lesion severity across all feet (WL-Severity).

		T1-Precalving	**T2-Calving**	**T3-Early**	**T4-Late**
DCT-MAX (mm)	Primiparous	5.98 (0.79)	5.92 (0.88)	6.26 (0.77)	7.01 (0.78)
	Multiparous	7.10 (0.93)	6.73 (0.86)	7.01 (0.90)	7.23 (0.91)
DCT-FT (mm)	Primiparous	4.95 (0.70)	4.61 (0.77)	5.23 (0.83)	5.80 (0.80)
	Multiparous	5.93 (0.99)	5.43 (0.95)	5.80 (1.00)	6.04 (0.97)
SL-Severity (0-6)	Primiparous	0.12 (0.21)	0.18 (0.34)	0.48 (0.50)	0.24 (0.36)
	Multiparous	0.20 (0.39)	0.26 (0.55)	0.37 (0.49)	0.40 (0.57)
WL-Severity (0-3)	Primiparous	0.18 (0.28)	0.20 (0.32)	0.12 (0.22)	0.27 (0.33)
	Multiparous	0.12 (0.22)	0.17 (0.33)	0.18 (0.27)	0.29 (0.31)

3.3.2 Genetic parameters

Four traits (DCT-MAX, DCT-FT, SL-Severity, and WL-Severity) were analysed at four assessment time points with single-trait models, estimates of variance components and heritability are provided in **Table 3.4.** The genetic correlation was estimated with bivariate models. The two DCT traits (DCT-MAX and DCT-FT) were strongly genetically correlated with each other at each time point, with estimates ranging from 0.95 (± 0.03) to 1.00 (± 0.001). The genetic correlation between time points was between 0.92 (± 0.04) and 1.00 (± 0.001) for DCT-MAX, and between 0.88 (± 0.26) and 1.00 (± 0.01) for DCT-FT.

The genetic correlations between DCT traits (DCT-MAX and DCT-FT) and CHL traits (SL-Severity and WL-Severity), both within each time point and between time points, are provided in **Table 3.5** and **Table 3.6**. The genetic correlation between DCT-MAX and SL-Severity was generally negative; the 95% confidence interval of this estimate did not include zero on five occasions: between DCT-MAX at T2-Calving and SL-Severity at T3-Early and T4-Late (-0.33 and -0.37, respectively), between DCT-MAX at T3-Early and SL-Severity at T3-Early and T4-Late (-0.33 and -0.38, respectively), and between DCT-MAX and SL-Severity at T4-Late (-0.35). The genetic correlation between DCT-FT and SL-Severity followed a similar pattern but there were only two occasions where the 95% confidence interval of this estimate did not include zero: between DCT-FT at T2-Calving and SL-Severity at T3-Early and T4-Late (-0.44 and -0.30, respectively). The genetic correlation between DCT traits and WL-Severity was effectively zero (95% confidence interval of estimate included zero) on all occasions except between DCT-MAX at T3-Early and WL-Severity at T4-Late (0.29).

Table 3.4. Additive genetic variance (σ_a^2), residual variance (σ_e^2), and narrow-sense heritability (h2) estimates (standard error) from single-trait analysis at each time point for the maximum digital cushion thickness (DCT-MAX), the digital cushion thickness distal to the flexor tuberosity of the distal phalanx (DCT-FT), the mean sole lesion severity across all feet (SL-Severity), and the mean white line lesion severity across all feet (WL-Severity)

Trait	**Time point**	**Number of animals**	σ_a^2	σ_e^2	**h²**
DCT-MAX	T1-Precalving	2,091	0.18 (0.03)	0.61 (0.03)	0.23 (0.04)
	T2-Calving	2,066	0.21 (0.03)	0.52 (0.03)	0.29 (0.04)
	T3-Early	1,995	0.21 (0.03)	0.51 (0.03)	0.29 (0.04)
	T4-Late	1,380	0.32 (0.05)	0.40 (0.04)	0.44 (0.06)
DCT-FT	T1-Precalving	1,059	0.11 (0.05)	0.67 (0.05)	0.14 (0.06)
	T2-Calving	1,157	0.21 (0.05)	0.58 (0.05)	0.26 (0.06)
	T3-Early	1,020	0.12 (0.05)	0.68 (0.05)	0.15 (0.06)
	T4-Late	670	0.21 (0.08)	0.52 (0.07)	0.29 (0.10)
SL-Severity	T1-Precalving	2,277	0.018 (0.004)	0.09 (0.004)	0.16 (0.03)
	T2-Calving	2,185	0.028 (0.006)	0.21 (0.008)	0.12 (0.03)
	T3-Early	2,124	0.043 (0.009)	0.17 (0.008)	0.20 (0.04)
	T4-Late	1,931	0.038 (0.009)	0.20 (0.010)	0.16 (0.04)
WL-Severity	T1-Precalving	2,277	0.005 (0.002)	0.05 (0.002)	0.09 (0.03)
	T2-Calving	2,185	0.007 (0.003)	0.10 (0.004)	0.07 (0.03)
	T3-Early	2,124	0.007 (0.002)	0.05 (0.002)	0.11 (0.03)
	T4-Late	1,931	0.013 (0.001)	0.09 (0.004)	0.13 (0.04)

Table 3.5. Genetic correlation (standard error) between the maximum digital cushion thickness (DCT-MAX) or the digital cushion thickness distal to the flexor tuberosity of the distal phalanx (DCT-FT) and mean severity of sole lesions across all feet (SL-Severity). Values on the diagonal refer to the genetic correlation between traits which were both recorded at the same time point, above the diagonal refers to the genetic correlation between traits which were recorded at different time points.

		SL-Severity			
		T1-Precalving	T2-Calving	T3-Early	T4-Late
DCT-MAX	T1-Precalving	-0.12 (0.12)	-0.23 (0.12)	-0.17 (0.11)	-0.18 (0.12)
	T2-Calving		-0.12 (0.12)	-0.33 (0.10)*	-0.37 (0.11)*
	T3-Early			-0.33 (0.10)*	-0.38 (0.11)*
	T4-Late				-0.35 (0.11)*
		SL-Severity			
		T1-Precalving	T2-Calving	T3-Early	T4-Late
DCT-FT	T1-Precalving	0.17 (0.22)	0.06 (0.22)	-0.11 (0.22)	-0.09 (0.23)
	T2-Calving		-0.11 (0.15)	-0.44 (0.12)*	-0.30 (0.14)*
	T3-Early			-0.47 (0.46)	-0.07 (0.23)
	T4-Late				-0.37 (0.42)

**95% confidence interval does not include zero.*

Table 3.6. Genetic correlation (standard error) between the maximum digital cushion thickness (DCT-MAX) or the digital cushion thickness distal to the flexor tuberosity of the distal phalanx (DCT-FT) and mean severity of white line lesions across all feet (WL-Severity). Values on the diagonal refer to the genetic correlation between traits which were both recorded at the same time point, above the diagonal refers to the genetic correlation between traits which were recorded at different time points.

		WL-Severity			
		T1-Precalving	T2-Calving	T3-Early	T4-Late
DCT-MAX	T1-Precalving	0.32 (0.16)	0.04 (0.05)	0.23 (0.16)	-0.08 (0.14)
	T2-Calving		0.04 (0.17)	0.18 (0.13)	-0.04 (0.13)
	T3-Early			0.13 (0.14)	0.29 (0.14)*
	T4-Late				0.21 (0.14)
		WL-Severity			
		T1-Precalving	T2-Calving	T3-Early	T4-Late
DCT-FT	T1-Precalving	0.42 (0.47)	0.29 (0.55)	0.44 (0.54)	0.26 (0.28)
	T2-Calving		0.41 (0.27)	0.34 (0.18)	-0.03 (0.18)
	T3-Early			0.45 (0.32)	0.46 (0.30)
	T4-Late				0.40 (0.38)

**95% confidence interval does not include zero.*

3.3.3 Quantitative trait loci and functional analysis

GWA analysis was performed for each DCT trait (DCT-MAX, DCT-FT) at each time point. The genomic inflation factor ranged from 0.94 to 1.00 in the eight single-marker GWA analyses. Single-marker analyses revealed a polygenic background to both DCT-MAX and DCT-FT at each time point (**Figure 3.1**). From all GWA analyses, only one significant SNP was identified: for DCT-MAX at T3-Early on *Bos taurus* autosome 4 (BTA-4). Suggestive SNP were identified on BTA-3, BTA-5, BTA-6, BTA-13, BTA-14, BTA-23, and BTA-26 (**Table 3.7**). Positional candidate genes located closest to each suggestive or significant SNP, and within 0.2 Mb upstream or downstream of the marker, are presented in **Table 3.7**.

Window-based GWA analyses showed a similarly complex genetic background to DCT-MAX and DCT-FT (**Figure 3.2**). The same genomic window on BTA-3 (comprising 31 SNP) explained more than 1% of the total genetic variance for DCT-MAX at T2-Calving and T3-Early, and a neighbouring window (comprising 29 SNP) explained 0.72% of total genetic variance for DCT-MAX at T1-Precalving. Other genomic windows which explained more than 0.5% of the total genetic variance were identified on BTA-5, BTA-6, BTA-8, BTA-11, BTA-14, and BTA-21 (**Table 3.8**). All genes contained, or partially contained, within these genomic windows are presented in **Table 3.8**.

The set of positional candidate genes, identified from single-marker and window-based GWA analyses, were enriched in biological processes of five Gene Ontology (**GO**) terms: positive regulation of I-kappaB kinase/NF-kappaB signalling (GO:0043123); positive regulation of CREB transcription factor activity (GO: 0032793); cellular response to a mechanical stimulus (GO:0071260); cell adhesions (GO:0007155), and positive regulation of phosphatidylinositol 3-kinase signalling (GO: 0014068).

Figure 3.1. Manhattan plots and quantile-quantile plots of the maximum digital cushion thickness (DCT-MAX) and the digital cushion thickness distal to the flexor tuberosity of the distal phalanx (DCT-FT) at each time point; -log10 *P*-value of marker effects against marker position on the chromosome. The solid line represents the genome-wide significance threshold (P ≤ 7.67E-07, 0.05/number of tested markers) and the dashed line represents the suggestive threshold (P ≤ 1.53E-05, 1/number of tested markers).

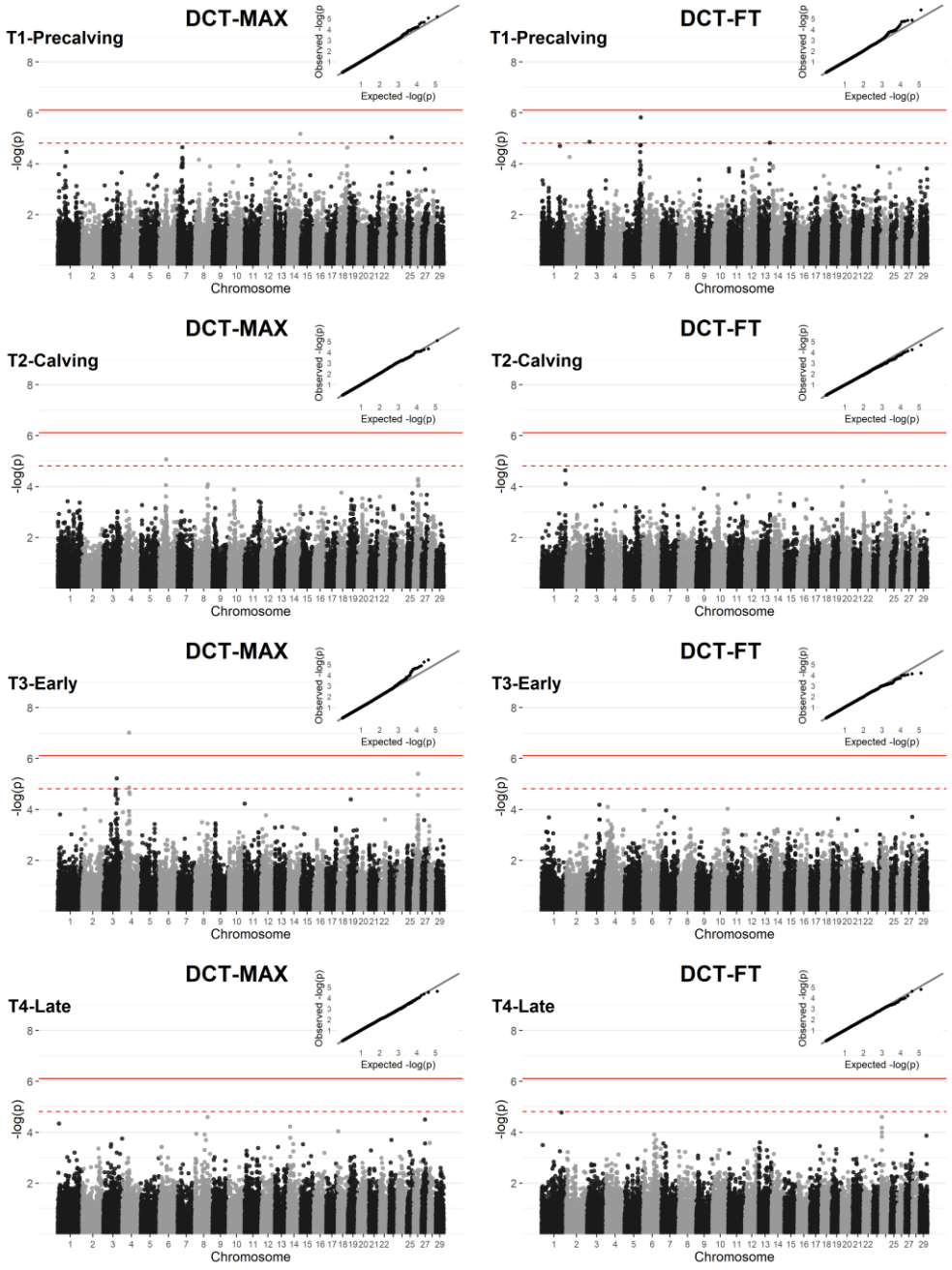


Table 3.7. Markers with a significant ($P \leq$ 7.67E-07, 0.05/number of tested markers) or suggestive effect ($P \leq$ 1.53E-05, 1/number of tested markers) on maximum digital cushion thickness (DCT-MAX) or digital cushion thickness distal to the flexor tuberosity of the distal phalanx (DCT-FT) at each time point, with the respective chromosome (BTA), position (bp), minor allele frequency (MAF), *P*-value of marker effect, and name and location of the closest gene up to a maximum of 0.2 Mb upstream or downstream from the marker.

Trait	Time point	BTA	Position (bp)	MAF	*P*-value	Gene	Gene location
DCT-MAX	T1-Precalving	14	81,367,974	0.25	6.58E-06	*DEPTORY*	81,286,837 - 81,402,413
		23	16,504,326	0.33	8.96E-06	*BICRAL**	16,471,820 - 16,506,078
	T2-Calving	6	42,273,485	0.19	8.43E-06	*GBA3**	42,263,666 - 42,395,508
	T3-Early	3	84,753,032	0.27	6.05E-06	*NFIA*	84,203,793 - 84,620,790
		4	44,839,097	0.32	9.56E-08	*RELN**	44,652,801 - 45,211,015
		26	28,997,874	0.48	3.96E-06	-	-
DCT-FT	T1-Precalving	3	15,443,604	0.08	1.37E-05	*TRIM46*	15,433,548 - 15,443,291
		5	104,055,417	0.49	1.51E-06	*TNFRSF1A*	104,024,027 - 104,036,846
		13	71,799,248	0.22	1.48E-05	-	-

**Marker was located inside candidate gene*

Figure 3.2. Proportion of the total additive genetic variance explained by sliding 0.65 Mb windows for the maximum digital cushion thickness (DCT-MAX) and the digital cushion thickness distal to the flexor tuberosity of the distal phalanx (DCT-FT) at each time point.

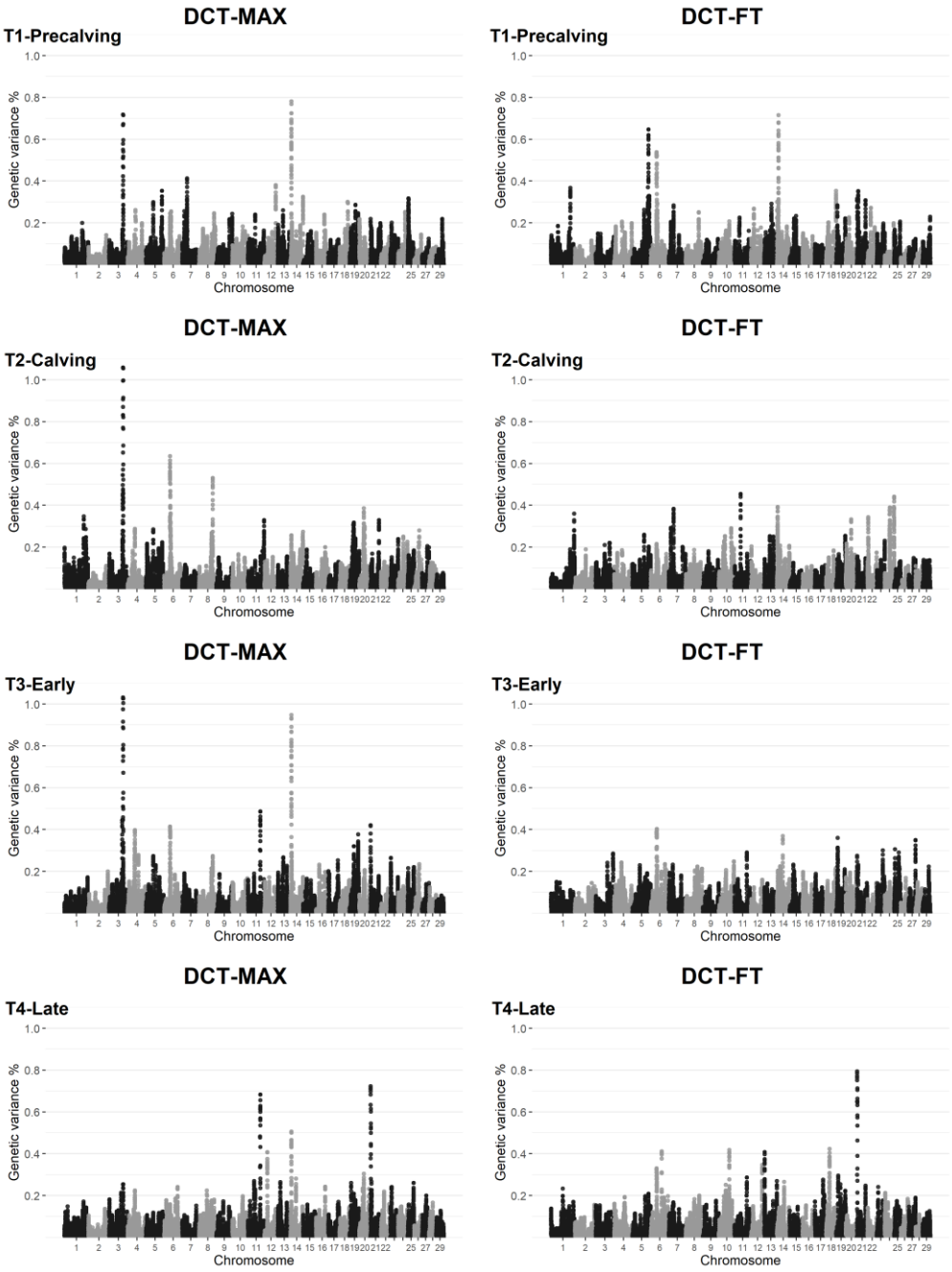


Table 3.8. Genomic regions which explained more than 0.5% of the total additive genetic variance for the maximum digital cushion thickness (DCT-MAX) and the digital cushion thickness distal to the flexor tuberosity of the distal phalanx (DCT-FT) at each time point, with the respective chromosome (BTA), window position (bp), the proportion of total genetic variance explained, and the name of gene(s) contained (or partially contained) within these windows.

Trait	Stage	BTA	Window position (bp)	Variance (%)	Gene(s)
DCT-MAX	T1-Precalving	3	90,742,849 - 91,356,392	0.72	*BSND, TMEM61*
		14	5,880,036 - 6,526,644	0.78	*KHDRBS3*
	T2-Calving	3	90,725,628 - 91,356,392	1.06	*BSND, TMEM61*
		6	37,690,172 - 38,332,952	0.64	-
		8	88,134,972 - 88,775,323	0.53	*GADD45G, SEMA4D*
	T3-Early	3	90,725,628 - 91,356,392	1.03	*BSND, TMEM61*
		14	5,880,036 - 6,526,644	0.95	*KHDRBS3*
	T4-Late	11	78,811,939 - 79,407,746	0.68	*LAPTM4A, TTC32, OSR1*
		14	5,998,335 - 6,619,386	0.51	*KHDRBS3*
		21	2,416,354 - 3,040,671	0.72	-
DCT-FT	T1-Precalving	5	103,798,423 - 104,430,699	0.65	*LPAR5, CHD4, NOP2, IFFO1, GAPDH, NCAPD2, MRPL51, VAMP1, TAPBPL, CD27, LTBR, SCNN1A, TNFRSF1A, CD9, VWF*
		6	37,796,921 - 38,426,291	0.54	-
		14	8,954,477 - 9,597,429	0.72	KCNQ3, EFR3A
	T4-Late	21	2,416,354 - 3,040,671	0.79	-

3.4 Discussion

3.4.1 Key results and interpretation: genetic parameters

We have corroborated the results of previous studies which demonstrated that DCT, as measured using ultrasonography, is a heritable trait in Holstein cows (Oikonomou et al., 2014; Sánchez-Molano et al., 2019; Stambuk et al., 2020a; b). Therefore, there could be scope to increase the average thickness of the digital cushion in a population through selective breeding, and this may translate to a reduced incidence of CHL (Machado et al., 2011; Toholj et al., 2014; Newsome et al., 2017b; Stambuk et al., 2019; Griffiths et al., 2020).

The heritability estimates in our study tended to be higher for the maximum DCT measurement (DCT-MAX, heritability: 0.23 - 0.44) compared to the DCT measurement taken distal to the flexor tuberosity of distal phalanx (DCT-FT, heritability: 0.14 – 0.29). The DCT-FT measurement is arguably more clinically relevant than DCT-MAX for sole lesion development, as this corresponds to the predilection site for these lesions and DCT-FT has been shown, phenotypically, to correlate with CHL risk (Machado et al., 2011; Toholj et al., 2014; Newsome et al., 2017b; Griffiths et al., 2020). We also observed a trend whereby the heritability of the DCT was dependent on the stage of lactation; the heritability of DCT (both DCT-MAX and DCT-FT) was lowest at T1-Precalving and highest at T4-Late. These differences are partially a reflection of changes in environmental variance; however, the additive genetic variance was highest at the T4-Late assessment. This trend is difficult to explain biologically and previous studies have not reported genetic parameters for DCT from different lactation stages (Oikonomou et al., 2014; Sánchez-Molano et al., 2019; Stambuk et al., 2020a; b), therefore we do not know if this observation is consistent in other populations. Overall, the heritability estimates of DCT in our study (both DCT-MAX and DCT-FT) were broadly comparable to previous studies of Holsteins where estimates range from 0.23 to 0.33 (Oikonomou et al., 2014; Sánchez-Molano et al., 2019; Stambuk et al., 2020b).

A key objective of our study was to estimate the genetic correlation between DCT and CHL. The genetic correlation between DCT traits and WL-Severity was

not statistically different from zero, except between DCT-MAX at T3-Early and WL-Severity at T4-Late when it was positive (0.29 ± 0.14). This single positive genetic correlation should be interpreted cautiously due to the large standard errors of this estimate, and because this was the only non-zero genetic correlation between these traits. If there truly is a positive genetic correlation between DCT and WL, it may be a reflection of previous observations that there is a potentially positive genetic correlation between body condition and DCT (Oikonomou et al., 2014), a positive genetic correlation between body condition and body weight (Berry et al., 2003), and a positive phenotypic association between body weight and WL (Schöpke et al., 2013; Pérez-Cabal and Charfeddine, 2016). This is a convoluted explanation, in part because the direct relationship between body condition and WL has not been established. Therefore, more research would be beneficial to assess the relationship between body condition and WL, as well as the genetic and phenotypic relationships between DCT and WL.

Our results indicated that DCT traits were negatively correlated with SL-Severity at several time points, although the magnitude of the genetic correlation in these instances was relatively small. A negative genetic correlation between DCT and CHL incidence was reported by Oikonomou et al. (2014), our results imply that this relationship may have been due to a negative genetic correlation between DCT and sole lesions (SH and SU), rather than between DCT and WL. Correlated traits can be incorporated into a composite selection index to increase the accuracy of selection and improve genetic progress (Boettcher et al., 1998; Banos et al., 2006), therefore there could be a benefit to including DCT in selection indexes for claw health. However, DCT is more challenging to record than foot lesions, so this is unlikely to be practical unless DCT was recorded in an intensively monitored reference population (Pryce et al., 2012; Calus et al., 2013b; Coffey, 2020).

The strength of the genetic correlation between DCT traits and SL-Severity we observed was generally weak, with large standard errors, and in many cases, the 95% confidence interval included zero. Unbiased estimates of genetic

correlation are effectively impossible to obtain (Lynch and Walsh, 1998), therefore care should be taken not to over-interpret the magnitude of these estimates or extrapolate them across populations. However, we consider the occasions where the 95% confidence interval of the correlation estimate did not include zero to be the most persuasive evidence for a truly negative relationship between the additive genetic variance of both traits; these specific results are interesting in the context of the proposed pathogenesis of sole lesion development.

We observed a weak negative genetic correlation between the DCT immediately after calving and the severity of sole lesions later in lactation. It has been demonstrated that the suspensory apparatus of the distal phalanx is weaker around parturition which may result in compression of the underlying soft tissues, which include the digital cushion and corium (Tarlton et al., 2002; Knott et al., 2007). Compression of the corium is thought to be detrimental to claw horn production and to initiate the development of SH or SU (Lischer et al., 2002b; Lischer and Ossent, 2002). The weak negative genetic correlation between DCT immediately after calving and future sole lesions lends support to this hypothesis from a genetic perspective. However, correlation is a bidirectional relationship and therefore an alternative explanation also exists. The development of CHL has been hypothesised to use fatty acids from the digital cushion as inflammatory mediators, thereby reducing the adipose tissue in the digital cushion, causing a reduction in thickness, and a presumed impairment of its functionality (Ossent and Lischer, 1998; Lischer et al., 2002b; Räber et al., 2006). Development of CHL has also been shown to increase the risk of future CHL development (Hirst et al., 2002; Oikonomou et al., 2013); therefore, the weak genetic correlation we observed, between DCT immediately after calving and sole lesions later in lactation, could be due to the occurrence of previous sole lesions and the genetic background to these historic lesions could affect future DCT and sole lesion risk. As the lameness history of animals prior to the start of this study is unknown, we cannot differentiate between these potential explanations with this data set.

Periparturient compression of the digital cushion is an intuitive explanation for our observation that DCT was thinnest immediately after calving, a finding replicated elsewhere (Newsome et al., 2017a; Bach et al., 2021). Our results indicated that DCT was strongly genetically correlated between stages of lactation and the additive genetic variance estimates were similar at T2-Calving to other time points. Therefore, it would appear there is not a major genetic component which explains why the DCT is thinnest immediately after calving beyond the genetic background to DCT which exists generally. We would conclude from these results that the extent of periparturient laxity in the suspensory apparatus of the distal phalanx, and associated compression of soft tissues, is more likely to be determined by environmental factors than genetic.

We recorded the DCT to be thinner at T3-Early than it was at either T1-Precalving or T4-Late, in agreement with previous research (Bicalho et al., 2009; Newsome et al., 2017a; Griffiths et al., 2020). High-yielding dairy cows mobilise extreme quantities of fat in early lactation (McNamara, 1991; Drackley et al., 2006) and the digital cushion is primarily composed of adipose tissue (Räber et al., 2004, 2006). It has been proposed that lipolysis during early lactation depletes the adipose tissue of the digital cushion and this is responsible for the observed reduction in DCT at this time, which in turn, is linked to an increased risk of CHL development (Bicalho et al., 2009). We observed a weak negative genetic correlation between DCT at T3-Early and T4-Late, and the severity of sole lesions at both T3-Early lactation and T4-Late time points. This would suggest the genetic tendency to have a thin DCT in early and late lactation is correlated with the genetic predisposition to develop more severe sole lesions at these times. However, the phenotypic relationships between fat mobilisation, DCT, and CHL development are complicated and reduced subcutaneous backfat is also associated with increased CHL risk independent of DCT (Newsome et al., 2017b). Therefore, it would be interesting for future studies to consider the genetic relationships between subcutaneous fat, DCT, and CHL development.

3.4.2 Key results and interpretation: quantitative trait loci

Characterisation of the genetic background of DCT revealed a complex trait, in agreement with previous research (Sánchez-Molano et al., 2019; Stambuk et al., 2020a; b). The marker with the strongest evidence of an effect on DCT was observed on BTA-4 for DCT-MAX at T3-Early. This SNP is situated within the *RELN* (Reelin) gene. In cattle, *RELN* is primarily expressed in central nervous tissue and is involved in neuron development (Fang et al., 2020; The UniProt Consortium, 2021). Although there is not an immediately intuitive link between the central nervous system and the digital cushion, the *RELN* gene was also part of three out of the five enriched biological pathways from the analysis of all candidate genes. These biological pathways were CREB transcription factor activity, cell adhesion, and phosphatidylinositol 3-kinase signalling. In dairy cattle, CREB is associated with periparturient lipid metabolism and is considered to be a key regulator of adipogenesis (McNamara et al., 1992). Phosphatidylinositol 3-kinase is an enzyme that is involved in insulin signalling and mediates glucose and lipid metabolism (Shepherd et al., 1998). Therefore, CREB transcription factor activity and phosphatidylinositol 3-kinase signaling pathways could plausibly relate to the thickness of the digital cushion and may underlie the observed association between DCT and the *RELN* gene, although this requires further investigation.

Many of the candidate genes we identified have roles in inflammation. In humans, *RELN* has been associated with bone development due to inflammation (Garshasbi et al., 2020), as has another candidate gene, *SEMA4D* (semaphorin 4D), which was identified for DCT-MAX at T2-Calving (Lontos et al., 2018). Localised inflammation in the bovine hoof has been linked with the development of bone growth on the distal phalanx (Lischer et al., 2002b; Newsome et al., 2016), and genes relating to inflammation and bone growth have previously been associated with DCT in dairy cattle (Stambuk et al., 2020a; b). One of the enriched biological processes was I-κB kinase/NF-κB signalling, a complex pathway which is instrumental in a wide range of inflammatory responses (Ghosh and Hayden, 2008; Liu et al., 2017). This pathway is recognised to have an important role in inflammatory osteolysis in humans (Boyce et al., 2010; Abu-Amer, 2013), and inflammation in diseases linked to lipid metabolism (Barma et al., 2009; Baker et

al., 2011). In cattle, ketone bodies, which are produced from the metabolism of fatty acids, are reported to activate the NF-κB pathway in bovine hepatocytes (Shi et al., 2014). It is possible that genetic regulation of inflammation, particularly if also associated with bone changes or lipid metabolism, could directly or indirectly influence the digital cushion.

The NF-κB pathway is activated by lipopolysaccharide and this pathway has been linked to subacute ruminal acidosis and clinical mastitis in dairy cows (Fan et al., 2016; Khan et al., 2020). An in vitro study demonstrated lipopolysaccharide caused inflammation of the dermal cells in the bovine hoof (Tian et al., 2019), and systemic administration of lipopolysaccharide in vivo has been reported to induce histological changes in the laminae (Boosman et al., 1991). Inflammation of the laminae is hypothesised to cause laxity in the suspensory apparatus of the distal phalanx (Ossent and Lischer, 1998), which would result in compression of the digital cushion.

Although candidate genes or biological pathways with roles in inflammation may affect the digital cushion, the DCT has been reported to increase when CHL were present due to inflammation in the corium (Ossent and Lischer, 1998; Newsome et al., 2017a). Therefore, as the DCT measurement in our study included both the digital cushion and the corium, it is also possible that genes associated with inflammation affect DCT due to inflammation in the corium, rather than directly affecting the thickness of the digital cushion.

The genomic region which explained the greatest proportion of the total genetic variance for DCT at any time point was on BTA-3 (90.73 – 91.36 Mb). This region explained 1.06% and 1.03% of the total genetic variation of DCT-MAX at T2-Calving and T3-Early, respectively, and contained two candidate genes: *BSND* (barttin CLCNK type accessory subunit beta) and *TMEM61* (transmembrane protein 61). The *BSND* gene is associated with chloride transport and *TMEM61* is unannotated (The UniProt Consortium, 2021), therefore it is not clear how these genes may relate to the digital cushion. However, QTL in this window have previously been associated with fat percentage and mineral content of milk in dairy cattle (Buitenhuis et al., 2015; van den Berg et al., 2020), and with luteal

activity in early lactation (Tenghe et al., 2016). Therefore, this part of the genome may be worth further investigation in dairy cattle.

Other results of interest from GWA analyses include the genomic region on BTA-14 (5.88 – 6.53 Mb) which explained 0.78% and 0.95% of the total genetic variance for DCT-MAX at T1-Precalving and T3-Early, respectively. This window was also adjacent to a region (6.00 – 6.62 Mb) which explained 0.51% of the total genetic variance for DCT-MAX at T4-Late. The candidate gene in these windows was KHDRBS3 (KH RNA Binding Domain Containing, Signal Transduction Associated 3). This gene has been associated with average daily gain in cattle (Seabury et al., 2017), and is in LD with a neighbouring gene associated with intramuscular fat deposition (Barendse et al., 2004; Gibbs et al., 2009). The digital cushion is primarily composed of adipose tissue and *KHDRBS3* is expressed in both adipose and muscle tissue in cattle (Räber et al., 2006; Fang et al., 2020). Therefore, there is a biologically plausible association between *KHDRBS3* and DCT. The *KHDRBS3* gene was also associated with SU development in a GWA study of CHL using the same dataset (Chapter 2), so *KHDRBS3* may contribute to the genetic correlation between DCT and sole lesions.

The other candidate genomic region on BTA-14 (8.95 – 9.60 Mb), which explained 0.72% of the total genetic variation for DCT-FT at T1-Precalving, was also associated with SU development in GWA analysis of CHL using this dataset (Chapter 2). Candidate genes in this window were *KCNQ3* (potassium voltage-gated channel subfamily Q member 3) and *EFR3A* (EFR3 Homolog A). The *KCNQ3* gene has previously been associated with milk fat percentage and milk yield in Holsteins (Kolbehdari et al., 2008; Jiang et al., 2019), while *EFR3A* has previously been associated with subclinical ketosis, milk fat percentage, and milk fatty acid composition in Holsteins (Li et al., 2014; Jiang et al., 2019; Soares et al., 2021). The lipid composition of the digital cushion changes with age (Räber et al., 2006) and is correlated with body condition (Hiss-Pesch et al., 2019; Newsome et al., 2021). Although it is not clear how the composition of the digital cushion affects its physical properties, it is possible that the same genes which affect the fat and fatty acid content of milk could have a similar influence on the digital cushion.

Additionally, milk yield, body condition, and subclinical ketosis have all been linked to CHL development (Amory et al., 2008; Green et al., 2014; Sepúlveda-Varas et al., 2018), so *KCNQ3* and *EFR3A* may also contribute to the genetic correlation between DCT and sole lesions.

Two of the candidate genes highlighted for DCT have previously been associated with conformation traits in cattle. The *OSR1* (Odd-Skipped Related Transcription Factor 1) gene on BTA-11 (identified for DCT-MAX at T4-Late) has been linked to multiple conformation traits including feet and leg conformation, rear leg placement, and rump width (Cole et al., 2011). The *VWF* (von Willebrand factor) gene on BTA-5 (identified for DCT-FT at T1-Precalving) has previously been associated with foot angle (Kolbehdari et al., 2008). It has been shown that the volume of the digital cushion increases when growing calves are exercised on rough terrain (Gard et al., 2015), which implies the size of the digital cushion is affected by external forces. Therefore, it is conceivable that genes which affect limb conformation could influence DCT.

We did not identify any QTL or candidate genes which were highlighted by previous GWA studies of DCT (Stambuk et al., 2020a; b). However, one candidate gene was *TRIM46* (tripartite motif containing 46) on BTA-3 (identified for DCT-FT at T1-Precalving), and one of the candidate genes for DCT which was identified by Stambuk et al. (2020b) was *TRIM55* (tripartite motif containing 55). Although *TRIM46* and *TRIM55* are in different families (Short and Cox, 2006; Ozato et al., 2008), TRIM proteins are associated with immune responses (Yang and Xia, 2021) and therefore there is a potential link between the genetic control of the immune system and DCT which would benefit from future research. Additionally, we highlighted QTL on BTA-3 (90.73 – 91.36 Mb) and BTA-14 (81.37 Mb) which are relatively close to QTL reported by Stambuk et al. (2020a) on BTA-3 (95.85 – 95.93 Mb) and BTA-14 (80.04 – 80.66 Mb); these genomic regions may also be worth further exploration.

Overall, in the context of the limited previous research in this area, our results replicate some of the reported findings as well as provide additional data. Given the number of markers tested in eight GWA analyses, only a small number

of markers were associated with significant or suggestive effects on DCT. Our results did not corroborate any of the specific QTL reported in two previous GWA studies of DCT by Stambuk et al. (2020a; b). Although the biological grouping and function of highlighted genes were similar in our results to those reported by Stambuk et al. (2020a; b), speculation about candidate genes relies heavily on the existing understanding of the biology of the trait in question, therefore this agreement in terms of gene function would be expected.

3.4.3 Study strengths and limitations

We have estimated the genetic parameters and characterised the genetic background of digital cushion thickness using the largest dataset currently available. In addition to the size of the study population, the prospective cohort study design and accuracy of phenotype recording are further strengths of this study, however, there are some important limitations which we acknowledge.

One of the limitations of this study population was the small number of farms included, which could reduce estimates of environmental variance and inflate the estimated heritability. This is also a feature of previous research so we have limited context from which to speculate as to how strongly this would affect our results. It is also important to note that almost two-thirds of the study population were from a single herd; replication of results in a wider and more diverse population would strengthen the interpretation of our findings.

A relatively large proportion of animals had missing data for the DCT-FT measurement due to the absence or ambiguity of anatomical landmarks in the stored ultrasound images, we did not record DCT-FT from these images to maintain a high accuracy of this phenotype. We designed our study to collect and store images which were retrospectively blinded and measured. If we had measured images at the same time as data collection we could have reduced the number of missing measurements, however, subconscious biases can influence this process unless these measurements are taken blinded to factors such as stage of lactation, body condition and presence of lesions (Griffiths et al., 2020). We applied stringent criteria to all stored images to ensure all DCT

measurements were consistent and accurate, but this approach resulted in more missing DCT-FT measurements and reduced study power for this trait.

It has recently been shown, albeit in a small cohort of animals, that ultrasound measurements of DCT in weight-bearing and non-weight-bearing feet are only weakly correlated (Bach et al., 2021), therefore the key assumption that our DCT measurements translate to DCT during standing and walking is potentially undermined. There are also wider concerns about the interpretation of ultrasound measurements of the digital cushion. A recent study quantified the volume of the digital cushion in cadavers using magnetic resonance imaging (MRI) and found the volume of the digital cushion in the lateral claws of hindfeet to range from 0 – 30 mL, with the middle digital cushion pad often completely absent (Wilson et al., 2021). Wilson et al. (2021) consider ultrasonographic DCT measurements to therefore relate exclusively to the corium in many cases, particularly when targeting the middle fat pad (i.e. DCT-FT) (Wilson et al., 2021). Previous estimates of corium thickness are no more than approximately 3 - 4 mm on ultrasound images (Toholj et al., 2014; Newsome et al., 2017a). In our study, a DCT-FT measurement of 4 mm corresponded to the 5th percentile implying that in 95% of cases the DCT-FT measurements are unlikely to only represent the corium. We would consider it more likely that these measurements also include connective tissue which is reported to replace adipose tissue in the digital cushion (Ossent and Lischer et al., 2002b). This explanation would be more consistent with the negative correlation reported between the thickness and echotexture of the digital cushion (Bicalho et al., 2009), because the corium is anechoic (Kofler et al., 1999). Regardless, the relationship of the corium with CHL development is not fully understood. A thin corium has been associated with future CHL development (Toholj et al., 2014), whereas a thickened corium is associated with the presence of a concurrent CHL (Lischer et al., 2002b; Newsome et al., 2017a). Interestingly, the ultrasonographic thickness of the corium has also been shown to correlate to subcutaneous fat thickness (Newsome et al., 2017a), which presents a further complication to the hypothesised pathogenesis of CHL which appears to at least include the digital

cushion, corium, and subcutaneous fat, as well as the relationships between these factors.

In conclusion, it is reasonable to question whether DCT measured using ultrasonography is the most important property of the digital cushion in terms of force dissipation and CHL development. There have been studies which described the composition of the digital cushion and its relationship with body condition and foot lesions (Räber et al., 2006; Hiss-Pesch et al., 2019; İzci et al., 2019; Newsome et al., 2021), but results are still equivocal or preliminary in terms of implications for CHL development. There are limitations in defining the ability of the digital cushion to effectively dissipate forces in the foot by its thickness, and key questions about how to infer the functionality of the digital cushion from either physical dimensions or its composition remain unanswered. It is also fair to say the measurement of the digital cushion using ultrasound is likely to be an example of an observational bias, where its importance may have been overestimated due to the relative ease of measurement. Further studies should attempt to utilise different approaches to assess the functionality of the digital cushion; but unfortunately, no such techniques have yet been described which could be employed in the type of longitudinal study required to clarify the role of the digital cushion in the pathogenesis of CHL.

3.4.4 Generalisability

Caution is required to generalise the genetic parameters and QTL reported in this study, particularly given the polygenic nature of DCT and the small number of herds. Our results are from a population of Holstein cows on four dairy herds which were all commercially run with operating practices common to many UK dairy farms, but could not be considered representative of the full spectrum of dairy farms. Within these four herds, three were operating similar and relatively intensive systems of zero-grazing and three times a day milking. The overall period prevalence of lame cows (Mahendran et al., 2017), based on repeated mobility scores throughout this project, ranged from 18.5% to 33.3% across the four herds; the mean point prevalence of lameness from all time points ranged from 6% to 11.8% across the four herds (data not shown). Recent cross-sectional

studies in the UK reported that herd lameness prevalence ranged from 6% to 65%; this suggests the four herds in our study had a lower prevalence of lameness compared to many dairy herds in the UK (Griffiths et al., 2018; Randall et al., 2019).

3.5 Conclusions

The results from this prospective cohort study indicate that digital cushion thickness is a heritable trait which is weakly negatively genetically correlated with the severity of sole lesions, but not with white line lesions. The strength of the genetic correlation between digital cushion thickness and sole lesions depends on the stage of lactation at which both the digital cushion and sole lesions are assessed. Digital cushion thickness is a polygenic trait, and few QTL were associated with observable effects. Candidate genes identified for DCT are related to inflammation, fat metabolism and bone development. Further work is needed to investigate these candidate genes and establish the precise role of the digital cushion in the pathogenesis of sole lesions.

Chapter 4: Genetic parameters of sole lesion recovery in Holstein cows

4.1 Introduction

Lameness in dairy cattle is a conspicuously painful condition that is ranked as the most important indicator of animal welfare on dairy farms (Whay et al., 2003b; Bicalho and Oikonomou, 2013). Lameness is also a major barrier to productivity because it is associated with reduced milk production (Green et al., 2002; Amory et al., 2008), poorer fertility (Melendez et al., 2003; Garbarino et al., 2004), and increased risk of culling (Booth et al., 2004; Bicalho et al., 2007b). One reason lameness has such a severe impact on both welfare and productivity is the long duration of behavioural and physiological changes attributed to lameness, which can persist for weeks or even months (Whay et al., 1998; Almeida et al., 2008; Laven et al., 2008).

Lameness is highly prevalent in dairy cows, in the United Kingdom (**UK**) a recent meta-analysis, using data from 27 studies published between 2000 and 2020, estimated the national prevalence to be between 30% - 40% (Afonso et al., 2020). Approximately 50% of lame cows are chronically lame (Archer et al., 2010b; Reader et al., 2011), therefore to reduce the prevalence of lameness, efforts to ensure effective recovery are required alongside the prevention of new cases.

Lameness in dairy cattle is primarily associated with foot lesions (Murray et al., 1996; Bicalho et al., 2007a; van Huyssteen et al., 2020); two of the most prevalent and important lesions are sole haemorrhage and sole ulcers (Murray et al., 1996; Cramer et al., 2008; Somers and O'Grady, 2015). Sole haemorrhage (**SH**) and sole ulcers (**SU**), collectively referred to as sole lesions, are thought to represent different stages, or manifestations, of the same disease process (Offer et al., 2000; Lischer and Ossent, 2002). The incidence of sole lesions peaks from around three to four months after calving (Leach et al., 1997; Offer et al., 2000; Barker et al., 2009). Although the prevalence of mild SH in early lactation can be exceptionally high (Bergsten and Herlin, 1996; Capion et al., 2008; Maxwell et al.,

2015), only severe cases of SH are considered to be clinically significant (Leach et al., 1998), at least in the short-term.

Severe sole lesions, particularly SU, are time-consuming and expensive to treat (Charfeddine and Pérez-Cabal, 2017; Dolecheck et al., 2018). Even with treatment, high rates of recurrence are observed in consecutive lactations (Enevoldsen et al., 1991; Foditsch et al., 2016; Charfeddine and Pérez-Cabal, 2017). It is thought that inflammation due to sole lesions may cause new bone development on the distal phalanx which is responsible for the increased risk of recurrence (Lischer et al., 2002b; Newsome et al., 2016). Consequently, one of the primary goals of sole lesion treatment is to minimise the severity and duration of inflammation (Pedersen and Wilson, 2021).

The success of sole lesion treatment can be determined by visual assessment of lesion healing or the resolution of visible lameness, with both reported to have similar time frames. Approximately 60 - 70% of uncomplicated SU were covered in a layer of new horn after four weeks (Van Amstel et al., 2003; Klawitter et al., 2019). With prompt and effective treatment, more than three-quarters of cows with sole lesions were no longer lame after 35 days (Thomas et al., 2015), although this was only true for 15% of cows which were chronically lame when first treated (Thomas et al., 2016).

Genetic selection of dairy cattle has driven exceptional improvements in production, but there has been a genetic decline in the health of dairy cows and there is an urgent need to reverse this trend (Oltenacu and Algers, 2005; European Food Safety Authority (EFSA), 2009; Miglior et al., 2017). Genetic selection for lameness resistance has the potential to produce cumulative, long-term benefits to complement husbandry-based initiatives. But just as lameness control programmes include measures to ensure affected animals recover quickly, as well as to prevent new cases (Bell et al., 2009; Leach and Whay, 2009), breeding goals should also reflect this. The strongest foundation from which to reduce the intractably high prevalence of lameness on dairy farms may be to select for cows with a better ability to recover from lameness, as well as a greater resistance to lameness.

Historically, farmers wishing to reduce lameness in their herd through genetic improvement could only select on indirect traits such as conformation (McDaniel, 1998), but it is now recognised that it is likely to be more effective to select on direct health traits, such as foot lesion records (Egger-Danner et al., 2014). The first step toward developing selection indexes is to understand the additive genetic variance that exists in a population. The heritability of sole lesion susceptibility in dairy cattle has been estimated on the underlying liability scale to range from 0.02 – 0.09 for SH (Buch et al., 2011; Häggman et al., 2013; Malchiodi et al., 2017) and 0.02 – 0.18 for SU (Huang and Shanks, 1995; Ødegård et al., 2013). These heritability estimates highlight the possibility to reduce lesion susceptibility through breeding, and foot lesion records have been directly incorporated into national selection indexes in many countries (Stoop et al., 2010; Häggman and Juga, 2013; Malchiodi et al., 2020). The heritability of sole lesion recovery, however, is unknown.

4.1.1 Objectives

The objectives of this study were to estimate the genetic parameters relating to the recovery of sole lesions (sole haemorrhage and sole ulcers) in dairy cows and to consider how this trait relates to the genetic background of sole lesion susceptibility.

4.2 Materials and methods

4.2.1 Study design and population

The study was conducted following ethical approval by the University of Liverpool Research Ethics Committee (VREC269a, VREC466ab) and procedures regulated by the Animals (Scientific Procedures) Act were conducted under a UK Home Office License (P191F589B).

A prospective cohort study was designed to record SH and SU at four time points during a production cycle. Data collection was conducted on four dairy herds (A - D) in the northwest of the UK which were selected for convenience based on the practicalities of frequent visits and assessments. Herds A to C housed lactating cows all-year-round, milked cows three times daily and recorded 305-day milk yields of approximately 11,000 - 11,500 L. Herd D housed lactating cows all-year-round except for lower-yielding cows which were grazed during the summer; cows were milked twice daily and the 305-day milk yield was approximately 9,000 L. Parous cows on all herds were routinely foot-trimmed twice a year before drying off and 60 - 120 days after calving. On all herds, lactating cows were footbathed after milking. Herd A footbathed cows three times a week with either copper sulphate or formalin; herd B footbathed cows twice daily with formalin, herd C footbathed cows daily with either copper sulphate or formalin and herd D footbathed three times a week with formalin.

All animals which were registered as Holsteins and expected to calve between April and December 2019 were prospectively enrolled with no additional inclusion or exclusion criteria applied. Data were collected by qualified veterinary surgeons during weekly or twice weekly visits to each herd from February 2019 to July 2020. Animals were assessed at four time points: before parturition (**T1-Precalving**), immediately after parturition (**T2-Calving**), in early lactation close to peak milk yield (**T3-Early**), and in late lactation (**T4-Late**). The sample size was determined by resource constraints; all eligible animals were enrolled until the final assessments (T4-Late) began, at which point further enrolments ended as data collection at four time points simultaneously was not feasible.

