GENOTYPIC AND PHENOTYPIC ASSOCIATIONS WITH DIGITAL CUSHION THICKNESS IN DAIRY CATTLE

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GENOTYPIC AND PHENOTYPIC ASSOCIATIONS WITH DIGITAL CUSHION THICKNESS IN DAIRY CATTLE

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The bovine digital cushion is a compression pad between the distal phalanx and sole. Digital cushion thickness (DCT) is associated with lameness and claw horn disruption lesions (CHDL) and estimated to be moderately heritable. This dissertation focuses on understanding DCT phenotypically and genotypically to evaluate whether DCT has an opportunity to be utilized for indirect selection against lameness and CHDL.

To investigate whether DCT varied between digits, across lactation within the cow, or could be a predictor of CHDL or lameness, we collected DCT measurements at 4 time points across lactation for 183 Holstein cows from one farm. Digital cushion thickness varied within primiparous and multiparous cows based on stage in lactation and digit. Parity group and early lactation DCT measurements were predictors of CHDL and lameness during the subsequent lactation.

Applying our results, we sampled 502 Holstein cows from 5 farms at 2 time points corresponding to when DCT was thickest and thinnest. The phenotypic results indicated DCT varied by time point, sacral height, parity, claw, farm, body condition score group (BCSG), and wither height. Genome-wide association studies were conducted to explore associations of genetic markers with DCT. From the associated markers, *MC4R* and *DLG2* were identified as putative genes related to fat deposition and bone growth.

To characterize DCT across multiple breeds and sexes, we sampled 698 cows and 85 bulls (Holstein and Jersey) from 8 farms. The phenotypic results indicated DCT for cows varied by breed, age, and digit; DCT for bulls varied by breed, age, digit, and BCSG. Genome-wide association studies were conducted on 9 datasets either separating or combining breed and sex.

From the associated markers, *MC4R, SFRS18,* and *LRRFIP1* function in fat deposition, *DLG2*, *AHR*, *BZW2*, *EFNA5*, *USP45*, and *VAV3* in bone remodeling, and *SOSTDC1* in epidermal keratinocyte function.

Breeding programs can incorporate the markers from this work for marker assisted selection to reduce CHDL and lameness. Producers can apply the phenotypic results to adjust management practices or monitor animals with higher odds of developing CHDL and lameness. The results from this work will hopefully lead to future studies to identify the causal variants of DCT.

BIOGRAPHICAL SKETCH

Cassandra Stambuk grew up in Yorba Linda, California. She has always had a passion for animals, especially horses. Cassandra has had the opportunity to compete with her horses on a national level for years and has garnered many achievements and awards. She continues to ride and compete when she gets the time. Driven by her love of horses, Ms. Stambuk decided to travel across the country to Auburn University in Alabama where she earned her Bachelor of Science in Animal Science with a pre-veterinary option in 2014.

During her education at Auburn, she developed an interest in livestock species and their role in feeding the world. Cassandra never truly saw herself as a veterinarian, so she reached out to Dr. Terry Brandebourg, an assistant professor whose work focused on growth and development of adipose tissue, to gain some lab and research experience with his Mangalitsa pigs. Due to this experience and her history of genetic studies on horse coat color for science fairs, she decided to pursue a PhD at Cornell University in Animal Science studying dairy cattle genetics in Dr. Heather Huson's laboratory. Her research focused on evaluating lameness through use of digital cushion thickness in dairy cattle on commercial dairy farms in Upstate New York and the genetics underlying digital cushion thickness as a potential trait to include in genomic evaluations to reduce lameness, hence improving animal welfare and productivity.

Ms. Stambuk aspires for a career in the agriculture industry where she will be able not only to improve the quality of life of livestock species at the animal level, but also develop relationships with producers such that she is able to work with them and give valuable advice based on their personal farm systems. Upon graduation, Cassandra will commence a post-doctoral position in the lab of Dr. Anna Johnson at Iowa State University. In her spare time, Cassandra will continue to own and ride horses, hopefully having the time to get back into the competition ring.

For my Dida (grandfather), Dr. Uros Stambuk, who always believed and supported me in all my endeavors, which included horses. He was a man of faith and a servant of God. Cheers Dida. Your wisdom inspired a member of the younger Stambuk generation to earn a doctorate. I know you are smiling down on me with that wonderful grin of yours. Volim te.

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Heins for pushing me to be a great scientist and critical thinker, while also being patient with me. I may not understand everything the first time around, but I try and try again until I succeed. Dr. Cherney, thank you for your unwavering support and imparting on me your passion for animal welfare. Dr. McArt, thank you for always reminding me to think of the practicality of my research because the people I want to benefit most from my results are the farmers. Dr. Heins, thank you for not only your advice on statistical modelling, but also for always being there for me and having confidence in me.

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CHAPTER 1: INTRODUCTION

The digital cushion of any hoofed animal, particularly cattle, is an important structure consisting of adipose fat and connective tissue that extends forward from the heel bulb between the distal phalanx and the sole horn (Räber et al., 2004). Digital cushion thickness (DCT) is a strong predictor of lameness and is associated with the prevalence of two types of claw horn lesions: sole ulcers and white line disease (Bicalho et al., 2009). Lameness and claw horn lesions represent an animal welfare concern due to the pain the animals may experience (Vermunt, 2007; von Keyserlingk et al., 2009). Lameness also results in substantial economic loss: the cost to the US dairy industry from sole ulcers and white line disease alone amounts to approximately $467 million (using the estimates of 9.4 million dairy cows with a 23% incidence and a cost of $216 per case; Bicalho et al., 2008; Cha et al., 2010; USDA National Agricultural Statistics Services, 2019). Because DCT is associated with two major types of claw horn lesions, the approach of this dissertation for tackling lameness in the dairy industry is through characterization of, and genetic selection for, DCT. The overall goal of this dissertation is to improve upon the phenotypic knowledge of DCT and generate novel diagnostic markers of DCT in order to combat dairy cattle lameness on farms through improved accuracy of genetic selection.