4.2.2 Data collection

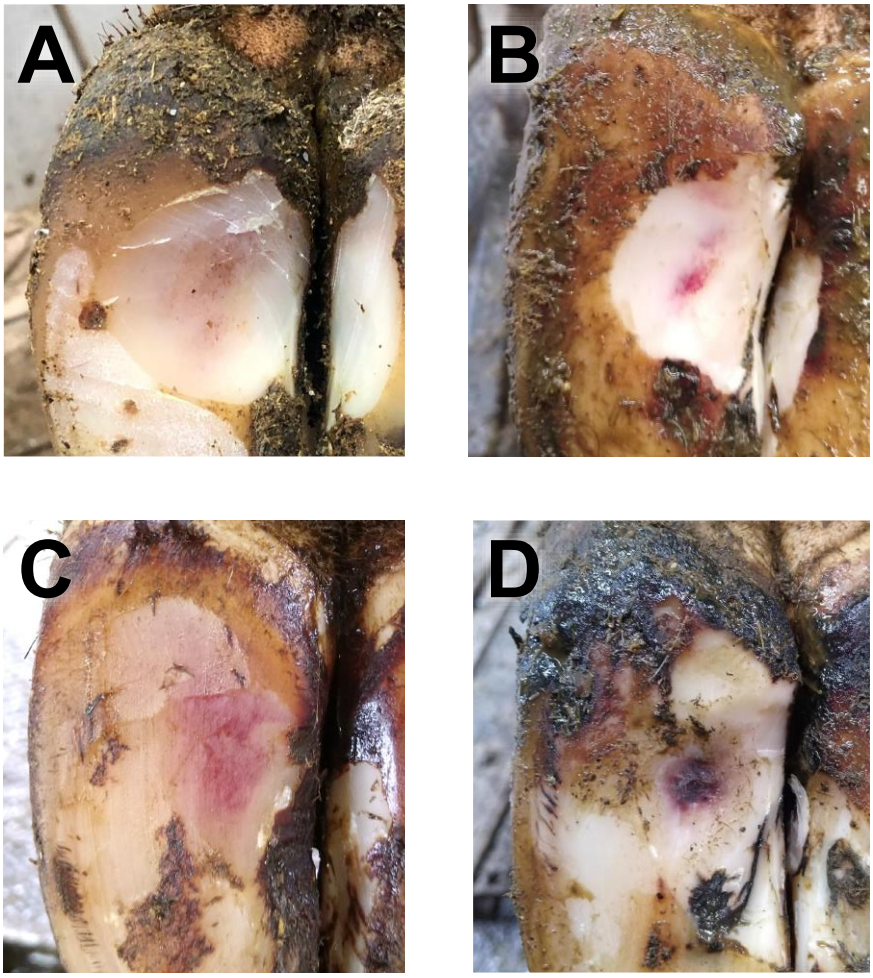
At each assessment, all cows were mobility scored according to a four-point system from 0 (sound) to 3 (severely lame) (Whay et al., 2003a; AHDB, 2020a). Animals were restrained in a foot-trimming crush and, if foot-trimming was not conducted during the visit, the claw horn on the sole of each foot was lightly trimmed to allow inspection of foot lesions. If lesions were visible initially but disappeared following the removal of the claw horn these were still recorded as present. On each claw, SH and SU were recorded using case definitions as described in the International Committee for Animal Recording (ICAR) claw health atlas (Egger-Danner et al., 2020). All foot lesions were examined and recorded by qualified veterinary surgeons; over 90% by a single researcher, and the remainder by three other researchers.

Sole haemorrhage was graded as either mild: light pink lesion < 2 cm diameter or diffuse discolouration of sole, or severe: light pink lesion ≥ 2 cm diameter or dark pink/purple lesion of any size (**Figure 4.1**). Sole ulcers were recorded as present or absent. This procedure was the same at all time points except in the case of T2-Calving on herd C, during which only hind feet were assessed to reduce the handling time of cows which had recently calved; this was only required on this herd due to the large numbers of cows calving each week.

When either severe SH or a SU were present, the claw was therapeutically trimmed by researchers (herd A and D), farm staff (herd C), or a combination of farm staff and professional foot-trimmers (herd B). All persons responsible for foot-trimming had completed specialist training in this area and had extensive experience. Regardless of the individual involved, a modified version of the five-step Dutch method was used (Toussaint Raven et al., 1985) which included wider and deeper modelling of the lateral claw on hind feet than the traditional method. In all cases, therapeutic foot-trimming aimed to create a concavity in the middle of the sole around the lesion and to reduce the heel of the affected claw to redistribute load onto the unaffected claw (Mahendran and Bell, 2015). Additionally, a hoof block was applied to the unaffected claw at the discretion of

the foot-trimmer; a block was applied if there was exposure of the corium, a pain response was elicited following digital pressure on the lesion (SH or SU), or the animal had impaired mobility attributable to the sole lesion.

Figure 4.1. Examples of sole haemorrhage (SH) severity grading. Mild sole haemorrhage: diffuse discolouration of sole (A) or a light pink lesion < 2 cm diameter (B); severe sole haemorrhage: light pink lesion ≥ 2 cm diameter (C) or dark pink/purple lesion of any size (D).



4.2.3 Trait definitions

Two genetic traits were defined to reflect the overall susceptibility to sole lesion development, and sole lesion recovery during lactation. The case definition for a sole lesion was: "the presence of severe sole haemorrhage or a sole ulcer".

Susceptibility to sole lesions. A binary trait (**SL-Susceptibility**) classified animals as being either "susceptible" or "resistant" to sole lesions using records from all claws across the whole study period. If animals were affected with a sole lesion (severe SH or any SU) at any assessment they were classified as "susceptible", regardless of the number of claws affected, the number of time points the lesion was present, or the total number of records for that animal. Animals were classified as "resistant" if they were unaffected with sole lesions (no or mild SH and no SU) at each assessment in a complete set of records from all four time points. Therefore, animals were unclassified by this trait if they were unaffected with a sole lesion but did not have records from all four time points. This resulted in a slight reduction in study power, due to a small proportion of incomplete lesion records for animals which had otherwise always been unaffected, but was intended to minimise misclassification bias by using all available information to increase confidence that these animals could be regarded as "resistant".

Recovery from sole lesions. A binary trait (**SL-Recovery**) defined animals which were affected with a sole lesion at the T3-Early assessment as having either "recovered" or remained "chronic" by T4-Late. The full dataset was filtered to only include animals affected with a sole lesion at T3-Early. Animals were excluded if lesion records were missing for any claw at either T3-Early or T4-Late. Animals were classified as "recovered" if all affected claws at T3-Early were no longer affected at T4-Late (no or mild SH and no SU), and "chronic" if at least one of the affected claws at T3-Early was still affected at T4-Late (severe SH or any SU).

4.2.4 Pedigrees and genotypes

Pedigree details for the study population were extracted from the national database of dairy cattle by tracing back seven generations for each animal. Blood

samples were collected from the coccygeal vein of each animal into EDTA vacutainers and used to genotype each animal with the Illumina BovineSNP50 BeadChip (Illumina Inc., USA). Genotypes were subsequently imputed to 80K single nucleotide polymorphism (**SNP**) genotypes by Edinburgh Genetic Evaluation Services (EGENES) using an in-house procedure which has been developed for all national genomic evaluations of dairy cattle in the UK. Briefly, this imputation process uses the Illumina BovineSNP50 BeadChip and Illumina BovineHD BeadChip (Illumina Inc., USA), in addition to other commercial genotyping arrays, extra gene tests, and large-effect sequence variants. Following imputation, genotypes included 79,051 SNP spanning the entire genome. Chromosomal locations of the imputed 80K SNP panel were drawn from the latest assembly of the *Bos taurus* genome (ARS-UCD 1.2) (Rosen et al., 2020).

Imputed genotypes were available for 2,250 animals. Genotype quality control was implemented using PREGSF90 (Aguilar et al., 2014) within the BLUPF90 software suite (Misztal et al., 2018). Quality control included the removal of SNP with a call rate < 0.90 (N = 10,977), SNP with a minor allele frequency < 0.05 (N = 3,008), monomorphic SNP (N = 36), or SNP showing a strong deviation (> 0.15) from Hardy-Weinberg equilibrium (N = 14) (Wiggans et al., 2009). Additionally, animals were removed if sample call rate < 0.90 (N = 63) or there were parent-progeny Mendelian conflicts (N = 20). Quality control procedures resulted in a final dataset of 2,167 animals with genotypes of 65,211 SNP.

4.2.5 Phenotypic analysis

At a claw-level, the differences in the proportion of claws that “recovered” or remained “chronic” were assessed with chi-square tests to compare forelimb to hindlimb lesions, severe SH to SU, and claws which had previously been affected with a sole lesion at T1-Precalving or T2-Calving to those which had been unaffected. At a cow-level, the relationship between clinical lameness (mobility score 2 or 3) and sole lesion recovery was also assessed with chi-

square tests. The duration between T3-Early and T4-Late was compared between animals classified as “recovered or “chronic” with a two-tailed *t*-test.

4.2.6 Genetic parameter estimation

Before genetic analyses of SL-Susceptibility and SL-Recovery, potential fixed effects were evaluated with multivariable logistic regression of each trait in R (R Core Team, 2021). The importance of each fixed effect was determined by finding the multivariable model with the lowest Akaike information criterion. In addition to the final model parameters, continuous variables of days in milk at T3-Early and T4-Late were evaluated in the SL-Recovery model, but neither improved model fit when the duration between T3-Early and T4-Late was included as a covariate. The effect of the researcher examining and recording lesions was tested but also increased Akaike information criterion.

Variance components were estimated for both traits (SL-Susceptibility and SL-Recovery) using threshold models to transform the binary observed phenotype to a latent liability scale (Gianola, 1982). A Markov chain Monte Carlo approach was used to obtain marginal posterior distributions for model parameters via the Gibbs sampling algorithm in THRGIBBS1F90 (Tsuruta and Misztal, 2006). Convergence of Gibbs sampling was assessed using the *coda* package in R (Plummer et al., 2006); a chain length of 500,000 samples with a 50,000 sample burn-in produced consistent results in both models. Lag correlation between consecutive samples was reduced with a thinning interval of 100, therefore genetic parameters were estimated from the posterior distribution of 4,500 Gibbs samples.

The animal threshold model used to separately analyse both traits (SL-Susceptibility and SL-Recovery) was:

$$\boldsymbol{\lambda} = \mathbf{Xb} + \boldsymbol{Z}_{hys}\mathbf{hys} + \boldsymbol{Z}_{a}\mathbf{a} + \mathbf{e} \quad (1)$$

where $\boldsymbol{\lambda}$ is a vector of unobserved liabilities for either SL-Susceptibility or SL-Recovery; **b** is a vector of the fixed effect of parity (3 levels: 1st parity, 2nd parity and ≥3rd parity) and the interval between the T3-Early and T4-Late assessments

in days as a continuous covariate (included in the model for SL-Recovery only, omitted from the model for SL-Susceptibility); **hys** is a vector of the random effects of herd-year-season of calving (**HYS,** 12 levels); **a** is a vector of random additive genetic effects for each animal; **e** is a vector of random residual effects, and $\mathbf{X}$, $\boldsymbol{Z}_{hys}$, and $\boldsymbol{Z}_{a}$ are incidence matrices for **b**, **hys**, and **a**, respectively. Model convergence was improved by treating **hys** as a random effect compared to a fixed effect. Random effects were assumed to be normally distributed with a mean of zero and covariance structure of:

$$var\begin{bmatrix}\boldsymbol{hys}\\ \boldsymbol{a}\\ \boldsymbol{e}\end{bmatrix} = \begin{bmatrix}\boldsymbol{I}\sigma^2_{hys} & 0 & 0\\ 0 & \boldsymbol{H}\sigma^2_{a} & 0\\ 0 & 0 & \boldsymbol{I}\sigma^2_{e}\end{bmatrix} \quad (2)$$

where σ^2_{hys} is the HYS variance; $\sigma^2_a$is the additive genetic variance; σ^2_e is the residual variance; $\boldsymbol{I}$ is an identity matrix, and $\boldsymbol{H}$ is the relationship matrix incorporating pedigree and genomic information in a single-step genomic analyses framework as defined by Legarra et al. (2009). The inverse of $\boldsymbol{H}$ is defined as (Aguilar et al., 2010; Christensen and Lund, 2010):

$$\boldsymbol{H}^{-1} = \boldsymbol{A}^{-1} + \begin{bmatrix}0 & 0\\ 0 & (\boldsymbol{G}^{-1} - \boldsymbol{A}_{22}^{-1})\end{bmatrix} \quad (3)$$

where $\boldsymbol{A}$ is the pedigree relationship matrix; $\boldsymbol{G}$ is the genomic relationship matrix, and $\boldsymbol{A}_{22}$ is the pedigree relationship matrix for genotyped animals. The $\boldsymbol{A}$ matrix includes inbreeding coefficients calculated from pedigree relationships (Meuwissen and Luo, 1992). The genomic relationship matrix, $\boldsymbol{G}$**,** was constructed as $0.95\boldsymbol{G}^* + 0.05\boldsymbol{A}_{22}$; $\boldsymbol{G}^*$ is defined according to VanRaden (2008) as:

$$\boldsymbol{G}^* = \frac{\boldsymbol{Z}\boldsymbol{Z}'}{2\sum_{i=1}^{M} p_i(1 - p_i)} \quad (4)$$

where $\boldsymbol{Z}$ is a centred matrix of genotype at each locus (*aa* = 0, *Aa* = 1, and *AA* = 2); M is the number of SNP, and p_i is the minor allele frequency at locus i.

To estimate the genetic correlation between SL-Susceptibility and SL-Recovery, a bivariate model was fit using both traits, based on the same model as Equation 1. Model convergence was not satisfactory despite extending the

chain length to one million samples. Therefore, the genomic estimated breeding value (**GEBV**) for each trait was estimated during Gibbs sampling, and the approximate genetic correlation between SL-Susceptibility and SL-Recovery was calculated after adjusting for the GEBV reliability, for animals which had both phenotypes recorded (Calo et al., 1973):

$$\tilde{r}_{g1,2} = r_{1,2} \times \frac{\sqrt{(\sum REL_1)(\sum REL_2)}}{\sum(REL_1 \times REL_2)} \quad (5)$$

where REL_1 and REL_2 are the reliabilities of GEBV for SL-Susceptibility and SL-Recovery, and $r_{1,2}$ is the Pearson correlation between GEBV for each trait. The reliability of GEBV for each trait was calculated as (Aguilar et al., 2020):

$$REL_i = 1 - \frac{PEV_i}{(1 + F_i)\sigma_a^2} \quad (6)$$

where PEV_i is the prediction error variance of the GEBV in animal i (calculated as the squared standard error of the GEBV), F_i is the inbreeding coefficient of animal i calculated from pedigree relationships (Meuwissen and Luo, 1992), and σ_a^2 is the additive genetic variance estimated with the threshold model (Equation 1). The standard error (SE) for the approximate genetic correlation was calculated as:

$$SE = \sqrt{\frac{1 - \tilde{r}^2{}_{g1,2}}{n - 2}} \quad (7)$$

where $\tilde{r}^2{}_{g1,2}$ is the squared approximate genetic correlation calculated in Equation 5, and n is the number of animals with records.

4.3 Results

4.3.1 Population and dataset description

A total of 2,352 animals were enrolled in this study: 132 animals from herd A, 432 animals from herd B, 1,549 animals from herd C, and 239 animals from herd D. The mean (standard deviation) timing of each assessment time point relative to parturition was T1-Precalving: -56.5 days (22.3), T2-Calving: +5.4 days (2.9), T3-Early: +84.0 days (13.9), and T4-Late: +199.5 days (30.5). At each of the four assessment time points, 2,341 animals had foot lesion records at T1-Precalving, 2,186 animals at T2-Calving, 2,132 animals at T3-Early, and 1,937 animals at T4-Late. The highest frequency of sole lesions was at T3-Early, details are provided in **Table 4.1**.

Table 4.1. Proportion (frequency) of animals with sole haemorrhage (SH) or sole ulcers (SU) at each assessment time point (using the most severe lesion from all claws). In all analyses, animals with no SH or SU, or mild SH were considered unaffected; animals with severe SH or a SU were considered affected.

Sole lesion	**Assessment time point**			
	T1-Precalving	T2-Calving	T3-Early	T4-Late
No SH or SU	0.66 (1,538)	0.66 (1,443)	0.41 (868)	0.45 (870)
Mild SH	0.24 (568)	0.26 (566)	0.32 (676)	0.35 (676)
Severe SH	0.06 (148)	0.06 (122)	0.21 (457)	0.14 (277)
SU	0.04 (87)	0.03 (55)	0.06 (131)	0.06 (114)
Total	2,341	2,186	2,132	1,937

The number of animals in the final study populations for the genetic analysis of each trait is provided in **Table 4.2**. A total of 2,025 animals were used to estimate the genetic parameters of sole lesion susceptibility (SL-Susceptibility). Not all animals were classified for this trait because animals were excluded if they were unaffected with a sole lesion but did not have complete records from all four time points. Genetic parameters of sole lesion recovery (SL-Recovery) used records from 498 animals. For analysis of this trait, the full dataset (N = 2,352) was first filtered to only include animals affected with a sole lesion at T3-Early, and which had been assessed again at T4-Late (N = 528). Finally, only animals which had lesion records from all eight claws at both T3-Early and T4-Late were included (N = 498). The final cohort of 498 animals corresponded to 694 affected claws.

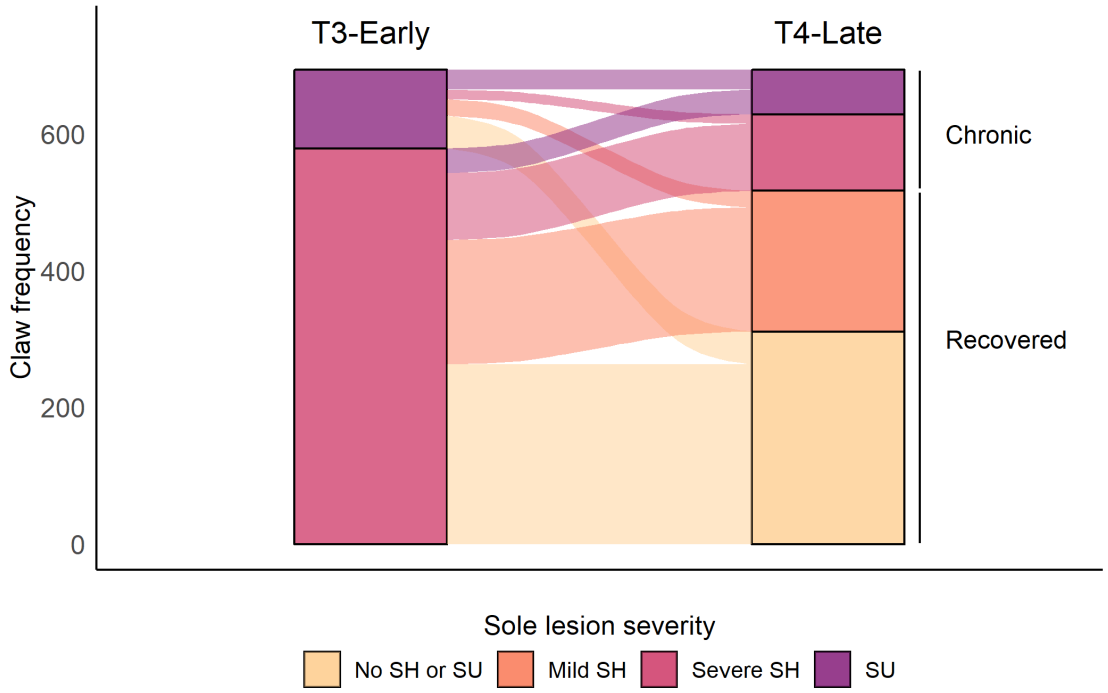
4.3.2 Phenotypic analysis

Of the claws affected with a sole lesion at T3-Early, 74.4% (517/694) had recovered by T4-Late; the outcome of each sole lesion is displayed in **Figure 4.2**. Forelimb claws was more like to recover than hindlimb claws (87.3% (96/110) vs 72.1% (421/584), χ^2 = 11.232, df = 1, P < 0.001); recovery of severe SH occurred more frequently than recovery of SU (76.9% (445/579) vs 62.6% (72/115), χ^2 = 10.251, df = 1, P = 0.001). Claws which had not been affected with a sole lesion at T1-Precalving or T2-Calving were more likely to have recovered between T3-Early and T4-Late compared to those which had been affected (78.4% (462/589) vs 52.3% (55/105), χ^2 = 31.846, df = 1, P < 0.001). The SL-Recovery trait was defined at the animal-level, animals were only considered to have recovered if all affected claws at T3-Early were no longer affected at T4-Late. Of the 352 animals in which all affected claws had recovered (SL-Recovery = “recovered”), 262 animals had only been affected on one claw, 79 animals on two claws, nine animals on three claws and two animals on four claws. Of the 146 animals which were considered to have been chronically affected (SL-Recovery = “chronic”), sole lesions were still present at T4-Late on all affected claws in 93 animals; 53 animals had claws which had recovered and claws which remained chronic, these animals were all classified as “chronic” for the SL-Recovery trait.

Table 4.2. Summary of the two genetic traits with details regarding trait classification and frequency of animals assigned to each class.

Trait	Phenotype	Definition	Frequency
Sole lesion susceptibility (SL-Susceptibility)	Resistant (= 0)	No sole ulcers and no/mild sole haemorrhage on all claws at all assessments with no missing records	1136
	Susceptible (= 1)	Severe sole haemorrhage and/or a sole ulcer on at least one claw at least one assessment	889
Sole lesion recovery (SL-Recovery)	Chronic (= 0)	Severe sole haemorrhage and/or a sole ulcer at the early lactation assessment (T3-Early) and severe sole haemorrhage and/or a sole ulcer still present on the same claw at the late lactation assessment (T4-Late)	146
	Recovered (= 1)	Severe sole haemorrhage and/or a sole ulcer at the early lactation assessment (T3-Early) and no sole ulcer and either no or only mild sole haemorrhage present on the same claw at the late lactation assessment (T4-Late)	352

Figure 4.2. The progression of sole lesion severity between the early lactation (T3-Early) and late lactation (T4-Late) assessments was used to define the sole lesion recovery trait (SL-Recovery). Data presented at claw-level for a total of 694 claws on 498 animals. SH = sole haemorrhage; SU = sole ulcer.



In the cohort of animals used to analyse SL-Recovery, 11.5% (56/485) of animals were lame according to mobility scoring (mobility score 2 or 3) at T3-Early and 12.0% (58/482) at T4-Late. A lower proportion of cows which were classified as "recovered" had been lame at T3-Early compared to cows classified as "chronic" (9.4% (32/340) vs 16.6% (24/145), χ^2 = 5.074, df = 1, *P* = 0.024). Similarly, a lower proportion of cows which were classified as "recovered" were lame at T4-Late compared to cows classified as "chronic" (7.6% (26/342) vs 22.9% (32/140), χ^2 = 21.838, df = 1, *P* < 0.001). The mean interval between the T3-Early assessment and T4-Late was 115.2 days (standard deviation (**SD**) 33.9). On average this interval was longer in "recovered "animals (mean 118.0 days, SD 35.6) compared to "chronic" animals (mean 108.5 days, SD 28.5); *t*(496) = 2.859, *P* = 0.004. The interval between T3-Early and T4-Late was correlated with the days in milk of T3-Early (Pearson correlation coefficient -0.47, 95% confidence interval (**CI**) -0.54 to -0.40).

4.3.3 Genetic parameters

The heritability was calculated during each round of Gibbs sampling, for SL-Susceptibility the posterior distribution had a mean of 0.25 (95% highest density interval (**HDI**) 0.16 to 0.34), for SL-Recovery the posterior distribution had a mean of 0.27 (95% HDI 0.02 to 0.52). Details of the variance component estimates are provided in **Table 4.3**. The bivariate model did not converge despite an extended chain length of 950,000 rounds (after a 50,000 burn-in), therefore the genetic correlation between SL-Susceptibility and SL-Recovery could not be directly estimated. The genetic correlation between SL-Susceptibility and SL-Recovery was approximated from the GEBV for the 498 animals with both phenotypes recorded, after adjusting for the GEBV reliabilities. The GEBV reliabilities were low for both traits with an average reliability of 0.32 for SL-Susceptibility and 0.21 for SL-Recovery. The approximate genetic correlation between SL-Susceptibility and SL-Recovery was -0.11 (95% CI -0.20 to -0.02).

Table 4.3. Additive genetic variance (σ_a^2), herd-year-season variance (σ_{hys}^2), residual variance (σ_e^2), and narrow-sense heritability (h^2) estimates for two traits: overall susceptibility to sole lesions (SL-Susceptibility) and recovery from sole lesions (SL-Recovery). Estimates refer to the posterior mean (95% highest density interval) from Gibbs sampling.

Trait	$\boldsymbol{\sigma_a^2}$	$\boldsymbol{\sigma_{hys}^2}$	$\boldsymbol{\sigma_e^2}$	$\mathbf{h^2}$
SL-Susceptibility	0.42 (0.25 – 0.63)	0.25 (0.04 – 0.60)	1.04 (0.91 – 1.09)	0.25 (0.16 – 0.34)
SL-Recovery	0.48 (0.02 – 1.20)	0.11 (0.0001 – 0.34)	1.02 (0.85 – 1.20)	0.27 (0.02 – 0.52)

4.4 Discussion

4.4.1 Key results and interpretation

We have used a dataset of accurately collected foot lesion records to define a novel trait relating to the recovery of sole lesions in Holstein cows (SL-Recovery). We estimate SL-Recovery to have a heritability of 0.27 on the liability scale, and therefore there could be potential to breed cows that can more effectively recover from sole lesions.

Reducing the prevalence of lameness is a key priority for the UK dairy industry (GB Cattle Health & Welfare Group, 2020; Rioja-Lang et al., 2020). It is suggested that producers aim for more than 75% of lame cows to recover between consecutive (e.g. monthly) mobility scores (Green, 2012); but as approximately 50% of lameness prevalence can be attributed to chronically lame cows, it is likely many farms do not currently achieve this (Archer et al., 2010b; Reader et al., 2011). While early identification and treatment of lame cows are rightly regarded as the most important interventions required to meet this target (Bell et al., 2009; Leach et al., 2012; Groenevelt et al., 2014), breeding cows that can recover more quickly and effectively from sole lesions would also be advantageous. Additionally, longevity is a now key priority of many breeding strategies because it is closely related to the environmental impact and profitability of dairy farming (Boulton et al., 2017; Grandl et al., 2019). As lameness has been associated with a greater risk of culling (Sprecher et al., 1997; Booth et al., 2004; Bicalho et al., 2007b), genetic selection for effective sole lesion recovery would also benefit those aiming to breed cows for a longer productive life.

The healing of sole lesions in cattle is similar to the secondary intention healing of cutaneous wounds (Azarabad et al., 2006; Shearer et al., 2015). The rate of ear punch hole closure has been used to investigate the genetics of cutaneous wound healing in mice, which has been characterised as a complex trait (Masinde et al., 2001) with an estimated heritability of 0.29 (Nicod et al., 2016). Wound healing is a “dynamic, interactive process involving soluble mediators, blood cells, extracellular matrix, and parenchymal cells” (Singer and

Clark, 1999), and therefore there is an abundance of opportunities for genetic influence. Notably, this genetic influence has been a therapeutic target since the late 1990s when gene therapy was considered a promising approach to promoting wound healing (Eming et al., 1997). However, single-gene targets (such as growth factors) showed only modest responses, attributed to the complexity of the healing process (Eming et al., 2014). As such, research in this field is now focused on the identification of the full spectrum of wound healing "driver genes" to advance this area (Tang et al., 2021); ultimately this may lead to a clearer understanding of the underlying gene pathways involved.

One possible explanation for the delayed recovery of SU is the development of complicating secondary infections with bacteria such as treponemes (Evans et al., 2011; Sykora et al., 2015), which are more frequently associated with bovine digital dermatitis. This complication could conceivably have a genetic background as genotype has been associated with the microbiome of chronic wounds in humans (Tipton et al., 2020) and the foot skin microbiome in cattle (Bay et al., 2021). Another possible mechanism for genetic influence on sole lesion recovery is via insulin-like growth factor-1 (**IGF-1**) which is a major promoter of wound healing (Garoufalia et al., 2021). In cattle, serum IGF-1 concentration has been demonstrated to be highly heritable (Davis and Simmen, 1997) and specific mutations have been identified in the *IGF-1* gene (Mullen et al., 2011). However, as IGF-1 concentration also correlates with negative energy balance and body condition, recovery of sole lesions could be affected by the timing of lesion development during lactation (Fenwick et al., 2008; Akbar et al., 2015). Future studies of sole lesion recovery in dairy cattle would therefore benefit from minimising the variation around the lactation stage at which healing is assessed.

We were unable to estimate the genetic correlation between SL-Susceptibility and SL-Recovery using a bivariate model. Consequently, we estimated the approximate genetic correlation between SL-Susceptibility and SL-Recovery by calculating the correlation between the GEBV for each trait and adjusting for the GEBV reliability. Correlation between breeding values is only

equivalent to genetic correlation when the accuracy of the GEBV is 100% (Koenig et al., 2005); given the low reliabilities of the GEBV, which were expected due to the small study population, we are cautious in our interpretation of this result. The approximate genetic correlation between SL-Susceptibility and SL-Recovery had a 95% confidence interval of -0.20 to -0.02. We interpret this result to suggest the genetic correlation between SL-Susceptibility and SL-Recovery is negative but very weak, therefore these traits appear to have relatively distinct genetic backgrounds. This result was unexpected and we anticipated these traits would be strongly genetically correlated because common biological pathways could plausibly underlie both traits. For example, genes related to keratinisation and inflammation pathways have previously been linked with sole lesion susceptibility (Swalve et al., 2014; Sánchez-Molano et al., 2019; Lai et al., 2021b), and these could conceivably also be involved in sole lesion healing (Hendry et al., 2001). Nevertheless, our results do not suggest sole lesion recovery is strongly genetically correlated with sole lesion susceptibility, although this result should be interpreted cautiously pending replication in further studies.

A practical implication of the apparently weak genetic correlation between SL-Susceptibility and SL-Recovery is that genetic selection for reduced susceptibility to sole lesions would not be expected to result in improved recovery (Shook, 1989). Therefore, to breed cows that can recover more quickly from sole lesions, this phenotype would need to be specifically recorded so that it could be utilised in national genetic evaluations. It is advised that cases of SU are re-examined following treatment within 30 days (Van Amstel et al., 2003), so one approach to obtain a phenotype of sole lesion recovery would be to record details of all follow-up assessments in farm records. Although this would be theoretically achievable, it is admittedly optimistic and, in general, lameness is poorly recorded on farms compared to other health conditions (Zwald et al., 2004; Leach et al., 2010; Parker Gaddis et al., 2014). A more realistic solution may be to use lesion records from professional foot-trimmers for national genetic evaluations, as is the case in other countries (Stoop et al., 2010; Häggman and Juga, 2013; Malchiodi et al., 2020). If repeated foot lesion records were available from the same lactation, assuming a reasonable accuracy and consistency in

recording, there would be scope to define a trait similar to SL-Recovery based on repeated records for each animal. Additionally, the inclusion of foot lesion records in genetic evaluations would be expected to improve existing genetic selection indexes for reduced lameness (Koenig et al., 2005; van der Linde et al., 2010; Ødegård et al., 2015).

The heritability of SL-Recovery had a large uncertainty estimate (95% HDI 0.02 to 0.52), so our results are also compatible with a very small or substantially larger true heritability, this would affect the expected response to selection and effectiveness of breeding programmes. The large uncertainty around the heritability estimate is due, in part, to the small study population used to analyse this trait (N = 498), but there are other sources of noise relating to this phenotype that could also contribute to this uncertainty, we discuss this further in the following section.

4.4.2 Study strengths and limitations

One of the strengths of this study was the accuracy and detail of foot lesion recording, which allowed the outcome of lesions to be determined from sequential assessments; however, there were some limitations to our study design which may have affected the accuracy of this classification.

Studies which have assessed the healing rate of sole lesions following different treatment protocols monitored lesion outcomes at multiple time points (Lischer et al., 2002a; Thomas et al., 2015; Klawitter et al., 2019). We recognise that this would be a more robust approach to judge the recovery of sole lesions than a single follow-up assessment, but our priority was to use our available resources to maximise the number of enrolled animals to allow the estimation of genetic parameters. For example, the largest sample size of animals with sole lesions with multiple follow-up assessments was 83 animals (Thomas et al., 2015); this would have been insufficient for our objectives. Therefore, we accept that a single follow-up assessment of a sole lesion has limitations regarding the ability to definitively determine healing progress.

We were not recording the spontaneous healing of sole lesions because all animals with sole lesions were therapeutically trimmed, and the unaffected claw was blocked when considered necessary. This treatment protocol reflects common practice on UK dairy farms (Horseman et al., 2013), but it does not represent the best approach to the treatment of sole lesions which includes administering non-steroidal anti-inflammatory drugs (Thomas et al., 2015; Pedersen and Wilson, 2021). One source of extraneous noise in our data may have been inconsistencies between foot-trimmers in terms of whether unaffected claws were blocked, as this is likely to affect the recovery rate (Thomas et al., 2015). As the person responsible for foot-trimming depended on the herd, we could not control for this in our analysis beyond the inclusion of herd in the statistical model.

Wound healing is a continuous process and the point at which a sole lesion could be regarded as having recovered is not absolute. A consensus is that, in the absence of complicating factors, mild-to-moderate SU should be covered by a thin layer of new horn after 30 days, and severe lesions after 40 to 60 days (Shearer and van Amstel, 2017). The mean interval between the T3-Early assessment and T4-Late was 115.2 days (SD 33.9) and the first percentile was 70 days, therefore we consider the interval to have been sufficiently long enough for uncomplicated sole lesions to appear visibly healed. However, the interquartile range of the duration between T3-Early and T4-Late was 90 to 126 days so in half of our study population there was a five-week or greater difference in the interval between lesion identification and outcome assessment. We observed a significant univariable association between this interval and whether animals were considered to have recovered. We included the interval between T3-Early and T4-Late as a covariate in the animal threshold model used to estimate the heritability of SL-Recovery which we believe will have mitigated the influence on the heritability estimate, at least to some extent. The association between the duration between T3-Early and T4-Late and SL-Recovery could also reflect how we classified the outcome of sole lesions, which meant that a SU was considered to have not fully recovered if severe SH was observed at T4-Late, despite the SU having potentially epithelialised. We grouped severe SH and SU to

create a trait which had sufficient numbers in each class to reasonably estimate the genetic parameters of sole lesion recovery, but there would be benefits from future studies of a sufficient sample size to assess SU independently of SH.

In addition to the variation in the interval between T3-Early and T4-Late, there was a dispersion of both assessment time points relative to parturition. We consider the timing of T3-Early to be of particular clinical relevance because it relates to when sole lesions developed during lactation and the timing of therapeutic intervention. It has been shown that cows which develop sole lesions in early lactation heal more quickly and respond better to corrective trimming and foot blocking, with this response declining over time (Thomas et al., 2015). It is also probable that lesions which were identified later in lactation were more likely to represent chronic lesions, and the recovery of chronic lesions is poorer than in acute cases (Leach et al., 2012; Groenevelt et al., 2014; Thomas et al., 2016). Furthermore, the moderate negative correlation between the timing of T3-Early and the interval between T3-Early and T4-Late meant that sole lesions which were recorded later in lactation also tended to have a shorter duration until the outcome was assessed, further complicating the interpretation of our results. In a similar vein, historic sole lesions may have influenced the risk of lesion development and recovery. We did not have a detailed lameness history for the study population, therefore there would be benefits in further studies of sufficient duration to record lesions over multiple lactations.

In conclusion, we acknowledge that the interpretation of sole lesion recovery in our data is complicated by several factors, and this is an important context in which to consider our results. However, if the recovery of sole lesions is ever going to be deducible from farm records, such that it could be incorporated into national genetic evaluations, the ability to recognise a heritable trait from an admittedly noisy dataset could be viewed as encouraging.

4.4.3 Generalisability

This study only included four dairy herds which, despite all being commercially run with operating practices common to many British dairy farms, could not be considered representative of the full spectrum of dairy farms. Within

these four herds, three were operating relatively intensive systems of zero-grazing and three times a day milking. We did not observe any differences in trends between these three farms and the remaining herd which was managed with a combination of housed and grazed groups and had a lower milk production.

The overall period prevalence of lame cows (Mahendran et al., 2017), based on repeated mobility scores throughout this project, ranged from 18.5% to 33.3% across the four herds; the mean point prevalence of lameness from all time points ranged from 6% to 11.8% across the four herds (data not shown). Recent cross-sectional studies in the UK reported that herd lameness prevalence ranged from 6% to 65%; this suggests the four herds in our study had a lower prevalence of lameness compared to many dairy herds in the UK (Griffiths et al., 2018; Randall et al., 2019). We observed the peak prevalence of sole lesions in early lactation (T3-Early) when 21.4% of cows had severe SH and 6.1% of cows had SU. The prevalence of sole lesions in peer-reviewed literature has historically only been reported in large numbers for lame animals or from foot-trimming records. Therefore, previous reports may not have a reliable numerator, due to under-reporting of mild lesions, or a reliable denominator due to over-representation of lame cows. In studies using foot-trimming records, the prevalence of SH has been reported to range from 5 – 59% (Capion et al., 2008; Malchiodi et al., 2017) and between 5 – 17% for SU (van der Waaij et al., 2005; König et al., 2008).

4.5 Conclusions

The results from this prospective cohort study indicate that recovery from sole lesions is a heritable trait in Holstein cows. This result requires replication in further studies, however, there could be potential to selectively breed cows which can recover more effectively from sole lesions. Our results also suggest that recovery from sole lesions is only weakly genetically correlated with the overall susceptibility to sole lesions, although this finding also requires corroboration. If sole lesion susceptibility and recovery are only weakly genetically correlated, selecting for resistance to sole lesions may have a limited impact on the ability of affected cows to recover, and a recovery trait would need to be evaluated specifically. Additionally, the apparent weakness of the genetic correlation between sole lesion recovery and susceptibility has interesting biological implications because the genetic background to each trait could be inferred to be largely independent.

Chapter 5: ^{1}H NMR-based serum metabolomic analysis of sole lesion development in Holstein cows

5.1 Introduction

Sole haemorrhage (**SH**) and sole ulcers (**SU**), referred to as sole lesions, are two of the most prevalent foot lesions in dairy cattle (Murray et al., 1996; Capion et al., 2008; Cramer et al., 2008; Somers and O'Grady, 2015). Sole lesions are thought to arise from contusions in the corium following a failure of the suspensory and supportive apparatus of the distal phalanx (Ossent and Lischer, 1998; Lischer et al., 2002b; Bicalho and Oikonomou, 2013). Tissue damage in the corium can cause blood staining of the newly forming sole horn resulting in SH; or the impairment of horn production resulting in SU (Hoblet and Weiss, 2001; Lischer and Ossent, 2002; Shearer and van Amstel, 2017).

The aetiopathogenesis of sole lesions is widely regarded as multifactorial and virtually all aspects of dairy cow management have been implicated in some form (Mülling, 2012). Consequently, the interconnecting relationships between causal factors and predisposing or exacerbating risks are complex, and many of these elements still lack a strong evidence base (Solano et al., 2015; Newsome et al., 2016). Historically, sole lesions were regarded as a consequence of subclinical laminitis caused by inappropriate nutrition (Greenough and Vermunt, 1991; Vermunt, 1992; Vermunt and Greenough, 1994); but due to a lack of robust, empirical support for this hypothesis (Randall et al., 2018b), current thinking now favours a predominantly biomechanical aetiopathogenesis (Mülling, 2019). However, there is still interest in the role of inflammation in sole lesion development (Thomas et al., 2015; Watson et al., 2022; Wilson et al., 2022), and it remains unclear whether there is any metabolic contribution to the disease process (Lean et al., 2013).

Sole lesions occur more frequently in hindlimbs, with a peak incidence from around three to four months after calving (Leach et al., 1997; Offer et al., 2000; Barker et al., 2009). As there is an estimated lag of approximately two months between instigating pathology in the corium and the detection of visible sole

lesions (Hoblet and Weiss, 2001), much of the research concerning the pathogenesis of sole lesions has focused on the transition period and early lactation. This represents a time of significant metabolic, management, and nutritional changes in dairy cattle (Goff and Horst, 1997; Drackley, 1999; Overton and Waldron, 2004; Cook and Nordlund, 2009).

The physiological events around calving are of particular interest for sole lesion pathogenesis because the biomechanical strength of the suspensory apparatus has been shown to reduce following parturition (Tarlton et al., 2002; Knott et al., 2007). Sole lesions have also been associated with poor body condition and high milk production (Amory et al., 2008; Green et al., 2014). Body condition and milk production both represent the biological endpoints of various interacting metabolic pathways, which include physiological processes such as carbohydrate, protein, and lipid metabolism (Tamminga et al., 1997; de Vries and Veerkamp, 2000; Roche et al., 2009; Megahed et al., 2019), and potentially pathophysiological processes such as periparturient inflammation and oxidative stress (Sordillo et al., 2009; Abuelo et al., 2015; Bradford et al., 2015; Mann, 2022). Therefore, there is an abundance of potential metabolic mechanisms which could have a role in the development of sole lesions; untangling causative relationships is difficult.

Measurement of metabolic markers in the blood has long been recognised as a valuable tool to monitor periparturient and early lactation dairy cows (Payne et al., 1970; Ingraham and Kappel, 1988). More recently, the field of metabolomics has developed using high-throughput platforms to detect large numbers of low-molecular-weight metabolites within a biological sample (Wishart, 2008). The primary technologies used in metabolomics are chromatographic separation coupled with mass spectrometry (**MS**), and proton nuclear magnetic resonance (**^{1}H NMR**) spectroscopy (Emwas et al., 2019).

Metabolomic techniques have only recently been directed toward studying lameness in dairy cows. To date, all research in this area comes from a single case-control study of six lame cows and 20 non-lame controls which has generated multiple ^{1}H NMR- and MS-based metabolic analyses of serum, milk,

and urine (Dervishi et al., 2019; Eckel et al., 2020; Zhang et al., 2020b; a; Zwierzchowski et al., 2020). These studies highlighted large numbers of metabolites which may be associated with lameness, both several weeks before and after the onset of clinical signs. Results also implied metabolic markers could be used to discriminate between lame and non-lame cows with almost perfect accuracy in all evaluated biofluids. However, as the cause of lameness was not described in these studies, it is unclear whether these results directly relate to sole lesions; additionally, there are other limitations to these studies, such as the small sample size, and the interpretation of results should be conditional on corroboration in further experiments. Nevertheless, there appears to be potential for metabolomic approaches to highlight metabolic pathways implicated in lameness, and this could make a valuable contribution toward understanding the aetiopathogenesis of sole lesions.

The development of SU is associated with an increased risk of recurrence in subsequent lactations (Hirst et al., 2002; Oikonomou et al., 2013), thought to be due to inflammation causing permanent and detrimental changes to the digital cushion and the distal phalanx (Lischer et al., 2002b; Räber et al., 2006; Newsome et al., 2016). Consequently, research into sole lesion pathogenesis often focuses on primiparous animals (Bell and Randall, 2021), because although heifers frequently develop mild SH during the rearing period, cases of SU before calving are extremely rare (Vermunt and Greenough, 1996; Randall et al., 2016). However, the limitation of focusing exclusively on primiparous animals is that results can only be extrapolated across parities without empirical evidence to support the assumption of consistent effects. Therefore, it is beneficial to consider both primiparous and multiparous animals, but with an understanding of the benefits and limitations of each cohort.

5.1.1 Objectives

This study aimed to compare the serum metabolome of dairy cows which developed sole lesions (sole haemorrhage and sole ulcers) in early lactation, to those which remained unaffected. We aimed to evaluate the metabolome (i) to determine the capacity of the serum metabolome to discriminate between

affected and unaffected animals, and (ii) to identify any metabolites related to sole lesions, which could infer a metabolic component of the pathogenesis. Within the overarching aims, specific objectives also included (a) assessment of the serum metabolome before the development of sole lesions, particularly around parturition; (b) assessment of the serum metabolome in animals with a concurrent sole lesion, and (c) specific evaluation of first parity animals, which were less likely to have had previous cases of severe sole lesions.

5.2 Materials and methods

The study was conducted following ethical approval by the University of Liverpool Research Ethics Committee (VREC269a, VREC466ab) and procedures regulated by the Animals (Scientific Procedures) Act were conducted under a United Kingdom (**UK**) Home Office Licence (P191F589B).

5.2.1 Study overview

We designed a two-stage observational study to evaluate the serum metabolome in dairy cattle which developed sole lesions in early lactation. The first stage followed a prospective cohort approach to record foot lesions and collect serum samples at repeated time points during a production cycle. In the second stage, a case-control sampling strategy was employed to select samples based on sole lesions recorded in early lactation to be analysed with ^{1}H NMR spectroscopy. The statistical analysis aimed to assess whether the serum metabolome could be used to differentiate between cases and controls, and to highlight any metabolites which were influential in this discrimination.

5.2.2 Stage one: cohort study

Herd description. Data collection was conducted on a single dairy herd in the northwest of the UK which was chosen for convenience and practical reasons, including the feasibility of frequent visits and assessments, herd size (> 1,000 cows), and the availability of a suitable environment on the farm in which to process serum samples. Animals were housed all year round in freestall barns of cubicles deep bedded with sand, milked three times daily, and recorded a 305-day milk yield of approximately 11,500 L. All animals were fed ad libitum with a total mixed ration formulated for each management group; no additional feed was provided during milking. All parous cows were routinely foot-trimmed before drying off and in early lactation. The timing of early lactation foot-trimming depended on parity; primiparous cows were foot-trimmed 60 – 70 days postpartum and multiparous cows were foot-trimmed 90 – 100 days postpartum. Cows were footbathed twice daily with a formalin solution.

Enrolment and sampling. All animals which were registered as Holsteins and expected to calve between May and December 2019 were prospectively enrolled with no additional inclusion or exclusion criteria applied. Data were collected by qualified veterinary surgeons during twice-weekly visits from April 2019 to July 2020. Animals were assessed at four time points: before parturition (**T1-Precalving**), immediately after parturition (**T2-Calving**), in early lactation (**T3-Early**), and in late lactation (**T4-Late**). The timing of T3-Early corresponded to the scheduled foot-trimming protocol on the farm, for that reason this time point was slightly earlier for first lactation animals compared to older animals. Serum samples were collected at the first three time points (T1-Precalving, T2-Calving, and T3-Early). The maximum daily milk yield during the first 100 days after calving was obtained from farm records for each animal.

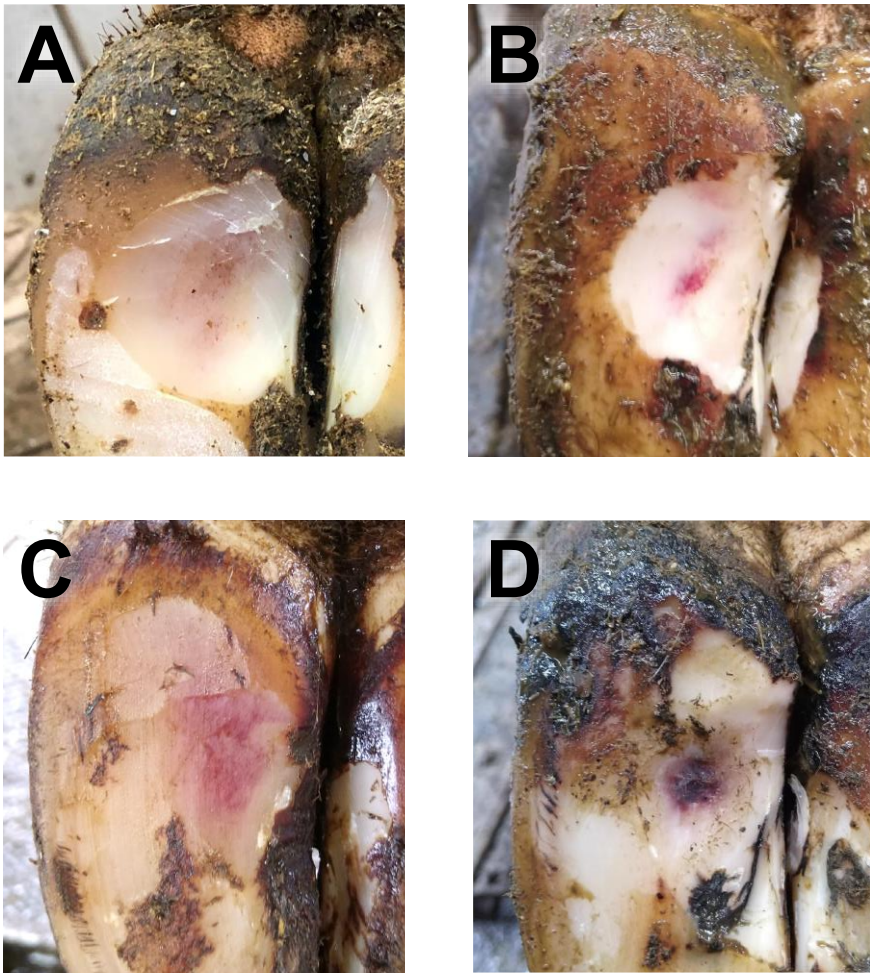
Study size. The sample size of this part of the study was determined by resource constraints. All eligible animals were enrolled until the final assessments (T4-Late) began, at which point further enrolments stopped as data collection at four time points simultaneously was not feasible.

Data collection procedures. Data collection was always conducted at the same time of day in nulliparous animals, and immediately after milking in parous animals; therefore, sampling times relative to feed access were consistent within contemporary groups. At each assessment, animals were restrained in a foot-trimming crush. Body condition score was recorded using a one to five scale with quarter-point intervals (Edmonson et al., 1989). Foot lesions were recorded either during routine foot-trimming (T1-Precalving and T3-Early) or after lightly trimming the sole horn to visualise lesions (T2-Calving and T4-Late). On each claw, all foot lesions were recorded using case definitions as described in the ICAR claw health atlas (Egger-Danner et al., 2020). All foot lesions were examined and recorded by qualified veterinary surgeons; over 95% by a single researcher, and the remainder by three other researchers. Sole haemorrhage was graded as either mild: light pink lesion < 2 cm diameter or diffuse discolouration of sole; or severe: light pink lesion ≥ 2 cm diameter or dark pink/purple lesion of any size (**Figure 5.1**). Sole ulcers were recorded as present or absent. Foot lesion recording was the same

at all time points except for T2-Calving, during which only hind feet were assessed to reduce the handling time of recently calved cows.

At the first three time points (T1-Precalving, T2-Calving, and T3-Early), blood samples were collected from the ventral coccygeal vein into plastic tubes coated with silica (Becton Dickinson Ltd, UK). Samples were mixed, allowed to clot at room temperature, and then centrifuged at 1,300 *g* for 20 minutes to separate the serum. Serum aliquots were either transported on ice directly to -80°C storage or placed into a -20°C freezer on the farm for up to eight hours before being transferred to storage at -80°C. The times between sampling and centrifugation and between centrifugation and storage at -80°C were recorded.

Figure 5.1. Examples of sole haemorrhage (SH) severity grading. Mild sole haemorrhage: diffuse discolouration of sole (A) or a light pink lesion < 2 cm diameter (B); severe sole haemorrhage: light pink lesion ≥ 2 cm diameter (C) or dark pink/purple lesion of any size (D).



5.2.3 Stage two: case-control study

Resources were available to analyse up to 600 serum samples using ^{1}H NMR spectroscopy, therefore a case-control approach was used to select samples for further analysis from the data collected during stage one of this study.

Data cleaning. The dataset was filtered to exclude animals with missing data. Missing data occurred if the animal left the herd during the study period, foot lesion records were incomplete from an assessment (minimum requirement of lesion records from both hindfeet), or a serum sample was missing from one of the first three time points. Animals were also excluded if data collection had occurred outside of an acceptable spread of sampling times, which was determined for each time point according to the expected variation at that stage in the production cycle: T1-Precalving = -90 to -21 days prepartum; T2-Calving = 1 to 10 days postpartum; T3-Early = 50 to 120 days postpartum, and T4-Late = 170 to 300 days postpartum.

Parity cohorts. We defined a cohort of first parity animals to specifically evaluate animals which were unlikely to have been affected by previous sole ulcers. A low incidence of both SH and SU were recorded in second parity animals, so these were excluded from subsequent analysis. Animals which were third parity or greater were grouped into a separate cohort.

Serum samples from T2-Calving and T3-Early were analysed in both cohorts (1^{st} parity and $\geq 3^{rd}$ parity), and samples from T1-Precalving were analysed in the primiparous cohort only. At T1-Precalving, nulliparous heifers were considered likely to be relatively homogenous, unlike parous animals, and therefore presented the best opportunity to evaluate possible differences in the metabolome before parturition.

Sample haemolysis. *S*evere serum haemolysis is associated with changes in the metabolome (Denihan et al., 2015). Before the final selection of cases and controls, serum samples were assessed for serum haemolysis using a 0 – 4 visual grading system (Akinyemi et al., 2018). A conservative threshold was

applied so that animals with samples which had haemolysis of grades 3 or 4 were excluded from further analysis.

Toe ulcers. Toe ulcers may develop from similar pathophysiological processes to sole ulcers, but can also occur due to over-wear or excessive trimming (Sanders et al., 2009; Kofler, 2017). Only a small number of cows were affected with toe ulcers (N = 5), so these were excluded to avoid potentially confounding effects.

Case and control definitions. Cases and controls were classified primarily by sole lesions at T3-Early, whilst taking into account prior and subsequent foot lesions. Animals with mild SH at T3-Early were excluded to promote the greatest differences between cases and control based on sole lesions. Cases were defined to reflect three separate outcomes relating to severe sole lesion development at T3-Early:

1. New sole haemorrhage: severe SH present at T3-Early; unaffected with severe SH or a SU at T1-Precalving and T2-Calving.
2. New sole ulcer: SU present at T3-Early; unaffected with severe SH or a SU at T1-Precalving and T2-Calving.
3. Chronic sole ulcer: affected with a SU at either T1-Precalving or T2-Calving, in addition to T3-Early.

Control animals were defined as those without mild or severe SH, or a SU at T3-Early; in addition to being unaffected with severe SH or a SU at T1-Precalving, T2-Calving, and T4-Late. Once cases had been defined, the same number of controls were randomly selected from eligible animals within each parity cohort.

5.2.4 ^{1}H NMR metabolomics

NMR spectra acquisition. Samples were processed in a randomised order (by sorting on a randomly generated number) to reduce systematic effects of measurement or instrument variability. Phosphate buffer (pH = 7.4) was prepared using dibasic and monobasic sodium phosphate in deuterated water. Once thawed, serum samples were diluted with equal volumes of phosphate buffer to minimise chemical shift due to pH fluctuations. Samples were vortexed,

centrifuged at 12,000 *g* at 4°C for 5 minutes, and then 600 µL was transferred into 5 mm diameter NMR tubes. Spectra were acquired using a Bruker Ascend 700 MHz spectrometer fitted with a 5 mm TCI Cryoprobe, AVANCE III HD console, and a SampleJet automated sample changer (Bruker Corp., USA). Before acquisition, spectrometer quality assurance was performed to establish temperature accuracy and stability (+/- 0.1°C) and shim quality (< 1 Hz line width at half height for DSS in Bruker supplied standard sucrose sample). Quality assurance was performed every 72 hours throughout the sample acquisition. Each spectrum was acquired at 37°C and one-dimensional ^{1}H NMR spectra were recorded using a Carr-Purcell Meiboom-Gill (**CPMG**) pulse sequence (cpmgpr1d, Bruker). The CPMG pulse program was selected because it attenuates signals from macromolecules to highlight small-molecule metabolites (Soininen et al., 2009).

NMR spectra processing. Spectra were processed via an automated pipeline (Bruker macro apk0.noe) to ensure consistent Fourier transformation, window function, and phasing. Spectral quality was manually assessed using TopSpin software (version 3.6; Bruker Corp., USA) according to the Metabolomics Standard Initiative (Sumner et al., 2007). Spectra were aligned to the alpha-glucose anomeric doublet at 5.24 ppm (indirectly referenced to trimethylsilyl propionate) because the presence of albumin in serum precluded the use of commonly used reference compounds (Pearce et al., 2008). Quality control assessments included the measurement of the half-height linewidth of glucose at 5.24 ppm, as well as a visual determination that water suppression, baseline stability, and signal-to-noise ratio were acceptable. If a sample failed quality control, then the spectra acquisition process was repeated a maximum of five times per sample, after which samples were excluded from further analysis.

NMR spectra binning and annotation. Spectral features were binned using the tameNMR toolkit on an in-house galaxy server (Afgan et al., 2018; Tools for Analysis of Metabolomic NMR (tameNMR), 2021) and provisionally annotated using Chenomx software (version 8.2; Chenomx Inc, Canada). Annotations were based on chemical shift and multiplicity using the Chenomx reference library of mammalian metabolites and cross-referenced with the Bovine Metabolome

Database (Foroutan et al., 2020). All spectra were overlaid to allow the visualisation of all small and consistent signals. Bin boundaries were manually specified to capture annotated peaks; if metabolite annotation was not possible then boundaries were selected to include individual peaks, distinct multiplets, or regions of consistent deviation from the baseline in all overlaid spectra. The peaks within each bin were integrated to calculate the relative intensity, and data were imported into R for subsequent analysis (R Core Team, 2021). Any negative values, due to noise at the baseline, were replaced with 1/5 of the minimum positive value for each bin. Data from each sample were normalised by the total spectral intensity to reduce technical variance between samples. A total of 211 bins were defined which contained all spectral peaks unaffected by residual water signal; 118 bins were putatively annotated to 34 different metabolites.

Bin selection. Twenty-four metabolites were represented by multiple bins in the spectrum. A single bin was manually selected which was highly correlated with other bins of the same metabolite label, and therefore considered to be representative of that metabolite (Grosman, 2019). Representative peaks were also selected based on the confidence of annotation and visually appraised to select a bin with minimal visible overlap from neighbouring signals.

There were 93 bins which could not be annotated with a candidate metabolite. Unlabelled bins were excluded if they were strongly correlated with a labelled bin (Pearson correlation coefficient ≥ 0.9). The remaining unlabelled bins were assessed for distinct clusters based on Euclidean distances using complete-linkage hierarchical clustering. The number of clusters was selected so that bins in different clusters had a Pearson correlation coefficient < 0.9; a single bin within each cluster was selected based on the clarity of peak isolation, as before.

The result of bin selection was to define the spectrum by a total of 85 bins. All annotated metabolites were represented by a single bin, and the remaining unlabelled bins were not strongly correlated with labelled or other unlabelled bins. The relative intensities calculated for these 85 bins were used for subsequent statistical analysis, referred to hereafter as (explanatory) variables.

5.2.5 Statistical analysis

Statistical analysis was conducted in R using *tidyverse* and *tidymodels* packages (Wickham et al., 2019; Kuhn and Wickham, 2020) unless otherwise specified.

Preliminary analysis. Principal component analysis (**PCA**) was conducted with the *GGally* package (Schloerke et al., 2018). The first five principal components, of centred and autoscaled data (van den Berg et al., 2006), were assessed for clustering or correlation due to technical or biological confounders. Technical confounders assessed were the timing of sampling relative to parturition, the time between sampling and centrifugation, the time between centrifugation and storage at -80°C, and the degree of serum haemolysis. Biological confounders assessed were body condition score, maximum milk yield, and the presence of other foot lesions.

General approach to statistical analysis. The aims of statistical analysis were to (i) determine whether the serum metabolome could discriminate between unaffected animals and those with one of four outcomes: **new SH**, **new SU**, either new SH or new SU (**new SH/SU**), and either new SU or chronic SU (**all SU**); and (ii) to identify any metabolites which had an informative association with one of these four outcomes. We, therefore, based our analysis on three methods which could be used for class prediction and automated variable selection.

To address our objectives, we split the dataset into 17 subsets pertaining to each comparison of interest. We analysed serum samples from animals at T1-Precalving, T2-Calving, and T3-Early, but did not incorporate repeated measures from each animal in our analysis, so each assessment time point (T1-Precalving, T2-Calving, T3-Early) was evaluated separately. In all subsets, the comparison group was cows which were not affected with severe SH or SU at any time point in our study (controls). The analysis included a separate evaluation of the two parity cohorts (1st parity and ≥3rd parity) and two outcomes corresponding to new sole lesions (new SH and new SU). We then repeated the analysis after pooling new SH and new SU together (new SH/SU), and then after pooling both parity

cohorts together. Finally, we used data from T3-Early to compare unaffected cows to all animals which had either a chronic or a new SU at that time (all SU).

5.2.6 Univariate analysis

Density plots were used to visualise the distribution of the relative intensity of each metabolite in each of the predefined subsets. Differences between case and control groups were assessed with a two-tailed, unpaired Wilcoxon signed-rank test as the distribution was non-gaussian in many instances.

5.2.7 Class prediction

The three statistical methods used were: partial least squares discriminant analysis (**PLSDA**), least absolute shrinkage and selection operator (**Lasso**) regression, and random forest classification; these were applied separately to each predefined subset. In all cases, model predictive performance was assessed using balanced accuracy, calculated as the average sensitivity and specificity of class prediction. Model hyperparameters were tuned to optimise balanced accuracy via 5-fold cross-validation repeated 20 times; the balanced accuracy of class prediction was assessed from the out-of-fold prediction results.

PLSDA. Partial least squares discriminant analysis is well suited to datasets with a large number of highly correlated variables, and as such is one of the most frequently used approaches in the analysis of metabolomics data (Gromski et al., 2015). This method finds a linear subspace of explanatory variables (referred to as components or latent variables) which maximises the covariance between the outcome and explanatory variables. The PLSDA model was fit on centred and autoscaled data using the *mixOmics* package (Rohart et al., 2017); the number of components in the model was tuned between 1 and 20.

Lasso regression. Regression models with a large number of explanatory variables can generalise poorly due to overfitting, this can be mitigated by including an additional penalty to shrink the absolute magnitude of the regression coefficients (Tibshirani, 1996). A logistic regression model with the lasso penalty was fit using the *glmnet* package (Friedman et al., 2010) on centred and

autoscaled data; the degree of penalisation was tuned on a grid of 20 values between 0.000001 and 10.

Random forest. Random forests combine the results from multiple decision trees which have each been fit on a random subsample of the data using a randomly selected subset of explanatory variables (Breiman, 2001). Random forests were fit using the *ranger* package (Wright and Ziegler, 2017) with 1000 individual trees and tuned via a grid of 100 combinations of *mtry* (number of variables selected in each split, 1 to 20) and terminal node size (2 to 40).

5.2.8 Variable selection

The same three methods were also used to highlight explanatory variables with an informative association with the outcome in each subset. Variables were considered to be "selected" as follows:

PLSDA. The variable importance in projection (**VIP**) reflects the influence of individual variables in a PLSDA model; scores greater than one corresponding to the variable being more influential than the average variable (Mehmood et al., 2012). The VIP scores were calculated in the final PLSDA model, and a variable was considered to be selected if the VIP score was greater than one.

Lasso regression. The Lasso penalty shrinks regression coefficients to produce coefficients of zero in the least important variables. A variable was considered to be selected if there was a non-zero coefficient in the final model.

Random forest. The Boruta algorithm is a variable selection method which uses random forests and performs well despite small group sizes, highly-dimensional data, and collinearity among explanatory variables (Degenhardt et al., 2019). The Boruta algorithm creates permuted copies of each variable and fits a random forest using both the actual and permuted variables. Over multiple iterations, the influence of each variable on model accuracy is assessed, and actual variables which have significantly higher importance scores than permuted variables ($P < 0.01$) are considered to be selected. The Boruta algorithm was implemented using the *Boruta* package (Kursa and Rudnicki, 2010).

5.2.9 Selection stability

Automated variable selection can erroneously classify non-informative variables as important in highly-dimensional data, in the presence of collinearity between variables, and without informed scrutiny of the variables being assessed (Westerhuis et al., 2008; Fan and Lv, 2010; Heinze et al., 2018). We considered incorrect variable selection (i.e., type one error) to be a likely problem in our analysis due to some subsets having more explanatory variables than samples, the complex correlation structures between metabolites, and the limited scope to assess the biological plausibility of results due to the broad and largely speculative metabolic component of sole lesion pathogenesis, and the inclusion of unlabelled metabolites. We, therefore, employed additional steps to assist the interpretation of variable selection results.

Observed stability. The robustness of selected variables to small perturbations in the data was assessed by bootstrapping the data (random sampling of the data with replacement) and repeating the variable selection steps on each bootstrapped resample. The proportion of times a variable is selected is termed stability, and higher stabilities increase confidence in a selected variable being truly informative (Austin and Tu, 2004; Meinshausen and Bühlmann, 2010; Sauerbrei et al., 2015). Variable selection stability was calculated from 200 bootstrapped resamples of each subset; we averaged the stability from the three variable selection methods to calculate a single, combined stability for each variable as described by Lima, Hyde and Green (2021), termed the “observed stability”.

Baseline stability. In each subset, we calculated thresholds to formalise the interpretation of the observed stability of each variable. Following the approach described by Green, Lima and Hyde (2021), the outcome variable was permuted to create a dataset in which any existing association between explanatory variables and the outcome had been removed. This was repeated ten times to produce independent datasets with randomised class labels. In each of these permuted datasets, the previous steps of bootstrapping and variable selection were repeated using 20 bootstrapped resamples. As before, the variable

selection stability in the bootstrapped resamples was calculated for each variable and then averaged over the three variable selection methods to determine the "baseline stability".

In each permuted dataset, the 99th and 100th percentiles of the baseline stabilities were taken from the distribution of baseline stabilities from all individual variables and averaged over the ten permuted datasets to define two thresholds, T_{99} and T_{100}, respectively. Therefore, on average 1% of variables in permuted datasets had a baseline stability which exceeded the T_{99} threshold, analogous to an expected false positive rate of 1%; similarly, the T_{100} threshold translates to an expected false positive rate of 0%.

In summary, the following steps were taken for variable selection in each specified subset:

1. Start with the original dataset
    a. Bootstrap 200 resamples
        i. Apply the three variable selection methods (PLSDA, Lasso regression, Boruta) to each resample
        ii. For each variable selection method - calculate the selection stability of each variable as the selection frequency divided by the number of bootstrap resamples
        iii. For each variable - take the mean of the selection stabilities from the three variable selection methods = "observed stability"
2. Return to the original dataset
    a. Generate ten datasets with permuted outcomes (remove the existing relationship between the explanatory variable and the outcome)
        i. Bootstrap 20 resamples of each permuted dataset
            - Apply the three variable selection methods (PLSDA, Lasso regression, Boruta) to each resample
            - For each variable selection method - calculate the selection stability of each variable in the permuted

dataset as the selection frequency divided by the number of bootstrap resamples

- For each variable - take the mean of the selection stabilities from the three variable selection methods in the permuted dataset = “baseline stability”

ii. For each permuted dataset – take the 99^{th} and 100^{th} percentile of the baseline stabilities

iii. Take the mean of the 99^{th} and 100^{th} percentiles of baseline stabilities from the ten permuted datasets to define two thresholds: T_{99} and T_{100}.

5.3 Results

A total of 1,169 cows were prospectively enrolled. After data cleaning, there were 737 animals which had foot lesion records from all time points, serum samples from the first three time points, and which had been assessed per the planned sampling time frame; the process and results of data cleaning are shown in **Figure 5.2**. In the dataset of 737 animals, 99.9% (736/737), 99.3% (732/737), and 96.6% (712/737) of animals had lesion records from all four feet at T1-Precalving, T3-Early, and T4-Late, respectively; all animals had lesion records from both hind feet at T2-Calving.

Details of foot lesion frequency at each assessment time point are provided in **Table 5.1**. At T3-Early, 5% (12/249) of primiparous animals and 11% (27/243) of animals in ≥ 3rd parity had SU; only 1% (2/245) of 2nd parity animals had SU at this time point. Not including those animals which also had SU, 26% (65/249) of primiparous animals and 19% (46/243) of ≥ 3rd parity animals had severe SH at T3-Early; only 9% (21/245) of 2nd parity animals had severe SH at T3-Early.

Figure 5.2. Data handling workflow to create the final case-control study population used in the analysis. The reason for animal exclusion is given in the grey box on the right.

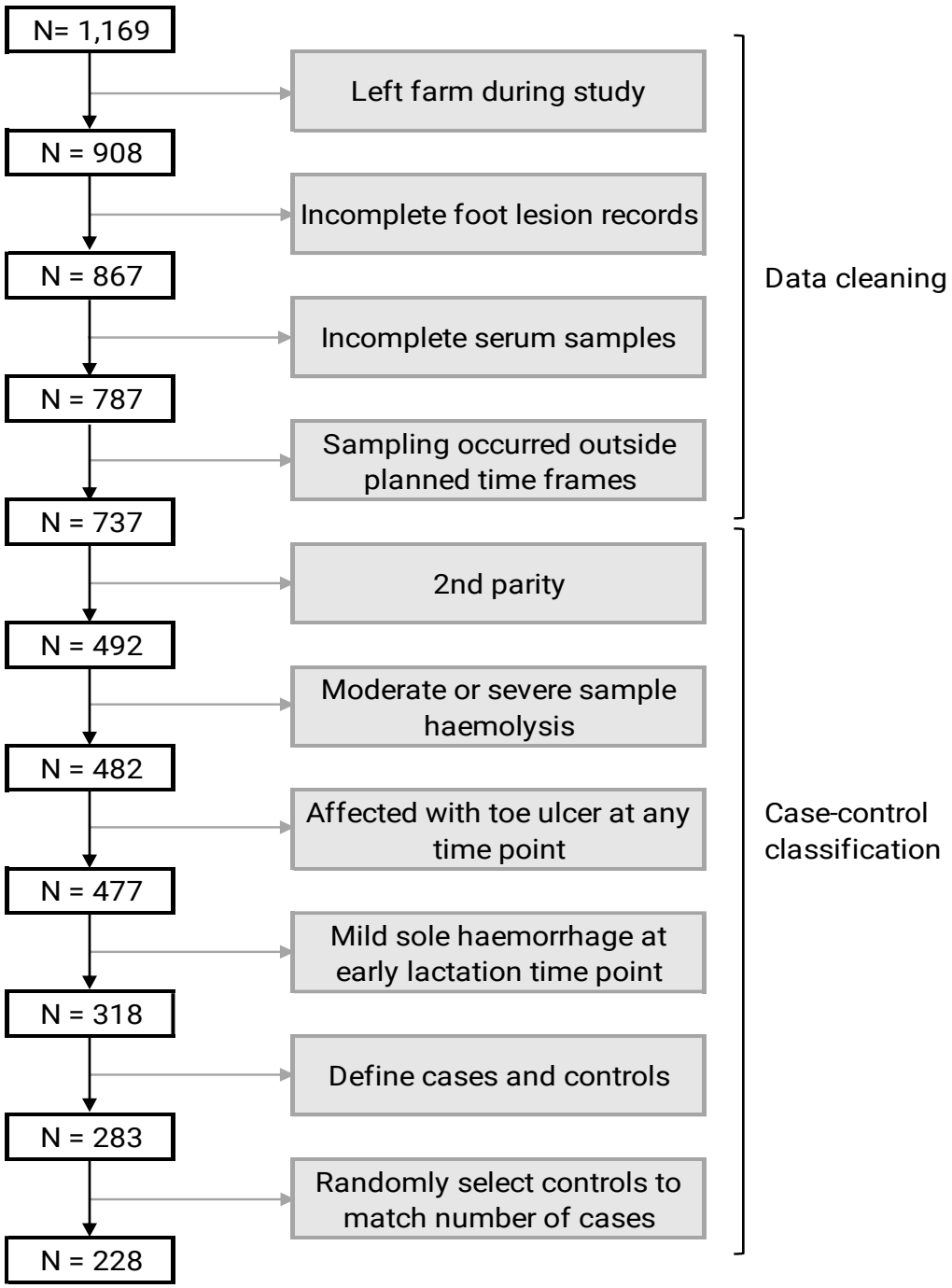


Table 5.1. Proportion (frequency) of animals affected by sole haemorrhage (SH) and sole ulcers (SU) at each time point in the study population following data cleaning (N = 737). Note: animals are defined according to the most severe sole lesion on all claws.

	No sole lesion	**Mild SH**	**Severe SH**	**SU**
1st parity				
T1-Precalving	0.74 (184)	0.24 (59)	0.02 (5)	< 0.01 (1)
T2-Calving	0.74 (185)	0.20 (51)	0.05 (12)	< 0.01 (1)
T3-Early	0.37 (92)	0.32 (80)	0.26 (65)	0.05 (12)
T4-Late	0.61 (152)	0.33 (83)	0.04 (11)	0.01 (3)
2nd parity				
T1-Precalving	0.90 (221)	0.09 (22)	< 0.01 (1)	< 0.01 (1)
T2-Calving	0.87 (214)	0.11 (27)	0.02 (4)	0.00 (0)
T3-Early	0.62 (151)	0.29 (71)	0.09 (21)	0.01 (2)
T4-Late	0.61 (149)	0.31 (77)	0.05 (13)	0.02 (6)
≥ 3rd parity				
T1-Precalving	0.63 (153)	0.23 (57)	0.06 (15)	0.07 (18)
T2-Calving	0.69 (167)	0.24 (58)	0.03 (7)	0.05 (11)
T3-Early	0.37 (91)	0.33 (79)	0.19 (46)	0.11 (27)
T4-Late	0.41 (100)	0.35 (84)	0.14 (34)	0.10 (25)

A total of 114 animals were classified as cases and an equal number of controls were randomly selected from eligible animals. In the 1st parity cohort, the mean (standard deviation) maximum daily milk yield was 36.9 kg (5.0) and 38.3 kg (4.8) in cases and controls, respectively; in the ≥3rd parity cohort, the mean (standard deviation) maximum daily milk yield was 54.5 kg (6.4) and 54.7 kg (6.4), respectively. Details of other potentially confounding factors in cases and controls are provided in **Table 5.2** and **Table 5.3**. Serum samples from the 1st parity cohort at T1-Precalving, T2-Calving, and T3-Early, and from the ≥3rd parity cohort at T2-Calving and T3-Early, were analysed using ^{1}H NMR spectroscopy. Fourteen samples repeatedly failed quality control procedures and were excluded from further analysis. Thirteen of these samples were from the T3-Early assessment time point, the other sample was from T2-Calving; seven of these samples were from 1st parity animals and the other seven from ≥ 3rd parity animals. The final dataset used for statistical analysis comprised 567 samples from 228 animals. Spectra were split into 211 bins, representative bins from labelled metabolites and clusters of unlabelled metabolites were selected, resulting in 85 selected bins (**Table 5.4** and **Table 5.5)**. Potential technical and biological confounders were assessed with PCA, no clustering or correlation attributable to these confounders was evident in the first five principal components.

Table 5.2. Details of potential technical confounders in the case and control groups.

		N	Mean (SD) assessment timing relative to parturition (days)	Mean (SD) time between sampling and serum separation (minutes)	Mean (SD) time between serum separation and -80°C storage (minutes)	Proportion (frequency) of samples by haemolysis grade (0 – 4)		
						Grade 0	Grade 1	Grade 2
1st parity								
T1-Precalving	Control	64	-52.6 (12.7)	119.8 (28.5)	405.1 (73.2)	0.70 (45)	0.25 (16)	0.05 (3)
	Case	61	-53.3 (13.9)	120.1 (20)	405.6 (61.3)	0.61 (37)	0.31 (19)	0.08 (5)
T2-Calving	Control	64	4.5 (2.0)	107.8 (64.8)	149.8 (81.6)	0.73 (47)	0.17 (11)	0.09 (6)
	Case	61	5.8 (2.3)	92.7 (48.7)	171.9 (83.6)	0.70 (43)	0.18 (11)	0.11 (7)
T3-Early	Control	60	67.0 (4.2)	49.5 (18.9)	143.9 (81.1)	0.60 (36)	0.35 (21)	0.05 (3)
	Case	58	67.1 (7.9)	52.0 (24.6)	133.4 (68.8)	0.66 (38)	0.28 (16)	0.07 (4)
≥ 3rd parity								
T2-Calving	Control	49	5.4 (2.2)	72.6 (38.6)	192.5 (86.6)	0.78 (38)	0.16 (8)	0.06 (3)
	Case	52	5.8 (2.4)	71.5 (25.8)	194.4 (81.1)	0.75 (39)	0.17 (9)	0.08 (4)
T3-Early	Control	48	95.7 (6.6)	74.3 (32.2)	185.8 (83.5)	0.79 (38)	0.12 (6)	0.08 (4)
	Case	50	95.0 (4.5)	83.7 (26.2)	190.5 (83.2)	0.82 (41)	0.12 (6)	0.06 (3)

SD: standard deviation

Table 5.3. Details of potential biological confounders in the case and control groups. Results are displayed as proportion affected (frequency) unless otherwise stated.

		Median (IQR) body condition score (1 - 5)	**White line lesion**	**Thin sole**	**Double sole**	**Digital dermatitis**	**Heel horn erosion**
1st parity							
T1-Precalving	Control	4 (4 – 4.25)	0.03 (2)	0.02 (1)	0.05 (3)	0.52 (33)	0.02 (1)
	Case	4 (4 – 4.25)	0.07 (4)	0.03 (2)	0.05 (3)	0.51 (31)	0.02 (1)
T2-Calving	Control	3.5 (3.5 – 3.75)	0.03 (2)	0.00 (0)	0.05 (3)	0.31 (20)	0.08 (5)
	Case	3.5 (3.5 – 3.75)	0.03 (2)	0.00 (0)	0.03 (2)	0.26 (16)	0.02 (1)
T3-Early	Control	3.25 (3 – 3.25)	0.03 (2)	0.12 (7)	0.13 (8)	0.18 (11)	0.12 (7)
	Case	3.25 (3 – 3.5)	0.03 (2)	0.07 (4)	0.05 (3)	0.14 (8)	0.12 (7)
≥ 3rd parity							
T2-Calving	Control	3.5 (3.25 – 3.5)	0.02 (1)	0.00 (0)	0.04 (2)	0.18 (9)	0.08 (4)
	Case	3.25 (3 – 3.5)	0.10 (5)	0.04 (2)	0.04 (2)	0.19 (10)	0.17 (9)
T3-Early	Control	3.25 (3 – 3.25)	0.08 (4)	0.08 (4)	0.29 (14)	0.19 (9)	0.21 (10)
	Case	3 (2.75 – 3.25)	0.14 (7)	0.10 (5)	0.32 (16)	0.20 (10)	0.34 (17)

IQR: interquartile range

Table 5.4. Details of the spectral bins selected as representative of each annotated metabolite and used in statistical analysis, including the Bovine Metabolome database identification number (BMDB ID).

Bin label	**BMDB ID**	**Bin range (ppm)**	**Bin label**	**BMDB ID**	**Bin range (ppm)**
1-Methylhistidine	0000001	8.00 - 7.97	Histidine	0000177	7.83 - 7.76
3-Hydroxybutyrate	0000357	2.31 - 2.29	Isoleucine	0000172	1.02 - 1.00
Acetate	0000042	1.93 - 1.91	Lactate	0000190	4.12 - 4.09
Acetoacetate	0000060	2.29 - 2.28	Leucine	0000687	0.98 - 0.96
Acetone	0001659	2.24 - 2.22	Leucine-Lysine	0000687/ 0000182	1.74 - 1.70
Alanine	0000161	1.49 - 1.47	Mannose	0000169	5.20 - 5.18
Arginine	0000517	3.25 - 3.24	Methionine	0000696	2.65 - 2.64
Aspartate	0000191	2.94 - 2.92	Methylsuccinate	0001844	1.08 - 1.06
Choline	0000097	3.20 - 3.20	Mobile lipids	-	0.90 - 0.80
Citrate	0000094	2.56 - 2.52	NMDA	0002393	3.16 - 3.15
Creatine	0000064	3.94 - 3.92	Phenylalanine	0000159	7.45 - 7.40
Creatinine	0000562	4.06 - 4.05	Proline	0000162	3.34 - 3.33
Formate	0000142	8.47 - 8.45	Propylene glycol	0001881	1.15 - 1.13
Glucose	0000122	3.54 - 3.53	Threonine	0000167	3.59 - 3.59
Glutamate	0000148	2.11 - 2.09	Tiglylglycine	0000959	1.83 - 1.82
Glutamine	0000641	2.48 - 2.43	Tyrosine	0000158	7.21 7.17
Glycine	0000123	3.57 - 3.56	Valine	0000883	0.99 - 0.98

Table 5.5. Details of the unlabelled spectral bins (not annotated to a metabolite) which were used in the statistical analysis.

Bin label	Bin range (ppm)	Bin label	Bin range (ppm)
Unlabelled_1	7.92 - 7.88	Unlabelled_27	3.20 - 3.17
Unlabelled_2	7.88 - 7.85	Unlabelled_28	3.08 - 3.05
Unlabelled_3	7.85 - 7.83	Unlabelled_29	3.04 - 3.03
Unlabelled_4	7.76 - 7.71	Unlabelled_30	2.95 - 2.94
Unlabelled_5	7.71 - 7.66	Unlabelled_31	2.92 - 2.87
Unlabelled_6	7.66 - 7.58	Unlabelled_32	2.81 - 2.77
Unlabelled_7	7.56 - 7.54	Unlabelled_33	2.60 - 2.57
Unlabelled_8	7.54 - 7.53	Unlabelled_34	2.57 - 2.56
Unlabelled_9	7.05 - 7.03	Unlabelled_35	2.38 - 2.36
Unlabelled_10	7.01 - 6.95	Unlabelled_36	2.28 - 2.24
Unlabelled_11	6.84 - 6.82	Unlabelled_37	2.21 - 2.19
Unlabelled_12	6.82 - 6.78	Unlabelled_38	2.18 - 2.17
Unlabelled_13	6.75 - 6.72	Unlabelled_39	2.17 - 2.16
Unlabelled_14	5.93 - 5.88	Unlabelled_40	2.06 - 2.03
Unlabelled_15	5.81 - 5.70	Unlabelled_41	1.91 - 1.90
Unlabelled_16	5.65 - 5.60	Unlabelled_42	1.89 - 1.88
Unlabelled_17	5.41 - 5.39	Unlabelled_43	1.87 - 1.83
Unlabelled_18	4.47 - 4.45	Unlabelled_44	1.64 - 1.54
Unlabelled_19	4.36 - 4.29	Unlabelled_45	1.47 - 1.45
Unlabelled_20	4.21 - 4.17	Unlabelled_46	1.45 - 1.43
Unlabelled_21	4.08 - 4.07	Unlabelled_47	1.42 - 1.39
Unlabelled_22	4.03 - 4.02	Unlabelled_48	1.19 - 1.18
Unlabelled_23	3.71 - 3.70	Unlabelled_49	1.17 - 1.15
Unlabelled_24	3.53 - 3.52	Unlabelled_50	1.13 - 1.11
Unlabelled_25	3.33 - 3.32	Unlabelled_51	0.91 - 0.90
Unlabelled_26	3.31 - 3.30		

The full dataset was split by time point, parity cohort, and outcome definition to create 17 subsets which related to the specific comparisons of interests (**Table 5.6**). The number of animals ranged from 58 to 211 in each subset. Univariate analysis of each metabolite in each subset was conducted with a Wilcoxon signed-rank test to compare cases and controls (**Supplementary Table 5.1.**). The balanced accuracy of class prediction was low in all subsets regardless of the statistical method, the average balanced accuracy of the three methods ranged from 50% to 62% (**Table 5.6**).

The stability of variable selection in bootstrapped resamples was calculated for each variable in each of the 17 subsets, resulting in 1,445 observed stabilities (**Supplementary Table 5.2**). Baseline stability thresholds were calculated in each subset after permuting the outcome; T_{99} and T_{100} thresholds ranged from 73.7% to 91.8% and 81.8% to 94.3% respectively (**Supplementary Table 5.2**).

As an example, **Figure 5.3** shows the distribution of observed stabilities from the subset relating to all parities at T3-Early, comparing all cases of SU (new SU and chronic SU) to unaffected cows. Across all subsets (i.e., 1,445 observed stabilities), 20 variables had an observed stability above the T_{99} threshold for that subset, corresponding to 15 different metabolites (**Table 5.7**). The distribution in relative intensities of these metabolites in case and control samples are displayed in **Figure 5.4**. Only nine variables had an observed stability greater than the T_{100} threshold, corresponding to phenylalanine and four unlabelled metabolites. **Figure 5.5** displays the spectra and bin boundaries of the four unlabelled metabolites represented by variables with an observed stability greater than the T_{100} threshold.

Table 5.6. Balanced accuracy of class prediction using partial least squares discriminant analysis (PLSDA), Lasso regression (Lasso), random forests (RF), and the average of all three methods (Combined). Data were analysed in 17 prespecified subsets split by time point, parity, and outcome definition; the control group in all cases were animals without sole lesions, cases were either cases of new sole haemorrhage (New SH), cases of new sole ulcers (New SU), those two groups combined (New SH/SU) or all cases of sole ulcers (All SU).

	Control (N)	**Case (N)**	**Outcome**	**Balanced accuracy of class prediction**			
				PLSDA	**Lasso**	**RF**	**Combined**
1st parity							
T1-Precalving	64	50	New SH	0.52	0.51	0.51	0.51
		11	New SU	0.50	0.50	0.50	0.50
T2-Calving	64	50	New SH	0.55	0.55	0.50	0.53
		11	New SU	0.56	0.57	0.50	0.54
T3-Early	60	47	New SH	0.57	0.53	0.47	0.52
		11	New SU	0.60	0.57	0.50	0.56
≥ 3rd parity							
T2-Calving	49	25	New SH	0.63	0.54	0.58	0.58
		12	New SU	0.71	0.54	0.53	0.59
T3-Early	48	26	New SH	0.57	0.54	0.50	0.54
		10	New SU	0.71	0.55	0.53	0.60
All parities							
T2-Calving	113	75	New SH	0.56	0.53	0.56	0.55
		23	New SU	0.53	0.53	0.50	0.52
		98	New SH/SU	0.57	0.54	0.57	0.56
T3-Early	108	73	New SH	0.56	0.56	0.50	0.54
		21	New SU	0.64	0.63	0.50	0.59
		94	New SH/SU	0.60	0.58	0.52	0.57
		35	All SU	0.66	0.65	0.54	0.62

Figure 5.3. Variable selection stability for all cases of sole ulcers compared to unaffected cows in all parities in early lactation (T3-Early). The solid line is the T_{100} threshold, and the dashed line is the T_{99} threshold.

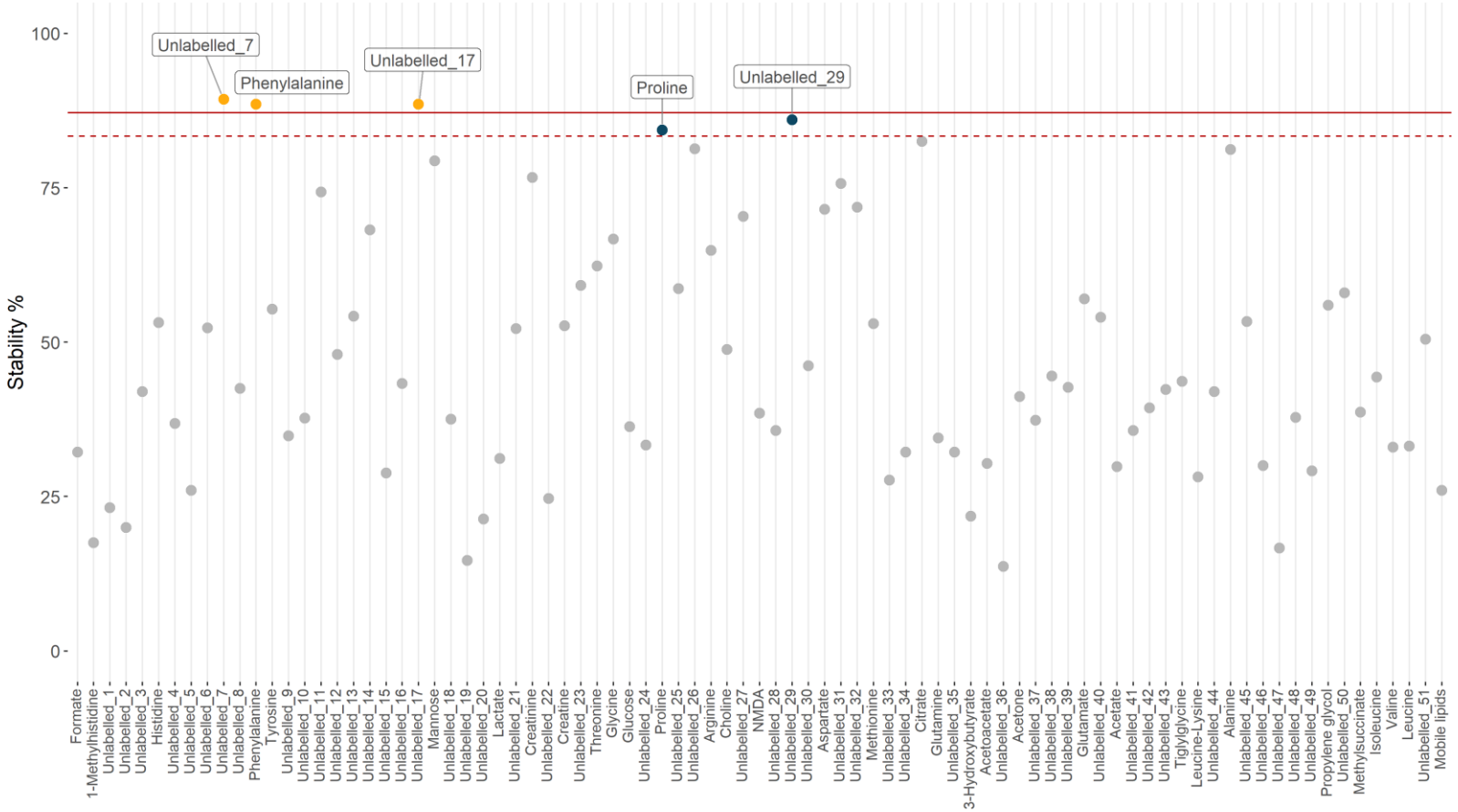


Table 5.7. Variable selection stability of variables which had a stability greater than the T_{99} threshold, * denotes variables with a stability greater than the T_{100}. Details include the mean log (base 2) fold-change (Log2FC) and the baseline stability thresholds (T_{99} and T_{100}, equivalent to an expected 1% and 0% false positive rate, respectively). Data were analysed in 17 prespecified subsets split by time point, parity, and outcome definition; the control group in all cases were animals without sole lesions, cases were either cases of sole new haemorrhage (New SH), cases of new sole ulcers (New SU), those two groups combined (New SH/SU) or all cases of sole ulcers (All SU).

Time point	Outcome	Metabolite	Log2FC	Stability (%)	T_{99} (%)	T_{100} (%)
1st parity						
T1-Precalving	New SH	Alanine	0.07	88.2	84.8	89.2
	New SU	Citrate	0.14	77.5	75.8	82.8
T2-Calving	New SH	Unlabelled_7*	-0.15	93.2	87.3	91.5
	New SU	Unlabelled_47*	0.09	83.7	73.7	81.8
T3-Early	New SU	Phenylalanine	0.20	81.2	75.6	83.7
		Unlabelled_26*	0.35	92.5		
≥ 3rd parity						
T3-Early	New SH	Unlabelled_24	0.09	78.3	77.5	85.3
		Propylene glycol	0.17	80.5		
	New SU	Unlabelled_43	0.13	75.3	74.6	82.3
All parities						
T2-Calving	New SH	Formate	-0.14	90.8	89.4	91.3
	New SU	Unlabelled_10	0.12	80.8	78.8	82.7
		Valine	-0.13	82.0		
	New SH/SU	Unlabelled_7*	-0.08	93.7	91.8	93.5
	New SU	Phenylalanine*	0.16	94.2	81.8	84.0
		Unlabelled_26*	0.18	86.3		
T3-Early	All SU	Unlabelled_7*	-0.11	89.3	83.4	87.2
		Phenylalanine*	0.08	88.5		
		Unlabelled_17*	-0.17	88.5		
		Proline	-0.13	84.3		
		Unlabelled_29	-0.08	86.0		

Figure 5.4. Density plots of standardised and mean-centred relative intensities of the metabolites in Table 5.7.

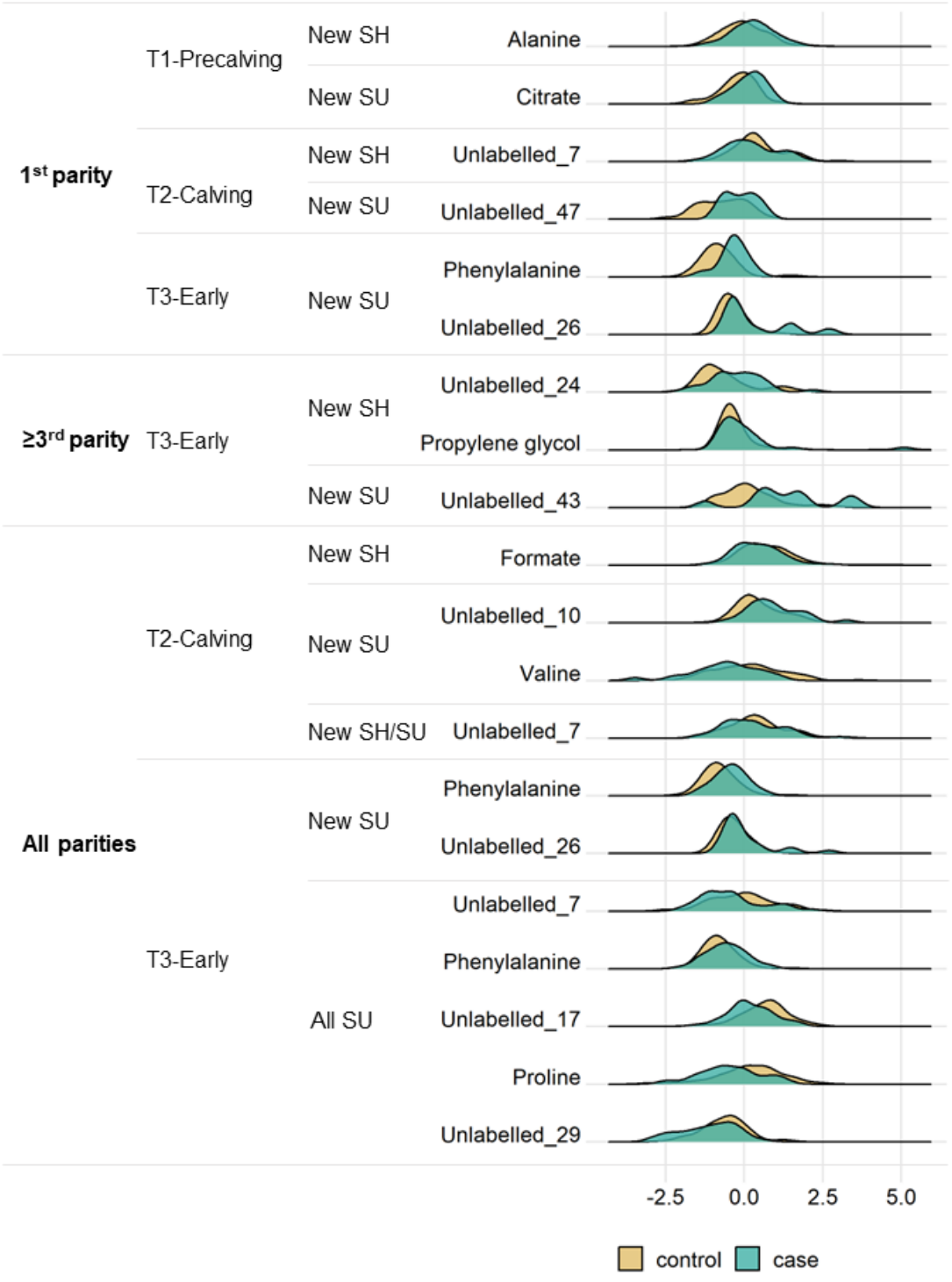
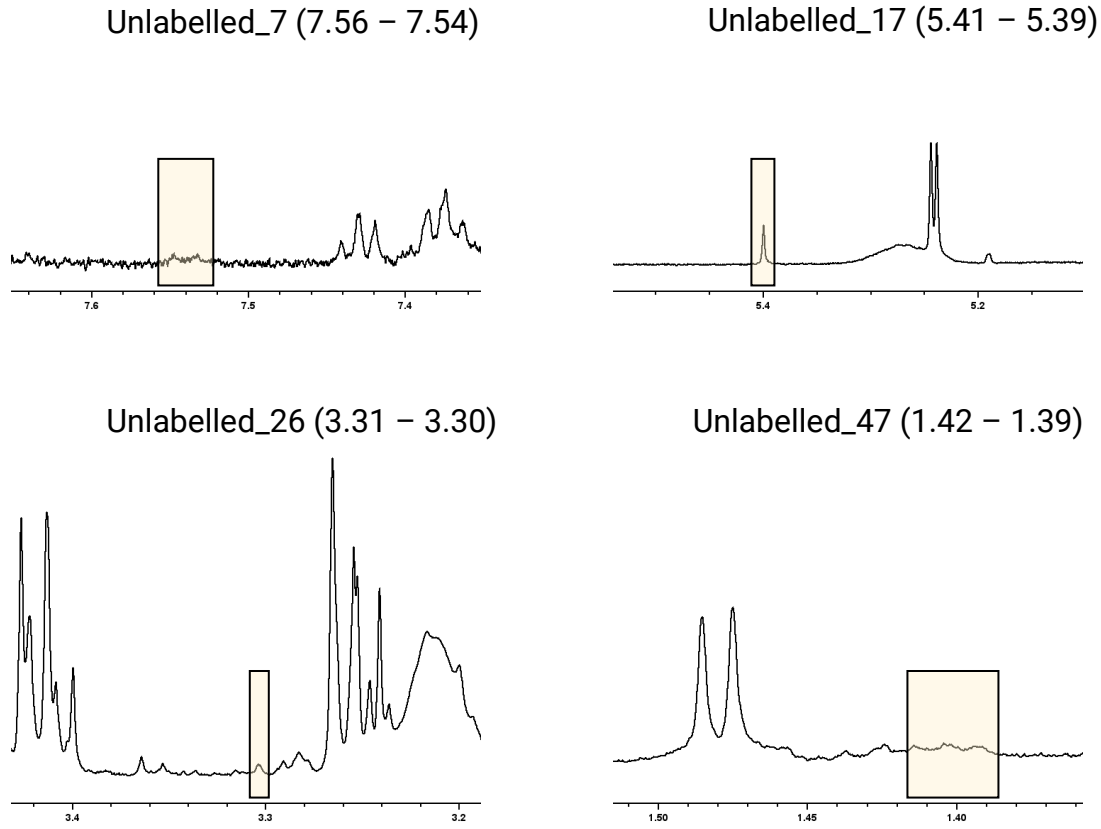


Figure 5.5. Example spectra and bin boundaries (yellow shading) for the four unlabelled metabolites which had an observed stability greater than the T_{100} threshold (Table 5.7).



5.4 Discussion

We designed a study to explore the association between the serum metabolome and the development of sole lesions in Holstein cows. To minimise variation due to factors other than our outcome of interest, data were analysed in subsets split by time point, parity, and case definition. As a consequence, analysis was conducted in relatively small subsets (range of observations: 58 to 211), often with imbalanced class sizes. We, therefore, limited the scope of our analysis to a broad screen of the discriminatory power of the serum metabolome in each subset, followed by further analysis to highlight informative variables. Our results indicated the serum metabolome, as characterised by ^{1}H NMR spectroscopy, could not reliably discriminate between animals based on the presence, or future development, of sole lesions. Additionally, we only identified a small number of metabolites which may be associated with sole lesion development. Taken together, there is limited support from this study for a major metabolic component in serum to the pathogenesis of sole lesions, however, we draw this conclusion cautiously because there are several important caveats to these results.

5.4.1 Key results and interpretation

***Class prediction*.** The average balanced accuracy of predictive modelling did not exceed 62% in any subset, although the balanced accuracy of PLSDA reached 71% on two occasions. Furthermore, as the predictive performance was assessed from cross-validation during the tuning of model parameters, estimates of the balanced accuracy may be upwardly biased (Varma and Simon, 2006). Balanced accuracy was highest in subsets relating to concurrent SU, compared to time points before lesion development or when the outcome was SH rather than SU, however, our results suggest a very limited predictive capacity of the serum metabolome for sole lesions.

The poor balanced accuracy of class prediction we observed is in contrast to results reported from two studies which analysed the serum metabolome of lame cows using MS-based techniques (Dervishi et al., 2019; Zhang et al., 2020b); but, as the cause of lameness in these cows was not described, these results

might not be comparable to our study. Nevertheless, both studies (Dervishi et al., 2019; Zhang et al., 2020b) reported near-perfect diagnostic accuracy of selected serum metabolites to differentiate between lame and non-lame cows before and after the onset of clinical lameness (area under the receiver operating characteristic curve > 0.99). The metabolites which had the highest VIP scores in these PLSDA models were lysine, leucine, and isoleucine (Zhang et al., 2020b) and valine, mannose, and phosphoric acid (Dervishi et al., 2019); except for phosphoric acid, these metabolites were represented by variables in our dataset but not highlighted as influential in our analysis. It should be noted that the predictive accuracy reported in these studies (Dervishi et al., 2019; Zhang et al., 2020b) may be over-optimistic due to the risk of feature selection bias with the analytical approach described (Xia et al., 2013; Kuhn and Johnson, 2018).

Compared to other farmed species and humans, dairy cattle have exceptionally high metabolic demands in early lactation (Webster, 2020), and individual-specific responses in this turbulent period could present a challenge to metabolomic studies of dairy cows. For example, Ghaffari *et al.* (2019) used MS-based analysis to determine differences in the serum metabolome between over-conditioned dairy cows and those in typical body condition at multiple time points. Four classification models showed reasonable prediction accuracy between groups (71% to 76%) when cows were also being fed different diets, however, in early lactation when diets were the same but differences in body condition remained, prediction accuracy was substantially lower (52% to 65%). In contrast, human studies have demonstrated that obesity is strongly associated with changes in the serum metabolome (Moore et al., 2018; Cirulli et al., 2019), and therefore the modest prediction accuracy observed by Ghaffari *et al.* (2019) may illustrate the challenges of assessing the serum metabolome in early lactation dairy cows, even due to factors which are known to have strong metabolic effects.

Variable selection. Across all subsets, only nine variables had an observed stability greater than the T_{100} threshold, these corresponded to five different metabolites: phenylalanine and four unlabelled metabolites (**Table 5.7**). Selected

variables should be interpreted in the context of the poor balanced accuracy of class prediction which suggests that even if these variables are truly associated with sole lesions, they only explain a small part of the differences between affected and unaffected animals.

Phenylalanine was highlighted as informative in subsets related to concurrent SU, and had a higher concentration in animals with SU compared to unaffected cows; Dervishi et al., (2019) observed the same trend between lame and non-lame cows. Phenylalanine has been reported to be increased in humans due to inflammation and oxidative stress associated with conditions such as trauma, sepsis, and burns (Rath et al., 1987; Ploder et al., 2008). There is interest in the role periparturient inflammation and oxidative stress may have on sole lesion development in dairy cattle (Al-Qudah and Ismail, 2012; Zhao et al., 2015; Watson et al., 2022; Wilson et al., 2022).

Unlabelled metabolites were spectral features which could not be annotated from a mammalian metabolite library. Example spectra of the four unlabelled metabolites which had an observed stability greater than the T_{100} threshold are shown in **Figure 5.5**, two of these unlabelled metabolites represent distinct single peaks (Unlabelled_17 and Unlabelled_26), whereas the other two (Unlabelled_7 and Unlabelled_47) are much more poorly defined. By overlaying the spectra from all 567 samples it was possible to visualise small, but consistent, changes in the baseline. These weak signals could relate to metabolites at the lower limit of detection by ^{1}H NMR spectroscopy, metabolites occluded by the presence of albumin in serum, or signals attenuated during the CPMG pulse program; either way interpretation of these peaks requires caution due to the low signal-to-noise ratio. Such metabolites may be more reliably detected and identified using serum extracts (which separate metabolite from albumin), other NMR pulse sequences, two-dimensional NMR, or MS-based techniques (Beckonert et al., 2007; Marchand et al., 2017; Emwas et al., 2019).