LAMENESS AND ITS CONSEQUENCES

Lameness is defined as the clinical presentation of impaired locomotion and is the result of a combination of factors such as infectious agents, claw disorders, injury, housing, environment, management, and genetics (Vermunt and Greenough, 1994; Archer et al., 2010; Shearer et al., 2012). Lameness in dairy cattle greatly contributes to economic loss for the producers and dairy industry. The early symptoms of lameness disorders are subtle, which makes it difficult for producers to observe or recognize them in a timely manner; thus, lameness

disorders are often neglected until they become severe (Whay et al., 2003). Detection is also impaired by the cow's natural instinct to disguise her discomfort (Shearer et al., 2012). Ultimately, failure of early detection leads to treatment delays and the development of more serious conditions that increase animal suffering and monetary costs. Lameness was determined to be the second most costly disease in the dairy industry after mastitis (Kossaibati and Esslemont, 1997). The mean prevalence of lameness ranged from 14 to 37% for intensely managed dairy cows worldwide (Cook and Nordlund, 2009; Barker et al., 2010; Bennett et al., 2014; Foditsch et al., 2016). Cha et al. (2010) determined the average cost per case for lameness based on sole ulcers, digital dermatitis, and foot rot to be $216, $133, and $121, respectively. The cost to the producer not only results from the treatments but also the relationships between lameness and decreased milk production, decreased fertility, increased postpartum health problems, and early culling (Enting et al., 1997; Kossaibati and Esslemont, 1997; Bruijnis et al., 2010; Vergara et al., 2014).

Lameness in dairy cattle is not only a significant cause of economic loss but also a serious animal welfare concern. According to Vermunt (2007), lameness is one of the most important welfare issues of high-producing dairy cows in North America. Animal welfare is an area for improvement in the agricultural industry beneficial to both animals and producers. While not new, this concern has more recently focused on the pain or distress that animals might experience as a result of widely accepted management practices (von Keyserlingk et al., 2009). Whay et al. (1998) demonstrated that lame cows were hyperalgesic, exhibiting an exaggerated sensitivity to pain, even up to 28 days after treatment. Pain associated with foot disorders causes impaired health and functioning, suffering, and affects the cows' ability to perform natural behavior (Bruijnis et al., 2012). There is also a growing concern for the wellbeing of animals in

response to the demand of consumers for welfare-certified products (Bicalho et al., 2009). Consumers have become more concerned about the origin of their food and the welfare of livestock (Bicalho et al., 2007; Machado et al., 2011). Lameness has been determined to be the most representative animal-based indicator of compromised welfare in dairy cattle because it is the most visible (Whay et al., 2003). Preventing lameness is one of the most crucial steps to reduce the negative welfare implications of the dairy industry and lessen costs for farmers.

Claw Disorders and Lameness

Claw disorders account for 70 to 90% of lameness in dairy cattle (Vermunt and Greenough, 1994; Murray et al., 1996; Shearer et al., 2012). Disorders affecting the bovine claw are split into two groups: infectious and noninfectious. Infectious disorders are those caused by infectious agents such as bacteria or fungi, while noninfectious disorders are those related to the physiology of the claw (Shearer and van Amstel, 2011). Three of the most common infectious lesions are digital dermatitis , interdigital dermatitis, and foot rot. Infectious lesions affect the skin of the interdigital space, heel bulbs, and interdigital cleft and are often due to housing and environmental hygiene (Toussaint Raven, 1989; Shearer and van Amstel, 2011). Two of the most common noninfectious lesions are sole ulcers and white line disease. Noninfectious lesions affect the claw horn integrity and are predisposed by metabolic and mechanical factors (Shearer and van Amstel, 2011). This chapter delves deeper into a discussion of sole ulcers and white line disease because of their relationship with DCT.

Sole ulcers and white line disease are collectively known as claw horn disruption lesions (CHDL) because, even though they may result from multiple etiologies, the response of the keratinocytes is relatively nonspecific, causing lesions to appear the same regardless of cause (Hoblet and Weiss, 2001). Together they account for over 65% of all lesions diagnosed in lame

cows with a combined incidence of around 23% (Murray et al., 1996; Bicalho et al., 2007; Bicalho et al., 2008). Risk factors associated with CHDL include subclinical laminitis (inflammation of the corium of the claw), hormonal changes around parturition, claw horn quality, environment, facilities and cow comfort, hoof trimming practices, feeding and nutrition management, other diseases, and individual cow characteristics such as age, milk yield, conformation, and genetics (Toussaint Raven 1989; Vermunt and Greenough, 1994; Hoblet and Weiss, 2001; Amory et al., 2008; Bicalho and Oikonomou, 2013).

Ulcers. Ulcers are defined as a full-thickness defect or break in the epidermis, which exposes the corium (Figure 1.1; Shearer and van Amstel, 2011; Shearer et al., 2015). Sole ulcers develop when the displacement of the distal phalanx compresses the corium between the flexor tuberosity of the distal phalanx and the sole horn (Toussaint Raven, 1989). This displacement is a result of a compromised suspensory apparatus allowing the distal phalanx to move more excessively (Lischer et al., 2002). Many factors contribute to the development of sole ulcers. Displacement of the distal phalanx may be attributed to metabolic factors such as rumen acidosis and laminitis, as well as the activation of metalloproteinase-2 by the enzyme hoofase, and changes in the levels of estrogen and relaxin around the time of calving (Tarlton et al., 2002; Shearer and van Amstel, 2011). Mechanical factors, such as abnormal overgrowth of the claw horn, can cause changes to the distribution of weight within and between claws and may contribute to the sinking and rotation of the distal phalanx (Toussaint Raven, 1989).

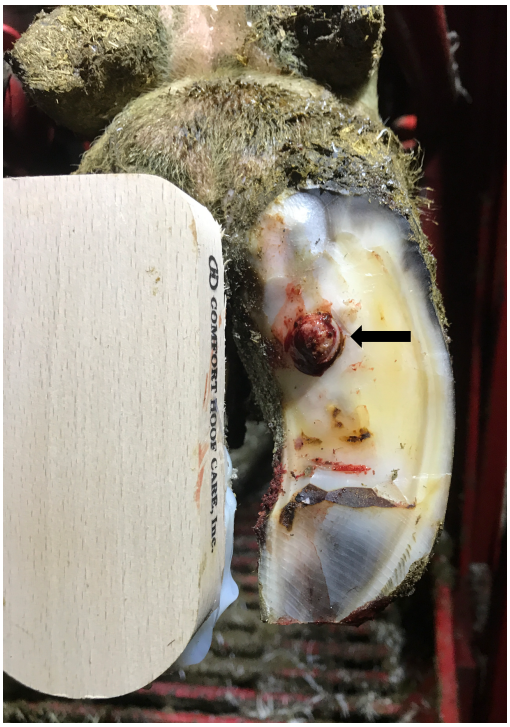

Figure 1.1 Image of a sole ulcer at the typical sole ulcer site beneath the flexor tuberosity in the medial aspect of the middle pad indicated by the black arrow.