5.4.2 Reflections and implications

***Sample processing*.** One of the challenges we experienced was ensuring serum separation was rapid enough to minimise changes in metabolites due to

ongoing cellular metabolism post-collection. Before the start of data collection, we attempted to establish a standard operating procedure for sample acquisition, processing, and storage (Stringer et al., 2016). To promote rapid clot formation, it is recommended that serum samples for metabolomics analysis are collected in glass tubes (Barri and Dragsted, 2013), however, this was not a practical option. We instead elected to use plastic serum tubes coated with silica and checked these did not produce any strong residual signals in NMR spectra. We set up a centrifuge on the farm and initially centrifuged blood samples 30 minutes after collection, as recommended for metabolomics research (Beckonert et al., 2007; Bernini et al., 2011). Unfortunately, a large proportion of samples had not adequately clotted by this time, resulting in gelatinous or low volumes of haemolysed serum. As cattle have variable and prolonged clotting times compared to other species (Osbaldiston et al., 1970), we were forced to adopt a less rigid protocol to allow time for clot formation and retraction. Consequently, there was some unavoidable variation in the time between collection and centrifugation due to samples which took a long time to clot. Although we did not observe any effect of the time between sampling and centrifugation with PCA, it is possible that this step in sample processing introduced extraneous variance to the data. Furthermore, clotting times can vary in periparturient cattle depending on the time since parturition (Heuwieser et al., 1990), creating a potential interaction between sampling time relative to calving and the efficiency of serum separation. Processing of plasma is more reproducible than serum (Hernandes et al., 2017), which may be one reason why plasma is more frequently analysed than serum in metabolomics studies of cattle (Goldansaz et al., 2017); this would be a worthwhile consideration in future studies.

Spectrum binning and annotation. For purposes of analysis, the serum metabolome was characterised by 85 components of the NMR spectrum. To reach this point, the spectrum was first manually subdivided into 211 bins to capture all potentially relevant spectral signals. One benefit of this approach was that bins could include entire multiplets which were annotated to the same metabolite, and bin boundaries could be flexible to accommodate small spectral

shifts in individual peaks; this is more challenging to achieve with equidistant binning (Emwas et al., 2018). However, there are also limitations to manually binning the spectrum due to the risk of introducing bias in the process of subjectively deciding bin boundaries and annotating metabolites.

Spectral features were annotated to a total of 34 different metabolites which is comparable to other NMR-based studies of serum in cattle (Chen et al., 2013; Blakebrough-Hall et al., 2020), and only slightly fewer than typically identified in NMR spectra acquired from human serum (Psychogios et al., 2011). As some metabolites had multiple signals, we selected a representative bin for each annotated metabolite (Grosman, 2019). This approach has advantages because it reduces the dimensionality of the data and eliminates highly correlated bins which relate to the same metabolite, although naturally occurring correlation structures between metabolites in interacting metabolic pathways remain. However, we acknowledge that this process also has limitations because of the potential loss of information in the unselected bins. Before bin selection, we used all 211 bins, corresponding to all signals in the spectra, in class prediction models and did not observe any improvement in model performance; therefore, we consider it unlikely that the poor balanced accuracy is attributable to the loss of resolution in the selection of representative bins.

Class prediction. It is possible that we were unable to reliably differentiate between cases and controls using the serum metabolome because there were too many extraneous sources of spectral variation. We highlighted some technical causes of potential variation, however, PCA did not indicate these to be influential in this regard. Teahan *et al.* (2006) examined the effect of several different experimental factors on NMR spectra and found the inter-individual variation to be much more influential than experimental factors. We anticipate that there were numerous biological differences between individual animals which may have affected the serum metabolome. No strong differences in body condition and milk yield were observed between cases and controls, and these factors also did not appear influential with PCA. However, the serum metabolome is known to be affected by a range of clinical and subclinical metabolic diseases,

for example, retained placentas and subclinical ketosis, which may occur parallel to sole lesion development in early lactation (Sun et al., 2014; Dervishi et al., 2016, 2017; Yong et al., 2021). As it was not feasible to monitor the study population closely enough to record all such conditions, it is unknown how their incidence may have affected our results.

Variable selection. We applied an approach to variable selection which aimed to minimise false positive results. It is suggested that data analysis should include multiple methods which have different and unrelated sources of bias to avoid the interpretation of spurious results as informative (Munafò and Davey Smith, 2018). This concept of triangulation has been further developed with variable selection stability by Lima *et al*. (2020), and subsequently become popular in recent veterinary epidemiological studies (Lewis et al., 2021; Browne et al., 2022). We followed the approach described by Green, Lima and Hyde (2021) by taking the average variable stability from multiple variable selection methods and comparing this observed stability to the distribution of baseline stabilities in permuted data. This approach was demonstrated to be robust to false positive results from variable selection (Green et al., 2021), however, we used different statistical methods, such as PLSDA and Boruta, so we cannot claim to have replicated this method exactly. Nevertheless, we would expect the principles of stability, triangulation, and permuted baseline stabilities to be generalisable across a range of statistical methods.

The T_{99} threshold was calculated from the 99th percentile in the distribution of baseline stabilities; therefore, we considered this threshold to translate to an expected false positive rate of 1%. By analysing 85 explanatory variables in 17 subsets, we calculated a total of 1,445 observed stabilities; if no variable had a relationship with the outcome, by definition, we would still expect 1% of variables to have an observed stability greater than the T_{99} threshold. Across all subsets, 20 variables had an observed stability which exceeded the T_{99} threshold, which is, therefore, more than would be expected in the complete absence of any informative explanatory variables. However, many of these variables could still be false positives, so we focused our interpretation on the metabolites which had

an observed stability above the T_{100} threshold, as these are the least likely to be artefactual. Nevertheless, we have reported all variables with an observed stability greater than the T_{99} threshold so that future studies can determine whether any of these more equivocal results are verifiable.

The T_{100} threshold was calculated for each subset as the average maximum baseline stability from the ten datasets in which the outcome had been permuted. It should be noted, however, that as we took the average maximum baseline stability, this threshold does not represent the absolute maximum baseline stability of any variable in the permuted datasets. In fact, in every subset, the maximum baseline stability calculated was greater than the highest observed stability. Therefore, we recognise that despite variables having an observed stability high enough to infer probable importance, it is still possible to obtain similar results in a dataset where the relationship between outcome and explanatory variable has been removed, although there is only a small chance of this occurring.

Future metabolomic studies. We annotated 118 bins to 34 different metabolites. Approximately 350 different metabolites have been detected in bovine serum, predominantly from MS-based analyses, although the full complement of low molecular weight metabolites is likely to be considerably greater (Goldansaz et al., 2017). It is recommended that metabolomics studies use multiple analytical platforms to improve metabolite coverage, although most published studies of livestock metabolomics only use a single approach (Goldansaz et al., 2017). Even within a single analytical platform, annotation of metabolites from spectral signals depends on the coverage of reference libraries, and this is a major bottleneck in biological inference (Viant et al., 2017). To date, the majority of metabolomics studies in cattle have analysed serum, plasma, urine, rumen fluid, or milk (Goldansaz et al., 2017). Serum and plasma are useful reflections of generalised physiological (or pathophysiological) states, but other biofluids, such as urine, have distinct metabolite compositions which can provide valuable complementary information regarding metabolic processes (Wishart, 2019; Kim et al., 2021; Anderson, 2022). Therefore, to characterise any metabolic

component in the pathogenesis of sole lesions it may be necessary to consider the parallel analysis of multiple sample types using multiple metabolomic platforms.

Generalisability. We enrolled cows from a single herd and, as a consequence, the generalisability of our study is intrinsically limited. The herd in our study operated under a relatively intensive management system of zero-grazing, and this is an important context from which to interpret our results. The housing environment is a major risk factor for sole lesions (Cook et al., 2004; Cook and Nordlund, 2009; Bergsten et al., 2015), and the aetiology and frequency of lameness differ between grazed and housed herds (Hund et al., 2019). High-input with high-output dairy systems are becoming increasingly common and there has been a global trend in recent years toward year-round housing of dairy cattle (Knaus, 2016). In a recent survey of 53 randomly selected dairy herds in the UK, 36% housed milking cows all year round (Thompson et al., 2020); another survey of 863 dairy herds, also in the UK, reported that 23% of herds housed early lactation and high-yielding groups all year round (March et al., 2014).

The average herd prevalence of lame cows in the UK has recently been reported from cross-sectional studies to be approximately 30% (Griffiths et al., 2018; Randall et al., 2019); in our study, we recorded the prevalence of lame cows to be between 7 - 9% depending on the time point of the study (data not shown). Previous studies in the UK which recorded foot lesions in first parity animals observed more than 95% of heifers were affected with sole lesions in early lactation (Leach et al., 1997; Maxwell et al., 2015; Randall et al., 2016); 63% of heifers were affected with a sole lesion at the T3-Early assessment in our study. Taken together, we would conclude that our study population was from a herd with a better overall management of lameness than the many UK dairy herds.

Luke et al. (2020) used ^{1}H NMR spectroscopy to analyse serum samples from dairy cows in thirteen herds with similar management systems; results indicated that inter-herd differences were responsible for 57% of the overall spectral variation. Therefore, it is likely that even if our study was repeated in seemingly comparable herds there could be differences in the results. Although

metabolomic approaches may ultimately progress understanding of the pathogenesis of sole lesions, a considerable body of further research is still needed.

5.5 Conclusions

We compared the serum metabolome in dairy cows which developed sole lesions in early lactation to those which were unaffected. Analysis of the serum metabolome could not reliably discriminate between animals based on the presence, or future development, of sole lesions. We also only highlighted a small number of metabolites which may be associated with sole lesion development. We conclude that the serum metabolome, as characterised by ^{1}H NMR spectroscopy, is not strongly associated with sole lesion development; but any true association may have been masked by variation between individual animals or from other experimental sources. The application of metabolomics undoubtedly has the potential to reveal underlying mechanisms of sole lesion aetiopathogenesis in dairy cows, however, our results were equivocal in this respect and further studies would be beneficial.

Chapter 6: Association between a genetic index for lameness resistance and the incidence of claw horn lesions in Holstein cows

6.1 Introduction

Farmers and veterinary surgeons regard lameness as one of the most important health and welfare concerns in dairy cattle (More et al., 2010; Bauman et al., 2016), and lameness has been identified as the most pressing problem affecting the modern dairy industry in Europe (European Food Safety Authority (EFSA), 2009). Foot lesions are the major cause of lameness in dairy cows (Murray et al., 1996; Bicalho et al., 2007a; van Huyssteen et al., 2020) and directly impact the longevity, productivity, and fertility of affected animals (Booth et al., 2004; Machado et al., 2010; Omontese et al., 2020).

Sole haemorrhage (**SH**), sole ulcers (**SU**), and white line lesions (**WL**) are often grouped under the collective term “claw horn lesions” (Murray et al., 1996; Offer et al., 2000). Claw horn lesions (**CHL**) have a high prevalence in dairy cattle (Murray et al., 1996; Laven and Lawrence, 2006; Somers and O’Grady, 2015) and, relative to other foot lesions, CHL have been associated with the most severe pain responses (Whay et al., 1998; Pastell et al., 2010), economic impacts (Amory et al., 2008; Bruijnis et al., 2010; Dolecheck et al., 2019), and environmental consequences (Mostert et al., 2018).

The phenotypic variation of CHL in a population represents the underlying risk of animals developing these lesions, this variation can be partitioned into genetic and environmental components (Tenesa and Haley, 2013). The proportion of phenotypic variation explained by genetic differences is referred to as heritability. The heritability of CHL, based on underlying risk, has been estimated in a large number of studies which have recently been summarised by Heringstad et al. (2018) as 0.07 – 0.09 for SH, 0.07 – 0.18 for SU, and 0.06 – 0.10 for WL; the heritability of lameness diagnosed from locomotion scoring has been estimated to be 0.15 (Weber et al., 2013). Therefore, although these heritability

estimates are low, genetic selection could produce cumulative, long-term benefits to complement husbandry-based initiatives to reduce lameness.

Dairy farmers are generally motivated to reduce lameness (Bruijnis et al., 2013; Bennett et al., 2014; Dutton-Regester et al., 2019) and lameness traits ranked highly when farmers were surveyed about their genetic selection preferences (Martin-Collado et al., 2015). Genetic traits relating to lameness can be considered as either direct traits, such as foot lesions, or indirect traits which include breed society classification traits such as leg conformation and gait assessment (Heringstad et al., 2018).

Historically, farmers wishing to reduce lameness in their herd through improved genetics could only select on indirect traits (McDaniel, 1998), but it is now recognised more broadly that selection on direct health traits could accelerate genetic gains (Egger-Danner et al., 2014). Consequently, two approaches have evolved in recent years to develop effective genetic selection indexes which can reduce the incidence of lameness in dairy herds. Some countries have incorporated foot lesion records directly into selection indexes (Egger-Danner et al., 2014), but other countries, where the infrastructure to record foot-trimming lesions on a large scale has not been established, have instead utilised farm records of lameness (Zwald et al., 2004; Pritchard et al., 2013; Parker Gaddis et al., 2014). In the UK, Pritchard et al. (2013) demonstrated that farm records could be used as phenotypes for both clinical mastitis and lameness, however, there were concerns regarding the quality of farm lameness records. In that study (Pritchard et al., 2013), only a third of cow records used for the mastitis analysis were included in the lameness evaluations due to a lack of lameness recording in individual herds. Furthermore, across the herds which were recording lameness, there was an apparent incidence of 15.8% over the first three lactations, which is lower than the average national prevalence of 34.9% (Afonso et al., 2020), and dramatically lower than more directly comparable annual incidence rates (Clarkson et al., 1996; Hedges et al., 2001). A further concern is that farm records may be skewed towards lesions which are consistently or severely associated with lameness (Archer et al., 2010a), for

example, SU are associated with more severe lameness than SH (Tadich et al., 2010), and therefore farm records may not reflect SH and SU with equal accuracy.

In 2018, a genetic selection index for lameness, termed "Lameness Advantage", was published by the UK Agricultural and Horticultural Development Board (AHDB). The Lameness Advantage index is calculated using lameness events from farm records (collected via milk recording organisations) in combination with traits from type classification: Bone Quality, Locomotion, Feet and Legs (an overall assessment by the classifier incorporating Foot Angle, Rear Legs Side View, Locomotion, and Bone Quality), and Digital Dermatitis (AHDB, 2020b). Higher values of this index are associated with better genetic merit for lameness and an expected reduction in the incidence of lameness compared to lower values, however, this index has not yet been evaluated in independent data. A recent study in Ireland reported that cows with a positive genetic index for lameness, in this case reflecting an increased genetic susceptibility, had a 37.5% increase in the odds of lameness compared to animals with a negative genetic index (Browne et al., 2022).

6.1.1 Objectives

It was hypothesised that the association between Lameness Advantage genetic index and the actual frequency of claw horn lesions would be weak due to the quality of farm lameness records; therefore, the primary objective of our study was to quantify this relationship in a cohort of dairy cattle with accurate foot lesion records. A further objective was to screen for associations between other selection indexes and claw horn lesions or lameness, to evaluate whether selection on type traits could still be utilised to reduce lameness.

6.2 Materials and methods

6.2.1 Study design and population

The study was conducted following ethical approval by the University of Liverpool Research Ethics Committee (VREC269a, VREC466ab). A prospective, cohort study on four dairy herds in the UK was designed to record foot lesions at four time points during a lactation cycle. Herds were selected based on the convenience and practicalities of frequent visits and data collection. Herds A to C housed lactating cows all-year-round, milked cows three times daily and recorded 305-day milk yields of approximately 11,000 - 11,500 L. Herd D housed early lactation and high-yielding cows all-year-round and lower-yielding cows were grazed during the summer; cows were milked twice daily and the 305-day milk yield was approximately 9,000 L. Parous cows on all herds were routinely foot-trimmed twice a year before drying off and 60 - 120 days after calving. On all herds, lactating cows were regularly footbathed after milking. Herd A footbathed cows three times a week with either copper sulphate or formalin; herd B footbathed cows twice daily with formalin, herd C footbathed cows daily with either copper sulphate or formalin and herd D footbathed three times a week with formalin.

6.2.2 Data collection

A total of 2,352 Holstein cows which were expected to calve between April and December 2019 were prospectively enrolled before calving with no additional inclusion or exclusion criteria applied. Data were collected by qualified veterinary surgeons during weekly or twice weekly visits to each herd from February 2019 to July 2020. Animals were assessed at four time points relative to their calving date: before calving (mean: -55 days, standard deviation (SD): 18), immediately after calving (mean: +5 days, SD: 3), in early lactation (mean: +84 days, SD: 14), and finally in late lactation (mean: +200 days, SD: 31). Enrolments continued until the final assessments in late lactation began, at which point additional enrolments stopped as data collection at four time points simultaneously was not feasible.

All cows were mobility scored according to the AHDB system from 0 (sound) to 3 (severely lame) (Whay et al., 2003a; AHDB, 2020a). Cows were restrained in a foot-trimming crush and, depending on the assessment time point and the foot-trimming schedule in each herd, either functionally foot-trimmed or lightly trimmed to allow visualisation of foot lesions. In either case, CHL on each claw were recorded based on the ICAR claw health atlas (Egger-Danner et al., 2020). Over 90% of foot lesion identification and recording were performed by a single researcher.

All cows were genotyped and genetic indexes for cows and their sires were provided by the AHDB in the form of genomic predicted transmitting abilities, following calculation in the August 2021 national evaluation. In addition to the Lameness Advantage index, the other genetic indexes available for analysis were: Profitable Lifetime Index (**£PLI**), Lifespan, Type Merit, Digital Dermatitis, Feet and Legs, Locomotion, Condition Score, Milk (kg), Fat (kg), Protein (kg), Fat (%), Protein (%), Somatic Cell Count (SCC), Mammary, Mastitis, Fertility Index, TB Advantage, Calf Survival, Maintenance, Stature, Chest Width, Body Depth, Angularity, Rump Angle, Rump Width, Rear Leg Side View, Foot Angle, Fore Udder Attachment, Rear Udder Height, Udder Support, Udder Depth, Front Teat Placement, Rear Teat Placement, Teat Position Side, Temperament, and Milking Speed.

6.2.3 Statistical analysis

Four independent outcomes were defined to reflect the susceptibility or resistance of an animal to SH, SU, WL, or lameness. Animals which were affected by a lesion or lameness at any time point were regarded as susceptible, and animals which were unaffected at every assessment were regarded as resistant. Therefore, repeated records from each animal were used to reduce misclassification bias by increasing the robustness of a “resistant” classification. Statistical analysis aimed to quantify the association between the four outcomes and genetic indexes by fitting logistic regression models in a descriptive capacity.

Lameness data collected during the study were matched by cow ear tag or herd book number to their published genetic indexes. Matched records were available for 2,107 cows out of the 2,352 with lameness data. Descriptive and statistical analyses were conducted in R (R Core Team, 2021).

Lesion records from all assessments were used to categorise cows as either affected (i.e., susceptible) if the lesion had been present on any foot at any time point during the study, or unaffected (i.e., resistant) if the lesion had been absent throughout. At each assessment, cows were considered affected with SU if there was any ulceration in the sole area of the foot; cows were considered affected with SH if the haemorrhage was ≥2cm diameter or dark pink/purple; cows were considered affected with WL if there had been discolouration or separation of the white line which was still present after limited trimming. Similarly, mobility scores from each time point were summarised by the maximum recorded mobility score across the whole study period. This maximum mobility score was dichotomised to indicate either the animal had always been recorded as "non-lame" (maximum mobility score 0 or 1) or the animal had been recorded as "lame" at least once (maximum mobility score 2 or 3). Finally, when adjusting for the confounding effects of parity, the parity of each cow was grouped into an ordinal variable (1 to 5) where the top level included the 5th parity or greater.

The Pearson correlation coefficient between the Lameness Advantage index and other genetic indexes was calculated. The unadjusted relationship between Lameness Advantage index and period prevalence (Mahendran et al., 2017) of CHL and lameness was calculated by binning the index, based on the distribution within our dataset, into: ≤ -2.0, > -2.0 ≤ -1.0, > -1.0 ≤ -0.5, > -0.5 ≤ +0.5, > +0.5 ≤ +1.0, > +1.0 ≤ +2.0, and > +2.0. This relationship was further evaluated after adjusting for the effects of herd and parity by fitting a multivariable logistic regression model with herd, parity, and Lameness Advantage index as covariates; herd and parity were included as categorical variables and Lameness Advantage index as a continuous variable. The same model was fit to the four different outcomes which categorised cows as unaffected/affected by SH, SU, WL, and

lameness as described above. The assumptions of logistic regression were assessed using the *performance* package (Lüdecke et al., 2021). Specifically, log-linearity was assessed by inspecting scatter plots of the genetic index against logit values; multicollinearity was assessed by calculating the variance inflation factor for each explanatory variable, and residual distribution was assessed by examining binned residual plots. The goodness of fit was assessed using the Hosmer-Lemeshow test and the explanatory power of the model was assessed by calculating the coefficient of discrimination (Tjur's R^2) (Tjur, 2009). The model-adjusted probabilities of each outcome for different values of the Lameness Advantage index were calculated using the *ggeffects* package (Lüdecke, 2018), this was displayed for different herds whilst averaging the effect of parity, and for different parities whilst averaging the effect of the herd.

The same approach of fitting multivariable logistic regression models to each of the four outcomes was repeated using the sire Lameness Advantage index in place of the animal's index. The only change made to this model was to reduce the parity variable to three levels to maintain adequate numbers of observations per level, consequently, cows were considered as either first parity, second parity, or third parity and greater. Finally, all other genetic indexes in the dataset were screened for an association with one of the four outcomes by fitting each one in turn in multivariable logistic regression models which also included farm and parity (five levels) as before. Given the lower prior probability of finding an association during this final part of the analysis, and the large number of hypotheses tested for each outcome (36 genetic indexes), associations were only considered statistically significant if the regression coefficient for the genetic index had a *P*-value lower than 0.05 following Bonferroni correction (i.e., 0.05/36). Therefore, the adjusted significance level for the association between a genetic index, other than Lameness Advantage, and one of the four outcomes was set at 0.0014.

6.3 Results

Lameness data and genetic indexes were available for 2,107 cows, representing 90% of the cows with lameness data. The reasons for missing genetic index data were either genotyping failures or mismatches between pedigree and genotyping information such as parental identification. A total of 1,818 cows could be matched to sires which had a published Lameness Advantage index, resulting in 280 different sires in this dataset.

The parity distribution and period prevalence of lesions and lameness were similar in the 2,352 cows with lameness data we had collected and the 2,107 cows with both lameness data and genetic indexes (**Table 6.1**). The mean Lameness Advantage index was +0.5 (SD 1.1) and ranged from -3.1 to +4.4; younger animals tended to have higher values than older animals (**Table 6.2**). The reliability of the Lameness Advantage index, calculated in the validation step of the genetic evaluations as the squared correlation between genomic merit and average relative performance, ranged from 0.46 to 0.60 (mean 0.55, SD 0.03). The mean Lameness Advantage index in sires represented in this dataset was +1.0 (SD 1.9) and ranged from -5.8 to +6.3. In cows which had a Lameness Advantage index value close to the genetic average (-0.5 to +0.5), the period prevalence of SH, SU, WL, and lameness was 33.0%, 11.1%, 21.3%, and 23.5% respectively. In all cases, there was a clear trend that cows with a lower value of Lameness Advantage had a higher period prevalence of each outcome and vice versa (**Table 6.3**).

Table 6.1. Period prevalence of claw horn lesions and lameness in the whole population of animals with lameness data and the final study population used for analysis.

		Whole study population with lesion records (N = 2,352)				
		N	SH	SU	WL	Lameness
Parity	1st	610	38.2%	4.6%	15.9%	6.1%
	2nd	730	22.2%	4.0%	20.5%	14.7%
	3rd	394	35.8%	12.4%	20.6%	27.0%
	4th	315	43.2%	22.9%	26.0%	40.5%
	5th	303	45.5%	30.4%	35.3%	51.2%
		Population with lesion records and genetic indexes (N = 2,107)				
		N	SH	SU	WL	Lameness
Parity	1st	583	37.2%	4.5%	16.1%	6.0%
	2nd	589	23.3%	4.8%	20.7%	14.6%
	3rd	362	34.8%	13.0%	20.2%	25.2%
	4th	290	39.7%	23.1%	24.1%	38.1%
	5th	283	44.5%	29.3%	35.3%	51.2%

SH: sole haemorrhage, SU: sole ulcer, WL: white line lesion; Lameness: mobility score 2 or 3.

Table 6.2. The average and distribution of Lameness Advantage index in female animals by year of birth.

Year of birth	N	Lameness Advantage	
		Mean (SD)	Range
2007 - 2012	159	-0.44 (1.17)	-2.88 – +2.21
2013 - 2014	415	-0.07 (1.17)	-2.62 – +3.59
2015 - 2016	940	+0.61 (1.03)	-3.10 – +3.72
2017 - 2018	593	+0.90 (0.84)	-1.43 – +4.40

SD: standard deviation

Table 6.3. Unadjusted period prevalence of claw horn lesions and lameness for ranges of Lameness Advantage index.

Lameness Advantage index	N	SH	SU	WL	Lameness
≤ -2.0	39	48.7%	56.4%	59.0%	61.5%
> -2.0 ≤ -1.0	174	45.4%	23.0%	29.9%	42.5%
> -1.0 ≤ -0.5	187	44.4%	23.5%	20.9%	32.3%
> -0.5 ≤ +0.5	630	33.0%	11.1%	21.3%	23.5%
> +0.5 ≤ +1.0	364	33.5%	9.3%	17.3%	16.5%
> +1.0 ≤ +2.0	554	30.9%	6.0%	20.0%	13.7%
> +2.0	159	24.5%	5.0%	23.3%	15.1%

SH: sole haemorrhage, SU: sole ulcer, WL: white line lesion; Lameness: mobility score 2 or 3

The multivariable logistic regression models were intended to quantify the relationship between the Lameness Advantage index and each outcome after adjusting for the effects of parity and herd. There were no violations in the assumptions regarding log-linearity, multicollinearity, and residual distributions. In all models, the Hosmer-Lemeshow test statistic was not statistically significant ($P > .05$) indicating an acceptable fit to the data. The explanatory power of each model was generally low, the coefficients of discrimination (Tjur's R^2) were 0.07, 0.11, 0.04, and 0.15 for SH, SU, WL, and lameness, respectively. The odds ratios (95% confidence intervals (**CI**)) for the Lameness Advantage index were 0.79 (95% CI 0.72 - 0.86), 0.68 (95% CI 0.59 – 0.78), 0.94 (95% CI 0.84 – 1.04), and 0.82 (95% CI 0.74 – 91) for SH, SU, WL, and lameness, respectively (**Table 6.4**). Model-adjusted probabilities were calculated and indicated an average relative risk increase between a Lameness Advantage index of -1 compared to +1 of 29% (absolute risk increase (**ARI**) 12%), 100% (ARI 10%), 12% (ARI 3%), and 33% (ARI 7%) for SH, SU, WL, and lameness, respectively. Subsequently, model-adjusted probabilities of each outcome were displayed for each herd after averaging the effect of parity (**Figure 6.1**), and for each parity after averaging the effect of the herd (**Figure 6.2**).

Table 6.4. Multivariable logistic regression for claw horn lesion presence or lameness based on mobility score using the animal's own Lameness Advantage index (N = 2,107).

		SH			SU			WL			Lameness		
		OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Lameness Advantage (animal)		0.79	0.72 – 0.86	<.001	0.68	0.59 – 0.78	<.001	0.94	0.84 – 1.04	.210	0.82	0.74 – 0.91	<.001
Parity	1	Reference											
	2	0.41	0.31 – 0.53	<.001	0.96	0.55 – 1.68	.889	1.24	0.91 – 1.68	.170	2.72	1.81 – 4.16	<.001
	3	0.73	0.55 – 0.97	.032	2.88	1.73 – 4.87	<.001	1.30	0.91 – 1.84	.143	5.05	3.34 – 7.80	<.001
	4	0.84	0.62 – 1.15	.284	4.91	2.99 – 8.24	<.001	1.59	1.10 – 2.29	.012	8.49	5.57 – 13.21	<.001
	≥5	0.96	0.70 – 1.32	.802	6.10	3.70 – 10.29	<.001	2.72	1.90 – 3.89	<.001	13.85	9.05 – 21.66	<.001
Herd	A	Reference											
	B	0.30	0.19 – 0.48	<.001	0.36	0.19 – 0.71	.002	0.32	0.20 – 0.51	<.001	0.85	0.47 – 1.64	.618
	C	0.68	0.41 – 1.11	.123	0.35	0.17 – 0.73	.004	0.53	0.32 – 0.88	.014	0.57	0.30 – 1.14	.098
	D	0.77	0.72 – 0.86	<.001	0.26	0.59 – 0.78	.001	0.27	0.15 – 0.48	<.001	0.77	0.39 – 1.559	.470

The intercept (standard error) for each model was: SH: 0.65 (0.24); SU: -1.80 (0.36); WL: -0.58 (0.25), and Lameness: -2.39 (0.34).

OR: Odds ratio; CI: confidence interval; SH: sole haemorrhage, SU: sole ulcer, WL: white line lesion; Lameness: mobility score 2 or 3.

Figure 6.1. The model-adjusted probabilities of sole haemorrhage (SH), sole ulcer (SU), white line lesion (WL) and lameness based on mobility score (Lameness). The probability of each outcome is displayed against the animal's Lameness Advantage genetic index for each herd using the average effect of parity.

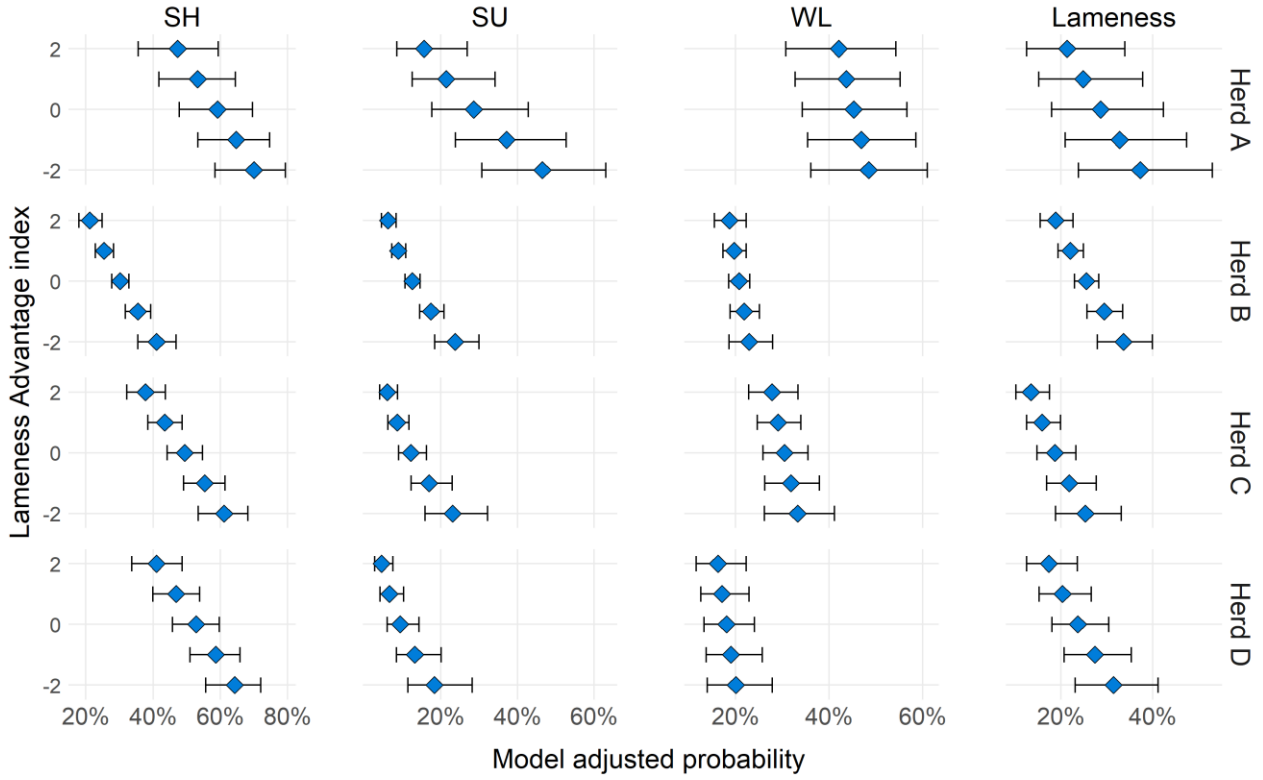
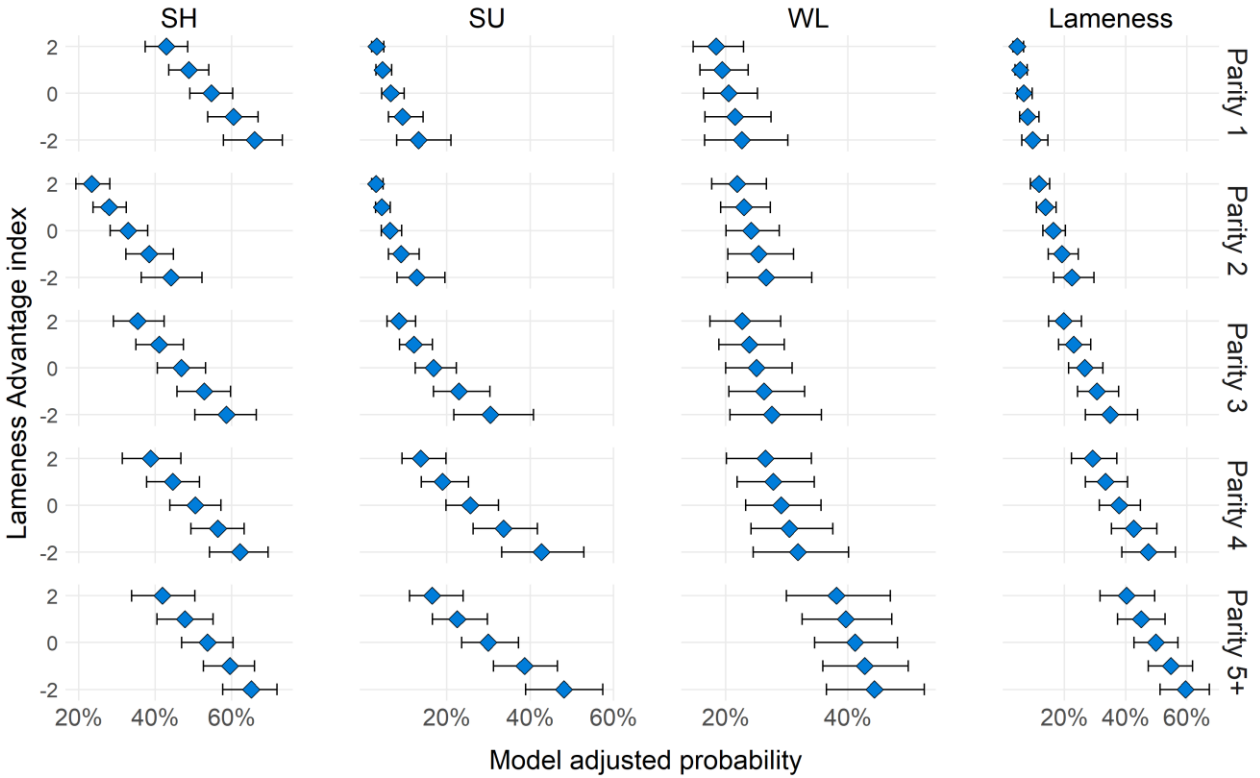


Figure 6.2. The model-adjusted probabilities of sole haemorrhage (SH), sole ulcer (SU), white line lesion (WL) and lameness based on mobility score (Lameness). The probability of each outcome is displayed against the animal's Lameness Advantage genetic index for each parity using the average effect of the herd.



The results of the multivariable logistic regression models which included the sire's Lameness Advantage index in place of the animal's index are presented in **Table 6.5**. Except for WL, there was a generally weaker effect of the sire's Lameness Advantage index compared to the animal's index, although 95% confidence intervals overlapped.

The correlation between all published genetic indexes was calculated. Only three indexes had a Pearson correlation coefficient with Lameness Advantage greater than 0.4: Digital Dermatitis, £PLI and Lifespan. Correlations between all genetic indexes are provided as supplementary materials (**Supplementary Table 6.1**), but of note was the low and positive correlation (r 0.09, 95% CI 0.04 – 0.13) between Lameness Advantage and the genetic index for milk production. All remaining genetic indexes were screened for an association with one of the four outcomes after adjusting for the effect of farm and parity (**Supplementary Table 6.2**). Genetic indexes which had a statistically significant association after adjusting for multiple testing (significance level: 0.0014) are presented in **Table 6.6**.

Table 6.5. Multivariable logistic regression for claw horn lesion presence or lameness based on mobility score using the Lameness Advantage index of the sire of each animal (N = 1,818).

		SH			SU			WL			Lameness		
		OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Lameness Advantage (sire)		0.90	0.84 – 0.95	<.001	0.82	0.75 – 0.88	<.001	0.91	0.85 – 0.97	.003	0.89	0.83 – 0.94	<.001
Parity	1	*Reference*											
	2	0.43	0.35 – 0.57	<.001	1.04	0.58 – 1.84	.906	1.35	0.97 – 1.88	.076	2.81	1.83 – 4.41	<.001
	≥3	0.93	0.73 – 1.19	.570	5.06	3.28 – 8.14	<.001	1.86	1.39 – 2.52	<.001	9.21	6.31 – 13.86	<.001
Herd	A	*Reference*											
	B	0.31	0.19 – 0.50	<.001	0.35	0.19 – 0.70	.002	0.32	0.20 – 0.52	<.001	0.84	0.46 – 1.60	.570
	C	0.73	0.44 – 1.22	.232	0.34	0.17 – 0.72	.004	0.48	0.28 – 0.81	.006	0.53	0.27 – 1.06	.063
	D	0.93	0.52 – 1.66	.804	0.21	0.08 – 0.53	.001	0.32	0.17 – 0.60	<.001	0.58	0.27 – 1.29	.175

The intercept (standard error) for each model was: SH: 0.60 (0.25); SU: -1.73 (0.35); WL: -0.52 (0.25), and Lameness: -2.33 (0.35).

OR: Odds ratio; CI: confidence interval; SH: sole haemorrhage, SU: sole ulcer, WL: white line lesion; Lameness: mobility score 2 or 3

Table 6.6. Multivariable logistic regression screening for associations between all genetic indexes and claw horn lesion presence or lameness (N = 2,107). Results are presented as the odds ratio and 95% confidence interval for the genetic index, adjusted for parity and herd. Only genetic indexes with at least one association which was statistically significant at the adjusted significance level of 0.0014 are presented, denoted with *.

	OR (95% CI) from multivariable model including parity and herd			
	SH	SU	WL	Lameness
Angularity	1.066 (0.947 – 1.201)	1.188 (0.990 – 1.429)	1.031 (0.900 – 1.181)	1.316* (1.138 – 1.525)
Digital dermatitis	0.584* (0.453 – 0.751)	0.490* (0.345 – 0.693)	0.978 (0.739 – 1.295)	0.606 (0.455 – 0.807)
Fertility index	0.977 (0.958 – 0.997)	0.958 (0.931 – 0.986)	0.987 (0.965 – 1.009)	0.957* (0.935 – 0.980)
Legs and Feet	1.031 (0.896 – 1.187)	1.081 (0.879 – 1.331)	1.043 (0.890 – 1.224)	0.753* (0.637 – 0.889)
Lifespan	0.999 (0.996 – 1.001)	0.996 (0.993 – 0.999)	1.001 (0.998 – 1.004)	0.994* (0.991 – 0.997)
Locomotion	1.084 (0.943 – 1.246)	1.137 (0.927 – 1.395)	1.075 (0.919 – 1.259)	0.751* (0.636 – 0.885)
£PLI	1.000 (0.999 – 1.000)	0.998 (0.997 – 1.000)	1.000 (0.999 – 1.001)	0.998* (0.997 – 0.999)
SCC	1.017 (1.003 – 1.031)	1.040* (1.018 – 1.062)	1.003 (0.987 – 1.018)	1.026 (1.009 – 1.044)

OR: Odds ratio; CI: confidence interval; SH: sole haemorrhage, SU: sole ulcer, WL: white line lesion; Lameness: mobility score 2 or 3; £PLI: Profitable Lifetime Index; SCC: Somatic Cell Count

6.4 Discussion

6.4.1 Key results

Our primary objective was to evaluate the Lameness Advantage genetic index with respect to claw horn lesion development in a cohort of dairy cows. The Lameness Advantage index is calculated from national genetic evaluations and primarily determined by an animal's genotype, with additional information from the animal's pedigree, farmer-recorded lameness events, and breed society classifying results. As the foot lesions and mobility scores recorded during our study were independent of the Lameness Advantage index calculation, we used these records to independently assess this genetic index.

Our results showed the Lameness Advantage index was associated with CHL development and lameness; for every one-point increase in Lameness Advantage, there were reduced odds of an animal having SH, SU, or lameness during our study. We observed a similar, but generally weaker, trend using the sire's index in place of the animal's index. It should be noted that as 95% confidence intervals overlapped between the odds ratios of an animal's own and animal's sire Lameness Advantage index, our results are also compatible with the Lameness Advantage index of both animal and sire having equivalent effects, although this is less likely. The strength of association between Lameness Advantage and CHL, using either the animal's index or the sire's, followed the general trend in heritability estimates of CHL, where SU is typically reported to have the highest heritability and WL the lowest (Heringstad et al., 2018). These results highlight the potential of genetic selection on the Lameness Advantage index to complement strategies to reduce the incidence of SH and SU in UK dairy herds.

6.4.2 Interpretation

In this study, the odds of SU decreased by 32% for every one-point increase in the Lameness Advantage index (OR 0.68, 95% CI 0.59 – 0.78), after adjusting for the effects of parity and herd (**Table 6.4**); the odds of SU decreased by 18% (OR 0.82, 95% CI 0.75 – 0.88) when the sire Lameness Advantage index was assessed in the same way (**Table 6.5**). For context, a recent study reported a 20%

reduction in odds of a SU in cows which had been preventatively foot-trimmed before drying-off (Thomsen et al., 2019). Preventive foot-trimming is widely considered a key part of SU prevention, so on the strength of the association between SU and the Lameness Advantage index, we believe it is advisable to also include genetic selection as part of SU prevention programmes. Furthermore, one of the major barriers to lameness control is often cited to be the cost of interventions (Leach et al., 2010; Bruijnis et al., 2013; Bennett et al., 2014; Dutton-Regester et al., 2019). However, the direct costs of selecting on a genetic index, particularly from a bull proof as opposed to an animal's own genotype, are negligible in comparison to other interventions which often include re-designing housing or increasing foot-trimming frequency.

The magnitude of the potential reduction in SU incidence that could be achieved through genetic selection could result in a substantial improvement in both animal welfare and farm efficiency. From an animal welfare perspective, SU are recognised as one of the major causes of lameness in dairy cattle, a condition which is painful and highly representative of their welfare (Whay et al., 1997, 2003b; Whay and Shearer, 2017). Additionally, this reduction in SU could have an economic benefit with every case of SU costing farmers between $232 and $622 depending on the severity of the lesion (Charfeddine and Pérez-Cabal, 2017).

All animals in our study had been genotyped and we observed the strongest associations between an animal's own Lameness Advantage index and the odds of SU development. Genomic testing and selection of females have risen over recent years (VanRaden, 2020) and improved profitability can offset the costs of genotyping (Weigel et al., 2012; Davenport et al., 2018), particularly when combined with breeding programmes which use sexed or beef semen (Hjortø et al., 2015; García-Ruiz et al., 2016; Thomasen et al., 2016; Newton et al., 2018; Clasen et al., 2021). The results of this study indicate an additional financial return from the genomic selection of heifers may include the reduction in SU incidence, and this could be realised, at least in part, during the first lactation. As genetic gains are slow, farmers can be reluctant to engage with genetic selection for lameness reduction (Bruijnis et al., 2013; Nielsen et al., 2014); therefore the

reduced chance of SU development within the first lactation could present a compelling incentive to consider this approach.

The relationship between Lameness Advantage and SU frequency appeared to be strongest in older cows (**Figure 6.2**). Our interpretation of this trend is that genetic resistance to SU may become increasingly important in older cows because the risk of CHL development increases cumulatively with age (Sanders et al., 2009; Newsome et al., 2016). Therefore, as there is a drive to increase the longevity of dairy cows (Boulton et al., 2017; Grandl et al., 2019), breeding cows with good genetic merit for lameness is a clear priority.

Genetic selection requires accurate selection indexes to be available and in turn, genetic selection indexes are reliant on phenotype accuracy. The Lameness Advantage index utilises farmer-recorded lameness to allow more direct selection for lameness reduction than through conformation traits alone (Pritchard et al., 2013), however, the recording of lameness in farm records has repeatedly been highlighted to be poorer than other health conditions (Zwald et al., 2004; Pritchard et al., 2013; Parker Gaddis et al., 2014). Despite the promising results we observed, we still believe it is important to encourage better recording of lameness on farms to provide useful phenotypes for national genetic evaluations. In the future, direct lesion traits, such as foot-trimming records, may be available for genetic evaluations, as they are in other countries (Stoop et al., 2010; Häggman and Juga, 2013; Malchiodi et al., 2020), and ultimately this is likely to be the approach which maximises genetic improvements to reduce lameness (Egger-Danner et al., 2014).

We observed associations between genetic indexes other than Lameness Advantage and the development of CHL or lameness. There were associations between lameness and Fertility Index, Lifespan, and £PLI, and likewise between SCC index and SU development. However, despite the low *P*-values, the magnitude of these associations was negligible, and we do not consider these results to be of particular importance. It is worth noting, however, the lack of association between £PLI and CHL development. Therefore, although Lameness Advantage is included in the £PLI, selection on £PLI alone is unlikely to result in

reductions in CHL incidence. Of the top ten Holstein bulls for £PLI listed on the AHDB website in November 2021 (Anon), four have a Lameness Advantage index greater than +2.0 so it is possible to select for both high £PLI and good Lameness Advantage; this is the approach we would advise to farms looking to use breeding decisions as part of lameness reduction programmes. Having said that, the correlations between Lameness Advantage and production indexes were low and positive, indicating that selecting on Lameness Advantage alone does not risk sacrificing productivity.

Lameness, as determined by mobility scoring, was associated with three genetic indexes other than Lameness Advantage: Angularity (OR 1.32, 95% CI 1.14 – 1.53), Legs and Feet (OR 0.75, 95% CI 0.64 – 0.89), and Locomotion (OR 0.75, 95% CI 0.64 – 0.89). Angularity, also called "dairy form", refers to the openness between ribs and is recognised to correlate with body condition (higher angularity is associated with lower body condition) and locomotion (Battagin et al., 2013), therefore this association seems plausible. The Feet and Legs index includes Locomotion, in addition to other linear conformation traits, and unsurprisingly these two genetic indexes are highly correlated with each other (**Supplementary Table 6.1**). The association between these three genetic indexes and lameness, but not CHL development, could be explained by an association with foot lesions other than CHL, which we did not evaluate in this study, or these genetic indexes could relate more closely to gait than the development of painful foot lesions. The absence of an association with CHL development suggests that although selecting on type traits such as Angularity, Legs and Feet, and Locomotion may reduce the prevalence of visibly lame cows, it is unlikely to reduce the incidence of CHL. A study which compared farmers' stated preferences for genetic selection with actual selection practices reported that although farmers reported health traits to be the most important, selection on these traits was less frequent, and the opposite effect was observed for type traits (Paakala et al., 2020). Our data indicate that type traits alone will not be as effective at reducing CHL frequency and this result, alongside the validation of the Lameness Advantage index, should be communicated to farmers wishing to breed for reduced lameness.

We observed a strong and unexpected association between the Digital Dermatitis genetic index and SH and SU development. This result warrants further investigation. The most straightforward explanation for this result would be if SH and SU are highly genetically correlated with digital dermatitis; however, previously reported genetic correlations have ranged from -0.15 to 0.12 for digital dermatitis and SH and from -0.19 to 0.56 for digital dermatitis and SU (Heringstad et al., 2018). Although the standard errors of previous genetic correlation estimates are large, the magnitude of these correlations suggests this explanation of our results is unlikely. Furthermore, the highest positive correlation reported of 0.56 (Koenig et al., 2005) is an outlier among previous studies with the next highest correlation reported to be 0.15 (van der Linde et al., 2010), and the genetic correlation between digital dermatitis and SH and SU is frequently reported to be negative (van der Waaij et al., 2005; Buch et al., 2011; Malchiodi et al., 2020) making it harder to accept this as an explanation for the association we observed in our study.

Digital dermatitis may be a risk factor for SH and SU in the absence of a shared genetic background. For this mechanism to exist, the digital dermatitis index must first have a strong association with digital dermatitis development, and a preliminary analysis of our data indicates that this could be the case (data not shown). However, it would then be necessary for digital dermatitis development to substantially increase the risk of SH and SU development, which previous studies have not identified, although analysis of sufficiently longitudinal and detailed foot lesion data is lacking. It is therefore theoretically possible that digital dermatitis in younger animals, such that it is identified during breed society classification, increases the risk of the animal subsequently developing SH or SU; but there are reasons to be sceptical about this hypothesis including the low rates of concurrent digital dermatitis and SH or SU reported (van der Waaij et al., 2005; van der Spek et al., 2013; Malchiodi et al., 2017) and the implausibly high attributable risk required for this mechanism to hold. We consider it more likely, therefore, that SH and SU may be conditionally associated with digital dermatitis via an unknown factor which has an association with both digital dermatitis and SH and SU, but is only genetically correlated with digital dermatitis.

6.4.3 Limitations

As only four herds were included in this study, the applicability of the results to other herds requires careful interpretation (further discussed in the "Generalisability" section). Genetic indexes were only available for 90% of cows with CHL and mobility score records and although the genetic merit of the missing animals is unknown, the distribution of lesion prevalence across parities appeared to be similar with and without these animals (**Table 6.1**). This study analysed the relationship between the Lameness Advantage index and the risk of CHL or lameness development during a single lactation. It, therefore, does not relate to the performance of an animal over its entire lifetime which would be a more appropriate phenotype to fully assess the influence of genetic merit, but one that is logistically much more challenging to obtain. Equally, although the accuracy of our phenotypes was improved by using repeated records for each animal, it is still possible that lesions could have been missed if they occurred transiently between assessment time points. Multivariable models were designed to be descriptive and therefore not evaluated as predictive models. We would expect the predictive performance of these models to be poor, as suggested by the low coefficients of discrimination; this is because it would be unlikely that a genetic index could predict the phenotype when the heritability estimates of these lesions suggest that the majority of phenotypic variance is not due to genetics (Heringstad et al., 2018).