White Line Disease. The white line is produced by laminar corium and is characterized by an outer, intermediate, and inner zone (Mülling, 2002). The outer and intermediate zones consist of laminar horn while the inner zone is a combination of laminar horn and loosely arranged tubules. The white line is the softest and least resistant part of the claw capsule easily damaged by mechanical forces and penetration by bacteria or foreign objects. White line disease is thus a result of the breakdown of the horn joining the sole and horn wall (Figure 1.2; Archer et al., 2010). In mild cases, white line lesions begin as cracks causing a slight separation between the sole and the hoof wall that become filled with stones or other organic matter. However, in severe cases, the lesion becomes infected causing an abscess to form inducing severe lameness (Shearer and van Amstel, 2011). White line disease can begin as a consequence of the same metabolic and mechanical factors as sole ulcers. However, mechanical and physical forces seem

to have a more important role in the development of white line disease, particularly related to bruising from uneven hard surfaces and flight movements during cow flow (Archer et al., 2010).

Figure 1.2 Image of white line disease causing an abscess from the breakdown of the horn joining the sole and hoof wall indicated by the black oval.

DIGITAL CUSHION

The bottom of the distal phalanx is supported by the digital cushion above the sole wall (Lischer et al., 2002). The digital cushion is a complex structure composed mostly of adipose and connective tissue (Räber et al., 2004). Approximately 90% of the digital cushion lies behind the navicular bone in the heel area while only 10% extends forward beneath the distal phalanx (Mülling and Greenough, 2006b). It is an important structure in absorbing and dissipating forces during weight bearing and protecting the germinal epithelium that produces the sole horn (Lischer et al., 2002; Räber et al., 2004).

The distal phalanx is suspended and held into position in the claw capsule by collagen fibers attached to the epidermal lamellae lining the hoof wall, known as the suspensory apparatus. Due to the fact that the bovine suspensory apparatus is less well developed than the

equine, the digital cushion must support a much greater proportion of the body weight (Räber et al., 2004). The suspensory apparatus and digital cushion are essential in the biomechanics of movement. For example, while a cow is walking, the ground contact and load on the hind foot begins at the proximal area of the heel causing the claw walls to expand sideways and the digital cushion to absorb most of the pressure as it is transferred forward towards the toe (Räber et al., 2004). The retinaculum of collagen fibers enveloping the digital cushion holds the cushion together and assists in restoring the cushion to its normal configuration once the compressive force is reduced (Mülling and Greenough, 2006b). However, physiological and hormonal changes around the time of calving make the suspensory apparatus within the claw lax, causing the distal phalanx to sit lower in the hoof capsule exerting more pressure on the digital cushion (Tarlton et al., 2002; Knott et al., 2007; Newsome et al., 2017). The mechanical load, particularly around first parturition, is considered a predisposing factor to changes in the suspensory apparatus and digital cushion because it increases disproportionately to the bearing surface of the hind claws due to the rapid weight gain of the udder and the fetus (Räber et al., 2006). Hormonal changes include increased estrogen and relaxin (Tarlton et al., 2002). Relaxin is known to distend the reproductive tract for parturition and can have an effect on other structures throughout the body involving connective tissue, likely affecting the digital cushion (Tarlton et al., 2002; Newsome et al., 2017).

A major factor affecting the composition of the digital cushion is age. Räber et al. (2004) discovered that the amount of adipose tissue in the digital cushion of primiparous heifers was less than in cows of two or three parities, with the amount reducing again after three parities. Primiparous heifers had cushions containing more white resilient rubbery loose connective tissue, while cows of two or three parities had cushions with more smooth yellow adipose tissue.

After three parities, the digital cushion contained more collagenous connective tissue (Räber et al., 2004). Multiparous cows also had significantly higher lipid content compared with primiparous heifers, and the fatty acid composition differed between cows and heifers (Räber et al., 2006). Additionally, the digital cushion of primiparous cows was thinner than that of multiparous cows at the typical sole ulcer site below the flexor tuberosity of the distal phalanx (Bicalho et al., 2009). Interestingly, rearing differences can have an influence on the size of the digital cushion. When calves were challenged with exercise before six months of age, their digital cushions had a greater volume and surface area than the calves that did not exercise (Gard et al., 2015).

Another factor affecting the digital cushion is differences in weight distribution. The weight distribution is relatively equal between the two digits in the forelimbs, as opposed to localizing mostly to the lateral claw in the hind limbs (Van der Tol et al., 2003). It is known that 60% of a cow's body weight is borne by the forelimbs, but in a high-producing cow near peak lactation, it is nearer to 50% on the hind claws (Mülling and Greenough, 2006b). The hind claws are significantly smaller than the front claws and the digital cushions of the front claws have been shown to contain more fat. Particularly in the front feet, the digital cushion in the lateral claws contained more fat than the medial claws, while the opposite was true in the hind feet (Räber et al., 2004). Furthermore, the hind medial claw is frequently smaller than its lateral counterpart partly because prolonged exposure to concrete surfaces causes the solear surface of the hind lateral claw to flatten and increase in width, changing the dynamics inside the claw. Instead of weight bearing being confined to the wall, part of the weight load is transferred to the central part of the sole creating abnormal pressure on the dermis of the sole (Mülling and

Greenough, 2006a). These observations support the conclusion that increased load correlates with decreasing fat content (Räber et al., 2004).

A third factor affecting DCT is the loss of body condition. Digital cushion thickness is positively associated with body condition score (BCS) such that DCT increases as BCS increases (Bicalho et al., 2009; Machado et al., 2011). Dairy cows experience loss of BCS in the early lactation period as a result of mobilizing adipose tissue toward the mammary gland to support milk production, especially during negative energy balance (Rastani et al., 2001). It is biologically plausible that lactating dairy cows are not only mobilizing adipose tissue from other parts of the body, such as subcutaneous fat and muscle, but also from the digital cushion, because it is mainly composed of adipose tissue (Bicalho et al., 2009; Newsome et al., 2017). Loss of body condition has also been reported to precede the onset of lameness measured by visual detection and CHDL treatment (Green et al., 2014; Lim et al., 2015; Randall et al., 2015).

The Relationship between DCT and CHDL

Digital cushion thickness has been reported to be a strong predictor of lameness and CHDL (Bicalho et al., 2009; Machado et al., 2011; Newsome et al., 2017). Cows in the highest quartile of DCT have an adjusted prevalence of lameness 15 percentage points lower than the lowest quartile, and cows with low DCT of the hind limbs are at a higher risk of sole ulcers and white line disease (Bicalho et al., 2009). Cows diagnosed with a CHDL in the subsequent lactation had significantly lower DCT at dryoff compared to non-affected cows (Machado et al., 2011). Cows that developed sole ulcers had thinner sole soft tissues than those that did not develop sole ulcers, except when the sole ulcer was present, possibly due to inflammation of the underlying tissues (Newsome et al., 2017). Sole ulcers and white line diseases were reported to be the most prevalent noninfectious claw lesions observed in lactating dairy cattle; thus, it is

important to find solutions to decrease their prevalence, such as genetically selecting for thicker DCT (Manske et al., 2002; Oikonomou et al., 2014). Oikonomou et al. (2014) determined the heritability of average DCT to be 0.33 ± 0.09 and the genetic correlation between CHDL and average DCT to be -0.60 ± 0.29 suggesting it is possible to genetically select for DCT to decrease the prevalence of CHDL. Thus, genetically selecting for DCT has a larger potential for reducing lameness than targeting a single cause of lameness.

It is important to understand there are other sources affecting lameness, lesions, and the digital cushion than those mentioned. Historically, much importance was put on nutrition and feed affecting lameness and lesions (Vermunt and Greenough, 1994). However, recent studies focus on cow comfort, housing, functional hoof trimming, and the influence of parturition. Recent research has considered factors such as stocking density and time spent standing, flooring type, surface hygiene, adequate heat abatement, hormonal changes around parturition, and claw horn growth and wear (Manske et al., 2002; Tarlton et al., 2002; Cook and Nordlund, 2009). These influence of these environmental factors are important for understanding the complexity of lameness and lesions. The main focus of this dissertation is the genetic regulation of DCT and how changes over lactation may affect regulation.

BOVINE GENETICS

Through archaeology and genetic research, it has been established that modern cattle breeds are descended from multiple independent domestication events from wild aurochs (*Bos primigenius*) around 10,000 years ago. Two major cattle species, *Bos taurus* and *Bos indicus*, resulted from domestication events in the Middle East and the Indian subcontinent, respectively (MacHugh et al., 1997). Major phenotypic differences between taurine and indicine cattle are that indicine cattle have a hump at the withers and droopy ears and have adapted to tolerate

limited food and water and extreme heat compared with taurine breeds (Frisch and Vercoe, 1977; Grigson, 1991). European taurine cattle from the Middle East have been intensively selected for milk and meat production and ease of handling (McTavish et al., 2013). This dissertation focuses on two European *Bos taurus* breeds, Holstein and Jersey.

Bos taurus Genome Assembly

The bovine genome consists of 60 chromosomes: 29 pairs of autosomal chromosomes and two sex chromosomes, X and Y. In 1990, the Human Genome Project was initiated and completed in 2003 (Baylor College of Medicine Human Genome Sequencing Center, 2018b). Livestock genomics followed behind the human genome initiative, adapting its strategies and technologies with much smaller budgets. Livestock genomics adds to our knowledge of the human genome and contributes to our understanding of evolution (Womack, 2005). The Bovine Genome Project was initiated in 2003 and selected for sequencing due to the unique biology of ruminants, the importance of ruminants as a major source of food for humans, and social stability (Womack, 2005; Tellam et al., 2009; Wiggans et al., 2017).

In April 2009, the first two assemblies of the *Bos taurus* genome were published. The original sequencing was conducted by the Baylor College of Medicine Human Genome Sequencing Center and went through three iterations for improvement from 3X to 7.1X until the fourth assembly was released to the public in October 2007 (Baylor College of Medicine Human Genome Sequencing Center, 2018a; Zimin et al., 2009). The original whole-genome sequencing was mostly based on a single partially inbred Hereford cow named L1 Dominette 01449. Low-depth sequencing of individual animals from Holstein, Angus, Jersey, Limousin, Brahman, and Norwegian Red breeds were added to generate information for single-nucleotide polymorphism (SNP) assay development (Womack, 2005; Wiggans et al., 2017). The Bovine Genome

Sequencing and Analysis Consortium annotated and analyzed assemblies Btau_3.1 and Btau_4.0 for a publication in Science in 2009, while the Center for Bioinformatics and Computational Biology at the University of Maryland used the same genomic sequence data to generate an alternate assembly, UMD2, which was published in Genome Biology in 2009. Both assemblies used a mixture of whole-genome shotgun sequencing and bacterial artificial chromosome (BAC) sequences (The Bovine Genome Sequencing and Analysis Consortium et al., 2009; Zimin et al., 2009). The most recent assembly of the *Bos taurus* genome is ARS-UCD1.2, which was released in 2018 and used for this dissertation (NCBI, 2018).

The bovine genome consists of 2.86 billion base pairs (bp) in comparison to the 3.3 billion bp in the human genome (Zimin et al., 2009; Baylor College of Medicine Human Genome Sequencing Center, 2018b). The most common and economically viable high-throughput genotyping technology that efficiently gathers information on thousands of genetic markers at once is a SNP microarray chip (Pearson and Manolio, 2008). The first commercially available SNP chip was the Illumina BovineSNP50 BeadChip (Illumina, Inc, San Diego, CA) released in 2007 with 54,001 SNPs. In 2010, Illumina released the low-density Bovine3K chip with 2,900 SNPs and the BovineHD chip with 777,962 SNPs. In 2011, Illumina released its BovineLD chip with 6,909 SNPs. Since then, there have been a number of customized proprietary chips created based on the BovineLD chip (Wiggans et al., 2017). The Illumina SNP chips are bead arrays with different oligonucleotide sequences attached to each bead and thousands of beads on one chip. The complementary oligonucleotides that are present in the sample bind to the beads and are measured by using a fluorescent label (NCBI, 2017). There is an important balance between cost and potential use of the genetic information. For producers, SNP chips are economically viable and provide a vast amount of information, while researchers

prefer to have full coverage and test all genetic variants in relation to their phenotypes. As whole-genome sequencing (WGS) costs decrease, more researchers will use this technique instead of SNP chip panels because WGS provides more information by covering all nucleotides in the genome rather than specific SNPs across the genome (Wiggans et al., 2017; Wetterstrand, 2018).