6.4.4 Generalisability

Generalisability from a study which only includes four herds is limited, however, we discuss relevant details of the study herds to allow interpretation of the potential applicability of these results to other herds. This study included four dairy herds which were all commercially run with operating practices common to many British dairy farms, but could not be considered representative of the full spectrum of dairy farms. Within these four herds, three were operating relatively intensive systems of zero-grazing and three times a day milking. In a recent survey of 53 randomly selected dairy herds in the UK, 36% housed milking cows all year round (Thompson et al., 2020). A survey of 863 dairy herds in 2012, also

in the UK, reported that 23% of herds housed early lactation and high-yielding groups all year round (March et al., 2014). We did not observe any differences in trends between the three farms which housed all milking cows and the remaining herd which was managed with a combination of housed and grazed groups (**Figure 6.1**).

The overall period prevalence of lame cows (Mahendran et al., 2017), based on repeated mobility scores throughout this project, ranged from 18.5% to 33.3% across the four herds; the mean point prevalence of lameness from all time points ranged from 6% to 11.8% across the four herds (data not shown). Recent cross-sectional studies in the UK reported that herd lameness prevalence ranged from 6% to 65%; this suggests the four herds in our study had a lower prevalence of lameness compared to many dairy herds in the UK (Griffiths et al., 2018; Randall et al., 2019). The prevalence of CHL has historically only been reported for lame animals or from foot-trimming records. Therefore, previous reports may not have a reliable numerator, due to under-reporting of mild lesions, or a reliable denominator, due to over-representation of lame cows. In studies using foot-trimming records, the prevalence of CHL has been reported to range from 5 – 59% for SH, 5 – 19% for SU, and 4 – 18% for WL (Manske et al., 2002b; Koenig et al., 2005; van der Waaij et al., 2005; Capion et al., 2008; van der Linde et al., 2010; van der Spek et al., 2013; Croué et al., 2017; Malchiodi et al., 2017). It is therefore possible that our study had a population of cows with an unusually high prevalence of CHL despite an average or below average prevalence of lame cows. However, we think this is unlikely and would consider our results to accurately represent the true frequency of CHL in these herds as foot lesions were recorded at repeated time points in cows assessed specifically for this purpose.

6.5 Conclusions

The results of this study highlight the potential of the Lameness Advantage genetic index to facilitate breeding cows with better resistance to lameness. We found differences in the frequency of claw horn lesions and lameness in cattle associated with this index, particularly for sole haemorrhage and sole ulcers. In comparable populations, genetic selection on the Lameness Advantage index is likely to translate to a reduced risk of cows developing sole haemorrhage and sole ulcers, although we would expect the greatest reductions to occur through a combination of genetic selection and husbandry-based improvements.

Chapter 7: General discussion and future research

Lameness is a threat to the sustainability of dairy farming (European Food Safety Authority (EFSA), 2009) and there is little indication that the number of lame dairy cows in the United Kingdom (**UK**) is decreasing (Afonso et al., 2020). Claw horn lesions (**CHL**) are a major cause of lameness in dairy cows (Murray et al., 1996). Due to the risk of recurrence with CHL (Hirst et al., 2002; Oikonomou et al., 2013; Newsome et al., 2016), prevention of first cases is particularly important but this is hindered by an incomplete understanding of the aetiopathogenesis (Huxley, 2012).

This thesis aimed to explore the aetiopathogenesis of CHL, with a specific focus on genetic and metabolic aspects. To address this, data were collected in a prospective, longitudinal study of around 2,300 Holstein cows. The most important, and ambitious, part of this study was the collection of comprehensive foot lesion records at repeated time points during a production cycle, including times when cows' feet would not otherwise have been examined, such as in the week after calving. The nature of these foot lesion data facilitated genetic analyses of accurate CHL phenotypes and novel traits such as the recovery of severe sole lesions, in addition to the characterisation of the genetic relationship between CHL and digital cushion thickness (**DCT**). This dataset also supported metabolomic analyses relating to sole lesion development, enabling strict criteria for claw health to be applied when selecting control animals. Finally, existing genetic indexes were evaluated for the potential to reduce CHL through selective breeding, using robust, lesion-specific case definitions of healthy and affected animals.

7.1 Breeding for reduced claw horn lesions

In Chapter 2, the heritability of sole haemorrhage (**SH**), sole ulcers (**SU**) and white line lesions (**WL**) were estimated to be higher than reported in many previous studies (Heringstad et al., 2018), particularly for sole lesions (SH and SU). This finding could be due to various aspects of the study design which minimised residual variance. The heritability estimates of sole lesions had a

similar magnitude to the reported heritability of many production traits, and a greater magnitude than most linear type traits (Pryce et al., 2000a; Berry et al., 2004; Oliveira Junior et al., 2021). Although the heritability estimates of sole lesions are not directly comparable to other traits and may not generalise to other populations (Visscher et al., 2008; Tenesa and Haley, 2013), this context is useful to communicate the capability of selective breeding to reduce SH and SU.

The heritability estimates of WL were lower than those for sole lesions, but this does not preclude effective genetic selection (Berry et al., 2019). Genetic progress can be achieved despite a low heritability, particularly given the large volume of phenotype data used in genetic evaluations (which improve the selection accuracy), as well as the increased accuracy and reduced generation interval which is achievable with genomic testing (García-Ruiz et al., 2016; Berry et al., 2019; Coffey, 2020). This is evident in the improved reproduction observed in dairy herds which can be attributed to genetic selection (Coleman et al., 2009); all female fertility traits have a lower or similar estimated heritability to CHL (Berry et al., 2014). Therefore, if appropriate genetic indexes are available, dairy cows could be bred for increased resistance to SH, SU, and WL.

Direct traits such as foot lesion data are the most effective way to reduce CHL with genetic selection (van der Linde et al., 2010; Häggman and Juga, 2013; Egger-Danner et al., 2014). A centralised dataset of foot lesions could be used for genetic evaluations in the UK, as it is in other countries, as well as providing phenotypes for research (Croué et al., 2017; Malchiodi et al., 2017; Ring et al., 2018). Developing the infrastructure to collate foot-trimming data would be hugely beneficial for the UK dairy industry.

The lack of direct genetic indexes for CHL in the UK could limit opportunities to make use of the genetic variance of CHL in breeding strategies. In Chapter 6, the effectiveness of the current selection index for improved lameness resistance (Lameness Advantage) was evaluated. Although this index is based on farm records of lameness, so it is not specific to CHL and uses data which may be of limited accuracy (Pritchard et al., 2013), it was significantly associated with the chance of animals having SH and SU. This result suggests currently

available genetic indexes could be used in breeding programmes to strengthen husbandry-based initiatives to reduce CHL. The challenge now is to communicate this to those involved in breeding decisions, as well as those working to reduce lameness in dairy cattle. To support this communication, a useful follow-up study would be to use stochastic modelling to simulate genetic selection (Raphaka et al., 2018), which could quantify the likely effect on CHL incidence from selection on the Lameness Advantage index.

Sceptics may understandably argue that the potential effectiveness of the Lameness Advantage index shown in Chapter 6 requires replication in a wider and more varied population. Although this would be an important addition to this area of research, considerable resources were required to collect data for this project, and this could be prohibitive for similar follow-up studies. A compromise would be to collate professional foot-trimming records from a diverse range of dairy herds, along with nationally evaluated breeding values for the study population. This would likely be sufficient to evaluate the Lameness Advantage index and determine the replicability of the results reported in Chapter 6. Foot lesions with relatively clear case definitions, such as SU, should be recorded accurately enough in foot-trimming datasets to be used as the outcome in this type of analysis. Recording of SH is likely to be more variable in foot-trimming records. Although this should not be accepted as an immutable fact, the genetic correlation between SH and SU estimated in Chapter 2 was strongly positive, so for practical purposes, sole lesions could be regarded as a single genetic trait. Ultimately, however, to undeniably demonstrate that selection on Lameness Advantage can reduce CHL would require a long-term study comparing control and selection lines (Veerkamp et al., 1994; de Paula Freitas et al., 2021).

Although the aetiopathogenesis of SH, SU, and WL are considered to be broadly similar (Ossent and Lischer, 1998; Hoblet and Weiss, 2001; Shearer and van Amstel, 2017), the genetic correlation between CHL estimated in Chapter 2 suggests WL may have a distinct, or at least partially distinct, genetic background to sole lesions. Advice on the prevention of CHL is usually provided for CHL collectively (Bicalho and Oikonomou, 2013; AHDB, 2018b; Newsome et al., 2019).

Although this is a pragmatic approach, the epidemiological curve in lactation is different for WL (Leach et al., 1997; Offer et al., 2000), and different risk factors have been identified for WL compared to sole lesions (Sogstad et al., 2005; Barker et al., 2009; Cramer et al., 2009; Sanders et al., 2009; Moreira et al., 2019). There have been recent studies which suggest DCT has a weaker phenotypic association with WL than sole lesions (Newsome et al., 2017b; Griffiths et al., 2020), the estimated genetic correlations between sole lesions and DCT, and between WL and DCT, support this distinction (Chapter 3). As the aetiopathogenesis of WL could plausibly differ from sole lesions (Mülling, 2002), and there appears to be a different genetic background to WL (Chapter 2), it would be useful for future research to differentiate between WL and sole lesions, especially when the role of the digital cushion is being assessed.

Ideally, research into CHL aetiopathogenesis should be based on foot lesion records which have been collected from the whole study population, so that lesion-specific (i.e., aetiopathogenesis-specific) risk factors can be determined. Lifting cows' feet to record foot lesions is undoubtedly the most labour-intensive part of lameness research, therefore a frequently adopted compromise is to record foot lesions in lame animals. Locomotion scoring to detect painful foot lesions has a sensitivity of around 0.67 (Bicalho et al., 2007a), therefore this approach has limitations and is likely to miss mild CHL which may only cause small changes in locomotion (Tadich et al., 2010; Blackie et al., 2013). In the future, this methodology could be improved with automated lameness detection, such as video analysis, to monitor for subtle changes in gait or posture (Abdul Jabbar et al., 2017; Kang et al., 2020; Piette et al., 2020). This technology is still being refined and needs validation against foot lesions and not just locomotion scores, but it has the potential to be more sensitive than manual locomotion scoring, and it could be a valuable tool for lameness research. The uptake of automated lameness detection systems is likely to increase on dairy farms as practical aspects and costs improve (Van De Gucht et al., 2017, 2018). Automatic lameness detection could also generate a valuable phenotype for future genetic analysis which could ultimately lead to the development of a direct and accurate lameness genetic index.

7.2 Digital cushion thickness

Digital cushion thickness was analysed as a genetic trait in Chapter 3. Even though DCT is heritable and negatively genetically correlated with sole lesions, the scope for it to be a useful auxiliary trait to reduce sole lesion incidence is limited by the difficulty, and therefore the expense, of recording. As the phenotypic relationship between DCT and CHL is still not fully understood, it is hard to advocate the recording of DCT in an intensively phenotyped reference population (Pryce et al., 2012; Calus et al., 2013b; Coffey, 2020).

The digital cushion may have a structural role in CHL pathogenesis, or it may change as a consequence of CHL development (Lischer et al., 2002b; Räber et al., 2006; Bicalho et al., 2009; Machado et al., 2010; Newsome et al., 2017b). The complicating influence of previous CHL on the DCT could be mitigated, at least in part, by enrolling enough primiparous animals in a similar study to that described in Chapter 3 to assess the phenotypic and genetic associations between DCT and first lifetime cases of CHL. However, as CHL can develop in nulliparous heifers (Vermunt and Greenough, 1996; Randall et al., 2016), and the development of DCT is affected by the rearing environment (Gard et al., 2015), it may be necessary to enrol an even younger cohort of animals to fully characterise the relationship between DCT and CHL.

There are limitations to ultrasound measurements of the digital cushion in terms of accuracy, repeatability, and relevance to functionality (Bach et al., 2021; Wilson et al., 2021). However, the major advantage of ultrasonography is that repeated measurements in live animals allow the evaluation of the temporal relationship between DCT and CHL development (Newsome et al., 2017b; Griffiths et al., 2020). More advanced imaging modalities, such as computed-tomography scanning and magnetic resonance imaging, have been used with post-mortem specimens to describe changes in distal phalanx and soft tissues of the foot, and these findings have been linked to historic CHL records (Newsome et al., 2016; Wilson et al., 2021). In the future, there may be opportunities to apply these techniques to live animals so that the functional anatomy of the foot can be assessed longitudinally as animals develop CHL. This

could progress understanding of the aetiopathogenesis of CHL more than ultrasonographic measurements of the digital cushion or cross-sectional studies of cadavers.

7.3 Sole lesion recovery

The recovery of sole lesions appears to be a heritable trait (Chapter 4), but this analysis used a relatively small study population so this result should be interpreted cautiously pending corroboration by future research, as should the minimal genetic correlation between sole lesion recovery and susceptibility.

One of the reasons for the small study population was the relatively low incidence of severe sole lesions in the four herds used for this study. A meta-analysis of UK studies found the pooled incidence rate of SU was 53 cases per 100 cow-years (Afonso et al., 2020), therefore it might be possible to select herds with a higher incidence of SU in future studies. Alternatively, the collation of foot-trimming records from trained foot-trimmers could provide a database large enough for genetic analysis of SU recovery, however, the need for a reliable follow-up after lesion diagnosis would make this challenging to assess. Recurrence of SU within a single lactation may be difficult to determine from routine foot-trimming records unless herds adopted protocols to ensure treated cows were always re-assessed at a fixed time point. Recurrence of SU across multiple lactations could be more easily assessed, for example, if cows were routinely foot-trimmed in early lactation; but these data would likely be biased by culling of animals with chronic or severe cases. Another option would be to assess the survival following the diagnosis of SU. Longevity can be evaluated as a genetic trait in dairy cattle using a proportional hazard model (Jamrozik et al., 2008; Pritchard et al., 2013). The same approach, but with foot-trimming records to filter the dataset to cows diagnosed with SU, could be utilised to determine the genetic variance relating to the effectiveness of SU recovery in a larger study population.

Sole lesion recovery and susceptibility did not appear to be strongly genetically correlated, which has interesting implications for the genetic background of sole lesion recovery. A follow-up to the study described in Chapter

4 would be a genome-wide association (**GWA**) analysis of sole lesion recovery. This would be a useful starting point to understand the genetic background of this trait, but this is again dependent on the availability of a large enough dataset.

Techniques have been described to collect and analyse fluid from wounds in humans (Trengove et al., 1996), and more recently in horses (Bundgaard et al., 2016). The metabolome and proteome of wound fluid can be evaluated (Kalkhof et al., 2014). If this approach could be adapted for SU in cattle, this may provide insight into the inflammatory and metabolic processes involved in SU healing. Histologic changes and gene expression have been assessed in biopsies of wound tissue in horses (Jørgensen et al., 2020), and this technique could also be applied to SU in cattle. As debridement of claw lesions under regional anaesthesia is advocated as part of treatment (Shearer et al., 2015), it could be possible to biopsy SU lesions without impeding the natural healing process so that recovery rates could be compared.

Biopsies of the corium through the dorsal wall of the hoof have been described in cattle to obtain tissue for transcriptomic analysis (Osorio et al., 2012, 2016). Understanding how gene expression in the corium differs between healthy cows and cows with CHL could be a useful approach to investigating the aetiopathogenesis and genetic background of CHL. However, taking corium biopsies from healthy cows will impact tissues in the foot which could increase future CHL risk, therefore this approach would only provide cross-sectional data. In cows already affected with SU, the endpoint is the recovery, or lack of recovery, of the lesion. Therefore, it may be possible to biopsy the corium of cows with SU without expecting this to have a major influence on lesion healing.

7.4 Genome-wide association analysis of claw horn lesions

The only consistent conclusion from GWA studies of CHL traits over the past 15 years is that a large number of loci control the genetic variation of these lesions. The GWA analyses described in Chapter 2 for CHL and Chapter 3 for DCT, support this conclusion, and although quantitative trait loci (**QTL**) were highlighted, there was little direct replication of results from previous GWA studies. Therefore, conclusions regarding candidate genes and the inferred

biological background to these traits should be interpreted cautiously. The natural follow-up to GWA studies is fine mapping of candidate genomic regions to identify causal variants (Schaid et al., 2018), however, given the equivocality of results from GWA studies of CHL, this may be overly speculative at this stage.

The major challenge with GWA studies of complex traits is achieving sufficient study power to detect variants with small or weak effects on the phenotype. Study power increases for more heritable traits (Shin and Lee, 2015), therefore detecting QTL is likely to be more rewarding for SU compared to other CHL. Four GWA studies of SU have already been published (van der Spek et al., 2015; Croué et al., 2019; Sánchez-Molano et al., 2019; Lai et al., 2021b), which combined with the data from this thesis equates to around 1,200 cases of SU and over 14,000 genotyped animals. Pooling data in a meta-analysis increases the power of GWA analyses (Wang et al., 2019), and this could be worthwhile with the current body of research.

False positive results are common in GWA studies (Platt et al., 2010), and attempts to control false positives with corrections for multiple testing severely limit study power, increasing the number of false negative results (Tam et al., 2019). These factors both contribute to the lack of replicability in GWA analyses of CHL. There is a lot of discussion relating to statistical methods to overcome these issues, one such avenue may be through employing analytical approaches to try and separate true signals from noise, such as automated variable selection, bootstrapping, and permutation. These techniques were used with metabolomics data in Chapter 5, and these principles have been applied to GWA analysis (Faye et al., 2011; Papachristou et al., 2016; Yang et al., 2020). Statistical methods which more reliably detect QTL from GWA studies are likely to be utilised in the future.

Further work with the data from this thesis could include multi-trait GWA analyses, which have been shown to be effective when applied to CHL (Lai et al., 2021a). This methodology could be particularly interesting if phenotypes reflecting potentially causal mechanisms, such as periparturient inflammation, are analysed alongside CHL to highlight pleiotropic QTL. For example, early

lactation mastitis has been phenotypically linked to SU development (Watson et al., 2022), therefore a two-trait GWA analysis of clinical mastitis and SU might detect genomic regions associated with both traits. Equally, so-called “deep phenotypes” (Robinson, 2012), such as biomarkers of inflammation, could be included in multi-trait GWA analysis alongside CHL traits. Combining genomic and metabolomic analyses to characterise the genetic background of metabolites in cattle, as has been conducted in humans (Kettunen et al., 2012; Shin et al., 2014), would be a useful starting point to identify potential metabolic phenotypes; these could then be explored for any genetic association with CHL development.

7.5 Metabolomics

In Chapter 5, proton nuclear magnetic resonance (**^{1}H NMR**) spectroscopy was used to analyse serum before and after the development of sole lesions in early lactation. Although there was not a strong relationship between the serum metabolome and sole lesion development, metabolomic platforms have the potential to be useful for research into CHL aetiopathogenesis. As discussed in Chapter 5, future metabolomic studies of CHL could benefit from analyses of multiple biofluids such as plasma and urine, as well as the use of other metabolomic techniques such as mass spectroscopy. It is widely considered that changes around calving are fundamental to the aetiopathogenesis of CHL, whether this is laxity in the suspensory apparatus (Lischer et al., 2002b; Tarlton et al., 2002), systemic inflammation (Watson et al., 2022; Wilson et al., 2022), or behavioural and environmental changes (Webster, 2002; Proudfoot et al., 2010). There is scope for the application of -omics technologies, such as metabolomics and proteomics, to characterise the complex hormonal, inflammatory, and metabolic changes around parturition. Linking these results to CHL development in early lactation could provide valuable insights into CHL aetiopathogenesis.

The predictive capacity of the serum metabolome was greatest using serum collected when SU were present, rather than prior to SU development. This implies future metabolomics studies could have the most success in exploring the role of metabolite profiles as a diagnostic tool, with the ultimate goal of

identifying cows with CHL using biomarkers. This could support the early detection and treatment of individual cows or allow herd-level screening to audit lameness control. The convenience of collecting milk samples from dairy cattle makes milk the most attractive biofluid for diagnostic testing. Metabolomic analysis of milk with ^{1}H NMR spectroscopy and mass spectroscopy highlighted changes in the milk which could reliably differentiate between lame and non-lame cows (Zwierzchowski et al., 2020), although there are limitations to this study that temper the interpretation of these results. Mid-infrared spectroscopy of milk has been evaluated as a tool to predict lameness in dairy cattle (Bonfatti et al., 2020; Shahinfar et al., 2021). Although prediction accuracy was modest in these studies, further research is needed in herds with a higher lameness prevalence. Milk spectral data should also be evaluated for CHL, and even a moderate prediction accuracy could still have practical benefits on farms struggling to control lameness. Metabolomic studies could make a significant contribution to improving the understanding of CHL aetiopathogenesis, and this will be an interesting area of research to follow.

7.6 Conclusions

This thesis builds on the current understanding of the genetic background of CHL by estimating the genetic parameters of these lesions and related traits in a cohort of prospectively enrolled and accurately phenotyped Holstein cows. The results of these analyses corroborate those of previous studies which showed that SH, SU, and WL have a low to moderate heritability. The magnitude of the heritability estimates for SH and SU susceptibility are particularly encouraging for the success of breeding programmes to improve resistance to these lesions. The genetic correlation between SH and SU was strong and positive, implying that a correlated response would be expected through selection on one of these lesions. Successful genetic selection for WL resistance would require greater selection intensity, due to the lower heritability, and selection on a specific WL trait due to the weaker and more variable genetic correlation with SH and SU.

The thickness of the digital cushion is heritable and weakly negatively genetically correlated with the severity of sole lesions (SH and SU), but not with WL. Therefore, genetic selection to increase the DCT in a herd may result in less severe sole lesions. The recovery from sole lesions also appears to be heritable and only weakly genetically correlated to the overall susceptibility to sole lesions. Therefore, it may be possible to breed cows which recover better from sole lesions, but this trait would need to be evaluated directly.

The genetic background of CHL and DCT was highly polygenic, in agreement with previous studies. Candidate genes relating to lipid metabolism and inflammation were identified for CHL, and genes related to inflammation, lipid metabolism and bone development were highlighted for DCT. Additionally, the same candidate gene, *KHDRBS3*, was identified for both SU and DCT.

The metabolic background of sole lesions was explored with ^{1}H NMR spectroscopy. Analysis of the serum metabolome could not reliably discriminate between animals based on the presence, or future development, of sole lesions, however, extraneous variation between individual animals or from other experimental sources may have masked any differences. A small number of metabolites appeared to be associated with sole lesions, mainly with concurrent SU in early lactation. Further investigation of the metabolic background of sole lesions using metabolomic platforms would be useful.

Finally, the relationship between selection indexes in national genetic evaluations and CHL were evaluated. The Lameness Advantage index was significantly associated with SH and SU development and appears to be a promising option to help breed resistance to these lesions. Genetic selection alongside management and environmental changes should be considered as the optimal approach to reducing CHL in dairy cattle.

References

Abdul Jabbar, K., M.F. Hansen, M.L. Smith, and L.N. Smith. 2017. Early and non-intrusive lameness detection in dairy cows using 3-dimensional video. Biosyst. Eng. 153:63–69. doi:10.1016/j.biosystemseng.2016.09.017.

Abu-Amer, Y. 2013. NF-κB signaling and bone resorption. Osteoporos. Int. J. Establ. Result Coop. Eur. Found. Osteoporos. Natl. Osteoporos. Found. USA 24:10.1007/s00198-013-2313–x. doi:10.1007/s00198-013-2313-x.

Abuelo, A., J. Hernández, J.L. Benedito, and C. Castillo. 2015. The importance of the oxidative status of dairy cattle in the periparturient period: Revisiting antioxidant supplementation. J. Anim. Physiol. Anim. Nutr. 99:1003–1016. doi:10.1111/jpn.12273.

Aeberhard, K., R.M. Bruckmaier, U. Kuepfer, and J.W. Blum. 2001. Milk Yield and Composition, Nutrition, Body Conformation Traits, Body Condition Scores, Fertility and Diseases in High-Yielding Dairy Cows - Part 1. J. Vet. Med. Ser. Physiol. Pathol. Clin. Med. 48:97–110. doi:10.1046/j.1439-0442.2001.00292.x.

Afgan, E., D. Baker, B. Batut, M. van den Beek, D. Bouvier, M. Čech, J. Chilton, D. Clements, N. Coraor, B.A. Grüning, A. Guerler, J. Hillman-Jackson, S. Hiltemann, V. Jalili, H. Rasche, N. Soranzo, J. Goecks, J. Taylor, A. Nekrutenko, and D. Blankenberg. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res. 46:W537–W544. doi:10.1093/nar/gky379.

Afonso, J.S., M. Bruce, P. Keating, D. Raboisson, H. Clough, G. Oikonomou, and J. Rushton. 2020. Profiling Detection and Classification of Lameness Methods in British Dairy Cattle Research: A Systematic Review and Meta-Analysis. Front. Vet. Sci. 7:542. doi:10.3389/fvets.2020.00542.

Aguilar, C.A., A. Shcherbina, D.O. Ricke, R. Pop, C.T. Carrigan, C.A. Gifford, M.L. Urso, M.A. Kottke, and A. Meissner. 2015. In vivo Monitoring of Transcriptional Dynamics After Lower-Limb Muscle Injury Enables Quantitative Classification of Healing. Sci. Rep. 5:13885. doi:10.1038/srep13885.

Aguilar, I., E.N. Fernandez, A. Blasco, O. Ravagnolo, and A. Legarra. 2020. Effects of ignoring inbreeding in model-based accuracy for BLUP and SSGBLUP. J. Anim. Breed. Genet. 137:356–364. doi:10.1111/jbg.12470.

Aguilar, I., A. Legarra, F. Cardoso, Y. Masuda, D. Lourenco, and I. Misztal. 2019. Frequentist p-values for large-scale-single step genome-wide association, with an application to birth weight in American Angus cattle. Genet. Sel. Evol. 51:28–28. doi:10.1186/s12711-019-0469-3.

Aguilar, I., I. Misztal, D.L. Johnson, A. Legarra, S. Tsuruta, and T.J. Lawlor. 2010. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score1. J. Dairy Sci. 93:743–752. doi:10.3168/jds.2009-2730.

Aguilar, I., I. Misztal, S. Tsuruta, A. Legarra, and H. Wang. 2014. PREGSF90 – POSTGSF90: Computational Tools for the Implementation of Single-step Genomic Selection and Genome-wide Association with Ungenotyped Individuals in BLUPF90 Programs. Page 680 in Proceedings 10th Congress of Genetics Applied to Livestock Production World Congress on Genetics Applied to Livestock Production (WCGALP). American Society of Animal Science, Vancouver, Canada.

AHDB. 2018a. Lameness Advantage: Genetic index to reduce lameness.

AHDB. 2018b. An Introduction to the Healthy Feet Programme. Accessed January 21, 2022. https://ahdb.org.uk/healthy-feet.

AHDB. 2020a. Mobility Scoring. Accessed October 10, 2021. https://projectblue.blob.core.windows.net/media/Default/Dairy/Publications/Dairy%20Mobility%20Scoresheet_200427_WEB.pdf.

AHDB. 2020b. Breeding Briefs. Accessed October 10, 2021. https://projectblue.blob.core.windows.net/media/Default/Dairy/Publications/BreedingBriefs3050_200228_WEB.pdf.

Akbar, H., T.M. Grala, M. Vailati Riboni, F.C. Cardoso, G. Verkerk, J. McGowan, K. Macdonald, J. Webster, K. Schutz, S. Meier, L. Matthews, J.R. Roche, and J.J. Loor. 2015. Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows. J. Dairy Sci. 98:1019–1032. doi:10.3168/jds.2014-8584.

Akinyemi, R.O., K. Akinwande, S. Diala, O. Adeleye, A. Ajose, K. Issa, D. Owusu, I. Boamah, I.S. Yahaya, A.O. Jimoh, L. Imoh, G. Fakunle, A. Akpalu, F. Sarfo, K. Wahab, E. Sanya, L. Owolabi, R. Obiako, G. Osaigbovo, M. Komolafe, M. Fawale, P. Adebayo, P. Olowoyo, Y. Obiabo, T. Sunmonu, I. Chukwuonye, O. Balogun, B. Adeoye, F. Oladele, P. Olowoniyi, F. Adeyemi, A. Lezzi, A.T. Falayi, M. Fasanya, K. Ogunwale, O. Adeola, O. Olomu, O. Aridegbe, R. Laryea, E. Uvere, M. Faniyan, E. Melikam, R. Tagge, O. Akpa, J. Akinyemi, O. Arulogun, H.K. Tiwari, B. Ovbiagele, and M.O. Owolabi. 2018. Biobanking in a Challenging African Environment: Unique Experience from the SIREN Project. Biopreservation Biobanking 16:217–232. doi:10.1089/bio.2017.0113.

Ali, S. 2020. Prevalence and Associated Risk Factors of Lameness in Cows at Commercial Dairy Herds in Punjab, Pakistan. Pak. J. Zool. 53. doi:10.17582/journal.pjz/20200322100328.

Allain, B., R. Jarray, L. Borriello, B. Leforban, S. Dufour, W. Liu, P. Pamonsinlapatham, S. Bianco, J. Larghero, R. Hadj-Slimane, C. Garbay, F. Raynaud, and Y. Lepelletier. 2012. Neuropilin-1 regulates a new VEGF-induced gene, Phactr-1, which controls tubulogenesis and modulates lamellipodial dynamics in human endothelial cells.. Cell. Signal. 24:214–223. doi:10.1016/j.cellsig.2011.09.003.

Almeida, P.E., P.S.D. Weber, J.L. Burton, and A.J. Zanella. 2008. Depressed DHEA and increased sickness response behaviors in lame dairy cows with inflammatory foot lesions. Domest. Anim. Endocrinol. 34:89–99. doi:10.1016/j.domaniend.2006.11.006.

Al-Qudah, K.M., and Z.B. Ismail. 2012. The relationship between serum biotin and oxidant/antioxidant activities in bovine lameness. Res. Vet. Sci. 92:138–141. doi:10.1016/j.rvsc.2010.10.017.

Amin, N., C.M. van Duijn, and Y.S. Aulchenko. 2007. A Genomic Background Based Method for Association Analysis in Related Individuals. PLOS ONE 2:e1274. doi:10.1371/journal.pone.0001274.

Amory, J.R., Z.E. Barker, J.L. Wright, S.A. Mason, R.W. Blowey, and L.E. Green. 2008. Associations between sole ulcer, white line disease and digital dermatitis and the milk yield of 1824 dairy cows on 30 dairy cow farms in England and Wales from February 2003–November 2004. Prev. Vet. Med. 83:381–391. doi:10.1016/j.prevetmed.2007.09.007.

van Amstel, S.R., and J.K. Shearer. 2006. Review of Pododermatitis Circumscripta (Ulceration of the Sole) in Dairy Cows. J. Vet. Intern. Med. 20:805–811. doi:10.1111/j.1939-1676.2006.tb01789.x.

Anderson, J.R. 2022. Science-in-brief: Proteomics and metabolomics in equine veterinary science. Equine Vet. J. 54:449–452. doi:10.1111/evj.13550.

Anon. AHDB Dairy: Available Holstein Bulls. Accessed November 8, 2021. https://breedingdairy.ahdbdigital.org.uk/tables.asp?preview=1&t=SAC_Bull_report_pli_Int_semen_available_HOL&i=1&b=HOL.

Arango-Sabogal, J.C., A. Desrochers, R. Lacroix, A.-M. Christen, and S. Dufour. 2020. Prevalence of foot lesions in Québec dairy herds from 2015 to 2018.. J. Dairy Sci. 103:11659–11675. doi:10.3168/jds.2020-18191.

Archer, S., N. Bell, and J. Huxley. 2010a. Lameness in UK dairy cows: A review of the current status. In Pract. 32:492–504. doi:10.1136/inp.c6672.

Archer, S.C., M.J. Green, and J.N. Huxley. 2010b. Association between milk yield and serial locomotion score assessments in UK dairy cows. J. Dairy Sci. 93:4045–4053. doi:10.3168/jds.2010-3062.

Austin, P.C., and J.V. Tu. 2004. Bootstrap Methods for Developing Predictive Models. Am. Stat. 58:131–137.

Azarabad, H., I. Nowrouzia, E. Soleymani, G. Vakilgilan, and S.M. Seyedjavad. 2006. Wound Healing Process of Uncomplicated “Rusterholz” Ulcer, Following Treatment by Wooden Block and Hoofgel® in Bovine Hoof: Histopathological Aspects. Am. J. Anim. Vet. Sci. 1:27–30. doi:10.3844/ajavsp.2006.27.30.

Bach, A., M. Dinarés, M. Devant, and X. Carré. 2007. Associations between lameness and production, feeding and milking attendance of Holstein cows milked with an automatic milking system. J. Dairy Res. 74:40–46. doi:10.1017/S0022029906002184.

Bach, K., S.S. Nielsen, and N. Capion. 2021. Changes in the soft-tissue thickness of the claw sole in Holstein heifers around calving.. J. Dairy Sci. 104:4837–4846. doi:10.3168/jds.2020-19161.

Baker, R.G., M.S. Hayden, and S. Ghosh. 2011. NF-κB, inflammation and metabolic disease. Cell Metab. 13:11–22. doi:10.1016/j.cmet.2010.12.008.

Banos, G., M.P. Coffey, E. Wall, and S. Brotherstone. 2006. Genetic Relationship Between First-Lactation Body Energy and Later-Life Udder Health in Dairy Cattle. J. Dairy Sci. 89:2222–2232. doi:10.3168/jds.S0022-0302(06)72293-7.

Barendse, W., R. Bunch, M. Thomas, S. Armitage, S. Baud, and N. Donaldson. 2004. The TG5 thyroglobulin gene test for a marbling quantitative trait loci evaluated in feedlot cattle. Aust. J. Exp. Agric. 44:669–674. doi:10.1071/ea02156.

Barker, Z.E., J.R. Amory, J.L. Wright, S.A. Mason, R.W. Blowey, and L.E. Green. 2009. Risk factors for increased rates of sole ulcers, white line disease, and digital dermatitis in dairy cattle from twenty-seven farms in England and Wales. J. Dairy Sci. 92:1971–1978. doi:10.3168/jds.2008-1590.

Barker, Z.E., K.A. Leach, H.R. Whay, N.J. Bell, and D.C.J. Main. 2010. Assessment of lameness prevalence and associated risk factors in dairy herds in England and Wales. J. Dairy Sci. 93:932–941. doi:10.3168/jds.2009-2309.

Barma, P., S. Bhattacharya, A. Bhattacharya, R. Kundu, S. Dasgupta, A. Biswas, S. Bhattacharya, S.S. Roy, and S. Bhattacharya. 2009. Lipid induced overexpression of NF-κB in skeletal muscle cells is linked to insulin resistance. Biochim. Biophys. Acta BBA - Mol. Basis Dis. 1792:190–200. doi:10.1016/j.bbadis.2008.11.014.

Barri, T., and L.O. Dragsted. 2013. UPLC-ESI-QTOF/MS and multivariate data analysis for blood plasma and serum metabolomics: Effect of experimental artefacts and anticoagulant. Anal. Chim. Acta 768:118–128. doi:10.1016/j.aca.2013.01.015.

Bascom, S.S., and A.J. Young. 1998. A Summary of the Reasons Why Farmers Cull Cows. J. Dairy Sci. 81:2299–2305. doi:10.3168/jds.S0022-0302(98)75810-2.

Bathgate, R.A.D., M.L. Halls, E.T.V.D. Westhuizen, G.E. Callander, M. Kocan, and R.J. Summers. 2013. Relaxin family peptides and their receptors. Physiol. Rev. 93:405–480. doi:10.1152/physrev.00001.2012.

Battagin, M., C. Sartori, S. Biffani, M. Penasa, and M. Cassandro. 2013. Genetic parameters for body condition score, locomotion, angularity, and production traits in Italian Holstein cattle. J. Dairy Sci. 96:5344–5351. doi:10.3168/jds.2012-6352.

Bauman, C.A., H.W. Barkema, J. Dubuc, G.P. Keefe, and D.F. Kelton. 2016. Identifying management and disease priorities of Canadian dairy industry stakeholders. J. Dairy Sci. 99:10194–10203. doi:10.3168/jds.2016-11057.

Bay, V., A. Gillespie, E.K. Ganda, N. Evans, S. Carter, L. Lenzi, A. Lucaci, S. Haldenby, M. Barden, B.E. Griffiths, E. Sánchez-Molano, R. Bicalho, G. Banos, A. Darby, and G. Oikonomou. 2021. The Bovine Foot Skin Microbiota is Associated with Host Genotype and the Development of Infectious Digital Dermatitis Lesions. In Review.

Beckonert, O., H.C. Keun, T.M.D. Ebbels, J. Bundy, E. Holmes, J.C. Lindon, and J.K. Nicholson. 2007. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nat. Protoc. 2:2692–2703. doi:10.1038/nprot.2007.376.

Bell, N. 2016. Preventing and managing lameness in dairy cows. 1st ed. Burleigh Dodds Science Publishing.

Bell, N.J., M.J. Bell, T.G. Knowles, H.R. Whay, D.J. Main, and A.J.F. Webster. 2009. The development, implementation and testing of a lameness control programme based on HACCP principles and designed for heifers on dairy farms. Vet. J. 180:178–188. doi:10.1016/j.tvjl.2008.05.020.

Bell, N.J., and L. Randall. 2021. Lameness in dairy heifers: a narrative review of control strategies. Livestock 26:68–76. doi:10.12968/live.2021.26.2.68.

Bennett, R.M., Z.E. Barker, D.C.J. Main, H.R. Whay, and K.A. Leach. 2014. Investigating the value dairy farmers place on a reduction of lameness in their herds using a willingness to pay approach. Vet. J. 199:72–75. doi:10.1016/j.tvjl.2013.09.068.

van den Berg, I., R. Xiang, J. Jenko, H. Pausch, M. Boussaha, C. Schrooten, T. Tribout, A.B. Gjuvsland, D. Boichard, Ø. Nordbø, M.-P. Sanchez, and M.E. Goddard. 2020. Meta-analysis for milk fat and protein percentage using imputed sequence variant genotypes in 94,321 cattle from eight cattle breeds. Genet. Sel. Evol. 52:37. doi:10.1186/s12711-020-00556-4.

van den Berg, R.A., H.C. Hoefsloot, J.A. Westerhuis, A.K. Smilde, and M.J. van der Werf. 2006. Centering, scaling, and transformations: improving the biological information content of metabolomics data. BMC Genomics 7:142. doi:10.1186/1471-2164-7-142.

Bergsten, C., and B. Frank. 1996. Sole haemorrhages in tied primiparous cows as an indicator of periparturient laminitis: effects of diet, flooring and season.. Acta Vet. Scand. 37:383–94.

Bergsten, C., and A.H. Herlin. 1996. Sole Haemorrhages and Heel Horn Erosion in Dairy Cows: The Influence of Housing System on their Prevalence and Severity. Acta Vet. Scand. 37:395–408. doi:10.1186/BF03548079.

Bergsten, C., E. Telezhenko, and M. Ventorp. 2015. Influence of soft or hard floors before and after first calving on dairy heifer locomotion, claw and leg health. Animals 5:662–686. doi:10.3390/ani5030378.

Bernini, P., I. Bertini, C. Luchinat, P. Nincheri, S. Staderini, and P. Turano. 2011. Standard operating procedures for pre-analytical handling of blood and urine for metabolomic studies and biobanks. J. Biomol. NMR 49:231–243. doi:10.1007/s10858-011-9489-1.

Berry, D.P., F. Buckley, P. Dillon, R.D. Evans, M. Rath, and R.F. Veerkamp. 2003. Genetic Relationships among Body Condition Score, Body Weight, Milk Yield, and Fertility in Dairy Cows. J. Dairy Sci. 86:2193–2204. doi:10.3168/jds.S0022-0302(03)73809-0.

Berry, D.P., F. Buckley, P. Dillon, R.D. Evans, and R.F. Veerkamp. 2004. Genetic Relationships among Linear Type Traits, Milk Yield, Body Weight, Fertility and Somatic Cell Count in Primiparous Dairy Cows. Ir. J. Agric. Food Res. 43:161–176.

Berry, D.P., A.J. Twomey, R.D. Evans, A.R. Cromie, and S.C. Ring. 2019. Heritability - what is it, and what is it not; implications for improving cattle health.. Cattle Pract. 27:1–11.

Berry, D.P., E. Wall, and J.E. Pryce. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8:105–121. doi:10.1017/S1751731114000743.

Bicalho, R.C., S.H. Cheong, G. Cramer, and C.L. Guard. 2007a. Association between a visual and an automated locomotion score in lactating Holstein cows. J. Dairy Sci. 90:3294–3300. doi:10.3168/jds.2007-0076.

Bicalho, R.C., V.S. Machado, and L.S. Caixeta. 2009. Lameness in dairy cattle: A debilitating disease or a disease of debilitated cattle? A cross-sectional study of lameness prevalence and thickness of the digital cushion. J. Dairy Sci. 92:3175–3184. doi:10.3168/jds.2008-1827.

Bicalho, R.C., and G. Oikonomou. 2013. Control and prevention of lameness associated with claw lesions in dairy cows. Livest. Sci. 156:96–105. doi:10.1016/j.livsci.2013.06.007.

Bicalho, R.C., F. Vokey, H.N. Erb, and C.L. Guard. 2007b. Visual Locomotion Scoring in the First Seventy Days in Milk: Impact on Pregnancy and Survival. J. Dairy Sci. 90:4586–4591. doi:10.3168/jds.2007-0297.

Blackie, N., E.C.L. Bleach, J.R. Amory, and J.R. Scaife. 2013. Associations between locomotion score and kinematic measures in dairy cows with varying hoof lesion types. J. Dairy Sci. 96:3564–3572. doi:10.3168/jds.2012-5597.

Blakebrough-Hall, C., A. Dona, M.J. D'occhio, J. McMeniman, and L.A. González. 2020. Diagnosis of Bovine Respiratory Disease in feedlot cattle using blood 1H NMR metabolomics. Sci. Rep. 10:115. doi:10.1038/s41598-019-56809-w.

Blowey, R.W., C.L. Watson, V. Hedges, P. Ossent, L.E. Green, and A.J. Packinigton. 2000. Possible distinction between sole ulcers and heel ulcers as a cause of bovine lameness. Vet. Rec. 147:110–112. doi:10.1136/vr.147.4.110.

Boadi, D., C. Benchaar, J. Chiquette, and D. Massé. 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. Can. J. Anim. Sci. 84:319–335. doi:10.4141/A03-109.

Boettcher, P.J., J.C.M. Dekkers, and B.W. Kolstad. 1998. Development of an Udder Health Index for Sire Selection Based on Somatic Cell Score, Udder Conformation, and Milking Speed. J. Dairy Sci. 81:1157–1168. doi:10.3168/jds.S0022-0302(98)75678-4.

Bonfatti, V., P.N. Ho, and J.E. Pryce. 2020. Usefulness of milk mid-infrared spectroscopy for predicting lameness score in dairy cows.. J. Dairy Sci. 103:2534–2544. doi:10.3168/jds.2019-17551.

Boosman, R., C.W. Mutsaers, and A. Klarenbeek. 1991. The role of endotoxin in the pathogenesis of acute bovine laminitis.. Vet. Q. 13:155–162. doi:10.1080/01652176.1991.9694301.

Booth, C.J., L.D. Warnick, Y.T. Gröhn, D.O. Maizon, C.L. Guard, and D. Janssen. 2004. Effect of lameness on culling in dairy cows. J. Dairy Sci. 87:4115–4122. doi:10.3168/jds.S0022-0302(04)73554-7.

Boulton, A.C., J. Rushton, and D.C. Wathes. 2017. An empirical analysis of the cost of rearing dairy heifers from birth to first calving and the time taken to repay these costs. Anim. Int. J. Anim. Biosci. 11:1372–1380. doi:10.1017/S1751731117000064.

Boyce, B.F., Z. Yao, and L. Xing. 2010. Functions of NF-κB in Bone. Ann. N. Y. Acad. Sci. 1192:367–375. doi:10.1111/j.1749-6632.2009.05315.x.

Bradford, B.J., K. Yuan, J.K. Farney, L.K. Mamedova, and A.J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J. Dairy Sci. 98:6631–6650. doi:10.3168/jds.2015-9683.

Bramley, E., I.J.J. Lean, W.J.J. Fulkerson, M.A.A. Stevenson, A.R.R. Rabiee, and N.D.D. Costa. 2008. The Definition of Acidosis in Dairy Herds Predominantly Fed on Pasture and Concentrates. J. Dairy Sci. 91:308–321. doi:10.3168/jds.2006-601.

Bran, J.A., R.R. Daros, M.A.G. von Keyserlingk, S.J. LeBlanc, and M.J. Hötzel. 2018. Cow- and herd-level factors associated with lameness in small-scale grazing dairy herds in Brazil.. Prev. Vet. Med. 151:79–86. doi:10.1016/j.prevetmed.2018.01.006.

Breiman, L. 2001. Random Forests. Mach. Learn. 45:5–32. doi:10.1023/A:1010933404324.

Brickell, J.S., and D.C. Wathes. 2011. A descriptive study of the survival of Holstein-Friesian heifers through to third calving on English dairy farms. J. Dairy Sci. 94:1831–1838. doi:10.3168/jds.2010-3710.

Britt, J.H. 1985. Enhanced Reproduction and Its Economic Implications. J. Dairy Sci. 68:1585–1592. doi:10.3168/jds.S0022-0302(85)80997-8.

Britt, J.H., R.A. Cushman, C.D. Dechow, H. Dobson, P. Humblot, M.F. Hutjens, G.A. Jones, F.M. Mitloehner, P.L. Ruegg, I.M. Sheldon, and J.S. Stevenson. 2021. Review: Perspective on high-performing dairy cows and herds. Animal 15:100298. doi:10.1016/j.animal.2021.100298.

Brotherstone, S., R.F. Veerkamp, and W.G. Hill. 1998. Predicting breeding values for herd life of Holstein-Friesian dairy cattle from lifespan and type. Anim. Sci. 67:405–411. doi:10.1017/S135772980003280X.

Brown, K.L., B.G. Cassell, M.L. Mcgilliard, M.D. Hanigan, and F.C. Gwazdauskas. 2012. Hormones, metabolites, and reproduction in Holsteins, Jerseys, and their crosses. J. Dairy Sci. 95:698–707. doi:10.3168/jds.2011-4666.

Browne, N., C.D. Hudson, R.E. Crossley, K. Sugrue, E. Kennedy, J.N. Huxley, and M. Conneely. 2022. Cow- and herd-level risk factors for lameness in partly housed pasture-based dairy cows. J. Dairy Sci. 105:1418–1431. doi:10.3168/jds.2021-20767.

Bruckmaier, R.M., L. Gregoretti, F. Jans, D. Faissler, and J.W. Blum. 1998. Longissimus Dorsi Muscle Diameter, Backfat Thickness, Body Condition Scores and Skinfold Values Related to Metabolic and Endocrine Traits in Lactating Dairy Cows Fed Crystalline Fat or Free Fatty Acids. J. Vet. Med. 410:397–410.

Bruijnis, M., H. Hogeveen, C. Garforth, and E. Stassen. 2013. Dairy farmers' attitudes and intentions towards improving dairy cow foot health. Livest. Sci. 155:103–113. doi:10.1016/j.livsci.2013.04.005.

Bruijnis, M.R.N., B. Beerda, H. Hogeveen, and E.N. Stassen. 2012. Assessing the welfare impact of foot disorders in dairy cattle by a modeling approach. Animal 6:962–970. doi:10.1017/S1751731111002606.

Bruijnis, M.R.N., H. Hogeveen, and E.N. Stassen. 2010. Assessing economic consequences of foot disorders in dairy cattle using a dynamic stochastic simulation model. J. Dairy Sci. 93:2419–2432. doi:10.3168/jds.2009-2721.

Buch, L.H., A.C. Sørensen, J. Lassen, P. Berg, J.-Å. Eriksson, J.H. Jakobsen, and M.K. Sørensen. 2011. Hygiene-related and feed-related hoof diseases show different patterns of genetic correlations to clinical mastitis and female fertility.. J. Dairy Sci. 94:1540–1551. doi:10.3168/jds.2010-3137.

Budras, K.-D. 2003. Bovine Anatomy: An Illustrated Text. 1st ed. Schlütersche, Hannover, Germany.

Buitenhuis, A.J., M.S. Lund, J.R. Thomasen, B. Thomsen, V.H. Nielsen, C. Bendixen, and B. Guldbrandtsen. 2007. Detection of quantitative trait loci affecting lameness and leg conformation traits in Danish Holstein cattle. J. Dairy Sci. 90:472–481. doi:10.3168/jds.s0022-0302(07)72649-8.

Buitenhuis, B., N.A. Poulsen, L.B. Larsen, and J. Sehested. 2015. Estimation of genetic parameters and detection of quantitative trait loci for minerals in Danish Holstein and Danish Jersey milk. BMC Genet. 16:52. doi:10.1186/s12863-015-0209-9.

Bundgaard, L., E. Bendixen, M.Aa. Sørensen, V.M. Harman, R.J. Beynon, L.J. Petersen, and S. Jacobsen. 2016. A selected reaction monitoring-based analysis of acute phase proteins in interstitial fluids from experimental equine wounds healing by secondary intention. Wound Repair Regen. 24:525–532. doi:10.1111/wrr.12425.

Butty, A.M., T.C.S. Chud, D.F. Cardoso, L.S.F. Lopes, F. Miglior, F.S. Schenkel, A. Cánovas, I.M. Häfliger, C. Drögemüller, P. Stothard, F. Malchiodi, and C.F. Baes. 2021. Genome-wide association study between copy number variants and hoof health traits in Holstein dairy cattle.. J. Dairy Sci.. doi:10.3168/jds.2020-19879.

Cai, Z., B. Guldbrandtsen, M.S. Lund, and G. Sahana. 2018. Prioritizing candidate genes post-GWAS using multiple sources of data for mastitis resistance in dairy cattle. BMC Genomics 19:656. doi:10.1186/s12864-018-5050-x.

Calo, L.L., R.E. McDowell, L.D. VanVleck, and P.D. Miller. 1973. Genetic Aspects of Beef Production among Holstein-Friesians Pedigree Selected for Milk Production. J. Anim. Sci. 37:676–682. doi:10.2527/jas1973.373676x.

Calus, M.P.L., D.P. Berry, G. Banos, Y. de Haas, and R.F. Veerkamp. 2013a. Genomic selection: the option for new robustness traits?. Adv. Anim. Biosci. 4:618–625. doi:10.1017/s2040470013000186.

Calus, M.P.L., Y. De Haas, M. Pszczola, and R.F. Veerkamp. 2013b. Predicted accuracy of and response to genomic selection for new traits in dairy cattle. Animal 7:183–191. doi:10.1017/S1751731112001450.

Capion, N., S.M. Thamsborg, and C. Enevoldsen. 2008. Prevalence of foot lesions in Danish Holstein cows. Vet. Rec. 163:80–86. doi:10.1136/vr.163.3.80.

Capion, N., S.M. Thamsborg, and C. Enevoldsen. 2009. Prevalence and severity of foot lesions in Danish Holstein heifers through first lactation. Vet. J. 182:50–58. doi:10.1016/j.tvjl.2008.05.026.

Capper, J.L., R.A. Cady, and D.E. Bauman. 2009. The environmental impact of dairy production: 1944 compared with 20071. J. Anim. Sci. 87:2160–2167. doi:10.2527/jas.2009-1781.

Caro, D., S.J. Davis, S. Bastianoni, and K. Caldeira. 2014. Global and regional trends in greenhouse gas emissions from livestock. Clim. Change 126:203–216. doi:10.1007/s10584-014-1197-x.