Genome-Wide Association Studies

The goal of genome-wide association studies (GWAS) is to understand the variation in complex traits and diseases (Ball, 2013). These studies have been made possible through the sequencing of the bovine genome. They use the high-throughput genotyping technologies, most commonly SNPs, to relate the genetic variants to a particular trait or disease (Pearson and Manolio, 2008). Traits can be quantitative or dichotomous (Scherer and Christensen, 2016). The most common type of variant are SNPs which occur when there is a substitution of a single nucleotide that occurs at a specific position in the genome. Other variants include haplotypes that are an inherited series of markers and copy number variation where stretches of genomic sequence are deleted or duplicated in varying quantities among individuals (Nature.com, 2019). Genome-wide association studies rely on strong associations among SNPs located near each other on a chromosome. Alleles that tend to be inherited together more often than expected by chance are in linkage disequilibrium (LD) (Pearson and Manolio, 2008; Scherer and Christensen, 2016). The degree of LD in a population is determined by selection, recombination rate, and mutation rate, and non-zero levels of LD have been observed up to 1 Mb in cattle (Hayes, 2013; Scherer and Christensen, 2016). The SNPs evaluated in GWAS from SNP chips are typically not the causal variants for a phenotype. Instead, they can either contribute to the trait or are in LD with a causal variant (Wray et al., 2013). Thus far, there has not been a GWAS published in

literature for DCT. However, Iqbal et al. (2016) discovered nonpregnant dairy cows fed a higher energy diet had consistent upregulation of lipogenic genes within the digital cushion.

Despite the advantages of association studies, there are aspects that must be accounted for in the study design. These include sample size, the heritability and variation of the trait, statistical power, correction for multiple testing, and population structure (Scherer and Christensen, 2016). Livestock populations have undergone selection by humans, particularly in dairy cattle, through the practice of breeding to popular sires, thus creating population structures. It is important to account for these population structures because any unaccounted population structure will result in false-positives (Pritchard et al., 2000). One solution is to include principal components from principal components analysis (PCA) to stratify subjects based on genomic similarity (Scherer and Christensen, 2016). Another solution is to use the SNP markers themselves to infer the genomic relationship matrix which can be included as a random effect in mixed models, such as the efficient mixed-model association expedited (EMMAX). EMMAX is based on a linear mixed model with a genomic relationship matrix that estimates the contribution of the sample structure to the phenotype. Estimating the phenotypic variance contributed by the sample structure prevents overdispersion, undercorrection, or overcorrection (Kang et al., 2010; Hayes, 2013). EMMAX was used in all GWAS for this dissertation.

Statistical power and multiple test correction are very important for GWAS. False-positive associations are problematic with GWAS because thousands of SNPs are tested within the same model (Hayes, 2013). The most common solution to reduce the false-positive rate is by applying the Bonferroni correction where the P-value is divided by the number of tests performed. However, this correction is overly conservative because it assumes independent associations of each SNP with the trait when SNPs are known to be correlated to some degree

due to LD (Pearson and Manolio, 2008). Another way to reduce false-positives is using the false discovery rate (FDR) as a significance level which is the expected proportion of significant associations that are actually false-positive (Pearson and Manolio, 2008). Statistical power is the probability the GWAS will correctly reject the null hypothesis when a SNP effect does exist in the population (Hayes, 2013). Power is affected by the genetic complexity of the phenotype, the frequency of the disease allele, accuracy of phenotype measurements, and LD relationships between causal variants and genotyped SNPs (Scherer and Christensen, 2016). An insignificant result can occur due to no effect or inadequate statistical power (Sham and Purcell, 2014).

Validation and replication of results in an independent population is the only evidence that the significant association detected is truly associated (Wray et al., 2013). In livestock, the most convincing validation is across breeds because the population structure should be different. Although if the SNP fails, it could be because the SNP is not segregating in both breeds (Hayes, 2013). This dissertation evaluates DCT in and across two breeds, Holstein and Jersey. Once the associations between SNPs and a trait are validated, researchers may determine candidate genes near the SNP, study the function of the candidate genes, examine other variants in LD, evaluate gene expression in tissue samples or cell lines, and study the biological pathways related to the genes of interest (Pearson and Manolio, 2008; Scherer and Christensen, 2016).

It is important to remember that variation in a phenotype is the result of an interaction between genotype and the environment, especially with regards to complex traits (Wray et al., 2013). Genome-wide association studies are the first step in understanding the genetics underlying a trait; however, GWAS do not have information about environmental exposures and other non-genetic risk factors, which make it difficult to identify gene-environment interactions or alterations in the associations due to environmental factors (Pearson and Manolio, 2008;

Scherer and Christensen, 2016). Researchers are able to include environmental or non-genetic risk factors as covariates in GWAS that are known to be associated with the trait in order to control bias, increase power, and prevent false-positives (Mefford and Witte, 2012).

Genetic Predictions

Prior to genomics, much of the advancements in dairy cattle relied on pedigree information and phenotype performance data. Pedigree records can be traced back to the origin of breed societies in the late 1800s. The first U.S. association was created in 1905 to record milk weights and analyze butterfat. Then, in 1908, the Dairy Herd Improvement Association (DHIA) was formed by the United States Department of Agriculture (USDA) to organize local and state cow testing of milk and its components (Weigel et al., 2017). From the 1930s to the 1970s the sire received the most attention in selection because the heredity of milk production was most accurately indicated by his daughters' production (rather than the dams') (Graves, 1925; Miglior et al., 2017). Before the use of genomic selection, much of the improvement in dairy cattle was due to the global use of elite bulls through artificial insemination (AI) starting in the 1940s (Weigel et al., 2017). However, the improvement was limited due to the time it took for the bulls to be progeny-tested based on their daughter phenotypes. Bulls entered a progeny-test program to determine their breeding value around one year of age and were at least five years old when their semen could be marketed based on the progeny-test results (Wiggans et al., 2017). Through the use of genomic technologies, the generation interval has decreased drastically because animals can be genetically tested when they are born.

The discovery of decreased fertility and health in dairy cattle as a result of focusing only on milk production changed the outlook of the dairy industry towards one that puts more emphasis on nonproduction traits pertaining to health, behavior, function, and conformation,

such as feet and legs (Schrooten et al., 2000; Schmutz et al., 2001; Holmbeg and Andersson-Eklund, 2004). Genomic evaluations began following the assembly of the bovine genome and development of SNP chip assays to genetically select high performance individuals while also considering correlations between traits and economic value (Wiggans et al., 2017). However, not all animals are genotyped and are still selected using genetic evaluations based on pedigree and phenotype performance.

Genotypes for over 15,000 cattle were used to determine which SNPs should be used in U.S. genomic evaluations of dairy cattle and tested using genotypes of over 5,000 progeny-tested Holstein bulls (Wiggans et al., 2017). The first official USDA genomic evaluations were released January 2009 for Holsteins and Jerseys. Many other countries and breeds have since developed their own genomic evaluations. In 2013, the USDA transferred responsibilities of the genomic evaluations to the Council on Dairy Cattle Breeding (CDCB, Bowie, MD). Currently, there are over 1 million dairy cattle (predominately female) genotyped and included in genomic evaluations. As of 2017, 60,671 SNPs are used for U.S. genomic evaluations (Wiggans et al., 2017). The value of including a SNP as a predictor depends on the proportion of genetic variance the SNP explains (Wray et al., 2013).

Genetic evaluations combine multiple traits into a single numerical selection index or breeding value. The goal is to select high-performance individuals while also considering the correlations of traits to one another and the economic value for each trait. The primary performance index calculated by CDCB is lifetime net merit. All traits in the index are weighted the same regardless of breed. The three main categories of traits are production, health, and type/conformation. Net merit is the summation of the parent transmitting ability for each trait multiplied by the corresponding economic value (VanRaden et al., 2019). There are specific

indices for Holstein (known as TPI) and Jersey (known as JPI). Net merit, TPI, and JPI have the same main categories, but with different weights. For example, TPI weighs production at 46%, health and fertility at 28%, and conformation at 36% while JPI weighs production at 53%, health at 27%, and type at 20% (Bohnert, 2017; Holstein Association USA, 2019). There are a multitude of individual traits within each category. For example, production includes fat and protein while health includes productive life and daughter pregnancy rate (Bohnert, 2017; VanRaden et al., 2019; Holstein Association USA, 2019). This dissertation is focused on the type/conformation category, specifically the traits related to feet and legs. Net merit and TPI weigh feet and leg composite at 2.7% and 6%, respectively, while JPI weighs rear legs at -0.1% and foot angle at 0.1% (Bohnert, 2017; VanRaden et al., 2019; Holstein Association USA, 2019).

The conformation traits used to select against lameness in the US genetic evaluations are rear legs side view, rear legs rear view, foot angle, and feet and legs composite (Wiggans et al., 2017; VanRaden et al., 2019). However, other countries, such as Norway and the Netherlands, include claw health traits in the genetic evaluations, whether it be by lesion or grouped into laminitis-related lesions and infectious lesions. Studies on dairy cattle in these countries determined that using only feet and leg conformation traits as indicator traits for claw health is not an efficient approach for genetic improvement (van der Linde et al., 2010; Ødegård et al., 2014). It would be advantageous for U. S. genetic evaluations to include claw health traits and incorporate DCT as an indicator trait against lameness and CHDL, which is the main application of the results of this dissertation.

Current Genetics for Lameness

Feet and leg conformation traits in genomic evaluations are some of the most important indicators of productive life. If an animal has poor conformation affecting mobility, it has a

major adverse effect on longevity (Pérez-Cabal and Alenda, 2002). Feet and leg conformation traits are low to moderately heritable with ranges from 0.10 to 0.41 and can be used as indicators for lameness (Van Dorp et al., 1998; Pérez-Cabal and Alenda, 2002; van der Waaij et al., 2005; Buitenhuis et al., 2007). Heritability estimates for the common claw lesions defined as binary traits were low ranging from 0.01 to 0.14 (Heringstad et al., 2018). When grouped, infectious lesions had a higher heritability than noninfectious lesions and rear-leg lesions had a higher heritability than front-leg lesions (Chapinal et al., 2013; Dhakal et al., 2015).

The indirect selection against lameness and for hoof-lesion resistance through conformation traits has not been very effective. Genetic correlations between different hoof lesions and feet and leg conformation traits have a wide range from -0.67 to 0.82 while genetic correlations between hoof lesions and lameness defined by Sprecher et al. (1997) ranges from 0.60 to 0.95 (van der Waaij et al., 2005; van der Linde et al., 2010; Chapinal et al., 2013; Weber et al., 2013). Genetically, cattle that are lame or experience hoof lesions likely share similar genes. It is important to remember that genetic heritability and correlations vary based on the particular dataset, the statistical models used, and the trait definition. Moreover, conformation traits are mostly scored on the animal once as a heifer, not considering changes in these traits over time (Heringstad et al., 2018). Changes in conformation as the animal ages are related to claw disorders and lameness (Vermunt and Greenough, 1994; van der Waaij et al., 2005; van der Linde et al., 2010). Including claw disorders in genomic evaluations in addition to feet and leg conformation traits is advantageous for genetic improvement against lameness and claw disorders (Heringstad et al., 2018). Furthermore, most studies on the genetics of hoof lesions have been conducted in Europe; thus, more studies are needed in North America due to differences in environment, management, and genetic selection (Chapinal et al., 2013).