Carrazco, A.V., C.B. Peterson, Y. Zhao, Y. Pan, J.J. McGlone, E.J. DePeters, and F.M. Mitloehner. 2020. The Impact of Essential Oil Feed Supplementation on Enteric Gas Emissions and Production Parameters from Dairy Cattle. Sustainability 12:10347. doi:10.3390/su122410347.

Cerri, R.L.A., I.M. Thompson, I.H. Kim, A.D. Ealy, P.J. Hansen, C.R. Staples, J.L. Li, J.E.P. Santos, and W.W. Thatcher. 2012. Effects of lactation and pregnancy on gene expression of endometrium of Holstein cows at day 17 of the estrous cycle or pregnancy. J. Dairy Sci. 95:5657–5675. doi:https://doi.org/10.3168/jds.2011-5114.

Cha, E., J.A. Hertl, D. Bar, and Y.T. Gröhn. 2010. The cost of different types of lameness in dairy cows calculated by dynamic programming. Prev. Vet. Med. 97:1–8. doi:10.1016/j.prevetmed.2010.07.011.

Chagas, L.M., M.C. Lucy, P.J. Back, D. Blache, J.M. Lee, P.J.S. Gore, A.J. Sheahan, and J.R. Roche. 2009. Insulin resistance in divergent strains of Holstein-Friesian dairy cows offered fresh pasture and increasing amounts of concentrate in early lactation. J. Dairy Sci. 92:216–222. doi:10.3168/jds.2008-1329.

Chan, F.Y. 2012. Genome wide association studies for temperament in New Zealand dairy cattle : a thesis presented in partial fulfilment of the

requirements for the degree of Master of Science in Animal Science in Animal Breeding and Genetics at Massey University, Palmerston North, New Zealand. Thesis Thesis. Massey University,.

Chapinal, N., A. Koeck, A. Sewalem, D.F. Kelton, S. Mason, G. Cramer, and F. Miglior. 2013. Genetic parameters for hoof lesions and their relationship with feet and leg traits in Canadian Holstein cows. J. Dairy Sci. 96:2596–2604. doi:10.3168/jds.2012-6071.

Chapinal, N., Y. Liang, D.M. Weary, Y. Wang, and M.A.G. Von Keyserlingk. 2014. Risk factors for lameness and hock injuries in Holstein herds in China. J. Dairy Sci. 97:4309–4316. doi:10.3168/jds.2014-8089.

Charfeddine, N., and M.A. Pérez-Cabal. 2017. Effect of claw disorders on milk production, fertility, and longevity, and their economic impact in Spanish Holstein cows. J. Dairy Sci. 100:653–665. doi:10.3168/jds.2016-11434.

Chen, W., E. White, and N.M. Holden. 2016. The effect of lameness on the environmental performance of milk production by rotational grazing. J. Environ. Manage. 172:143–150. doi:10.1016/j.jenvman.2016.02.030.

Chen, Y., J. Wu, L. Tu, X. Xiong, X. Hu, J. Huang, Z. Xu, X. Zhang, C. Hu, X. Hu, A. Guo, Y. Wang, and H. Chen. 2013. 1H-NMR Spectroscopy Revealed Mycobacterium tuberculosis Caused Abnormal Serum Metabolic Profile of Cattle. PLOS ONE 8:e74507. doi:10.1371/journal.pone.0074507.

Chesterton, R., K. Lawrence, and R. Laven. 2008. A descriptive analysis of the foot lesions identified during veterinary treatment for lameness on dairy farms in north Taranaki. N. Z. Vet. J. 56:130–138. doi:10.1080/00480169.2008.36821.

Christen, M., C. Bergsten, J. Burgstaller, N. Capion, N. Charfeddine, J. Clarke, V. Daniel, D. Döpfer, A. Fiedler, T. Fjeldaas, B. Heringstad, G. Cramer, J. Kofler, K. Müller, P. Nielsen, E. Oakes, C. Ødegård, K. O'Driscoll, J. Pryce, A. Steiner, K. Stock, G. Thomas, K. Ulvshammar, M. Holzhauer, J. Cole, C. Egger-Danner, Z. Kowalski, N. Petreny, M. Burke, P. Buček, L. Journaux, M. Coffey, C. Hunlun, and D. Radzio. 2015. Recording of claw and foot disorders in dairy cattle: current role and prospects of the international harmonization initiative of ICAR. Page in Performance recording in the genotyped world ICAR Technical Meeting, Krakow, Poland.

Christensen, O.F., and M.S. Lund. 2010. Genomic prediction when some animals are not genotyped. Genet. Sel. Evol. 42:2. doi:10.1186/1297-9686-42-2.

Cirulli, E.T., L. Guo, C. Leon Swisher, N. Shah, L. Huang, L.A. Napier, E.F. Kirkness, T.D. Spector, C.T. Caskey, B. Thorens, J.C. Venter, and A. Telenti. 2019. Profound Perturbation of the Metabolome in Obesity Is Associated with Health Risk. Cell Metab. 29:488-500.e2. doi:10.1016/j.cmet.2018.09.022.

Clark, W., and M. Lenaghan. 2020. The Future of Food: Sustainable protein strategies around the world. Zero Waste Scotland. Stirling, Scotland, UK.

Clarkson, M.J., D.Y. Downham, W.B. Faull, J.W. Hughes, F.J. Manson, J.B. Merritt, R.D. Murray, W.B. Russell, J.E. Sutherst, and W.R. Ward. 1996. Incidence and prevalence of lameness in dairy cattle. Vet. Rec. 138:563–567. doi:10.1136/vr.138.23.563.

Clasen, J.B., M. Kargo, S. Østergaard, W.F. Fikse, L. Rydhmer, and E. Strandberg. 2021. Genetic consequences of terminal crossbreeding, genomic test, sexed semen, and beef semen in dairy herds. J. Dairy Sci. 104:8062–8075. doi:10.3168/jds.2020-20028.

Coffey, M. 2020. Dairy cows: in the age of the genotype, #phenotypeisking. Anim. Front. 10:19–22. doi:10.1093/af/vfaa004.

Cole, J.B., and P.M. VanRaden. 2018. Symposium review: Possibilities in an age of genomics: The future of selection indices. J. Dairy Sci. 101:3686–3701. doi:10.3168/jds.2017-13335.

Cole, J.B., G.R. Wiggans, L. Ma, T.S. Sonstegard, T.J. Lawlor, B.A. Crooker, C.P. Van Tassell, J. Yang, S. Wang, L.K. Matukumalli, and Y. Da. 2011. Genome-wide association analysis of thirty one production, health, reproduction and body conformation traits in contemporary U.S. Holstein cows. BMC Genomics 12. doi:10.1186/1471-2164-12-408.

Coleman, J., K.M. Pierce, D.P. Berry, A. Brennan, and B. Horan. 2009. The influence of genetic selection and feed system on the reproductive performance of spring-calving dairy cows within future pasture-based production systems. J. Dairy Sci. 92:5258–5269. doi:10.3168/jds.2009-2108.

Collard, B.L., P.J. Boettcher, J.C.M. Dekkers, D. Petitclerc, and L.R. Schaeffer. 2000. Relationships between energy balance and health traits of dairy cattle in early lactation. J. Dairy Sci. 83:2683–2690. doi:10.3168/jds.S0022-0302(00)75162-9.

Cook, N.B., and K. V Nordlund. 2009. The influence of the environment on dairy cow behavior, claw health and herd lameness dynamics. Vet. J. 179:360–369. doi:10.1016/j.tvjl.2007.09.016.

Cook, N.B., K. V. Nordlund, and G.R. Oetzel. 2004. Environmental influences on claw horn lesions associated with laminitis and subacute ruminal acidosis in dairy cows. J. Dairy Sci. 87:E36–E46. doi:10.3168/jds.S0022-0302(04)70059-4.

Cramer, G., K.D. Lissemore, C.L. Guard, K.E. Leslie, and D.F. Kelton. 2008. Herd- and cow-level prevalence of foot lesions in ontario dairy cattle. J. Dairy Sci. 91:3888–3895. doi:10.3168/jds.2008-1135.

Cramer, G., K.D. Lissemore, C.L. Guard, K.E. Leslie, and D.F. Kelton. 2009. Herd-level risk factors for seven different foot lesions in Ontario Holstein cattle housed in tie stalls or free stalls. J. Dairy Sci. 92:1404–1411. doi:10.3168/jds.2008-1134.

Crouch, D.J.M., and W.F. Bodmer. 2020. Polygenic inheritance, GWAS, polygenic risk scores, and the search for functional variants. Proc. Natl. Acad. Sci. 117:18924–18933. doi:10.1073/pnas.2005634117.

Croué, I., F. Fikse, K. Johansson, E. Carlén, G. Thomas, H. Leclerc, and V. Ducrocq. 2017. Genetic evaluation of claw health traits accounting for potential preselection of cows to be trimmed. J. Dairy Sci. 100:8197–8204. doi:10.3168/jds.2017-13002.

Croué, I., A. Michenet, H. Leclerc, and V. Ducrocq. 2019. Genomic analysis of claw lesions in Holstein cows: Opportunities for genomic selection, quantitative trait locus detection, and gene identification. J. Dairy Sci. 102:6306–6318. doi:10.3168/jds.2018-15979.

Cury, P.R., V.C. Araujo, F. Canavez, C. Furuse, and N.S. Araujo. 2007. Hydrocortisone Affects the Expression of Matrix Metalloproteinases (MMP-1, -2, -3, -7, and -11) and Tissue Inhibitor of Matrix Metalloproteinases (TIMP-1) in Human Gingival Fibroblasts. J. Periodontol. 1309–1315. doi:10.1902/jop.2007.060225.

Damgaard, R.B., J.A. Walker, P. Marco-Casanova, N. V Morgan, H.L. Titheradge, P.R. Elliott, D. McHale, E.R. Maher, A.N.J. McKenzie, and D. Komander. 2016. The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity.. Cell 166:1215-1230.e20. doi:10.1016/j.cell.2016.07.019.

Danscher, A.M., J.M.D. Enemark, E. Telezhenko, N. Capion, C.T. Ekstrøm, and M.B. Thoefner. 2009. Oligofructose overload induces lameness in cattle. J. Dairy Sci. 92:607–616. doi:10.3168/jds.2008-1271.

Danscher, A.M.M., T.H.H. Toelboell, and O. Wattle. 2010. Biomechanics and histology of bovine claw suspensory tissue in early acute laminitis. J. Dairy Sci. 93:53–62. doi:10.3168/jds.2009-2038.

Das, A., F. Panitz, V.R. Gregersen, C. Bendixen, and L.E. Holm. 2015. Deep sequencing of Danish Holstein dairy cattle for variant detection and insight into potential loss-of-function variants in protein coding genes. BMC Genomics 16:1043. doi:10.1186/s12864-015-2249-y.

Davenport, K.M., J.A. Spencer, J.J. Peak, J.C. Dalton, and B.M. Murdoch. 2018. Genomic testing of female Holsteins: a resource for selection and improvement. Transl. Anim. Sci. 2:S149–S154. doi:10.1093/tas/txy033.

Davis, M.E., and R.C. Simmen. 1997. Genetic parameter estimates for serum insulin-like growth factor I concentration and performance traits in Angus beef cattle.. J. Anim. Sci. 75:317. doi:10.2527/1997.752317x.

De Koster, J.D., and G. Opsomer. 2013. Insulin resistance in dairy cows. Vet. Clin. N. Am. - Food Anim. Pract. 29:299–322. doi:10.1016/j.cvfa.2013.04.002.

De Vries, A., and M.I. Marcondes. 2020. Review: Overview of factors affecting productive lifespan of dairy cows. Anim. Int. J. Anim. Biosci. 14:s155–s164. doi:10.1017/S1751731119003264.

Degenhardt, F., S. Seifert, and S. Szymczak. 2019. Evaluation of variable selection methods for random forests and omics data sets. Brief. Bioinform. 20:492–503. doi:10.1093/bib/bbx124.

Dekkers, J.C. 2004. Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons.. J. Anim. Sci. 82 E-Suppl. doi:10.2527/2004.8213_supplE313x.

Denihan, N.M., B.H. Walsh, S.N. Reinke, B.D. Sykes, R. Mandal, D.S. Wishart, D.I. Broadhurst, G.B. Boylan, and D.M. Murray. 2015. The effect of haemolysis on the metabolomic profile of umbilical cord blood. Clin. Biochem. 48:534–537. doi:10.1016/j.clinbiochem.2015.02.004.

Dervishi, E., G. Zhang, S.M. Dunn, R. Mandal, D.S. Wishart, and B.N. Ametaj. 2016. GC–MS Metabolomics Identifies Metabolite Alterations That Precede Subclinical Mastitis in the Blood of Transition Dairy Cows. J. Proteomics 16:433–446. doi:10.1021/acs.jproteome.6b00538.

Dervishi, E., G. Zhang, R. Mandal, D.S. Wishart, and B.N. Ametaj. 2017. Targeted metabolomics: new insights into pathobiology of retained placenta in dairy cows and potential risk biomarkers. Animal 12:1050–1059. doi:10.1017/S1751731117002506.

Dervishi, E., G. Zhang, G. Zwierzchowski, R. Mandal, D.S. Wishart, and B.N. Ametaj. 2019. Serum metabolic fingerprinting of pre-lameness dairy cows by GC-MS reveals typical profiles that can identify susceptible cows. J. Proteomics 213. doi:10.1016/j.jprot.2019.103620.

Dhakal, K., F. Tiezzi, J.S. Clay, and C. Maltecca. 2015. Short communication: Genomic selection for hoof lesions in first-parity US Holsteins. J. Dairy Sci. 98:3502–3507. doi:10.3168/jds.2014-8830.

Dillon, J.A., K.R. Stackhouse-Lawson, G.J. Thoma, S.A. Gunter, C.A. Rotz, E. Kebreab, D.G. Riley, L.O. Tedeschi, J. Villalba, F. Mitloehner, A.N. Hristov, S.L. Archibeque, J.P. Ritten, and N.D. Mueller. 2021. Current state of enteric methane and the carbon footprint of beef and dairy cattle in the United States. Anim. Front. Rev. Mag. Anim. Agric. 11:57–68. doi:10.1093/af/vfab043.

Dippel, S., M. Dolezal, C. Brenninkmeyer, J. Brinkmann, S. March, U. Knierim, and C. Winckler. 2009. Risk factors for lameness in freestall-housed dairy cows across two breeds, farming systems, and countries. J. Dairy Sci. 92:5476–5486. doi:10.3168/jds.2009-2288.

Dohoo, I., W. Martin, and H. Stryhn. 2012. Methods in Epidemiologic Research. Rev. VER Inc., Charlottetown, Prince edward Island.

Dolecheck, K.A., R.M. Dwyer, M.W. Overton, and J.M. Bewley. 2018. A survey of United States dairy hoof care professionals on costs associated with treatment of foot disorders. J. Dairy Sci. 101:8313–8326. doi:10.3168/jds.2018-14718.

Dolecheck, K.A., M.W. Overton, T.B. Mark, and J.M. Bewley. 2019. Use of a stochastic simulation model to estimate the cost per case of digital dermatitis, sole ulcer, and white line disease by parity group and incidence timing.. J. Dairy Sci. 102:715–730. doi:10.3168/jds.2018-14901.

Dong, S., S. Zhang, D. Wang, H. Wang, X. Shang, and P. Yan. 2015. Comparative proteomics analysis provide novel insight into laminitis in Chinese Holstein cows. BMC Vet. Res. 11:1–9. doi:10.1186/s12917-015-0474-x.

Donovan, G.A., C.A. Risco, G.M.D.C. Temple, T.Q. Tran, and H.H. Van Horn. 2004. Influence of transition diets on occurrence of subclinical laminitis in Holstein dairy cows. J. Dairy Sci. 87:73–84. doi:10.3168/jds.S0022-0302(04)73144-6.

Drackley, J.K. 1999. Biology of dairy cows during the transition period: the final frontier?. J. Dairy Sci. 82:2259–2273. doi:10.3168/jds.s0022-0302(99)75474-3.

Drackley, J.K., S.S. Donkin, and C.K. Reynolds. 2006. Major Advances in Fundamental Dairy Cattle Nutrition. J. Dairy Sci. 89:1324–1336. doi:10.3168/jds.S0022-0302(06)72200-7.

Durham, A.E., N. Frank, C.M. McGowan, N.J. Menzies-Gow, E. Roelfsema, I. Vervuert, K. Feige, and K. Fey. 2019. ECEIM consensus statement on equine metabolic syndrome. J. Vet. Intern. Med. 33:335–349. doi:10.1111/jvim.15423.

Dutton-Regester, K.J., J.D. Wright, A.R. Rabiee, and T.S. Barnes. 2019. Understanding dairy farmer intentions to make improvements to their management practices of foot lesions causing lameness in dairy cows. Prev. Vet. Med. 171:104767–104767. doi:10.1016/j.prevetmed.2019.104767.

Eastham, N. 2019. How can UK dairy farmers use genomics to breed a better herd? Taunton, UK.

Eckel, E.F., and B.N. Ametaj. 2016. Invited review : Role of bacterial endotoxins in the etiopathogenesis of periparturient diseases of transition dairy cows. J. Dairy Sci. 99:5967–5990. doi:10.3168/jds.2015-10727.

Eckel, E.F., G. Zhang, E. Dervishi, G. Zwierzchowski, R. Mandal, D.S. Wishart, and B.N. Ametaj. 2020. Urinary metabolomics fingerprinting around parturition identifies metabolites that differentiate lame dairy cows from healthy ones. Animal 14:2138–2149. doi:10.1017/S1751731120001172.

Eckersall, P.D., F.J. Young, C. Mccomb, C.J. Hogarth, S. Safi, A. Weber, T. Mcdonald, A.M. Nolan, and J.L. Fitzpatrick. 2001. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. Vet. Rec. 35–41.

Edmonson, A.J., I.J. Lean, L.D. Weaver, T. Farver, and G. Webster. 1989. A Body Condition Scoring Chart for Holstein Dairy Cows. J. Dairy Sci. 72:68–78. doi:10.3168/jds.S0022-0302(89)79081-0.

Egger-Danner, C., J.B. Cole, J.E. Pryce, N. Gengler, B. Heringstad, A. Bradley, and K.F. Stock. 2014. Invited review: Overview of new traits and phenotyping strategies in dairy cattle with a focus on functional traits. Animal 9:191–207. doi:10.1017/S1751731114002614.

Egger-Danner, C., P. Nielsen, A. Fiedler, A. Müller, T. Fjeldaas, D. Döpfer, V. Daniel, C. Bergsten, G. Cramer, A.M. Christen, K.F. Stock, G. Thomas, M. Holzhauer, Steiner. A, J. Clarke, N. Capion, N. Charfeddine, J. Pryce, E. Oakes, J. Burgstaller, B. Heringstad, C. Ødegård, J. Kofler, F. Egger, and J.B. Cole. 2020. ICAR Claw Health Atlas. 2nd ed. ICAR Technical Series. ICAR, Rome, Italy.

Elischer, M. 2014. History of Dairy Cow Breeds: Holstein. Accessed April 27, 2022. https://www.canr.msu.edu/news/history_of_dairy_cow_breeds_holstein.

Eming, S.A., P. Martin, and M. Tomic-Canic. 2014. Wound repair and regeneration: Mechanisms, signaling, and translation. Sci. Transl. Med. 6:265sr6. doi:10.1126/scitranslmed.3009337.

Eming, S.A., J.R. Morgan, and A. Berger. 1997. Gene therapy for tissue repair: approaches and prospects. Br. J. Plast. Surg. 50:491–500. doi:10.1016/S0007-1226(97)91297-2.

Emwas, A.-H., R. Roy, R.T. McKay, L. Tenori, E. Saccenti, G.A.N. Gowda, D. Raftery, F. Alahmari, L. Jaremko, M. Jaremko, and D.S. Wishart. 2019. NMR Spectroscopy for Metabolomics Research. Metabolites 9:123. doi:10.3390/metabo9070123.

Emwas, A.-H., E. Saccenti, X. Gao, R.T. McKay, V.A.P.M. dos Santos, R. Roy, and D.S. Wishart. 2018. Recommended strategies for spectral processing and post-processing of 1D 1H-NMR data of biofluids with a particular focus on urine. Metabolomics 14:31. doi:10.1007/s11306-018-1321-4.

Enevoldsen, C., Y.T. Gröhn, and I. Thysen. 1991. Sole Ulcers in Dairy Cattle: Associations with Season, Cow Characteristics, Disease, and Production. J. Dairy Sci. 74:1284–1298. doi:10.3168/jds.S0022-0302(91)78284-2.

Enting, H., D. Kooij, A.A. Dijkhuizen, R.B.M. Huirne, and E.N. Noordhuizen-Stassen. 1997. Economic losses due to clinical lameness in dairy cattle. Livest. Prod. Sci. 49:259–267. doi:10.1016/S0301-6226(97)00051-1.

Espejo, L.A., M.I. Endres, and J.A. Salfer. 2006. Prevalence of Lameness in High-Producing Holstein Cows Housed in Freestall Barns in Minnesota. J. Dairy Sci. 89:3052–3058. doi:10.3168/jds.S0022-0302(06)72579-6.

European Food Safety Authority (EFSA). 2009. Scientific report on the effects of farming systems on dairy cow welfare and disease. EFSA J. Eur. Food Saf. Auth. 7:1143r. doi:10.2903/j.efsa.2009.1143r.

Evans, N.J., R.W. Blowey, D. Timofte, D.R. Isherwood, J.M. Brown, R. Murray, R.J. Paton, and S.D. Carter. 2011. Association between bovine digital dermatitis treponemes and a range of “non-healing” bovine hoof disorders. Vet. Rec. 168:214–214. doi:10.1136/vr.c5487.

Fabian, J., R.A. Laven, and H.R. Whay. 2014. The prevalence of lameness on New Zealand dairy farms: A comparison of farmer estimate and locomotion scoring. Vet. J. 201:31–38. doi:10.1016/j.tvjl.2014.05.011.

Falconer, D.S., and T.F.C. Mackay. 1996. Introduction to Quantitative Genetics. 4th ed. Pearson Education Limited.

Fan, J., and J. Lv. 2010. A Selective Overview of Variable Selection in High Dimensional Feature Space. Stat. Sin. 20:101–148.

Fan, W.-J., H.-P. Li, H.-S. Zhu, S.-P. Sui, P.-G. Chen, Y. Deng, T.-M. Sui, and Y.-Y. Wang. 2016. NF-κB is involved in the LPS-mediated proliferation and apoptosis of MAC-T epithelial cells as part of the subacute ruminal acidosis response in cows. Biotechnol. Lett. 38:1839–1849. doi:10.1007/s10529-016-2178-0.

Fang, L., W. Cai, S. Liu, O. Canela-Xandri, Y. Gao, J. Jiang, K. Rawlik, B. Li, S.G. Schroeder, B.D. Rosen, C. Li, T.S. Sonstegard, L.J. Alexander, C.P.V. Tassell, P.M. VanRaden, J.B. Cole, Y. Yu, S. Zhang, A. Tenesa, L. Ma, and G.E. Liu. 2020. Comprehensive analyses of 723 transcriptomes enhance genetic and biological interpretations for complex traits in cattle. Genome Res.. doi:10.1101/gr.250704.119.

FAO. 2018. The future of food and agriculture - Alternative pathways to 2050 |. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAWC. 1993. Report on priorities for animal welfare: research and development.

Faye, L.L., L. Sun, A. Dimitromanolakis, and S.B. Bull. 2011. A flexible genome-wide bootstrap method that accounts for ranking and threshold-selection bias in GWAS interpretation and replication study design. Stat. Med. 30:1898–1912. doi:10.1002/sim.4228.

Fenwick, M.A., R. Fitzpatrick, D.A. Kenny, M.G. Diskin, J. Patton, J.J. Murphy, and D.C. Wathes. 2008. Interrelationships between negative energy balance (NEB) and IGF regulation in liver of lactating dairy cows. Domest. Anim. Endocrinol. 34:31–44. doi:10.1016/j.domaniend.2006.10.002.

Ferguson, J.D., D.T. Galligan, and N. Thomsen. 1994. Principal Descriptors of Body Condition Score in Holstein Cows. J. Dairy Sci. 77:2695–2703. doi:10.3168/jds.S0022-0302(94)77212-X.

Fetrow, J., K.V. Nordlund, and H.D. Norman. 2006. Invited Review: Culling: Nomenclature, Definitions, and Recommendations. J. Dairy Sci. 89:1896–1905. doi:10.3168/jds.S0022-0302(06)72257-3.

Fiil, B.K., R.B. Damgaard, S.A. Wagner, K. Keusekotten, M. Fritsch, S. Bekker-Jensen, N. Mailand, C. Choudhary, D. Komander, and M. Gyrd-Hansen. 2013. OTULIN restricts Met1-linked ubiquitination to control innate immune signaling.. Mol. Cell 50:818–830. doi:10.1016/j.molcel.2013.06.004.

Foditsch, C., G. Oikonomou, V.S. Machado, M.L. Bicalho, E.K. Ganda, S.F. Lima, R. Rossi, B.L. Ribeiro, A. Kussler, and R.C. Bicalho. 2016. Lameness prevalence and risk factors in large dairy farms in upstate New York. Model development for the prediction of claw horn disruption lesions. PLoS ONE 11:1–15. doi:10.1371/journal.pone.0146718.

Foote, R.H. 2002. The history of artificial insemination: Selected notes and notables. J. Anim. Sci. 80:1–10. doi:10.2527/animalsci2002.80E-Suppl_21a.

Foroutan, A., C. Fitzsimmons, R. Mandal, H. Piri-Moghadam, J. Zheng, A. Guo, C. Li, L.L. Guan, and D.S. Wishart. 2020. The Bovine Metabolome. Metabolites 10:E233. doi:10.3390/metabo10060233.

Fraser, D., D. Weary, E. Pajor, and B. Milligan. 1997. A Scientific Conception of Animal Welfare that Reflects Ethical Concerns. Ethics Anim. Welf. Collect.

Freebern, E., D.J.A. Santos, L. Fang, J. Jiang, K.L. Parker Gaddis, G.E. Liu, P.M. VanRaden, C. Maltecca, J.B. Cole, and L. Ma. 2020. GWAS and fine-mapping of livability and six disease traits in Holstein cattle. BMC Genomics 21:41. doi:10.1186/s12864-020-6461-z.

Friedman, J.H., T. Hastie, and R. Tibshirani. 2010. Regularization Paths for Generalized Linear Models via Coordinate Descent. J. Stat. Softw. 33:1–22. doi:10.18637/jss.v033.i01.

Garbarino, E.J., J.A. Hernandez, J.K. Shearer, C.A. Risco, and W.W. Thatcher. 2004. Effect of Lameness on Ovarian Activity in Postpartum Holstein Cows. J. Dairy Sci. 87:4123–4131. doi:10.3168/jds.S0022-0302(04)73555-9.

García-Ruiz, A., J.B. Cole, P.M. VanRaden, G.R. Wiggans, F.J. Ruiz-López, and C.P. Van Tassell. 2016. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. Proc. Natl. Acad. Sci. U. S. A. 113:E3995–E4004. doi:10.1073/pnas.1519061113.

Gard, J.A., D.R. Taylor, D.R. Wilhite, S.P. Rodning, M.L. Schnuelle, R.K. Sanders, R.J. Beyers, M.A. Edmondson, F.J. DeGraves, and E. van Santen. 2015. Effect of exercise and environmental terrain on development of the digital cushion and bony structures of the bovine foot. Am. J. Vet. Res. 76:246–252. doi:10.2460/ajvr.76.3.246.

Garoufalia, Z., A. Papadopetraki, E. Karatza, D. Vardakostas, A. Philippou, G. Kouraklis, and D. Mantas. 2021. Insulin-like growth factor-I and wound healing, a potential answer to non-healing wounds: A systematic review of the literature and future perspectives. Biomed. Rep. 15:66. doi:10.3892/br.2021.1442.

Garshasbi, M., M. Mahmoudi, E. Razmara, M. Vojdanian, S. Aslani, E. Farhadi, L.R. Jensen, S.M. Arzaghi, S. Poursani, A. Bitaraf, M. Eidi, E.E. Gharehdaghi, A.W. Kuss, and A. Jamshidi. 2020. Identification of RELN variant p.(Ser2486Gly) in an Iranian family with ankylosing spondylitis; the first association of RELN and AS. Eur. J. Hum. Genet. 28:754–762. doi:10.1038/s41431-020-0573-4.

GB Cattle Health & Welfare Group. 2020. GB Cattle Health & Welfare Group (CHAWG) - fifth report. CHAWG.

Gerber, P., T. Vellinga, C. Opio, and H. Steinfeld. 2011. Productivity gains and greenhouse gas emissions intensity in dairy systems. Livest. Sci. 139:100–108. doi:10.1016/j.livsci.2011.03.012.

Gernand, E., D.A. Döhne, and S. König. 2013. Genetic background of claw disorders in the course of lactation and their relationships with type traits. J. Anim. Breed. Genet. 130:435–444. doi:10.1111/jbg.12046.

Gernand, E., P. Rehbein, U.U. von Borstel, and S. König. 2012. Incidences of and genetic parameters for mastitis, claw disorders, and common health traits recorded in dairy cattle contract herds. J. Dairy Sci. 95:2144–2156. doi:10.3168/jds.2011-4812.

Ghaffari, M.H., A. Jahanbekam, H. Sadri, K. Schuh, G. Dusel, C. Prehn, J. Adamski, C. Koch, and H. Sauerwein. 2019. Metabolomics meets machine learning: Longitudinal metabolite profiling in serum of normal versus

overconditioned cows and pathway analysis. J. Dairy Sci. 102:11561–11585. doi:10.3168/jds.2019-17114.

Ghosh, S., and M.S. Hayden. 2008. New regulators of NF-κB in inflammation. Nat. Rev. Immunol. 8:837–848. doi:10.1038/nri2423.

Gianola, D. 1982. Theory and Analysis of Threshold Characters. J. Anim. Sci. 54:1079–1096. doi:10.2527/jas1982.5451079x.

Gibbs, R.A., J.F. Taylor, C.P. Van Tassell, W. Barendse, K.A. Eversole, C.A. Gill, R.D. Green, D.L. Hamernik, S.M. Kappes, S. Lien, L.K. Matukumalli, J.C. McEwan, L.V. Nazareth, R.D. Schnabel, G.M. Weinstock, D.A. Wheeler, P. Ajmone-Marsan, W. Barends, P.J. Boettcher, A.R. Caetano, J.F. Garcia, OlivierHanotte, P. Mariani, L.C. Skow, T.S. Sonstegard, J.L. Williams, B. Diallo, L. Hailemariam, O. Hanotte, M.L. Martinez, C.A. Morris, L.O.C. Silva, R.J. Spelman, W. Mulatu, K. Zhao, C.A. Abbey, M. Agaba, F.R. Araujo, R.J. Bunch, J. Burton, C. Gorni, B.E. Harrison, B. Luff, M.A. Machado, J. Mwakaya, G. Plastow, W. Sim, T. Smith, M.B. Thomas, A. Valentini, P. Williams, J. Womack, J.A. Woolliams, Y. Liu, X. Qin, K.C. Worley, C. Gao, H. Jiang, S.S. Moore, Y. Ren, X.Z. Song, C.D. Bustamante, R.D. Hernandez, D.M. Muzny, S. Patil, A.S. Lucas, Q. Fu, M.P. Kent, R. Vega, A. Matukumalli, S. McWilliam, G. Sclep, K. Bryc, J. Choi, H. Gao, J.J. Grefenstette, B. Murdoch, A. Stella, R. Villa-Angulo, M. Wright, J. Aerts, O. Jann, R. Negrini, M.E. Goddard, B.J. Hayes, D.G. Bradley, M.B. Da Silva, L.P.L. Lau, G.E. Liu, D.J. Lynn, F. Panzitta, and K.G. Dodds. 2009. Genome-wide survey of SNP variation uncovers the genetic structure of cattle breeds. Science 324:528–532. doi:10.1126/science.1167936.

Goddard, M.E., and B.J. Hayes. 2012. Genome-Wide Association Studies and Linkage Disequilibrium in Cattle.

Goff, J.P., and R.L. Horst. 1997. Physiological Changes at Parturition and Their Relationship to Metabolic Disorders 1 , 2. J. Dairy Sci. 80:1260–1268. doi:10.3168/jds.S0022-0302(97)76055-7.

Goldansaz, S.A., A.C. Guo, T. Sajed, M.A. Steele, G.S. Plastow, and D.S. Wishart. 2017. Livestock metabolomics and the livestock metabolome: A systematic review. PLOS ONE 12:e0177675. doi:10.1371/journal.pone.0177675.

Gomez, A., and N.B. Cook. 2010. Time budgets of lactating dairy cattle in commercial freestall herds. J. Dairy Sci. 93:5772–5781. doi:10.3168/jds.2010-3436.

González, L.A., B.J. Tolkamp, M.P. Coffey, A. Ferret, and I. Kyriazakis. 2008. Changes in Feeding Behavior as Possible Indicators for the Automatic Monitoring of Health Disorders in Dairy Cows. J. Dairy Sci. 91:1017–1028. doi:10.3168/jds.2007-0530.

Grandl, F., M. Furger, M. Kreuzer, and M. Zehetmeier. 2019. Impact of longevity on greenhouse gas emissions and profitability of individual dairy cows analysed with different system boundaries. Anim. Int. J. Anim. Biosci. 13:198–208. doi:10.1017/S175173111800112X.

Green, L.E., V.J. Hedges, Y.H. Schukken, R.W. Blowey, and A.J. Packington. 2002. The impact of clinical lameness on the milk yield of dairy cows. J. Dairy Sci. 85:2250–2256. doi:10.3168/jds.S0022-0302(02)74304-X.

Green, L.E., J.N. Huxley, C. Banks, and M.J. Green. 2014. Temporal associations between low body condition, lameness and milk yield in a UK dairy herd. Prev. Vet. Med. 113:63–71. doi:10.1016/j.prevetmed.2013.10.009.

Green, M., E. Lima, and R. Hyde. 2021. Selection Stability in High Dimensional Statistical Modelling: Defining a Threshold for Robust Model Inference. doi:10.21203/rs.3.rs-738092/v1.

Green, M.J. ed. . 2012. Dairy Herd Health. 1st ed. CABI Publishers, Wallingford, UK.

Greenough, P.R. 2007. Bovine Laminitis and Lameness. 1st ed. Saunders Elsevier, Edinburgh, UK.

Greenough, P.R., and J.J. Vermunt. 1991. Evaluation of subclinical laminitis in a dairy herd and observations on associated nutritional and management factors. Vet. Rec. 128:11–17. doi:10.1136/vr.128.1.11.

Griffiths, B.E., D. Grove-White, and G. Oikonomou. 2018. A cross-sectional study into the prevalence of dairy cattle lameness and associated herd-level risk factors in England and Wales. Front. Vet. Sci. 5:65–65. doi:10.3389/fvets.2018.00065.

Griffiths, B.E., P.J. Mahen, R. Hall, N. Kakatsidis, N. Britten, K. Long, L. Robinson, H. Tatham, R. Jenkin, and G. Oikonomou. 2020. A Prospective Cohort Study on the Development of Claw Horn Disruption Lesions in Dairy Cattle; Furthering our Understanding of the Role of the Digital Cushion. Front. Vet. Sci. 7:1–9. doi:10.3389/fvets.2020.00440.

Groenevelt, M., D.C.J. Main, D. Tisdall, T.G. Knowles, and N.J. Bell. 2014. Measuring the response to therapeutic foot trimming in dairy cows with fortnightly lameness scoring. Vet. J. 201:283–288. doi:10.1016/j.tvjl.2014.05.017.

Gromski, P.S., H. Muhamadali, D.I. Ellis, Y. Xu, E. Correa, M.L. Turner, and R. Goodacre. 2015. A tutorial review: Metabolomics and partial least squares-discriminant analysis - a marriage of convenience or a shotgun wedding. Anal. Chim. Acta 879:10–23. doi:10.1016/j.aca.2015.02.012.

Grosman, R. 2019. NMR METABOLIC PROFILING OF MOSQUITO SPECIES TO UNDERSTAND INSECTICIDE RESISTANCE. phd Thesis. University of Liverpool,.

Grove-White, D. 2004. Rumen Health in the Dairy Cow. In Pract. 297–304. doi:10.1002/9781118948538.ch30.

Guo, J., G. Chang, K. Zhang, L. Xu, D. Jin, M.S. Bilal, and X. Shen. 2017. Rumen-derived lipopolysaccharide provoked inflammatory injury in the liver of dairy cows fed a high-concentrate diet. Oncotarget 8:46769–46780. doi:10.18632/oncotarget.18151.

Hadley, G.L., C.A. Wolf, and S.B. Harsh. 2006. Dairy Cattle Culling Patterns, Explanations, and Implications. J. Dairy Sci. 89:2286–2296. doi:10.3168/jds.S0022-0302(06)72300-1.

Häggman, J., and J. Juga. 2013. Genetic parameters for hoof disorders and feet and leg conformation traits in Finnish Holstein cows. J. Dairy Sci. 96:3319–3325. doi:10.3168/jds.2012-6334.

Häggman, J., J. Juga, M.J. Sillanpää, and R. Thompson. 2013. Genetic parameters for claw health and feet and leg conformation traits in Finnish Ayrshire cows. J. Anim. Breed. Genet. 130:89–97. doi:10.1111/j.1439-0388.2012.01007.x.

Hayes, B.J., P.J. Bowman, A.J. Chamberlain, and M.E. Goddard. 2009. Invited review: Genomic selection in dairy cattle: Progress and challenges. J. Dairy Sci. 92:433–443. doi:10.3168/jds.2008-1646.

Hedges, J., R.W. Blowey, A.J. Packington, C.J. O'Callaghan, and L.E. Green. 2001. A Longitudinal Field Trial of the Effect of Biotin on Lameness in Dairy Cows. J. Dairy Sci. 84:1969–1975. doi:10.3168/jds.S0022-0302(01)74639-5.

Heinze, G., C. Wallisch, and D. Dunkler. 2018. Variable selection – A review and recommendations for the practicing statistician. Biom. J. 60:431–449. doi:https://doi.org/10.1002/bimj.201700067.

Hendry, K.A.K., A.J. Maccallum, C.H. Knight, and C.J. Wilde. 2001. Synthesis and distribution of cytokeratins in healthy and ulcerated bovine claw epidermis. J. Dairy Res. 68:525–537. doi:10.1017/S0022029901005052.

Heringstad, B., C. Egger-Danner, N. Charfeddine, J.E. Pryce, K.F. Stock, J. Kofler, A.M. Sogstad, M. Holzhauer, A. Fiedler, K. Müller, P. Nielsen, G. Thomas, N. Gengler, G. de Jong, C. Ødegård, F. Malchiodi, F. Miglior, M. Alsaaod, and J.B. Cole. 2018. Invited review: Genetics and claw health: Opportunities to enhance claw health by genetic selection. J. Dairy Sci. 101:4801–4821. doi:10.3168/jds.2017-13531.

Hernandes, V.V., C. Barbas, and D. Dudzik. 2017. A review of blood sample handling and pre-processing for metabolomics studies. ELECTROPHORESIS 38:2232–2241. doi:10.1002/elps.201700086.

Hernandez, J., J.K. Shearer, and D.W. Webb. 2001. Effect of lameness on the calving-to-conception interval in dairy cows. J. Am. Vet. Med. Assoc. 218:1611–1614. doi:10.2460/javma.2001.218.1611.

Heuwieser, W., J. Kautni, M. Biesel, and E. Grunert. 1990. Coagulation Profile of Dairy Cattle in the Periparturient Period. J. Vet. Med. Ser. A 37:8–15. doi:10.1111/j.1439-0442.1990.tb00870.x.

Hiendleder, S., H. Thomsen, N. Reinsch, J. Bennewitz, B. Leyhe-Horn, C. Looft, N. Xu, I. Medjugorac, I. Russ, C. Kühn, G.A. Brockmann, J. Blümel, B. Brenig, F. Reinhardt, R. Reents, G. Averdunk, M. Schwerin, M. Förster, E. Kalm, and G. Erhardt. 2003. Mapping of QTL for Body Conformation and Behavior in Cattle. J. Hered. 94:496–506. doi:10.1093/jhered/esg090.

Hill, W.G., M.E. Goddard, and P.M. Visscher. 2008. Data and theory point to mainly additive genetic variance for complex traits. PLoS Genet. 4:1000008. doi:10.1371/journal.pgen.1000008.

Hill, W.G., and G.J.T. Swanson. 1983. A selection index for dairy cows. Anim. Sci. 37:313–319. doi:10.1017/S0003356100001914.

Hirschberg, R.M., C.K.W. Mülling, and K.D. Budras. 2001. Pododermal angioarchitecture of the bovine claw in relation to form and function of the papillary body: A scanning electron microscopic study. Microsc. Res. Tech. 54:375–385. doi:10.1002/jemt.1150.

Hirst, W.M., R.D. Murray, W.R. Ward, and N.P. French. 2002. A mixed-effects time-to-event analysis of the relationship between first-lactation lameness and subsequent lameness in dairy cows in the UK. Prev. Vet. Med. 54:191–201. doi:10.1016/S0167-5877(02)00021-1.

Hiss-Pesch, S., J. Weise, B. Heitkönig, and H. Sauerwein. 2019. Short communication: Adipocyte sizes in the digital fat pad and their relationship to body condition in dairy cows. J. Dairy Sci. 102:6551–6554. doi:10.3168/jds.2018-15531.

Hjortø, L., J.F. Ettema, M. Kargo, and A.C. Sørensen. 2015. Genomic testing interacts with reproductive surplus in reducing genetic lag and increasing economic net return. J. Dairy Sci. 98:646–658. doi:10.3168/jds.2014-8401.

Hoblet, K.H., and W. Weiss. 2001. Metabolic hoof horn disease. Vet. Clin. North Am. Food Anim. Pract. 17. doi:10.1016/S0749-0720(15)30057-8.

Hoedemaker, M., D. Prange, and Y. Gundelach. 2009. Body condition change ante- and postpartum, health and reproductive performance in German

Holstein Cows. Reprod. Domest. Anim. 44:167–173. doi:10.1111/j.1439-0531.2007.00992.x.

Hoffman, A.C., D.A. Moore, J.R. Wenz, and J. Vanegas. 2013. Comparison of modeled sampling strategies for estimation of dairy herd lameness prevalence and cow-level variables associated with lameness. J. Dairy Sci. 96:5746–5755. doi:10.3168/jds.2013-6891.

Horseman, S.V., H.R. Whay, J.N. Huxley, N.J. Bell, and C.S. Mason. 2013. A survey of the on-farm treatment of sole ulcer and white line disease in dairy cattle. Vet. J. 197:461–467. doi:10.1016/j.tvjl.2013.02.027.

Hristov, A.N., T. Ott, J. Tricarico, A. Rotz, G. Waghorn, A. Adesogan, J. Dijkstra, F. Montes, J. Oh, E. Kebreab, S.J. Oosting, P.J. Gerber, B. Henderson, H.P.S. Makkar, and J.L. Firkins. 2013. SPECIAL TOPICS — Mitigation of methane and nitrous oxide emissions from animal operations: III. A review of animal management mitigation options1. J. Anim. Sci. 91:5095–5113. doi:10.2527/jas.2013-6585.

Huang, D.W., B.T. Sherman, and R.A. Lempicki. 2009a. Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 37:1–13. doi:10.1093/nar/gkn923.

Huang, D.W., B.T. Sherman, and R.A. Lempicki. 2009b. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4:44–57. doi:10.1038/nprot.2008.211.

Huang, Y.C., and R.D. Shanks. 1995. Within herd estimates of heritabilities for six hoof characteristics and impact of dispersion of discrete severity scores on estimates. Livest. Prod. Sci. 44:107–114. doi:10.1016/0301-6226(95)00061-3.

Hui, W., A.D. Rowan, and T. Cawston. 2001. Insulin-like growth factor 1 blocks collagen release and down regulates matrix metalloproteinase-1, -3, -8, and -13 mRNA expression in bovine nasal cartilage stimulated with oncostatin M in combination with interleukin 1-alpha. Ann. Rheum. Dis. 60:254–261.

Hund, A., J. Chiozza Logroño, R.D. Ollhoff, and J. Kofler. 2019. Aspects of lameness in pasture based dairy systems.. Vet. J. Lond. Engl. 1997 244:83–90. doi:10.1016/j.tvjl.2018.12.011.

Huxley, J.N. 2012. Lameness in cattle: An ongoing concern. Vet. J. 193:610–611. doi:10.1016/j.tvjl.2012.06.039.

Huxley, J.N. 2013. Impact of lameness and claw lesions in cows on health and production. Livest. Sci. 156:64–70. doi:10.1016/j.livsci.2013.06.012.

van Huyssteen, M., H.W. Barkema, S. Mason, and K. Orsel. 2020. Association between lameness risk assessment and lameness and foot lesion

prevalence on dairy farms in Alberta, Canada.. J. Dairy Sci. 103:11750–11761. doi:10.3168/jds.2019-17819.

Ingraham, R.H., and L.C. Kappel. 1988. Metabolic Profile Testing. Vet. Clin. North Am. Food Anim. Pract. 4:391–411. doi:10.1016/S0749-0720(15)31056-2.

Itle, A.J., J.M. Huzzey, D.M. Weary, and M.A.G.V. Keyserlingk. 2015. Clinical ketosis and standing behavior in transition cows. J. Dairy Sci. 98:128–134. doi:10.3168/jds.2014-7932.

İzci, C., İ. Çelik, M. Erol, and E. Gökşahin. 2019. Histochemical and Histomorphometrical Studies on the Digital Cushion of Heifers and Dairy Cows with Claw Horn Lesions. Concepts Dairy Vet. Sci. 3:254–262. doi:10.32474/CDVS.2019.03.000151.

Jamrozik, J., J. Fatehi, and L. r. Schaeffer. 2008. Comparison of models for genetic evaluation of survival traits in dairy cattle: a simulation study. J. Anim. Breed. Genet. 125:75–83. doi:10.1111/j.1439-0388.2007.00712.x.

Jarray, R., B. Allain, L. Borriello, D. Biard, A. Loukaci, J. Larghero, R. Hadj-Slimane, C. Garbay, Y. Lepelletier, and F. Raynaud. 2011. Depletion of the novel protein PHACTR-1 from human endothelial cells abolishes tube formation and induces cell death receptor apoptosis.. Biochimie 93:1668–1675. doi:10.1016/j.biochi.2011.07.010.

Jia, G., Y. Fu, X. Zhao, Q. Dai, G. Zheng, Y. Yang, C. Yi, T. Lindahl, T. Pan, Y.-G. Yang, and C. He. 2011. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO.. Nat. Chem. Biol. 7:885–887. doi:10.1038/nchembio.687.

Jiang, J., L. Ma, D. Prakapenka, P.M. VanRaden, J.B. Cole, and Y. Da. 2019. A Large-Scale Genome-Wide Association Study in U.S. Holstein Cattle. Front. Genet. 10:412. doi:10.3389/fgene.2019.00412.

Johansson, K., J.-åke Eriksson, U.S. Nielsen, and J. Pösö. 2011. Genetic evaluation of claw health in Denmark, Finland and Sweden. Interbull Bull. 0:224–228.

Jones, W.P., L.B. Hansen, and H. Chester-Jones. 1994. Response of Health Care to Selection for Milk Yield of Dairy Cattle. J. Dairy Sci. 77:3137–3152. doi:10.3168/jds.S0022-0302(94)77257-X.

Jørgensen, E., F.B. Hjerpe, H.P. Hougen, T. Bjarnsholt, L.C. Berg, and S. Jacobsen. 2020. Histologic changes and gene expression patterns in biopsy specimens from bacteria-inoculated and noninoculated excisional body and limb wounds in horses healing by second intention. Am. J. Vet. Res. 81:276–284. doi:10.2460/ajvr.81.3.276.

Kalkhof, S., Y. Förster, J. Schmidt, M.C. Schulz, S. Baumann, A. Weißflog, W. Gao, U. Hempel, U. Eckelt, S. Rammelt, and M. von Bergen. 2014. Proteomics

and Metabolomics for In Situ Monitoring of Wound Healing. BioMed Res. Int. 2014:934848. doi:10.1155/2014/934848.

Kang, X., X.D. Zhang, and G. Liu. 2020. Accurate detection of lameness in dairy cattle with computer vision: A new and individualized detection strategy based on the analysis of the supporting phase.. J. Dairy Sci. 103:10628–10638. doi:10.3168/jds.2020-18288.

Kaniyamattam, K., M.A. Elzo, J.B. Cole, and A. De Vries. 2016. Stochastic dynamic simulation modeling including multitrait genetics to estimate genetic, technical, and financial consequences of dairy farm reproduction and selection strategies. J. Dairy Sci. 99:8187–8202. doi:10.3168/jds.2016-11136.

Katz, L.M., and S.R. Bailey. 2012. A review of recent advances and current hypotheses on the pathogenesis of acute laminitis. Equine Vet. J. 44:752–761. doi:10.1111/j.2042-3306.2012.00664.x.

Kemper, K.E., and M.E. Goddard. 2012. Understanding and predicting complex traits: knowledge from cattle. Hum. Mol. Genet. 21:R45–R51. doi:10.1093/hmg/dds332.

Kempson, S., and D. Logue. 1993. Ultrastructural observations of hoof horn from dairy cows: the structure of the white line. Vet. Rec. 132:499–502. doi:10.1136/vr.132.20.499.

Kettunen, J., T. Tukiainen, A.-P. Sarin, A. Ortega-Alonso, E. Tikkanen, L.-P. Lyytikäinen, A.J. Kangas, P. Soininen, P. Würtz, K. Silander, D.M. Dick, R.J. Rose, M.J. Savolainen, J. Viikari, M. Kähönen, T. Lehtimäki, K.H. Pietiläinen, M. Inouye, M.I. McCarthy, A. Jula, J. Eriksson, O.T. Raitakari, V. Salomaa, J. Kaprio, M.-R. Järvelin, L. Peltonen, M. Perola, N.B. Freimer, M. Ala-Korpela, A. Palotie, and S. Ripatti. 2012. Genome-wide association study identifies multiple loci influencing human serum metabolite levels. Nat. Genet. 44:269–276. doi:10.1038/ng.1073.

von Keyserlingk, M.A.G., A. Barrientos, K. Ito, E. Galo, and D.M. Weary. 2012. Benchmarking cow comfort on North American freestall dairies: Lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. J. Dairy Sci. 95:7399–7408. doi:10.3168/jds.2012-5807.

von Keyserlingk, M.A.G., J. Rushen, A.M. de Passillé, and D.M. Weary. 2009. Invited review: The welfare of dairy cattle—Key concepts and the role of science. J. Dairy Sci. 92:4101–4111. doi:10.3168/jds.2009-2326.

Khafipour, E., D.O. Krause, and J.C. Plaizier. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92:1060–1070. doi:10.3168/jds.2008-1389.

Khan, M.Z., A. Khan, J. Xiao, J. Ma, Y. Ma, T. Chen, D. Shao, and Z. Cao. 2020. Overview of Research Development on the Role of NF-κB Signaling in Mastitis. Anim. Open Access J. MDPI 10:1625. doi:10.3390/ani10091625.

Kim, H.S., E.T. Kim, J.S. Eom, Y.Y. Choi, S.J. Lee, S.S. Lee, C.D. Chung, and S.S. Lee. 2021. Exploration of metabolite profiles in the biofluids of dairy cows by proton nuclear magnetic resonance analysis. PLOS ONE 16:e0246290. doi:10.1371/journal.pone.0246290.