Genetic research has identified quantitative trait loci (QTL) associated with feet and leg conformation traits and hoof lesions. They have been detected for the conformation traits of rear legs side view, rear legs rear view, feet and legs score, hock quality, bone quality, and foot angle (Ashwell et al., 1998; Schrooten et al., 2000; Boichard et al., 2003; Hiendleder et al., 2003; Ashwell et al., 2005; Schnabel et al., 2005; Buitenhuis et al., 2007; Cole et al., 2009). A few studies have detected QTL for specific claw lesions (Scholey et al., 2012; van der Spek et al., 2015; Wu et al., 2016). Swalve et al. (2014) identified an association between a SNP in the IQ motif-containing GTPase-activating protein 1 (*IQGAP1*) gene and sole hemorrhage. Scholey et al. (2013) found many keratin proteins to be downregulated in digital dermatitis.

Quantitative trait loci related to feet and leg conformation traits and hoof lesions provide an indirect means to select animals for reduced lameness. However, the accuracy of a QTL to estimate trait expression depends upon its association to the trait of interest, the amount of variation it explains, and its linkage with a causative mutation directly affecting expression (Wray et al., 2013). Most QTL for feet and leg conformation and hoof lesions have been identified using lower density microsatellite or SNP chips, which can produce larger QTL of greater distances from causative mutations, thus decreasing its accuracy. The purpose for this project was to identify QTL regions for DCT determined by significant SNPs from GWAS. All cows for this project were genotyped using the Bovine HD 777K SNP chip, which identified smaller regions likely linked more closely to causative mutations, while bull genotypes were shared from outside sources and sequenced on a variety of chip sizes from low density to 150K. Finding genomic regions associated with DCT is crucial for including the trait in genomic evaluations.

CONCLUSION

This dissertation utilizes modern genomic technology to understand genetic and environmental influences of DCT with the goal of breeding healthier animals. As previously mentioned, DCT is a strong predictor of lameness and associated phenotypically and genotypically with common noninfectious claw horn lesions. Being able to determine selection criteria to limit the incidence of hoof lesions and lameness in a herd is essential for increasing animal wellbeing and decreasing economic losses to the producer. Selecting for DCT may help shed more positive light on the cattle industry by increasing animal wellbeing in the eyes of consumers. The chapters presented in this work assess DCT in Holsteins and Jerseys of both sexes, illuminating animal-specific, environmental, and management characteristics, as well as determine genomic regions and candidate genes associated with DCT. Throughout this dissertation, DCT is the distance between the flexor tuberosity of the distal phalanx and the sole horn measured at the typical sole ulcer site using an ultrasound machine. The application of the results from these studies may be used not only to genetically select against lameness caused particularly by sole ulcers and white line disease but also to improve upon management practices on farm to decrease further the prevalence of lameness.

REFERENCES

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. https://doi.org/10.1016/j.prevetmed.2007.09.007.

Archer, S., N. Bell, and J. Huxley. 2010. Lameness in UK dairy cows: a review of the current status. In Practice. 32**:**492-504. https://doi.org/10.1136/inp.c6672.

Ashwell, M. S., Y. Da, P. M. vanRaden, C. E. Rexroad Jr, and R. H. Miller. 1998. Detection of putative loci affecting conformational type traits in an elite population of United States Holsteins using microsatellite markers. J. Dairy Sci. 81:1120-1125. https://doi.org/10.3168/jds.S0022-0302(98)75674-7.

Ashwell, M. S., D. W. Heyen, J. I. Weller, M. Ron, T. S. Sonstegard, C. P. Van Tassell, and H. A. Lewin. 2005. Detection of quantitative trait loci influencing conformation traits and calving ease in Holstein-Friesian cattle. J. Dairy Sci. 88:4111-4119. https://doi.org/10.3168/jds.S0022-0302(05)73095-2.

Ball, R.D. 2013. Designing a GWAS: Power, sample size, and data structure. Pages 37-98 in: Genome-Wide Association Studies and Genomic Prediction. Methods in Molecular Biology (Methods and Protocols). Vol. 1019. C. Gondro, J. van der Werf, and B. Hayes, ed. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-62703-447-0_3.

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. https://doi.org/10.3168/jds.2009-2309.

Baylor College of Medicine Human Genome Sequencing Center. 2018a. Bovine Genome Project. Available at: https://www.hgsc.bcm.edu/other-mammals/bovine-genome-project Accessed Apr. 27, 2019.

Baylor College of Medicine Human Genome Sequencing Center. 2018b. Human Genome Project. Available at: https://www.hgsc.bcm.edu/human-genome-project Accessed Apr. 27, 2019.

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. https://doi.org/10.1016/j.tvjl.2013.09.068.

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

Bicalho, R. C., L. D. Warnick, and C. L. Guard. 2008. Strategies to Analyze Milk Losses Caused by Diseases with Potential Incidence Throughout the Lactation: A Lameness Example. J. Dairy Sci. 91:2653-2661. https://doi.org/10.3168/jds.2007-0744.

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. https://doi.org/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. https://doi.org/10.1016/j.livsci.2013.06.007.

Bohnert, T. 2017. Introducing JPI 2017. Available at: http://us.altagenetics.com/dairy-news/introducing-jpi-2017/. Accessed Apr. 10, 2019.

Boichard, D., C. Grohs, F. Bourgeois, F. Cerqueira, R. Faugeras, A. Neau, R. Rupp, Y. Amigues, M. Y. Boscher, and H. Levéziel. 2003. Detection of genes influencing economic traits in three French dairy cattle breeds. Genet. Sel. Evol. 35:77-101. https://doi.org/10.1186/1297-9686-35-1-77.

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. https://doi.org/10.3168/jds.2009-2721.

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. Anim. 6:962-970. https://doi.org/10.1017/S1751731111002606.

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. https://doi.org/10.3168/jds.S0022-0302(07)72649-8.

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. Pre. Vet. Med. 97:1-8. https://doi.org/10.1016/j.prevetmed.2010.07.011.

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. https://doi.org/10.3168/jds.2012-6071.

Cole, J. B., P. M. VanRaden, J. R. O'Connell, C. P. Van Tassell, T. S. Sonstegard, R. D. Schnabel, J. F. Taylor, and G. R. Wiggans. 2009. Distribution and location of genetic effects for dairy traits. J. Dairy Sci. 92:2931-2946. https://doi.org/10.3168/jds.2008-1762.

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. http://dx.doi.org/10.1016/j.tvjl.2007.09.016.