King, M.T.M., S.J. LeBlanc, E.A. Pajor, and T.J. DeVries. 2017. Cow-level associations of lameness, behavior, and milk yield of cows milked in automated systems. J. Dairy Sci. 100:4818–4828. doi:10.3168/jds.2016-12281.

King, M.T.M., R.D. Matson, and T.J. DeVries. 2021. Connecting farmer mental health with cow health and welfare on dairy farms using robotic milking systems. Anim. Welf. 30:25–38. doi:10.7120/09627286.30.1.025.

Klawitter, M., T.B. Braden, and K.E. Müller. 2019. Randomized clinical trial evaluating the effect of bandaging on the healing of sole ulcers in dairy cattle. Vet. Anim. Sci. 8:100070. doi:10.1016/j.vas.2019.100070.

Knaus, W. 2009. Dairy cows trapped between performance demands and adaptability. J. Sci. Food Agric. 89:1107–1114. doi:10.1002/jsfa.3575.

Knaus, W. 2016. Perspectives on pasture versus indoor feeding of dairy cows. J. Sci. Food Agric. 96:9–17. doi:10.1002/jsfa.7273.

Knott, L., J.F. Tarlton, H. Craft, and A.J.F. Webster. 2007. Effects of housing, parturition and diet change on the biochemistry and biomechanics of the support structures of the hoof of dairy heifers. Vet. J. 174:277–287. doi:10.1016/j.tvjl.2006.09.007.

Köck, A., B. Fuerst-Waltl, J. Kofler, J. Burgstaller, F. Steininger, C. Fuerst, and C. Egger-Danner. 2019. Short communication: Use of lameness scoring to genetically improve claw health in Austrian Fleckvieh, Brown Swiss, and Holstein cattle. J. Dairy Sci. 102:1397–1401. doi:10.3168/jds.2018-15287.

Koenig, S., A.R. Sharifi, H. Wentrot, D. Landmann, M. Eise, and H. Simianer. 2005. Genetic parameters of claw and foot disorders estimated with logistic models. J. Dairy Sci. 88:3316–3325. doi:10.3168/jds.S0022-0302(05)73015-0.

Kofler, J. 2017. Pathogenesis and Treatment of Toe Lesions in Cattle Including “Nonhealing” Toe Lesions. Vet. Clin. N. Am. - Food Anim. Pract. 33:301–328. doi:10.1016/j.cvfa.2017.02.005.

Kofler, J., P. Kübber, and W. Henninger. 1999. Ultrasonographic Imaging and Thickness Measurement of the Sole Horn and the Underlying Soft Tissue Layer in Bovine Claws. Vet. J. 157:322–331. doi:10.1053/tvjl.1998.0315.

Kolbehdari, D., Z. Wang, J.R. Grant, B. Murdoch, A. Prasad, Z. Xiu, E. Marques, P. Stothard, and S.S. Moore. 2008. A whole-genome scan to map quantitative trait loci for conformation and functional traits in Canadian Holstein bulls. J. Dairy Sci. 91:2844–2856. doi:10.3168/jds.2007-0585.

König, S., X.L. Wu, D. Gianola, B. Heringstad, and H. Simianer. 2008. Exploration of Relationships Between Claw Disorders and Milk Yield in Holstein Cows via Recursive Linear and Threshold Models. J. Dairy Sci. 91:395–406. doi:10.3168/jds.2007-0170.

Kossaibati, M.A., and R.J. Esslemont. 1997. The costs of production diseases in dairy herds in England. Vet. J. 154:41–51. doi:10.1016/S1090-0233(05)80007-3.

Kuhn, M., and K. Johnson. 2018. Applied Predictive Modelling. Springer, New York, NY.

Kuhn, M., and H. Wickham. 2020. Tidymodels: a collection of packages for modeling and machine learning using tidyverse principles.

Kujala, M., T. Orro, and T. Soveri. 2010. Short Communications Serum acute phase proteins as a marker of inflammation in dairy cattle with hoof diseases. doi:10.1136/vr.b4770.

Kursa, M.B., and W.R. Rudnicki. 2010. Feature Selection with the Boruta Package. J. Stat. Softw. 36:1–13. doi:10.18637/jss.v036.i11.

Kuznetsova, A., P.B. Brockhoff, and R.H.B. Christensen. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. 82:1–26. doi:10.18637/jss.v082.i13.

de Laat, M.A., C.M. McGowan, M.N. Sillence, and C.C. Pollitt. 2010. Equine laminitis: Induced by 48 h hyperinsulinaemia in Standardbred horses. Equine Vet. J. 42:129–135. doi:10.2746/042516409X475779.

Lai, E., A.L. Danner, T.R. Famula, and A.M. Oberbauer. 2021a. Pleiotropic Loci Associated With Foot Disorders and Common Periparturient Diseases in Holstein Cattle. Front. Genet. 12.

Lai, E., A.L. Danner, T.R. Famula, and A.M. Oberbauer. 2021b. Genome-Wide Association Studies Reveal Susceptibility Loci for Noninfectious Claw Lesions in Holstein Dairy Cattle. Front. Genet. 12. doi:10.3389/fgene.2021.657375.

Lander, E., and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat. Genet. 11:241–247. doi:10.1038/ng1195-241.

Latruffe, L., A. Diazabakana, C. Bockstaller, Y. Desjeux, J. Finn, E. Kelly, M. Ryan, and S. Uthes. 2016. Measurement of sustainability in agriculture: a review of indicators. Stud. Agric. Econ. 118:123–130. doi:10.7896/j.1624.

Laven, R., K. Lawrence, J. Weston, K. Dowson, and K. Stafford. 2008. Assessment of the duration of the pain response associated with lameness in dairy cows, and the influence of treatment. N. Z. Vet. J. 56:210–217. doi:10.1080/00480169.2008.36835.

Laven, R.A., and K.R. Lawrence. 2006. An Evaluation of the Seasonality of Veterinary Treatments for Lameness in UK Dairy Cattle. J. Dairy Sci. 89:3858–3865. doi:10.3168/jds.S0022-0302(06)72428-6.

Laven, R.A., C.T. Livesey, and S.A. May. 2004. Relationship between acute phase proteins and hoof horn haemorrhages in postpatrum first-lactation heifers. Vet. Rec. 154:389–395. doi:10.1136/vr.154.13.389.

Leach, K.A., D.N. Logue, S.A. Kempson, J.E. Offer, H.E. Ternent, and J.M. Randalls. 1997. Claw lesions in dairy cattle: Development of Sole and white line haemorrhages during the first lactation. Vet. J. 154:215–225. doi:10.1016/S1090-0233(97)80024-X.

Leach, K.A., D.N. Logue, J.M. Randall, and S.A. Kempson. 1998. Claw lesions in dairy cattle: Methods for assessment of sole and white line lesions. Vet. J. 155:91–102. doi:10.1016/S1090-0233(98)80043-9.

Leach, K.A., D.A. Tisdall, N.J. Bell, D.C.J. Main, and L.E. Green. 2012. The effects of early treatment for hindlimb lameness in dairy cows on four commercial UK farms. Vet. J. 193:626–632. doi:10.1016/j.tvjl.2012.06.043.

Leach, K.A., and H.R. Whay. 2009. The Welfare Quality Lameness Control programme for Dairy Cattle. Welfare Quality Reports. University of Bristol, UK.

Leach, K.A., H.R. Whay, C.M. Maggs, Z.E. Barker, E.S. Paul, A.K. Bell, and D.C.J. Main. 2010. Working towards a reduction in cattle lameness: 1. Understanding barriers to lameness control on dairy farms. Res. Vet. Sci. 89:311–317. doi:10.1016/j.rvsc.2010.02.014.

Lean, I.J., and H.M. Golder. 2018. Ruminal Acidosis: Beyond pH and Rumen. Pages 127–145 Cornell Nutrition Conference, Syracuse, New York.

Lean, I.J., C.T. Westwood, H.M. Golder, and J.J. Vermunt. 2013. Impact of nutrition on lameness and claw health in cattle. Livest. Sci. 156:71–87. doi:10.1016/j.livsci.2013.06.006.

Lebacq, T., P.V. Baret, and D. Stilmant. 2013. Sustainability indicators for livestock farming. A review. Agron. Sustain. Dev. 33:311–327. doi:10.1007/s13593-012-0121-x.

Lee, S.H., N.R. Wray, M.E. Goddard, and P.M. Visscher. 2011. Estimating Missing Heritability for Disease from Genome-wide Association Studies. Am. J. Hum. Genet. 88:294–305. doi:10.1016/j.ajhg.2011.02.002.

Lee, Y.-L., H. Takeda, G.C.M. Moreira, L. Karim, E. Mullaart, W. Coppieters, T.G. Consortium, R. Appeltant, R.F. Veerkamp, M.A.M. Groenen, M. Georges, M. Bosse, T. Druet, A.C. Bouwman, and C. Charlier. 2021. A 12 kb multi-allelic copy number variation encompassing a GC gene enhancer is associated with mastitis resistance in dairy cattle. PLOS Genet. 17:e1009331. doi:10.1371/journal.pgen.1009331.

Legarra, A., I. Aguilar, and I. Misztal. 2009. A relationship matrix including full pedigree and genomic information. J. Dairy Sci. 92:4656–4663. doi:10.3168/jds.2009-2061.

Leitch, H.W. 1994. Comparison of international selection indices for dairy cattle breeding. Interbull Bull.

Lewis, K.E., M.J. Green, J. Witt, and L.E. Green. 2021. Multiple model triangulation to identify factors associated with lameness in British sheep flocks. Prev. Vet. Med. 193:105395. doi:10.1016/j.prevetmed.2021.105395.

Li, C., D. Sun, S. Zhang, S. Wang, X. Wu, Q. Zhang, L. Liu, Y. Li, and L. Qiao. 2014. Genome Wide Association Study Identifies 20 Novel Promising Genes Associated with Milk Fatty Acid Traits in Chinese Holstein. PLoS ONE 9:e96186. doi:10.1371/journal.pone.0096186.

Lim, P.Y., J.N. Huxley, J.A. Willshire, M.J. Green, A.R. Othman, and J. Kaler. 2015. Unravelling the temporal association between lameness and body condition score in dairy cattle using a multistate modelling approach. Prev. Vet. Med. 118:370–377. doi:10.1016/j.prevetmed.2014.12.015.

Lima, E., P. Davies, J. Kaler, F. Lovatt, and M. Green. 2020. Variable selection for inferential models with relatively high-dimensional data: Between method heterogeneity and covariate stability as adjuncts to robust selection. Sci. Rep. 10:8002. doi:10.1038/s41598-020-64829-0.

Lima, E., R. Hyde, and M. Green. 2021. Model selection for inferential models with high dimensional data: synthesis and graphical representation of multiple techniques. Sci. Rep. 11:1–15. doi:10.1038/s41598-020-79317-8.

van der Linde, C., G. de Jong, E.P.C. Koenen, and H. Eding. 2010. Claw health index for Dutch dairy cattle based on claw trimming and conformation data. J. Dairy Sci. 93:4883–4891. doi:10.3168/jds.2010-3183.

Lischer, Ch.J., U. Koller, H. Geyer, Ch. Mülling, J. Schulze, and P. Ossent. 2002a. Effect of Therapeutic Dietary Biotin on the Healing of Uncomplicated Sole Ulcers in Dairy Cattle – a Double Blinded Controlled Study. Vet. J. 163:51–60. doi:10.1053/tvjl.2001.0627.

Lischer, Ch.J., P. Ossent, M. Raber, and H. Geyer. 2002b. Suspensory structures and supporting tissues of the third phalanx of cows and their relevance to the development of typical sole ulcers (Rusterholz ulcers). Vet Rec 151:694–698.

Lischer, C.J., and P. Ossent. 2002. Pathogenesis of sole lesions attributed to laminitis in cattle. Pages 82–89 in Proceedings of the 12th International Symposium of Lameness in Ruminants International Conference on Lameness in Ruminants, Orlando, FL, USA.

Liu, T., L. Zhang, D. Joo, and S.-C. Sun. 2017. NF-κB signaling in inflammation. Signal Transduct. Target. Ther. 2:1–9. doi:10.1038/sigtrans.2017.23.

Livesey, C.T., J.A. Metcalf, and R.A. Laven. 2003. Effect of concentrate composition and cubicle bedding on the development of hoof haemorrhages in Holstein heifers after calving. Vet. Rec. 152:735–739. doi:10.1136/vr.152.24.735.

Loh, Z., S. Clark, and J.H.J. van der Werf. 2022. Effects of Experimental Design, Genetic Architecture and Threshold on Power and False Positive Rate of Genome-Wide Association Studies 2022.02.19.481168. doi:10.1101/2022.02.19.481168.

Lontos, K., J. Adamik, A. Tsagianni, D.L. Galson, J.M. Chirgwin, and A. Suvannasankha. 2018. The Role of Semaphorin 4D in Bone Remodeling and Cancer Metastasis. Front. Endocrinol. 9.

Lopes, F., G. Rosa, P. Pinedo, J.E.P. Santos, R.C. Chebel, K.N. Galvao, G.M. Schuenemann, R.C. Bicalho, R.O. Gilbert, S. Rodrigez-Zas, C.M. Seabury, and W. Thatcher. 2020. Genome-enable prediction for health traits using high-density SNP panel in US Holstein cattle. Anim. Genet. 51:192–199. doi:10.1111/age.12892.

Lüdecke, D. 2018. ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. J. Open Source Softw. 3:772. doi:10.21105/joss.00772.

Lüdecke, D., M.S. Ben-Shachar, I. Patil, P. Waggoner, and D. Makowski. 2021. performance: An R Package for Assessment, Comparison and Testing of Statistical Models. J. Open Source Softw. 6:3139. doi:10.21105/joss.03139.

Luke, T.D.W., J.E. Pryce, A.C. Elkins, W. J. Wales, and S.J. Rochfort. 2020. Use of Large and Diverse Datasets for 1H NMR Serum Metabolic Profiling of Early Lactation Dairy Cows. Metabolites 10:180. doi:10.3390/metabo10050180.

Lynch, M., and B. Walsh. 1998. Genetics and Analysis of Quantitative Traits. 1st ed. Sinauer, Sunderland.

Machado, V.S., L.S. Caixeta, and R.C. Bicalho. 2011. Use of data collected at cessation of lactation to predict incidence of sole ulcers and white line disease during the subsequent lactation in dairy cows. Am. J. Vet. Res. 72:1338–1343. doi:10.2460/ajvr.72.10.1338.

Machado, V.S., L.S. Caixeta, J. a. A. McArt, and R.C. Bicalho. 2010. The effect of claw horn disruption lesions and body condition score at dry-off on survivability, reproductive performance, and milk production in the subsequent lactation. J. Dairy Sci. 93:4071–4078. doi:10.3168/jds.2010-3177.

Magee, D.A., D.E. MacHugh, and C.J. Edwards. 2014. Interrogation of modern and ancient genomes reveals the complex domestic history of cattle. Anim. Front. 4:7–22. doi:10.2527/af.2014-0017.

Mahendran, S., and N. Bell. 2015. Lameness in cattle 2. Managing claw health through appropriate trimming techniques. In Pract. 37:231–242. doi:10.1136/inp.h2011.

Mahendran, S.A., J.N. Huxley, Y.M. Chang, M. Burnell, D.C. Barrett, H.R. Whay, T. Blackmore, C.S. Mason, and N.J. Bell. 2017. Randomised controlled trial to evaluate the effect of foot trimming before and after first calving on subsequent lameness episodes and productivity in dairy heifers. Vet. J. 220. doi:10.1016/j.tvjl.2017.01.011.

Malchiodi, F., J. Jamrozik, A.-M. Christen, A. Fleming, G.J. Kistemaker, C. Richardson, V. Daniel, D.F. Kelton, F.S. Schenkel, and F. Miglior. 2020. Symposium review: Multiple-trait single-step genomic evaluation for hoof health. J. Dairy Sci. 103:140–140. doi:10.3168/jds.2019-17755.

Malchiodi, F., A. Koeck, S. Mason, A.M. Christen, D.F. Kelton, F.S. Schenkel, and F. Miglior. 2017. Genetic parameters for hoof health traits estimated with linear and threshold models using alternative cohorts. J. Dairy Sci. 100:2828–2836. doi:10.3168/jds.2016-11558.

Mann, S. 2022. Symposium review: The role of adipose tissue in transition dairy cows: Current knowledge and future opportunities. J. Dairy Sci. 0. doi:10.3168/jds.2021-21215.

Manske, T., J. Hultgren, and C. Bergsten. 2002a. The effect of claw trimming on the hoof health of Swedish dairy cattle. Prev. Vet. Med. 54:113–129. doi:10.1016/S0167-5877(02)00020-X.

Manske, T., J. Hultgren, and C. Bergsten. 2002b. Prevalence and interrelationships of hoof lesions and lameness in Swedish dairy cows. Prev. Vet. Med. 54:247–263. doi:10.1016/S0167-5877(02)00018-1.

March, M.D., M.J. Haskell, M.G.G. Chagunda, F.M. Langford, and D.J. Roberts. 2014. Current trends in British dairy management regimens. J. Dairy Sci. 97:7985–7994. doi:10.3168/jds.2014-8265.

Marchand, J., E. Martineau, Y. Guitton, G. Dervilly-Pinel, and P. Giraudeau. 2017. Multidimensional NMR approaches towards highly resolved, sensitive and high-throughput quantitative metabolomics. Curr. Opin. Biotechnol. 43:49–55. doi:10.1016/j.copbio.2016.08.004.

Martin-Collado, D., T.J. Byrne, P.R. Amer, B.F.S. Santos, M. Axford, and J.E. Pryce. 2015. Analyzing the heterogeneity of farmers' preferences for improvements in dairy cow traits using farmer typologies. J. Dairy Sci. 98:4148–4161. doi:10.3168/jds.2014-9194.

Masinde, G.L., X. Li, W. Gu, H. Davidson, S. Mohan, and D.J. Baylink. 2001. Identification of wound healing/regeneration quantitative trait loci (QTL) at multiple time points that explain seventy percent of variance in (MRL/MpJ and SJL/J) mice F2 population. Genome Res. 11:2027–2033. doi:10.1101/gr.203701.

Maxwell, O.J.R., C.D. Hudson, and J.N. Huxley. 2015. Effect of early lactation foot trimming in lame and non-lame dairy heifers: a randomised controlled trial. Vet. Rec. 177:100–100. doi:10.1136/vr.103155.

McCarthy, M.I., G.R. Abecasis, L.R. Cardon, D.B. Goldstein, J. Little, J.P.A. Ioannidis, and J.N. Hirschhorn. 2008. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat. Rev. Genet. 9:356–369. doi:10.1038/nrg2344.

Mccawley, L.J., and L.M. Matrisian. 2001. Matrix metalloproteinases: they're not just for matrix anymore!. Curr. Opin. Cell Biol. 13:534–540.

McDaniel, B. 1998. Breeding programs to reduce foot and leg problems. Interbull 115–122.

McDougall, S., S.J. LeBlanc, and A. Heiser. 2017. Effect of prepartum energy balance on neutrophil function following pegbovigrastim treatment in periparturient cows. J. Dairy Sci. 100:7478–7492. doi:10.3168/jds.2017-12786.

McGuffey, R.K., and J.E. Shirley. 2011. Introduction | History of Dairy Farming. J.W. Fuquay, ed. Academic Press, San Diego.

McNamara, J.P. 1991. Regulation of Adipose Tissue Metabolism in Support of Lactation. J. Dairy Sci. 74:706–719. doi:10.3168/jds.S0022-0302(91)78217-9.

McNamara, J.P., B. Becker-Khaleel, and K.L. Parmley. 1992. Quantitative Relationships Between Cyclic Adenosine-3',5'- Monophosphate and

Lipolysis in Adipose Tissue During the Peripartum Period. J. Dairy Sci. 75:1901–1913. doi:10.3168/jds.S0022-0302(92)77950-8.

Megahed, A.A., M.W.H. Hiew, D. Ragland, and P.D. Constable. 2019. Changes in skeletal muscle thickness and echogenicity and plasma creatinine concentration as indicators of protein and intramuscular fat mobilization in periparturient dairy cows. J. Dairy Sci. 102:5550–5565. doi:10.3168/jds.2018-15063.

Mehmood, T., K.H. Liland, L. Snipen, and S. Sæbø. 2012. A review of variable selection methods in Partial Least Squares Regression. Chemom. Intell. Lab. Syst. 118:62–69. doi:10.1016/j.chemolab.2012.07.010.

Meinshausen, N., and P. Bühlmann. 2010. Stability selection. J. R. Stat. Soc. Ser. B Stat. Methodol. 72:417–473. doi:https://doi.org/10.1111/j.1467-9868.2010.00740.x.

Melendez, P., J. Bartolome, L.F. Archbald, and A. Donovan. 2003. The association between lameness, ovarian cysts and fertility in lactating dairy cows. Theriogenology 59:927–937. doi:10.1016/S0093-691X(02)01152-4.

Mellor, D.J. 2016. Moving beyond the “Five Freedoms” by Updating the “Five Provisions” and Introducing Aligned “Animal Welfare Aims”. Anim. Open Access J. MDPI 6:59. doi:10.3390/ani6100059.

Meuwissen, T., and Z. Luo. 1992. Computing inbreeding coefficients in large populations. Genet. Sel. Evol. 24:305. doi:10.1186/1297-9686-24-4-305.

Meuwissen, T.H.E., B.J. Hayes, and M.E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829.

Miglior, F., A. Fleming, F. Malchiodi, L.F. Brito, P. Martin, and C.F. Baes. 2017. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. J. Dairy Sci. 100:10251–10271. doi:10.3168/jds.2017-12968.

Mishra, B., K. Kizaki, T. Sato, A. Ito, and K. Hashizume. 2012. The Role of Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) in the Regulation of Bovine Endometrial Cell Functions. Biol. Reprod. 87:1–8. doi:10.1095/biolreprod.112.102152.

Misztal, I., S. Tsuruta, D. Lourenco, Y. Masuda, I. Aguilar, A. Legarra, and Z. Vitezica. 2018. Manual for BLUPF90 family programs. University of Georgia.

Moore, S.C., M.C. Playdon, J.N. Sampson, R.N. Hoover, B. Trabert, C.E. Matthews, and R.G. Ziegler. 2018. A Metabolomics Analysis of Body Mass Index and Postmenopausal Breast Cancer Risk. JNCI J. Natl. Cancer Inst. 110:588–597. doi:10.1093/jnci/djx244.

More, S.J., K. McKenzie, J. O'Flaherty, M.L. Doherty, A.R. Cromie, and M.J. Magan. 2010. Setting priorities for non-regulatory animal health in Ireland: Results from an expert Policy Delphi study and a farmer priority identification survey. Prev. Vet. Med. 95:198–207. doi:10.1016/j.prevetmed.2010.04.011.

Moreira, T.F., R.R. Nicolino, R.M. Meneses, G.V. Fonseca, L.M. Rodrigues, E.J. Facury Filho, and A.U. Carvalho. 2019. Risk factors associated with lameness and hoof lesions in pasture-based dairy cattle systems in southeast Brazil.. J. Dairy Sci. 102:10369–10378. doi:10.3168/jds.2018-16215.

Mostert, P.F., C.E. van Middelaar, I.J.M. de Boer, and E.A.M. Bokkers. 2018. The impact of foot lesions in dairy cows on greenhouse gas emissions of milk production. Agric. Syst. 167:206–212. doi:10.1016/j.agsy.2018.09.006.

Muehlhoff, E., A. Bennett, D. MacMahon, and Food and Agriculture Organization of the United Nations eds. . 2013. Milk and Dairy Products in Human Nutrition. Food and Agriculture Organization of the United Nations, Rome.

Mullen, M., D. Berry, D. Howard, M. Diskin, C. Lynch, L. Giblin, D. Kenny, D. Magee, K. Meade, and S. Waters. 2011. Single Nucleotide Polymorphisms in the Insulin-Like Growth Factor 1 (IGF-1) Gene are Associated with Performance in Holstein-Friesian Dairy Cattle. Front. Genet. 2.

Mülling, C.K.W. 2002. Theories on the pathogenesis of white line disease – an anatomical perspective. Page in Proceedings of the 12th International Symposium on Lameness in Ruminants, Florida, USA.

Mülling, C.K.W. 2012. Functional anatomy of the bovine foot - failure of key structures in pathogensis of claw disease. Pages 9–17 in Proceedings of the Cattle Lameness Conference Cattle Lameness Conference, Worcester, UK.

Mülling, C.K.W. 2019. Biomechanics of the bovine foot. Pages 32–40 in Proceedings of the 12th International Symposium on Lameness in Ruminants International Conference on Lameness in Ruminants, Tokyo, Japan.

Munafò, M.R., and G. Davey Smith. 2018. Robust research needs many lines of evidence. Nature 553:399–401. doi:10.1038/d41586-018-01023-3.

Murray, R.D., D.Y. Downham, M.J. Clarkson, W.B. Faull, J.W. Hughes, F.J. Manson, J.B. Merritt, W.B. Russell, J.E. Sutherst, and W.R. Ward. 1996. Epidemiology of lameness in dairy cattle: Description and analysis of foot lesions. Vet. Rec. 138:586–591. doi:10.1136/vr.138.24.586.

Naderi, S., M. Bohlouli, T. Yin, and S. König. 2018. Genomic breeding values, SNP effects and gene identification for disease traits in cow training sets. Anim. Genet. 49:178–192. doi:10.1111/age.12661.

Nalon, E., and P. Stevenson. 2019. Addressing lameness in farmed animals: An urgent need to achieve compliance with EU animal welfare law. Animals 9:3–7. doi:10.3390/ani9080576.

Navarro, G., L.E. Green, and N. Tadich. 2013. Effect of lameness and lesion specific causes of lameness on time budgets of dairy cows at pasture and when housed. Vet. J. 197:788–793. doi:10.1016/j.tvjl.2013.05.012.

Neuenschwander, T.F.O., F. Miglior, J. Jamrozik, O. Berke, D.F. Kelton, and L.R. Schaeffer. 2012. Genetic parameters for producer-recorded health data in Canadian Holstein cattle. Animal 6:571–578. doi:10.1017/S1751731111002059.

Newsome, R., M.J. Green, N.J. Bell, M.G.G. Chagunda, C.S. Mason, C.S. Rutland, C.J. Sturrock, H.R. Whay, and J.N. Huxley. 2016. Linking bone development on the caudal aspect of the distal phalanx with lameness during life. J. Dairy Sci. 99:4512–4525. doi:10.3168/jds.2015-10202.

Newsome, R., B. Reilly, and J. Reader. 2019. Management of claw horn lesions; a practitioner's guide through the literature. Livestock 24:6–12. doi:10.12968/live.2019.24.1.6.

Newsome, R.F. 2016. Involvement of the Digital Cushion and the Distal Phalanx in the Development and Reoccurrence of Claw Horn Disruption Lesions in Dairy Cattle. University of Nottingham,.

Newsome, R.F., M.J. Green, N.J. Bell, N.J. Bollard, C.S. Mason, H.R. Whay, and J.N. Huxley. 2017a. A prospective cohort study of digital cushion and corium thickness . Part 1 : Associations with body condition, lesion incidence, and proximity to calving. J. Dairy Sci. 100:4745–4758. doi:10.3168/jds.2016-12012.

Newsome, R.F., M.J. Green, N.J. Bell, N.J. Bollard, C.S. Mason, H.R. Whay, and J.N. Huxley. 2017b. A prospective cohort study of digital cushion and corium thickness. Part 2: Does thinning of the digital cushion and corium lead to lameness and claw horn disruption lesions?. J. Dairy Sci. 100:4759–4771. doi:10.3168/jds.2016-12013.

Newsome, R.F., M.J. Green, N.J. Bell, N.J. Bollard, C.S. Mason, H.R. Whay, and J.N. Huxley. 2017c. A prospective cohort study of digital cushion and corium thickness . Part 1 : Associations with body condition, lesion incidence, and proximity to calving. J. Dairy Sci. 100:4745–4758. doi:10.3168/jds.2016-12012.

Newsome, R.F., A. Mostyn, J.P. Wilson, A. Alibhai, N. Bollard, L. Randall, M.G.G. Chagunda, C.J. Sturrock, M. Keane, M. Green, J.N. Huxley, and C.S. Rutland. 2021. Morphology, adipocyte size, and fatty acid analysis of dairy cattle digital cushions, and the effect of body condition score and age.. J. Dairy Sci. 104:6238–6252. doi:10.3168/jds.2020-19388.

Newton, J.E., B.J. Hayes, and J.E. Pryce. 2018. The cost-benefit of genomic testing of heifers and using sexed semen in pasture-based dairy herds. J. Dairy Sci. 101:6159–6173. doi:10.3168/jds.2017-13476.

Nicod, J., R.W. Davies, N. Cai, C. Hassett, L. Goodstadt, C. Cosgrove, B.K. Yee, V. Lionikaite, R.E. McIntyre, C.A. Remme, E.M. Lodder, J.S. Gregory, T. Hough, R. Joynson, H. Phelps, B. Nell, C. Rowe, J. Wood, A. Walling, N. Bopp, A. Bhomra, P. Hernandez-Pliego, J. Callebert, R.M. Aspden, N.P. Talbot, P.A. Robbins, M. Harrison, M. Fray, J.-M. Launay, Y.M. Pinto, D.A. Blizard, C.R. Bezzina, D.J. Adams, P. Franken, T. Weaver, S. Wells, S.D.M. Brown, P.K. Potter, P. Klenerman, A. Lionikas, R. Mott, and J. Flint. 2016. Genome-wide association of multiple complex traits in outbred mice by ultra-low-coverage sequencing. Nat. Genet. 48:912–918. doi:10.1038/ng.3595.

Nielsen, H.M., P.R. Amer, and T.J. Byrne. 2014. Approaches to formulating practical breeding objectives for animal production systems. Acta Agric. Scand. Sect. — Anim. Sci. 64:2–12. doi:10.1080/09064702.2013.827237.

Nielsen, U.S., G.P. Aamand, and T. Mark. 2000. National genetic evaluation of udder health and other health traits in Denmark. Interbull Bull. 143–143.

Oberbauer, A.M., S.L. Berry, J.M. Belanger, R.M. McGoldrick, J.M. Pinos-Rodriquez, and T.R. Famula. 2013. Determining the heritable component of dairy cattle foot lesions.. J. Dairy Sci. 96:605–13. doi:10.3168/jds.2012-5485.

O'Connor, A.H., E.A.M. Bokkers, I.J.M. de Boer, H. Hogeveen, R. Sayers, N. Byrne, E. Ruelle, and L. Shalloo. 2019. Associating cow characteristics with mobility scores in pasture-based dairy cows.. J. Dairy Sci. 102:8332–8342. doi:10.3168/jds.2018-15719.

Ødegård, C., M. Svendsen, and B. Heringstad. 2013. Genetic analyses of claw health in Norwegian Red cows. J. Dairy Sci. 96:7274–7283. doi:10.3168/jds.2012-6509.

Ødegård, C., M. Svendsen, and B. Heringstad. 2015. Foot and leg conformation traits have a small effect on genomic predictions of claw disorders in Norwegian Red cows. J. Dairy Sci. 98:4139–4147. doi:10.3168/jds.2014-9186.

O'Driscoll, K.O., M. Mccabe, and B. Earley. 2015. Differences in leukocyte profile, gene expression, and metabolite status of dairy cows with or without sole ulcers. J. Dairy Sci. 98:1685–1695. doi:10.3168/jds.2014-8199.

Oehm, A.W., G. Knubben-Schweizer, A. Rieger, A. Stoll, and S. Hartnack. 2019. A systematic review and meta-analyses of risk factors associated with lameness in dairy cows. BMC Vet. Res. 15:1–14. doi:10.1186/s12917-019-2095-2.

Offer, J.E., K.A. Leach, S. Brocklehurst, and D.N. Logue. 2003. Effect of forage type on claw horn lesion development in dairy heifers. Vet. J. 165:221–227. doi:10.1016/S1090-0233(02)00171-5.

Offer, J.E., D.N. Logue, and D. McNulty. 2000. Observations of lameness, hoof conformation and development of lesions in dairy cattle over four lactations. Vet. Rec. 147:105–109. doi:10.1136/vr.147.4.105.

Offer, J.E., D.N. Logue, N.W. Offer, and M. Marsden. 2004. The effect of concentrate composition on lameness and hoof health in dairy cows. Vet. J. 167:111–113. doi:10.1016/j.tvjl.2003.08.003.

Oikonomou, G., G. Banos, V. Machado, L. Caixeta, and R.C. Bicalho. 2014. Short communication: Genetic characterization of digital cushion thickness. J. Dairy Sci. 97:532–536. doi:10.3168/jds.2013-7212.

Oikonomou, G., N.B. Cook, and R.C. Bicalho. 2013. Sire predicted transmitting ability for conformation and yield traits and previous lactation incidence of foot lesions as risk factors for the incidence of foot lesions in holstein cows. J. Dairy Sci. 96:3713–3722. doi:10.3168/jds.2012-6308.

Oliveira Junior, G.A., F.S. Schenkel, L. Alcantara, K. Houlahan, C. Lynch, and C.F. Baes. 2021. Estimated genetic parameters for all genetically evaluated traits in Canadian Holsteins. J. Dairy Sci. 104:9002–9015. doi:10.3168/jds.2021-20227.

Oltenacu, P.A., and B. Algers. 2005. Selection for increased production and the welfare of dairy cows: are new breeding goals needed?. Ambio 34:311–315.

Omontese, B.O., R. Bellet-Elias, A. Molinero, G.D. Catandi, R. Casagrande, Z. Rodriguez, R.S. Bisinotto, and G. Cramer. 2020. Association between hoof lesions and fertility in lactating Jersey cows.. J. Dairy Sci. 103:3401–3413. doi:10.3168/jds.2019-17252.

Onyiro, O.M., J. Offer, and S. Brotherstone. 2008. Risk factors and milk yield losses associated with lameness in Holstein-Friesian dairy cattle. Animal 2:1230–1237. doi:10.1017/S1751731108002279.

Osbaldiston, G.W., E.C. Stowe, and P.R. Griffith. 1970. Blood Coagulation: Comparative Studies in Dogs, Cats, Horses and Cattle. Br. Vet. J. 126:512–521. doi:10.1016/S0007-1935(17)48136-X.

Osorio, J.S., F. Batistel, E.F. Garrett, M.M. Elhanafy, M.R. Tariq, M.T. Socha, and J.J. Loor. 2016. Corium molecular biomarkers reveal a beneficial effect on

hoof transcriptomics in peripartal dairy cows supplemented with zinc, manganese, and copper from amino acid complexes and cobalt from cobalt glucoheptonate. J. Dairy Sci. 99:9974–9982. doi:10.3168/jds.2015-10698.

Osorio, J.S., B.C. Fraser, D.E. Graugnard, S.S. Singh, J.K. Drackley, E.F. Garrett, and J.J. Loor. 2012. Corium tissue expression of genes associated with inflammation, oxidative stress, and keratin formation in relation to lameness in dairy cows. J. Dairy Sci. 95:6388–6396. doi:10.3168/jds.2011-5143.

Ossent, P., and Ch.J. Lischer. 1998. Bovine lamninitis: the lesions and their pathogenesis. In Pract. 20:415–427. doi:10.1136/inpract.20.8.415.

Overton, M.W., and K.C. Dhuyvetter. 2020. Symposium review: An abundance of replacement heifers: What is the economic impact of raising more than are needed?. J. Dairy Sci. 103:3828–3837. doi:10.3168/jds.2019-17143.

Overton, T.R., and M.R. Waldron. 2004. Nutritional Management of Transition Dairy Cows: Strategies to Optimize Metabolic Health. J. Dairy Sci. 87:E105–E119. doi:10.3168/jds.S0022-0302(04)70066-1.

Ozato, K., D.-M. Shin, T.-H. Chang, and H.C. Morse. 2008. TRIM family proteins and their emerging roles in innate immunity. Nat. Rev. Immunol. 8:849–860. doi:10.1038/nri2413.

Paakala, E., D. Martín-Collado, A. Mäki-Tanila, and J. Juga. 2020. Farmers' stated selection preferences differ from revealed AI bull selection in Finnish dairy herds. Livest. Sci. 240:104117. doi:10.1016/j.livsci.2020.104117.

Pagenstecher, A., A.K. Stalder, C.L. Kincaid, B. Volk, and I.L. Campbell. 2000. Regulation of matrix metalloproteinases and their inhibitor genes in lipopolysaccharide-induced endotoxemia in mice. Am. J. Pathol. 157:197–210. doi:10.1016/S0002-9440(10)64531-2.

Paiano, R.B., D.B. Birgel, R.D. Ollhoff, E. Harry, and B. Junior. 2019. Biochemical Profile and Productive Performance in Dairy Cows With Lameness During Postpartum Period. Acta Sci. Vet. 55:1–7. doi:10.22456/1679-9216.93775.

Papachristou, C., C. Ober, and M. Abney. 2016. A LASSO penalized regression approach for genome-wide association analyses using related individuals: application to the Genetic Analysis Workshop 19 simulated data. BMC Proc. 10:221–226. doi:10.1186/s12919-016-0034-9.

Parker Gaddis, K.L., J.B. Cole, J.S. Clay, and C. Maltecca. 2014. Genomic selection for producer-recorded health event data in US dairy cattle. J. Dairy Sci. 97:3190–3199. doi:10.3168/jds.2013-7543.

Parks, W.C., C.L. Wilson, Y.S. López-boado, and W. Forest. 2004. Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat. Rev. Immunol. 4. doi:10.1038/nri1418.

Pastell, M., L. Hänninen, A.M. de Passillé, and J. Rushen. 2010. Measures of weight distribution of dairy cows to detect lameness and the presence of hoof lesions. J. Dairy Sci. 93:954–960. doi:10.3168/jds.2009-2385.

Patterson-Kane, J.C., N.P. Karikoski, and C.M. McGowan. 2018. Paradigm shifts in understanding equine laminitis. Vet. J. 231:33–40. doi:10.1016/j.tvjl.2017.11.011.

de Paula Freitas, A., M.L. Santana Júnior, F.S. Schenkel, M.E.Z. Mercadante, J.N. dos S.G. Cyrillo, and C.C.P. de Paz. 2021. Different selection practices affect the environmental sensitivity of beef cattle. PLoS ONE 16:e0248186. doi:10.1371/journal.pone.0248186.

Payne, J.M., S.M. Dew, R. Manston, and M. Faulks. 1970. The use of a metabolic profile test in dairy herds. Vet. Rec. 87:150–158. doi:10.1136/vr.87.6.150.

Pearce, J.T.M., T.J. Athersuch, T.M.D. Ebbels, J.C. Lindon, J.K. Nicholson, and H.C. Keun. 2008. Robust Algorithms for Automated Chemical Shift Calibration of 1D 1H NMR Spectra of Blood Serum. Anal. Chem. 80:7158–7162. doi:10.1021/ac8011494.

Pedersen, S., and J. Wilson. 2021. Early detection and prompt effective treatment of lameness in dairy cattle. Livestock 26:115–121. doi:10.12968/live.2021.26.3.115.

Pereira, P.C. 2014. Milk nutritional composition and its role in human health. Nutrition 30:619–627. doi:10.1016/j.nut.2013.10.011.

Pérez-Cabal, M.A., and N. Charfeddine. 2016. Short communication: Association of foot and leg conformation and body weight with claw disorders in Spanish Holstein cows. J. Dairy Sci. 99:9104–9108. doi:10.3168/jds.2016-11331.

Pérez-Cabal, M.A.A., and N. Charfeddine. 2015. Models for genetic evaluations of claw health traits in Spanish dairy cattle. J. Dairy Sci. 98:8186–8194. doi:doi.org/10.3168/jds.2015-9562.

Perneger, T.V. 1998. What's wrong with Bonferroni adjustments. Br. Med. J. 316:1236–1238. doi:10.1136/bmj.316.7139.1236.

Piette, D., T. Norton, V. Exadaktylos, and D. Berckmans. 2020. Individualised automated lameness detection in dairy cows and the impact of historical window length on algorithm performance. Animal 14:409–417. doi:10.1017/S1751731119001642.

Plaizier, J.C., M.D. Mesgaran, H. Derakhshani, H. Golder, E. Khafipour, J.L. Kleen, I. Lean, J. Loor, G. Penner, and Q. Zebeli. 2018. Review: Enhancing gastrointestinal health in dairy cows. Animal 12:S399–S418. doi:10.1017/S1751731118001921.

Platt, A., B.J. Vilhjálmsson, and M. Nordborg. 2010. Conditions Under Which Genome-Wide Association Studies Will be Positively Misleading. Genetics 186:1045–1052. doi:10.1534/genetics.110.121665.

Ploder, M., G. Neurauter, A. Spittler, K. Schroecksnadel, E. Roth, and D. Fuchs. 2008. Serum phenylalanine in patients post trauma and with sepsis correlate to neopterin concentrations. Amino Acids 35:303–307. doi:10.1007/s00726-007-0625-x.

Plummer, M., N. Best, K. Cowles, and K. Vines. 2006. CODA: convergence diagnosis and output analysis for MCMC. R News 6:7–11.

Pritchard, T., M. Coffey, R. Mrode, and E. Wall. 2013. Genetic parameters for production, health, fertility and longevity traits in dairy cows. Animal 7:34–46. doi:10.1017/S1751731112001401.

Proudfoot, K.L., D.M. Weary, and M.A.G.V. Keyserlingk. 2010. Behavior during transition differs for cows diagnosed with claw horn lesions in mid lactation. J. Dairy Sci. 93:3970–3978. doi:10.3168/jds.2009-2767.

Pryce, J.E., J. Arias, P.J. Bowman, S.R. Davis, K.A. Macdonald, G.C. Waghorn, W.J. Wales, Y.J. Williams, R.J. Spelman, and B.J. Hayes. 2012. Accuracy of genomic predictions of residual feed intake and 250-day body weight in growing heifers using 625,000 single nucleotide polymorphism markers. J. Dairy Sci. 95:2108–2119. doi:10.3168/jds.2011-4628.

Pryce, J.E., M.P. Coffey, and S. Brotherstone. 2000a. The Genetic Relationship between Calving Interval, Body Condition Score and Linear Type and Management Traits in Registered Holsteins. J. Dairy Sci. 83:2664–2671. doi:10.3168/jds.S0022-0302(00)75160-5.

Pryce, J.E., G. Simm, P.R. Amer, M.P. Coffey, and A.W. Stott. 2000b. Returns from genetic improvement in dairy cattle over a twenty year horizon. Proc. Br. Soc. Anim. Sci. 2000:37–37. doi:10.1017/S1752756200000387.

Psychogios, N., D.D. Hau, J. Peng, A.C. Guo, R. Mandal, S. Bouatra, I. Sinelnikov, R. Krishnamurthy, R. Eisner, B. Gautam, N. Young, J. Xia, C. Knox, E. Dong, P. Huang, Z. Hollander, T.L. Pedersen, S.R. Smith, F. Bamforth, R. Greiner, B. McManus, J.W. Newman, T. Goodfriend, and D.S. Wishart. 2011. The Human Serum Metabolome. PLOS ONE 6:e16957. doi:10.1371/journal.pone.0016957.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M.A.R. Ferreira, D. Bender, J. Maller, P. Sklar, P.I.W. de Bakker, M.J. Daly, and P.C. Sham. 2007. PLINK:

A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. Am. J. Hum. Genet. 81:559–575.

Qanbari, S. 2020. On the Extent of Linkage Disequilibrium in the Genome of Farm Animals. Front. Genet. 10.

Qanbari, S., E.C.G. Pimentel, J. Tetens, G. Thaller, P. Lichtner, A.R. Sharifi, and H. Simianer. 2010. The pattern of linkage disequilibrium in German Holstein cattle. Anim. Genet. 41:346–356. doi:10.1111/j.1365-2052.2009.02011.x.

R Core Team. 2021. R: A Language and Environment for Statistical Computing.

Räber, M., C.J. Lischer, H. Geyer, and P. Ossent. 2004. The bovine digital cushion - A descriptive anatomical study. Vet. J. 167:258–264. doi:10.1016/S1090-0233(03)00053-4.

Räber, M., M.R.L. Scheeder, P. Ossent, C.J. Lischer, and H. Geyer. 2006. The content and composition of lipids in the digital cushion of the bovine claw with respect to age and location - A preliminary report. Vet. J. 172:173–177. doi:10.1016/j.tvjl.2005.03.009.

Raboisson, D., M. Mounié, and E. Maigné. 2014. Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: A meta-analysis and review. J. Dairy Sci. 97:7547–7563. doi:10.3168/jds.2014-8237.

Randall, L. V., M.J. Green, M.G.G. Chagunda, C. Mason, S.C. Archer, L.E. Green, and J.N. Huxley. 2015. Low body condition predisposes cattle to lameness: An 8-year study of one dairy herd. J. Dairy Sci. 98:3766–3777. doi:10.3168/jds.2014-8863.

Randall, L.V., M.J. Green, M.G.G. Chagunda, C. Mason, L.E. Green, and J.N. Huxley. 2016. Lameness in dairy heifers; impacts of hoof lesions present around first calving on future lameness, milk yield and culling risk. Prev. Vet. Med. 133:52–63. doi:10.1016/j.prevetmed.2016.09.006.

Randall, L.V., M.J. Green, L.E. Green, M.G.G. Chagunda, C. Mason, S.C. Archer, and J.N. Huxley. 2018a. The contribution of previous lameness events and body condition score to the occurrence of lameness in dairy herds: A study of 2 herds. J. Dairy Sci. 101:1311–1324. doi:10.3168/jds.2017-13439.

Randall, L.V., M.J. Green, and J.N. Huxley. 2018b. Use of statistical modelling to investigate the pathogenesis of claw horn disruption lesions in dairy cattle. Vet. J. 238:41–48. doi:10.1016/j.tvjl.2018.07.002.

Randall, L.V., H.J. Thomas, J.G. Remnant, N.J. Bollard, and J.N. Huxley. 2019. Lameness prevalence in a random sample of UK dairy herds. Vet. Rec. 184:350–350. doi:10.1136/vr.105047.

Ranjbar, S., A.R. Rabiee, A. Gunn, and J.K. House. 2016. Identifying risk factors associated with lameness in pasture-based dairy herds. J. Dairy Sci. 99:7495–7505. doi:10.3168/jds.2016-11142.

Raphaka, K., E. Sánchez-Molano, S. Tsairidou, O. Anacleto, E.J. Glass, J.A. Woolliams, A. Doeschl-Wilson, and G. Banos. 2018. Impact of Genetic Selection for Increased Cattle Resistance to Bovine Tuberculosis on Disease Transmission Dynamics. Front. Vet. Sci. 5.

Rath, T., E. Roth, R. Keidl, and G. Meissl. 1987. Phenylalanine: total amino acid ratio in 45 burn patients. Scand. J. Plast. Reconstr. Surg. Hand Surg. 21:297–300. doi:10.3109/02844318709086465.

Reader, J.D., M.J. Green, J. Kaler, S.A. Mason, and L.E. Green. 2011. Effect of mobility score on milk yield and activity in dairy cattle. J. Dairy Sci. 94:5045–5052. doi:10.3168/jds.2011-4415.

Refaai, W., M. Van Aert, A.M. Abd El-Aal, A.E. Behery, and G. Opsomer. 2013. Infectious diseases causing lameness in cattle with a main emphasis on digital dermatitis (Mortellaro disease). Livest. Sci. 156:53–63. doi:10.1016/j.livsci.2013.06.004.

Rendel, J.M., and A. Robertson. 1950. Estimation of genetic gain in milk yield by selection in a closed herd of dairy cattle. J. Genet. 50:1–8. doi:10.1007/BF02986789.

van Rheenen, W., W.J. Peyrot, A.J. Schork, S.H. Lee, and N.R. Wray. 2019. Genetic correlations of polygenic disease traits: from theory to practice. Nat. Rev. Genet. 20:567–581. doi:10.1038/s41576-019-0137-z.

Richardson, C.M., P.R. Amer, C. Quinton, J. Crowley, F.S. Hely, I. van den Berg, and J.E. Pryce. 2022. Reducing greenhouse gas emissions through genetic selection in the Australian dairy industry. J. Dairy Sci. 0. doi:10.3168/jds.2021-21277.

Richardson, I.W., D.P. Berry, H.L. Wiencko, I.M. Higgins, S.J. More, J. McClure, D.J. Lynn, and D.G. Bradley. 2016. A genome-wide association study for genetic susceptibility to Mycobacterium bovis infection in dairy cattle identifies a susceptibility QTL on chromosome 23. Genet. Sel. Evol. 48:19. doi:10.1186/s12711-016-0197-x.

van Rijssel, J., J. Kroon, M. Hoogenboezem, F.P.J. van Alphen, R.J. de Jong, E. Kostadinova, D. Geerts, P.L. Hordijk, and J.D. van Buul. 2012. The Rho-guanine nucleotide exchange factor Trio controls leukocyte transendothelial migration by promoting docking structure formation. Mol. Biol. Cell 23:2831–2844. doi:10.1091/mbc.e11-11-0907.

Ring, S.C., A.J. Twomey, N. Byrne, M.M. Kelleher, T. Pabiou, M.L. Doherty, and D.P. Berry. 2018. Genetic selection for hoof health traits and cow mobility

scores can accelerate the rate of genetic gain in producer-scored lameness in dairy cows. J. Dairy Sci. 101:10034–10047. doi:10.3168/jds.2018-15009.

Rioja-Lang, F.C., M. Connor, H.J. Bacon, A.B. Lawrence, and C.M. Dwyer. 2020. Prioritization of Farm Animal Welfare Issues Using Expert Consensus. Front. Vet. Sci. 6:495. doi:10.3389/fvets.2019.00495.

Robinson, M.R., N.R. Wray, and P.M. Visscher. 2014. Explaining additional genetic variation in complex traits. Trends Genet. 30:124–132. doi:10.1016/j.tig.2014.02.003.

Robinson, P.N. 2012. Deep phenotyping for precision medicine. Hum. Mutat. 33:777–780. doi:10.1002/humu.22080.

Roche, J.R., P.G. Dillon, C.R. Stockdale, L.H. Baumgard, and M.J. VanBaale. 2004. Relationships among international body condition scoring systems. J. Dairy Sci. 87:3076–3079. doi:10.3168/jds.S0022-0302(04)73441-4.

Roche, J.R., N.C. Friggens, J.K. Kay, M.W. Fisher, K.J. Stafford, and D.P. Berry. 2009. Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92:5769–5801. doi:10.3168/jds.2009-2431.

Rodríguez-Ramilo, S.T., J. Fernández, M.A. Toro, D. Hernández, and B. Villanueva. 2015. Genome-Wide estimates of coancestry, inbreeding and effective population size in the spanish holstein population. PLoS ONE 10. doi:10.1371/journal.pone.0124157.

Rogers, G.W., J.A.M. Van Arendonk, and B.T. McDaniel. 1988. Influence of Involuntary Culling on Optimum Culling Rates and Annualized Net Revenue. J. Dairy Sci. 71:3463–3469. doi:10.3168/jds.S0022-0302(88)79952-X.

Rohart, F., B. Gautier, A. Singh, and K.-A. Lê Cao. 2017. mixOmics: An R package for 'omics feature selection and multiple data integration. PLOS Comput. Biol. 13:e1005752. doi:10.1371/journal.pcbi.1005752.

Rosen, B.D., D.M. Bickhart, R.D. Schnabel, S. Koren, C.G. Elsik, E. Tseng, T.N. Rowan, W.Y. Low, A. Zimin, C. Couldrey, R. Hall, W. Li, A. Rhie, J. Ghurye, S.D. McKay, F. Thibaud-Nissen, J. Hoffman, B.M. Murdoch, W.M. Snelling, T.G. McDaneld, J.A. Hammond, J.C. Schwartz, W. Nandolo, D.E. Hagen, C. Dreischer, S.J. Schultheiss, S.G. Schroeder, A.M. Phillippy, J.B. Cole, C.P. Van Tassell, G. Liu, T.P.L. Smith, and J.F. Medrano. 2020. De novo assembly of the cattle reference genome with single-molecule sequencing. GigaScience 9:1–9. doi:10.1093/gigascience/giaa021.

Rotz, A. 2020. Environmental Sustainability of Livestock Production. Meat Muscle Biol. 4. doi:10.22175/mmb.11103.

Rusterholz, A. 1920. Das spezifisch-traumatische klauensohlengeschwür des rindes (sole ulcer of traumatic origin in cattle). Schweiz. Arch. Tierheilkd. 62:505–525.

Sadiq, M.B., S.Z. Ramanoon, R. Mansor, S.S. Syed-Hussain, and W.M. Shaik Mossadeq. 2017. Prevalence of lameness, claw lesions, and associated risk factors in dairy farms in Selangor, Malaysia. Trop. Anim. Health Prod. 49:1741–1748. doi:10.1007/s11250-017-1387-4.

Samuel, C.S., J.P. Coghlan, and J.F. Bateman. 1998. Effects of relaxin, pregnancy and parturition on collagen metabolism in the rat pubic symphysis. J. Endocrinol. 159:117–125.

Sánchez-Molano, E., V. Bay, R.F. Smith, G. Oikonomou, and G. Banos. 2019. Quantitative Trait Loci Mapping for Lameness Associated Phenotypes in Holstein–Friesian Dairy Cattle. Front. Genet. 10:926–926. doi:10.3389/fgene.2019.00926.

Sanders, A.H., J.K. Shearer, and A. de Vries. 2009. Seasonal incidence of lameness and risk factors associated with thin soles, white line disease, ulcers, and sole punctures in dairy cattle. J. Dairy Sci. 92:3165–3174. doi:10.3168/jds.2008-1799.

Sárová, R., I. Stéhulová, P. Kratinová, P. Firla, and M. Špinka. 2011. Farm managers underestimate lameness prevalence in Czech dairy herds. Anim. Welf. 20:201–204.

Sartori, R., J.M. Haughian, R.D. Shaver, G.J.M. Rosa, and M.C. Wiltbank. 2004. Comparison of Ovarian Function and Circulating Steroids in Estrous Cycles of Holstein Heifers and Lactating Cows. J. Dairy Sci. 87:905–920. doi:10.3168/jds.S0022-0302(04)73235-X.

Sato, S. 2016. Pathophysiological evaluation of subacute ruminal acidosis (SARA) by continuous ruminal pH monitoring. Anim. Sci. J. 87:168–177. doi:10.1111/asj.12415.

Sauerbrei, W., A. Buchholz, A.-L. Boulesteix, and H. Binder. 2015. On stability issues in deriving multivariable regression models. Biom. J. 57:531–555. doi:10.1002/bimj.201300222.

Schaeffer, L.R. 2006. Strategy for applying genome-wide selection in dairy cattle. J. Anim. Breed. Genet. 123:218–223. doi:10.1111/j.1439-0388.2006.00595.x.

Schaid, D.J., W. Chen, and N.B. Larson. 2018. From genome-wide associations to candidate causal variants by statistical fine-mapping. Nat. Rev. Genet. 19:491–504. doi:10.1038/s41576-018-0016-z.

Schloerke, B., J. Crowley, D. Cook, F. Briatte, M. Marbach, E. Thoen, A. Elberg, and J. Larmarange. 2018. Ggally: Extension to ggplot2.

Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9:671–675. doi:10.1038/nmeth.2089.

Schöpke, K., S. Weidling, R. Pijl, and H.H. Swalve. 2013. Relationships between bovine hoof disorders, body condition traits, and test-day yields. J. Dairy Sci. 96:679–689. doi:10.3168/jds.2012-5728.

Schuler, G., R. Fürbass, and K. Klisch. 2018. Placental contribution to the endocrinology of gestation and parturition. Page in 10th International Ruminant Reproduction Symposium.

Seabury, C.M., D.L. Oldeschulte, M. Saatchi, J.E. Beever, J.E. Decker, Y.A. Halley, E.K. Bhattarai, M. Molaei, H.C. Freetly, S.L. Hansen, H. Yampara-Iquise, K.A. Johnson, M.S. Kerley, J. Kim, D.D. Loy, E. Marques, H.L. Neibergs, R.D. Schnabel, D.W. Shike, M.L. Spangler, R.L. Weaber, D.J. Garrick, and J.F. Taylor. 2017. Genome-wide association study for feed efficiency and growth traits in U.S. beef cattle. BMC Genomics 18:386. doi:10.1186/s12864-017-3754-y.

Semba, R.D. 2016. The Rise and Fall of Protein Malnutrition in Global Health. Ann. Nutr. Metab. 69:79–88. doi:10.1159/000449175.

Sepúlveda-Varas, P., J. Lomb, M.A.G. Von Keyserlingk, R. Held, H. Bustamante, and N. Tadich. 2018. Claw horn lesions in mid-lactation primiparous dairy cows under pasture-based systems: Association with behavioral and metabolic changes around calving. J. Dairy Sci. 101:9439–9450. doi:10.3168/jds.2018-14674.

Shabalina, T., T. Yin, and S. König. 2020. Influence of common health disorders on the length of productive life and stayability in German Holstein cows. J. Dairy Sci. 103:583–596. doi:10.3168/jds.2019-16985.

Shahinfar, S., M. Khansefid, M. Haile-Mariam, and J.E. Pryce. 2021. Machine learning approaches for the prediction of lameness in dairy cows. Animal 15:100391. doi:10.1016/j.animal.2021.100391.

Shearer, J.K., P.J. Plummer, and J.A. Schleining. 2015. Perspectives on the treatment of claw lesions in cattle. Vet. Med. Res. Rep. 6:273–292. doi:10.2147/VMRR.S62071.

Shearer, J.K.K., and S.R. van Amstel. 2017. Pathogenesis and Treatment of Sole Ulcers and White Line Disease. Vet. Clin. North Am. Food Anim. Pract. 33:283–300. doi:10.1016/j.cvfa.2017.03.001.

Shepherd, P.R., D.J. Withers, and K. Siddle. 1998. Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling.. Biochem. J. 333:471–490.

Shi, X., X. Li, D. Li, Y. Li, Y. Song, Q. Deng, J. Wang, Y. Zhang, H. Ding, L. Yin, Y. Zhang, Z. Wang, X. Li, and G. Liu. 2014. Beta-hydroxybutyrate Activates the

NF-κB Signaling Pathway to Promote the Expression of Pro-Inflammatory Factors in Calf Hepatocytes. Cell. Physiol. Biochem. 33:920–932. doi:10.1159/000358664.

Shin, J., and C. Lee. 2015. Statistical power for identifying nucleotide markers associated with quantitative traits in genome-wide association analysis using a mixed model. Genomics 105:1–4. doi:10.1016/j.ygeno.2014.11.001.

Shin, S.-Y., E.B. Fauman, A.-K. Petersen, J. Krumsiek, R. Santos, J. Huang, M. Arnold, I. Erte, V. Forgetta, T.-P. Yang, K. Walter, C. Menni, L. Chen, L. Vasquez, A.M. Valdes, C.L. Hyde, V. Wang, D. Ziemek, P. Roberts, L. Xi, E. Grundberg, M. Waldenberger, J.B. Richards, R.P. Mohney, M.V. Milburn, S.L. John, J. Trimmer, F.J. Theis, J.P. Overington, K. Suhre, M.J. Brosnan, C. Gieger, G. Kastenmüller, T.D. Spector, and N. Soranzo. 2014. An atlas of genetic influences on human blood metabolites. Nat. Genet. 46:543–550. doi:10.1038/ng.2982.

Shook, G.E. 1989. Selection for Disease Resistance. J. Dairy Sci. 72:1349–1362. doi:10.3168/jds.S0022-0302(89)79242-0.

Short, K.M., and T.C. Cox. 2006. Subclassification of the RBCC/TRIM Superfamily Reveals a Novel Motif Necessary for Microtubule Binding *. J. Biol. Chem. 281:8970–8980. doi:10.1074/jbc.M512755200.

Siivonen, J., S. Taponen, M. Hovinen, M. Pastell, B.J. Lensink, S. Pyörälä, and L. Hänninen. 2011. Impact of acute clinical mastitis on cow behaviour. Appl. Anim. Behav. Sci. 132:101–106. doi:10.1016/j.applanim.2011.04.005.

Silva, A.A., D.A. Silva, F.F. Silva, C.N. Costa, H.T. Silva, P.S. Lopes, R. Veroneze, G. Thompson, and J. Carvalheira. 2020. GWAS and gene networks for milk-related traits from test-day multiple lactations in Portuguese Holstein cattle. J. Appl. Genet. 61:465–476. doi:10.1007/s13353-020-00567-3.

Simm, G. 2000. Genetic Improvement of Cattle and Sheep. 1st ed. Farming Press, Ipswich.

Singer, A.J., and R.A.F. Clark. 1999. Cutaneous Wound Healing. N. Engl. J. Med. 341:738–746. doi:10.1056/NEJM199909023411006.

Smemo, S., J.J. Tena, K.-H. Kim, E.R. Gamazon, N.J. Sakabe, C. Gómez-Marín, I. Aneas, F.L. Credidio, D.R. Sobreira, N.F. Wasserman, J.H. Lee, V. Puviindran, D. Tam, M. Shen, J.E. Son, N.A. Vakili, H.-K. Sung, S. Naranjo, R.D. Acemel, M. Manzanares, A. Nagy, N.J. Cox, C.-C. Hui, J.L. Gomez-Skarmeta, and M.A. Nóbrega. 2014. Obesity-associated variants within FTO form long-range functional connections with IRX3. Nature 507:371–375. doi:10.1038/nature13138.

Smith, B.I., J. Kauffold, and L. Sherman. 2010. Serum haptoglobin concentrations in dairy cattle with lameness due to claw disorders. Vet. J. 186:162–165. doi:10.1016/j.tvjl.2009.08.012.

Soares, R.A.N., G. Vargas, T. Duffield, F. Schenkel, and E.J. Squires. 2021. Genome-wide association study and functional analyses for clinical and subclinical ketosis in Holstein cattle. J. Dairy Sci. 104:10076–10089. doi:10.3168/jds.2020-20101.

Sogstad, Å., T. Fjeldaas, and O. Østerås. 2005. Lameness and Claw Lesions of the Norwegian Red Dairy Cattle Housed in Free Stalls in Relation to Environment, Parity and Stage of Lactation. Acta Vet. Scand. 46:203–217. doi:10.1186/1751-0147-46-203.

Soininen, P., A.J. Kangas, P. Würtz, T. Tukiainen, T. Tynkkynen, R. Laatikainen, M.R. Järvelin, M. Kähönen, T. Lehtimäki, J. Viikari, O.T. Raitakari, M.J. Savolainen, and M. Ala-Korpela. 2009. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst 134:1781–1785. doi:10.1039/b910205a.

Solano, L., H.W. Barkema, E.A. Pajor, S. Mason, S.J. LeBlanc, J.C. Zaffino Heyerhoff, C.G.R. Nash, D.B. Haley, E. Vasseur, D. Pellerin, J. Rushen, A.M. de Passillé, and K. Orsel. 2015. Prevalence of lameness and associated risk factors in Canadian Holstein-Friesian cows housed in freestall barns. J. Dairy Sci. 98:6978–6991. doi:10.3168/jds.2015-9652.

Somers, J., and L. O'Grady. 2015. Foot lesions in lame cows on 10 dairy farms in Ireland. Ir. Vet. J. 68:10. doi:10.1186/s13620-015-0039-0.

Somers, J.G.C.J., K. Frankena, E.N. Noordhuizen-Stassen, and J.H.M. Metz. 2003. Prevalence of Claw Disorders in Dutch Dairy Cows Exposed to Several Floor Systems. J. Dairy Sci. 86:2082–2093. doi:10.3168/jds.S0022-0302(03)73797-7.

Sordillo, L.M., G.A. Contreras, and S.L. Aitken. 2009. Metabolic factors affecting the inflammatory response of periparturient dairy cows†. Anim. Health Res. Rev. 10:53–63. doi:10.1017/S1466252309990016.

Sordillo, L.M., and W. Raphael. 2013. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. Vet. Clin. N. Am. - Food Anim. Pract. 29:267–278. doi:10.1016/j.cvfa.2013.03.002.

Southey, B.R., S.L. Rodriguez-zas, and J.V. Sweedler. 2009. Characterization of the prohormone complement in cattle using genomic libraries and cleavage prediction approaches. BMC Genomics 11:1–11. doi:10.1186/1471-2164-10-228.

Speed, D., and D.J. Balding. 2015. Relatedness in the post-genomic era: Is it still useful?. Nat. Rev. Genet. 16:33–44. doi:10.1038/nrg3821.

Speed, D., N. Cai, U. Consortium, M.R. Johnson, and D.J. Balding. 2017. Re-evaluation of SNP heritability in complex human traits. Nat. Genet. 49:986–992. doi:10.1038/ng.3865.Re-evaluation.

van der Spek, D., J.A.M. van Arendonk, and H. Bovenhuis. 2015. Genome-wide association study for claw disorders and trimming status in dairy cattle. J. Dairy Sci. 98:1286–1295. doi:10.3168/jds.2014-8302.

van der Spek, D., J.A.M. van Arendonk, A.A.A. Vallée, and H. Bovenhuis. 2013. Genetic parameters for claw disorders and the effect of preselecting cows for trimming. J. Dairy Sci. 96:6070–6078. doi:10.3168/jds.2013-6833.

Sprecher, D.J., D.E. Hostetler, and J.B. Kaneene. 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 4:1179–1187.

Stambuk, C.R., J.A.A. Mcart, R.C. Bicalho, A.M. Miles, and H.J. Huson. 2019. A longitudinal study of digital cushion thickness and its function as a predictor for compromised locomotion and hoof lesions in Holstein cows. Transl. Anim. Sci. 3:74–83. doi:10.1093/tas/txy107.

Stambuk, C.R., E.A. Staiger, B.J. Heins, and H.J. Huson. 2020a. Exploring physiological and genetic variation of digital cushion thickness in Holstein and Jersey cows and bulls. J. Dairy Sci. 103:9177–9194. doi:10.3168/jds.2020-18290.

Stambuk, C.R., E.A. Staiger, A. Nazari-Ghadikolaei, B.J. Heins, and H.J. Huson. 2020b. Phenotypic characterization and genome-wide association studies of digital cushion thickness in Holstein cows. J. Dairy Sci. 103:3289–3303. doi:10.3168/jds.2019-17409.

Steele, M.A., G.B. Penner, F. Chaucheyras-Durand, and L.L. Guan. 2016. Development and physiology of the rumen and the lower gut: Targets for improving gut health. J. Dairy Sci. 99:4955–4966. doi:10.3168/jds.2015-10351.

Steinfeld, H., P. Gerber, T. Wassenaar, V. Castel, M. Rosales, and C. de Haan. 2006. Livestock's long shadow.

Stoop, V.M., G. de Jong, M.L. van Pelt, and C. van der Linde. 2010. Implementation of a claw health index in The Netherlands. Interbull Bull. 95–95.

Stott, A.W., M.P. Coffey, and S. Brotherstone. 2005. Including lameness and mastitis in a profit index for dairy cattle. Anim. Sci. 80:41–52. doi:10.1079/ASC40520041.

Strandén, I., and D.J. Garrick. 2009. Technical note: Derivation of equivalent computing algorithms for genomic predictions and reliabilities of animal merit. J. Dairy Sci. 92:2971–2975. doi:10.3168/jds.2008-1929.

Stringer, K.A., R.T. McKay, A. Karnovsky, B. Quémerais, and P. Lacy. 2016. Metabolomics and Its Application to Acute Lung Diseases. Front. Immunol. 7.

Suchocki, T., C. Egger-Danner, H. Schwarzenbacher, and J. Szyda. 2020. Two-stage genome-wide association study for the identification of causal variants underlying hoof disorders in cattle. J. Dairy Sci. 4483–4494. doi:10.3168/jds.2019-17542.

Sumner, L.W., A. Amberg, D. Barrett, M.H. Beale, R. Beger, C.A. Daykin, T.W.-M. Fan, O. Fiehn, R. Goodacre, J.L. Griffin, T. Hankemeier, N. Hardy, J. Harnly, R. Higashi, J. Kopka, A.N. Lane, J.C. Lindon, P. Marriott, A.W. Nicholls, M.D. Reily, J.J. Thaden, and M.R. Viant. 2007. Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). Metabolomics Off. J. Metabolomic Soc. 3:211–221. doi:10.1007/s11306-007-0082-2.

Sun, L.W., H.Y. Zhang, L. Wu, S. Shu, C. Xia, C. Xu, and J.S. Zheng. 2014. 1H-Nuclear magnetic resonance-based plasma metabolic profiling of dairy cows with clinical and subclinical ketosis. J. Dairy Sci. 97:1552–1562. doi:10.3168/jds.2013-6757.

Swalve, H.H., H. Alkhoder, and R. Pijl. 2008. Estimates of breeding values for sires based on diagnoses recorded at hoof trimming: Relationships with EBV for conformation traits. Interbull 38:87–90.

Swalve, H.H., C. Floren, M. Wensch-Dorendorf, K. Schöpke, R. Pijl, K. Wimmers, and B. Brenig. 2014. A study based on records taken at time of hoof trimming reveals a strong association between the IQ motif-containing GTPase-activating protein 1 (IQGAP1) gene and sole hemorrhage in Holstein cattle. J. Dairy Sci. 97:507–519. doi:10.3168/jds.2013-6997.

Sykora, S., J. Kofler, J. Glonegger-Reichert, J. Dietrich, G. Auersperg, and S. Brandt. 2015. Treponema DNA in bovine 'non-healing' versus common sole ulcers and white line disease. Vet. J. 205:417–420. doi:10.1016/j.tvjl.2015.05.023.

Tadich, N., E. Flor, and L. Green. 2010. Associations between hoof lesions and locomotion score in 1098 unsound dairy cows. Vet. J. 184:60–65. doi:10.1016/j.tvjl.2009.01.005.

Takagi, M., D. Yamamoto, M. Ohtani, and A. Miyamoto. 2007. Quantitative Analysis of Messenger RNA Expression of Matrix Metalloproteinases (MMP-2 and MMP-9), Tissue Inhibitor-2 of Matrix Metalloproteinases (TIMP-2), and Steroidogenic Enzymes in Bovine Placentomes During Gestation and Postpartum. Mol. Reprod. Dev. 74:801–807. doi:10.1002/mrd.

Tam, V., N. Patel, M. Turcotte, Y. Bossé, G. Paré, and D. Meyre. 2019. Benefits and limitations of genome-wide association studies. Nat. Rev. Genet. 20:467–484. doi:10.1038/s41576-019-0127-1.

Tamminga, S., P.A. Luteijn, and R.G.M. Meijer. 1997. Changes in composition and energy content of liveweight loss in dairy cows with time after parturition. Livest. Prod. Sci. 52:31–38. doi:10.1016/S0301-6226(97)00115-2.

Tang, X., M. Hao, C. Chang, A. Bhatia, K. O'Brien, M. Chen, D.G. Armstrong, and W. Li. 2021. Wound Healing Driver Gene and Therapeutic Development: Political and Scientific Hurdles. Adv. Wound Care 10:415–435. doi:10.1089/wound.2019.1143.

Tarlton, J.F., D.E. Holah, K.M. Evans, S. Jones, G.R. Pearson, and A.J.F. Webster. 2002. Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving. Vet. J. 163:196–204. doi:10.1053/tvjl.2001.0651.

Taylor, J.F., K.H. Taylor, and J.E. Decker. 2016. Holsteins are the genomic selection poster cows. Proc. Natl. Acad. Sci. 113:7690–7692. doi:10.1073/pnas.1608144113.

Teahan, O., S. Gamble, E. Holmes, J. Waxman, J.K. Nicholson, C. Bevan, and H.C. Keun. 2006. Impact of Analytical Bias in Metabonomic Studies of Human Blood Serum and Plasma. Anal. Chem. 78:4307–4318. doi:10.1021/ac051972y.

Tenesa, A., and C.S. Haley. 2013. The heritability of human disease: Estimation, uses and abuses. Nat. Rev. Genet. 14:139–149. doi:10.1038/nrg3377.

Tenghe, A.M.M., A.C. Bouwman, B. Berglund, E. Strandberg, D.J. de Koning, and R.F. Veerkamp. 2016. Genome-wide association study for endocrine fertility traits using single nucleotide polymorphism arrays and sequence variants in dairy cattle. J. Dairy Sci. 99:5470–5485. doi:10.3168/jds.2015-10533.

The UniProt Consortium. 2021. UniProt: The universal protein knowledgebase in 2021. Nucleic Acids Res. 49:D480–D489. doi:10.1093/nar/gkaa1100.

Thoefner, M.B., C.C. Pollitt, A.W. Van Eps, G.J. Milinovich, D.J. Trott, O. Wattle, and P.H. Andersen. 2004. Acute bovine laminitis: A new induction model using alimentary oligofructose overload. J. Dairy Sci. 87:2932–2940. doi:10.3168/jds.S0022-0302(04)73424-4.

Thomas, H.J., G.G. Miguel-Pacheco, N.J. Bollard, S.C. Archer, N.J. Bell, C. Mason, O.J.R. Maxwell, J.G. Remnant, P. Sleeman, H.R. Whay, and J.N. Huxley. 2015. Evaluation of treatments for claw horn lesions in dairy cows in a randomized controlled trial. J. Dairy Sci. 98:4477–4486. doi:10.3168/jds.2014-8982.

Thomas, H.J., J.G. Remnant, N.J. Bollard, A. Burrows, H.R. Whay, N.J. Bell, C. Mason, and J.N. Huxley. 2016. Recovery of chronically lame dairy cows following treatment for claw horn lesions: A randomised controlled trial. Vet. Rec. 178:116–116. doi:10.1136/vr.103394.

Thomasen, J.R., A. Willam, C. Egger-Danner, and A.C. Sørensen. 2016. Reproductive technologies combine well with genomic selection in dairy breeding programs. J. Dairy Sci. 99:1331–1340. doi:10.3168/jds.2015-9437.

Thompson, J.S., J.N. Huxley, C.D. Hudson, J. Kaler, J. Gibbons, and M.J. Green. 2020. Field survey to evaluate space allowances for dairy cows in Great Britain. J. Dairy Sci. 103:3745–3759. doi:10.3168/jds.2019-17004.

Thomsen, P.T., L. Foldager, P. Raundal, and N. Capion. 2019. Lower odds of sole ulcers in the following lactation in dairy cows that received hoof trimming around drying off. Vet. J. 254:105408. doi:10.1016/j.tvjl.2019.105408.

Tian, M., J. Fan, Z. Zhuang, F. Dai, C. Wang, H. Hou, and Y. Ma. 2019. Effects of silymarin on p65 NF- κB, p38 MAPK and CYP450 in LPS-induced hoof dermal inflammatory cells of dairy cows. BMC Vet. Res. 15:1–8.

Tibshirani, R. 1996. Regression Shrinkage and Selection Via the Lasso. J. R. Stat. Soc. Ser. B Methodol. 58:267–288. doi:10.1111/j.2517-6161.1996.tb02080.x.

Tipton, C.D., R.D. Wolcott, N.E. Sanford, C. Miller, G. Pathak, T.K. Silzer, J. Sun, D. Fleming, K.P. Rumbaugh, T.D. Little, N. Phillips, and C.D. Phillips. 2020. Patient genetics is linked to chronic wound microbiome composition and healing. PLOS Pathog. 16:e1008511. doi:10.1371/journal.ppat.1008511.

Tjur, T. 2009. Coefficients of Determination in Logistic Regression Models—A New Proposal: The Coefficient of Discrimination. Am. Stat. 63:366–372. doi:10.1198/tast.2009.08210.

Toholj, B., M. Cincović, M. Stevančević, J. Spasojevic, V. Ivetić, and A. Potkonjak. 2014. Evaluation of ultrasonography for measuring solar soft tissue thickness as a predictor of sole ulcer formation in Holstein-Friesian dairy cows. Vet. J. 199:290–294. doi:10.1016/j.tvjl.2013.11.005.

van der Tol, P.P.J., J.H.M. Metz, E.N. Noordhuizen-Stassen, W. Back, C.R. Braam, and W.A. Weijs. 2002. The pressure distribution under the bovine claw during square standing on a flat substrate. J. Dairy Sci. 85:1476–1481. doi:10.3168/jds.S0022-0302(02)74216-1.

van der Tol, P.P.J., J.H.M. Metz, E.N. Noordhuizen-Stassen, W. Back, C.R. Braam, and W.A. Weijs. 2003. The vertical ground reaction force and the pressure distribution on the claws of dairy cows while walking on a flat substrate. J. Dairy Sci. 86:2875–2883. doi:10.3168/jds.S0022-0302(03)73884-3.

Tomlinson, I. 2013. Doubling food production to feed the 9 billion: A critical perspective on a key discourse of food security in the UK. J. Rural Stud. 29:81–90. doi:10.1016/j.jrurstud.2011.09.001.

Tools for Analysis of Metabolomic NMR (tameNMR). 2021. . Accessed February 2, 2022. https://github.com/PGB-LIV/tameNMR.

Tothova, C., O. Nagy, and G. Kovac. 2014. Acute phase proteins and their use in the diagnosis of diseases in ruminants: a review. Vet. Med. (Praha) 59:163–180.

Toussaint Raven, E., R.T. Haalstra, D.J. Peterse, and A. Lurvink. 1985. Cattle Footcare and Claw Trimming. 1st ed. Farming Press, Ipswich.

Trengove, N.J., S.R. Langton, and M.C. Stacey. 1996. Biochemical analysis of wound fluid from nonhealing and healing chronic leg ulcers. Wound Repair Regen. 4:234–239. doi:10.1046/j.1524-475X.1996.40211.x.

Tsuruta, S., and I. Misztal. 2006. THRGIBBS1F90 for estimation of variance components with threshold and linear models.. Proc. 8th World Congr. Genet. Appl. Livest. Prod. Belo Horiz. Minas Gerais Braz. 13-18 August 2006 27.31.

Van Amstel, S.R., J.K. Shearer, and F.L. Palin. 2003. Clinical response to treatment of pododermatitis circumscripta (ulceration of the sole) in dairy cows. Bov. Pract. Vol. 37:143-150 Pages. doi:10.21423/BOVINE-VOL37NO2P143-150.

Van De Gucht, T., W. Saeys, J. Van Meensel, A. Van Nuffel, J. Vangeyte, and L. Lauwers. 2018. Farm-specific economic value of automatic lameness detection systems in dairy cattle: From concepts to operational simulations.. J. Dairy Sci. 101:637–648. doi:10.3168/jds.2017-12867.

Van De Gucht, T., S. Van Weyenberg, A. Van Nuffel, L. Lauwers, J. Vangeyte, and W. Saeys. 2017. Supporting the Development and Adoption of Automatic Lameness Detection Systems in Dairy Cattle: Effect of System Cost and Performance on Potential Market Shares. Anim. Open Access J. MDPI 7:77. doi:10.3390/ani7100077.

Vandenbroucke, R.E., and C. Libert. 2014. Is there new hope for therapeutic matrix metalloproteinase inhibition?. Nat. Rev. Drug Discov. 13:904–927. doi:10.1038/nrd4390.

Vanholder, T., J. Papen, R. Bemers, G. Vertenten, and A.C.B. Berge. 2015. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. J. Dairy Sci. 98:880–888. doi:10.3168/jds.2014-8362.

VanRaden, P.M. 2008. Efficient Methods to Compute Genomic Predictions. J. Dairy Sci. 91:4414–4423. doi:10.3168/jds.2007-0980.

VanRaden, P.M. 2020. Symposium review: How to implement genomic selection. J. Dairy Sci. 103:5291–5301. doi:10.3168/jds.2019-17684.

VanRaden, P.M., D.J. Null, M. Sargolzaei, G.R. Wiggans, M.E. Tooker, J.B. Cole, T.S. Sonstegard, E.E. Connor, M. Winters, J.B.C.H.M. van Kaam, A. Valentini, B.J. Van Doormaal, M.A. Faust, and G.A. Doak. 2013. Genomic imputation and evaluation using high-density Holstein genotypes. J. Dairy Sci. 96:668–678. doi:10.3168/jds.2012-5702.

Varma, S., and R. Simon. 2006. Bias in error estimation when using cross-validation for model selection. BMC Bioinformatics 7:91. doi:10.1186/1471-2105-7-91.

Veerkamp, R.F., W.G. Hill, A.W. Stott, S. Brotherstone, and G. Simm. 1995. Selection for longevity and yield in dairy cows using transmitting abilities for type and yield. Anim. Sci. 61:189–197. doi:10.1017/S1357729800013710.

Veerkamp, R.F., G. Simm, and J.D. Oldham. 1994. Effects of interaction between genotype and feeding system on milk production, feed intake, efficiency and body tissue mobilization in dairy cows. Livest. Prod. Sci. 39:229–241. doi:10.1016/0301-6226(94)90202-X.

Vermunt, J.J. 1992. “Subclinical” laminitis in dairy cattle. N. Z. Vet. J. 40:133–141. doi:10.1080/00480169.1992.35718.

Vermunt, J.J. 2007. One step closer to unravelling the pathophysiology of claw horn disruption: For the sake of the cows’ welfare. Vet. J. 174:219–220. doi:10.1016/j.tvjl.2006.10.006.

Vermunt, J.J., and P.R. Greenough. 1994. Predisposing factors of laminitis in cattle. Br. Vet. J. 150:151–164. doi:10.1016/S0007-1935(05)80223-4.

Vermunt, J.J., and P.R. Greenough. 1996. Sole haemorrhages in dairy heifers managed under different underfoot and environmental conditions. Br. Vet. J. 152:57–73. doi:10.1016/s0007-1935(96)80086-8.

Viant, M.R., I.J. Kurland, M.R. Jones, and W.B. Dunn. 2017. How close are we to complete annotation of metabolomes?. Curr. Opin. Chem. Biol. 36:64–69. doi:10.1016/j.cbpa.2017.01.001.

Vickery, H.B. 1950. The Origin of the Word Protein. Yale J. Biol. Med. 22:387–393.

Villot, C., B. Meunier, J. Bodin, C. Martin, and M. Silberberg. 2018. Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows. Animal 12:481–490. doi:10.1017/S1751731117001677.

Visscher, P.M., W.G. Hill, and N.R. Wray. 2008. Heritability in the genomics era - Concepts and misconceptions. Nat. Rev. Genet. 9:255–266. doi:10.1038/nrg2322.

Visscher, P.M., S.E. Medland, M.A.R. Ferreira, K.I. Morley, G. Zhu, B.K. Cornes, G.W. Montgomery, and N.G. Martin. 2006. Assumption-free estimation of heritability from genome-wide identity-by-descent sharing between full siblings. PLoS Genet. 2:0316–0325. doi:10.1371/journal.pgen.0020041.

Visscher, P.M., J.A. Woolliams, D. Smith, and J.L. Williams. 2002. Estimation of pedigree errors in the UK dairy population using microsatellite markers and the impact on selection. J. Dairy Sci. 85:2368–2375. doi:10.3168/jds.S0022-0302(02)74317-8.

Visscher, P.M., N.R. Wray, Q. Zhang, P. Sklar, M.I. McCarthy, M.A. Brown, and J. Yang. 2017. 10 Years of GWAS Discovery: Biology, Function, and Translation. Am. J. Hum. Genet. 101:5–22. doi:10.1016/j.ajhg.2017.06.005.

de Vries, M.J., and R.F. Veerkamp. 2000. Energy Balance of Dairy Cattle in Relation to Milk Production Variables and Fertility. J. Dairy Sci. 83:62–69. doi:10.3168/jds.S0022-0302(00)74856-9.

van der Waaij, E.H., M. Holzhauer, E. Ellen, C. Kamphuis, and G. De Jong. 2005. Genetic parameters for claw disorders in Dutch Dairy Cattle and correlations with conformation traits. J. Dairy Sci. 88:3672–3678. doi:10.3168/jds.S0022-0302(05)73053-8.

Wakefield, J. 2008. Reporting and interpretation in genome-wide association studies. Int. J. Epidemiol. 37:641–653. doi:10.1093/ije/dym257.

Wall, E., M.P. Coffey, and G.E. Pollott. 2012. The effect of lactation length on greenhouse gas emissions from the national dairy herd. animal 6:1857–1867. doi:10.1017/S1751731112000936.

Walter, I., and A. Boos. 2001. Matrix Metalloproteinases (MMP-2 and MMP-9) and Tissue Inhibitor-2 of Matrix Metalloproteinases (TIMP-2) in the Placenta and Interplacental Uterine Wall in Normal Cows and in Cattle with Retention of Fetal Membranes. Placenta 22:473–483.

Wang, H., I. Misztal, I. Aguilar, A. Legarra, R.L. Fernando, Z. Vitezica, R. Okimoto, T. Wing, R. Hawken, and W.M. Muir. 2014. Genome-wide association mapping including phenotypes from relatives without genotypes in a single-step (ssGWAS) for 6-week body weight in broiler chickens. Front. Genet. 5:1–10. doi:10.3389/fgene.2014.00134.

Wang, H., I. Misztal, I. Aguilar, A. Legarra, and W.M. Muir. 2012. Genome-wide association mapping including phenotypes from relatives without genotypes. Genet. Res. 94:73–83. doi:10.1017/S0016672312000274.

Wang, M.H., H.J. Cordell, and K. Van Steen. 2019. Statistical methods for genome-wide association studies. Semin. Cancer Biol. 55:53–60. doi:10.1016/j.semcancer.2018.04.008.

Warnick, L.D., D. Janssen, C.L. Guard, and Y.T. Gröhn. 2001. The effect of lameness on milk production in dairy cows. J. Dairy Sci. 84:1988–1997. doi:10.3168/jds.S0022-0302(01)74642-5.

Wathes, D.C., Z. Cheng, N. Bourne, V.J. Taylor, M.P. Coffey, and S. Brotherstone. 2007. Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. Domest. Anim. Endocrinol. 33:203–225. doi:10.1016/j.domaniend.2006.05.004.

Wathes, D.C., Z. Cheng, M.A. Fenwick, R. Fitzpatrick, and J. Patton. 2011. Influence of energy balance on the somatotrophic axis and matrix metalloproteinase expression in the endometrium of the postpartum dairy cow. Reproduction 141:269–281. doi:10.1530/REP-10-0177.

Watson, C., M. Barden, B.E. Griffiths, A. Anagnostopoulos, H.M. Higgins, C. Bedford, S. Carter, A. Psifidi, G. Banos, and G. Oikonomou. 2022. Prospective cohort study of the association between early lactation mastitis and the presence of sole ulcers in dairy cows. Vet. Rec. e1387. doi:10.1002/vetr.1387.

Weber, A., E. Stamer, W. Junge, and G. Thaller. 2013. Genetic parameters for lameness and claw and leg diseases in dairy cows. J. Dairy Sci. 96:3310–3318. doi:10.3168/jds.2012-6261.

Webster, A.J.F. 2001. Effects of Housing and Two Forage Diets on the Development of Claw Horn Lesions in Dairy Cows at First Calving and in First Lactation. Vet. J. 162:56–65. doi:10.1053/tvjl.2001.0569.

Webster, A.J.F. 2002. Effects of housing practices on the development of foot lesions in dairy heifers in early lactation. Vet. Rec. 151:9–12. doi:10.1136/vr.151.1.9.

Webster, J. 2020. Understanding the Dairy Cow. 3rd ed. John Wiley & Sons, Ltd, Hoboken, NJ, USA.

Weigel, K.A., P.C. Hoffman, W. Herring, and T.J. Lawlor. 2012. Potential gains in lifetime net merit from genomic testing of cows, heifers, and calves on commercial dairy farms. J. Dairy Sci. 95:2215–2225. doi:10.3168/jds.2011-4877.

Weigel, K.A., R.W. Palmer, and D.Z. Caraviello. 2003. Investigation of Factors Affecting Voluntary and Involuntary Culling in Expanding Dairy Herds in Wisconsin using Survival Analysis. J. Dairy Sci. 86:1482–1486. doi:10.3168/jds.S0022-0302(03)73733-3.

Weigel, K.A., P.M. VanRaden, H.D. Norman, and H. Grosu. 2017. A 100-Year Review: Methods and impact of genetic selection in dairy cattle—From

daughter–dam comparisons to deep learning algorithms. J. Dairy Sci. 100:10234–10250. doi:10.3168/jds.2017-12954.

van der Werf, J., and J. Pryce. 2019. Advances in Breeding Dairy Cattle. 1st ed. Burleigh Dodds Science Publishing, Cambridge, UK.

Westerhuis, J.A., H.C.J. Hoefsloot, S. Smit, D.J. Vis, A.K. Smilde, E.J.J. Velzen, J.P.M. Duijnhoven, and F.A. Dorsten. 2008. Assessment of PLSDA cross validation. Metabolomics 4:81–89. doi:10.1007/s11306-007-0099-6.

Whay, H. 2002. Locomotion scoring and lameness detection in dairy cattle. In Pract. 24:444–449. doi:10.1136/inpract.24.8.444.

Whay, H.R., D.C.J. Main, L.E. Green, and A.J.F. Webster. 2003a. Assessment of the welfare of dairy cattle using animal-based measurements: Direct observations and investigation of farm records. Vet. Rec. 153:197–202. doi:10.1136/vr.153.7.197.

Whay, H.R., D.C.J. Main, L.E. Green, and A.J.F. Webster. 2003b. Animal-based measures for the assessment of welfare state of diary cattle, pigs and laying hens: Consensus of expert opinion. Anim. Welf. 12:205–217.

Whay, H.R., and J.K. Shearer. 2017. The Impact of Lameness on Welfare of the Dairy Cow. Vet. Clin. N. Am. - Food Anim. Pract. 33:153–164. doi:10.1016/j.cvfa.2017.02.008.

Whay, H.R., A.E. Waterman, and A.J.F. Webster. 1997. Associations between locomotion, claw lesions and nociceptive threshold in dairy heifers during the peri-partum period. Vet. J. 154:155–161. doi:10.1016/S1090-0233(97)80053-6.

Whay, H.R., A.E. Waterman, A.J.F. Webster, and J.K. O'Brien. 1998. The influence of lesion type on the duration of hyperalgesia associated with hindlimb lameness in dairy cattle. Vet. J. 156:23–29. doi:10.1016/S1090-0233(98)80058-0.

Wickham, H., M. Averick, J. Bryan, W. Chang, L.D. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T.L. Pedersen, E. Miller, S.M. Bache, K. Müller, J. Ooms, D. Robinson, D.P. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, and H. Yutani. 2019. Welcome to the Tidyverse. J. Open Source Softw. 4:1686. doi:10.21105/joss.01686.

Wiggans, G.R., J.B. Cole, S.M. Hubbard, and T.S. Sonstegard. 2017. Genomic Selection in Dairy Cattle: The USDA Experience. Annu. Rev. Anim. Biosci. 5:309–327. doi:10.1146/annurev-animal-021815-111422.

Wiggans, G.R., T.S. Sonstegard, P.M. VanRaden, L.K. Matukumalli, R.D. Schnabel, J.F. Taylor, F.S. Schenkel, and C.P. Van Tassell. 2009. Selection of single-nucleotide polymorphisms and quality of genotypes used in genomic

evaluation of dairy cattle in the United States and Canada. J. Dairy Sci. 92:3431–3436. doi:10.3168/jds.2008-1758.

Wilhelm, K., J. Wilhelm, and M. Fürll. 2017. Claw disorders in dairy cattle – an unexpected association between energy metabolism and sole haemorrhages. J. Dairy Res. 84:54–60. doi:10.1017/S0022029916000510.

Willett, W., J. Rockström, B. Loken, M. Springmann, T. Lang, S. Vermeulen, T. Garnett, D. Tilman, F. DeClerck, A. Wood, M. Jonell, M. Clark, L.J. Gordon, J. Fanzo, C. Hawkes, R. Zurayk, J.A. Rivera, W.D. Vries, L.M. Sibanda, A. Afshin, A. Chaudhary, M. Herrero, R. Agustina, F. Branca, A. Lartey, S. Fan, B. Crona, E. Fox, V. Bignet, M. Troell, T. Lindahl, S. Singh, S.E. Cornell, K.S. Reddy, S. Narain, S. Nishtar, and C.J.L. Murray. 2019. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. The Lancet 393:447–492. doi:10.1016/S0140-6736(18)31788-4.

Wilson, J.P. 2021. Furthering our Understanding of the Aetiopathogenesis of Claw Horn Lesions. Doctoral dissertation Thesis. University of Nottingham,.

Wilson, J.P., M.J. Green, L.V. Randall, C.S. Rutland, N.J. Bell, H. Hemingway-Arnold, J.S. Thompson, N.J. Bollard, and J.N. Huxley. 2022. Effects of routine treatment with nonsteroidal anti-inflammatory drugs at calving and when lame on the future probability of lameness and culling in dairy cows: A randomized controlled trial. J. Dairy Sci. 0. doi:10.3168/jds.2021-21329.

Wilson, J.P., L.V. Randall, M.J. Green, C.S. Rutland, C.R. Bradley, H.J. Ferguson, A. Bagnall, and J.N. Huxley. 2021. A history of lameness and low body condition score is associated with reduced digital cushion volume, measured by magnetic resonance imaging, in dairy cattle. J. Dairy Sci. 0. doi:10.3168/jds.2020-19843.

Wiltbank, M., H. Lopez, and R. Sartori. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 65:17–29. doi:10.1016/j.theriogenology.2005.10.003.

Winkler, B., and J.K. Margerison. 2012. Mechanical properties of the bovine claw horn during lactation. J. Dairy Sci. 95:1714–1728. doi:10.3168/jds.2011-4489.

Wishart, D.S. 2008. Metabolomics: applications to food science and nutrition research. Trends Food Sci. Technol. 19:482–493. doi:10.1016/j.tifs.2008.03.003.

Wishart, D.S. 2019. Metabolomics for Investigating Physiological and Pathophysiological Processes. Physiol. Rev. 99:1819–1875. doi:10.1152/physrev.00035.2018.

Wray, N.R., J. Yang, B.J. Hayes, A.L. Price, M.E. Goddard, and P.M. Visscher. 2013. Pitfalls of predicting complex traits from SNPs. Nat. Rev. Genet. 14:507–515. doi:10.1038/nrg3457.

Wright, M.N., and A. Ziegler. 2017. ranger: A Fast Implementation of Random Forests for High Dimensional Data in C++ and R. J. Stat. Softw. 77:1–17. doi:10.18637/jss.v077.i01.

Wu, X., B. Guldbrandtsen, M.S. Lund, and G. Sahana. 2016. Association analysis for feet and legs disorders with whole-genome sequence variants in 3 dairy cattle breeds. J. Dairy Sci. 99:7221–7231. doi:10.3168/jds.2015-10705.

Xia, J., D.I. Broadhurst, M. Wilson, and D.S. Wishart. 2013. Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics Off. J. Metabolomic Soc. 9:280–299. doi:10.1007/s11306-012-0482-9.

Yang, L., and H. Xia. 2021. TRIM Proteins in Inflammation: from Expression to Emerging Regulatory Mechanisms. Inflammation 44:811–820. doi:10.1007/s10753-020-01394-8.

Yang, S., J. Wen, S.T. Eckert, Y. Wang, D.J. Liu, R. Wu, R. Li, and X. Zhan. 2020. Prioritizing genetic variants in GWAS with lasso using permutation-assisted tuning. Bioinformatics 36:3811–3817. doi:10.1093/bioinformatics/btaa229.

Yong, K., Z.Z. Luo, Q. Luo, Q.W. Yang, Y.X. Huang, X.X. Zhao, Y. Zhang, and S.Z. Cao. 2021. Plasma metabolome alteration in dairy cows with left displaced abomasum before and after surgical correction. J. Dairy Sci. 104:8177–8187. doi:10.3168/jds.2020-19761.

Yu, S.-L., S.-M. Lee, M.-J. Kang, H.-J. Jeong, B.-C. Sang, J.-T. Jeon, and J.-H. Lee. 2009. Identification of Differentially Expressed Genes Between Preadipocytes and Adipocytes Using Affymetrix Bovine Genome Array. J. Anim. Sci. Technol. 51:443–452. doi:10.5187/JAST.2009.51.6.443.

Zenger, K.R., M.S. Khatkar, J.A.L.L. Cavanagh, R.J. Hawken, and H.W. Raadsma. 2007. Genome-wide genetic diversity of Holstein Friesian cattle reveals new insights into Australian and global population variability, including impact of selection. Anim. Genet. 38:7–14. doi:10.1111/j.1365-2052.2006.01543.x.

Zhang, G., E. Dervishi, G. Zwierzchowski, R. Mandal, D.S. Wishart, and B.N. Ametaj. 2020a. Urinary Metabolomics around Parturition Identifies

Metabolite Alterations in Dairy Cows Affected Postpartum by Lameness: Preliminary Study. Dairy 1:2. doi:10.3390/dairy1010002.

Zhang, G., D. Hailemariam, E. Dervishi, Q. Deng, S.A. Goldansaz, S.M. Dunn, and B.N. Ametaj. 2015. Alterations of Innate Immunity Reactants in Transition Dairy Cows before Clinical Signs of Lameness. Animals 5:717–747. doi:10.3390/ani5030381.

Zhang, G., G. Zwierzchowski, R. Mandal, D.S. Wishart, and B.N. Ametaj. 2020b. Serum metabolomics identifies metabolite panels that differentiate lame dairy cows from healthy ones. Metabolomics 16:73. doi:10.1007/s11306-020-01693-z.

Zhao, X.-J., X.-Y. Wang, J.-H. Wang, Z.-Y. Wang, L. Wang, and Z.-H. Wang. 2015. Oxidative Stress and Imbalance of Mineral Metabolism Contribute to Lameness in Dairy Cows. Biol. Trace Elem. Res. 164:43–49. doi:10.1007/s12011-014-0207-1.

Zheng, J., L. Sun, S. Shu, K. Zhu, C. Xu, J. Wang, and H. Wang. 2016. Nuclear magnetic resonance-based serum metabolic profiling of dairy cows with footrot. J Vet Med Sci 78:1421–1428. doi:10.1292/jvms.15-0720.

Zhou, J., J. Wan, X. Gao, X. Zhang, S.R. Jaffrey, and S.-B. Qian. 2015. Dynamic m(6)A mRNA methylation directs translational control of heat shock response.. Nature 526:591–594. doi:10.1038/nature15377.

Zhou, X., and M. Stephens. 2012. Genome-wide efficient mixed-model analysis for association studies. Nat. Genet. 44:821–824. doi:10.1038/ng.2310.

Zhou, X., and M. Stephens. 2014. Efficient multivariate linear mixed model algorithms for genome-wide association studies. Nat. Methods 11:407–409. doi:10.1038/nmeth.2848.

Zimin, A. V., A.L. Delcher, L. Florea, D.R. Kelley, M.C. Schatz, D. Puiu, F. Hanrahan, G. Pertea, C.P. Van Tassell, T.S. Sonstegard, G. Marçais, M. Roberts, P. Subramanian, J.A. Yorke, and S.L. Salzberg. 2009. A whole-genome assembly of the domestic cow, Bos taurus. Genome Biol. 10:R42. doi:10.1186/gb-2009-10-4-r42.

Zinicola, M., C.P. Batista, L. Bringhenti, E.B.S. Meira, F.S. Lima, S.P. McDonough, and R.C. Bicalho. 2019a. Effects of recombinant bovine interleukin-8 (rbIL-8) treatment on health, metabolism, and lactation performance in Holstein cattle IV: Insulin resistance, dry matter intake, and blood parameters. J. Dairy Sci. 102:10340–10359. doi:10.3168/jds.2019-16337.

Zinicola, M., H. Korzec, A.G.V. Teixeira, E.K. Ganda, L. Bringhenti, A.C.C.H. Tomazi, R.O. Gilbert, and R.C. Bicalho. 2018. Effects of pegbovigrastim administration on periparturient diseases, milk production, and

reproductive performance of Holstein cows. J. Dairy Sci. 101:11199–11217. doi:10.3168/jds.2018-14869.

Zinicola, M., P.R. Menta, B.L. Ribeiro, Y. Boisclair, and R.C. Bicalho. 2019b. Effects of recombinant bovine interleukin-8 (rbIL-8) treatment on health, metabolism, and lactation performance in Holstein cattle III: Administration of rbIL-8 induces insulin resistance in bull calves. J. Dairy Sci. 102:10329–10339. doi:10.3168/jds.2019-16336.

Zlobin, A.S., P.S. Nikulin, N.A. Volkova, N.A. Zinovieva, B.S. Iolchiev, V.A. Bagirov, P.M. Borodin, T.I. Aksenovich, and Y.A. Tsepilov. 2021. Multivariate Analysis Identifies Eight Novel Loci Associated with Meat Productivity Traits in Sheep. Genes 12:367. doi:10.3390/genes12030367.

Zwald, N.R., K.A. Weigel, Y.M. Chang, R.D. Welper, and J.S. Clay. 2004. Genetic Selection for Health Traits Using Producer-Recorded Data. I. Incidence Rates, Heritability Estimates, and Sire Breeding Values. J. Dairy Sci. 87:4287–4294. doi:10.3168/jds.S0022-0302(04)73573-0.

Zwierzchowski, G., G. Zhang, R. Mandal, D.S. Wishart, and B.N. Ametaj. 2020. Milk Metabotyping Identifies Metabolite Alterations in the Whole Raw Milk of Dairy Cows with Lameness. J. Agric. Food Chem. 68:4507–4514. doi:10.1021/acs.jafc.9b08312.

Supplementary materials

All supplementary materials are available at this repository:

https://data.mendeley.com/datasets/w82dw5skn8/1

Chapter 5

Supplementary Table 5.1. Results of univariable analysis with Wilcoxon signed-rank tests. Data were analysed in 17 prespecified subsets split by time point, parity, and outcome definition; the control group in all cases were animals without sole lesions, cases were either cases of sole new haemorrhage (New SH), cases of new sole ulcers (New SU), those two groups combined (New SH/SU) or all cases of sole ulcers (All SU). *P*-values were corrected for multiple testing within each subset with a Bonferroni correction to (i.e. 0.05/number of variables).

Supplementary Table 5.2. Variable selection stability including the mean log (base 2) fold-change (Log2FC) and the baseline stability thresholds (T_{99} and T_{100}, equivalent to an expected 1% and 0% false positive rate, respectively). Data were analysed in 17 prespecified subsets split by time point, parity, and outcome definition; the control group in all cases were animals without sole lesions, cases were either cases of sole new haemorrhage (New SH), cases of new sole ulcers (New SU), those two groups combined (New SH/SU) or all cases of sole ulcers (All SU).

Chapter 6

Supplementary Table 6.1. Pearson correlation between genetic indexes.

Supplementary Table 6.2. Multivariable logistic regression screening for associations between all genetic indexes and claw horn lesion presence or lameness (N = 2,107). Results are presented as the odds ratio and 95% confidence interval for the genetic index, adjusted for parity and herd.