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. https://doi.org/10.3168/jds.2014-8830.

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. https://doi.org/10.1016/S0301-6226(97)00051-1.

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:e0146718. https://doi.org/10.1371/journal.pone.0146718.

Frisch, J. E., and J. E. Vercoe. 1977. Food intake, eating rate, weight gains, metabolic rate and efficiency of feed utilization in Bos Taurus and Bos Indicus crossbred cattle. Animal Sci. 25:1977343–358. https://doi.org/10.1017/S0003356100016755.

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. https://doi.org/10.2460/ajvr.76.3.246.

Graves, R. R. 1925. Improving dairy cattle by the continuous use of the proved sire. J. Dairy Sci. 5:391-404. https://doi.org/10.3168/jds.S0022-0302(25)93972-0.

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. https://doi.org/10.1016/j.prevetmed.2013.10.009.

Grigson, C. 1991. An African origin for African cattle? - Some archaeological evidence. Afr. Archaeol. Rev. 9:119–144. https://doi.org/10.1007/BF01117218.

Hayes, B. 2013. Overview of statistical methods for genome-wide association studies (GWAS). Pages 149-169 in: Genome-Wide Association Studies and Genomic Prediction. Methods in Molecular Biology (Methods and Protocols). Vol. 1019. C. Gondro, J. van der Werf, and B. Hayes, ed. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-62703-447-0_6.

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. https://doi.org/10.3168/jds.2017-13531.

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. https://doi.org/10.1093/jhered/esg090.

Hoblet, K. H., and W. Weiss. 2001. Metabolic hoof horn disease claw horn disruption. Vet. Clin. North Am. Food Anim. Pract. 17:111-127. https://doi.org/10.1016/S0749-0720(15)30057-8.

Holmbeg, M., and L. Andersson-Eklund. 2004. Quantitative trait loci affecting health traits in Swedish dairy cattle. J. Dairy Sci. 87:2653-2659. https://doi.org/10.3168/jds.S0022-0302(04)73391-3.

Holstein Association USA. 2019. TPI formula - August 2017. Available at: http://www.holsteinusa.com/genetic_evaluations/ss_tpi_formula.html Accessed Apr. 10, 2019.

Iqbal, Z. M., H. Akbar, A. Hosseini, E. B. R. Forteguerri, J. S. Osorio, and J. J. Loor. 2016. Digital cushion fatty acid composition and lipid metabolism gene network expression in Holstein dairy cows fed a high-energy diet. PLoS One 11:e0159536. https://doi.org/10.1371/journal.pone.0159536.

Kang, H. M., J. H. Sul, S. K. Service, N. A. Zaitlen, S. Y. Kong, and N. B. Freimer. 2010. Variance component model to account for sample structure in genome-wide association studies. Nat. Genet. 42:348-354. https://doi.org/10.1038/ng.548.

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. https://doi.org/10.1016/j.tvjl.2006.09.007.

Kossaibati M. A., and R. J. Esslemont. 1997. The costs of production diseases in dairy herds in England. Vet. J. 154:41-51. https://doi.org/10.1016/S1090-0233(05)80007-3.

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. https://doi.org/10.1016/j.prevetmed.2014.12.015.

Lischer, C.J., P. Ossent , M. Räber, and H. Geyer. 2002. 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. https://doi.org/10.1136/vr.151.23.694.

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. https://doi.org/10.2460/ajvr.72.10.1338.

MacHugh, D. E., M. D. Shriver, R. T. Loftus, P. Cunningham, and D. G. Bradley. 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (Bos taurus and Bos indicus). Genetics. 146:1071-86.

Manske, T., J. Hultgren, and C. Bergsten. 2002. Prevalence and interrelationships of hoof lesions and lameness in Swedish dairy cows. Prev. Vet. Med. 54:247-263. https://doi.org/10.1016/S0167-5877(02)00018-1.

McTavish, E. J., J. E. Decker, R. D. Schnabel, J. F. Taylor, and D. M. Hillis. 2013. Origins of New World cattle. PNAS. 110:E1398-E1406. https://doi.org/10.1073/pnas.1303367110.

Mefford, J., and J. S. Witte. 2012. The covariate's dilemma. PLoS Genet. 8:e1003096. https://doi.org/10.1371/journal.pgen.1003096.

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. https://doi.org/10.3168/jds.2017-12968.

Mülling, C. K. W. 2002. Theories on the pathogeneisis of white line disease—An anatomical perspective. Pages 90-96 in Proc. 12th Int. Symp. Lameness in Ruminants. Orlando, FL.

Mülling, C. K. W., and P. R. Greenough. 2006a. Applied physiopathology of the foot. In: XXIV World Buiatrics Congress. Nice, France. World Association for Buiatrics, Paris, France. Available at: http://www.ivis.org/proceedings/wbc/wbc2006/mulling.pdf?LA=1 Accessed Apr. 27, 2019.

Mülling, C. K. W., and P. R. Greenough. 2006b. Functional synergism of the biomechanical systems of the bovine claw. Pages 39-42 in: 14th International Symposium and 6th Conference on Lameness in Ruminants. Uruguay. Available at: http://www.ivis.org/proceedings/rumlameness/2006/block1/mulling.pdf?la=1 Accessed Apr. 27, 2019.

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.

Nature.com. 2019. Learn Science at Scitable. Available at: https://www.nature.com/scitable/glossary Accessed Apr. 27, 2019.

NCBI. 2017. Bead arrays. Available at: https://www.ncbi.nlm.nih.gov/probe/docs/techbeadarray/ Accessed Apr. 27, 2019.

NCBI. 2018. NCBI Bos taurus annotation release 106. Available at: https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Bos_taurus/106/ Accessed Apr. 27, 2019.

Newsome, R. F., M. J. Green, N. J. Bell, N. J. Bollard, C. S. Mason, H. R. Whay, and J. N. Huxley. 2017. 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. https://doi.org/10.3168/jds.2016-12012.

Ødegård, C., M. Svendsen, and B. Heringstad. 2014. Genetic correlations between claw health and feet and leg conformation in Norwegian Red cows. J. Dairy Sci., 97:4522-4529. https://doi.org/10.3168/jds.2013-7837.

Oikonomou, G., G. Banos, V. Machado, L. Caixeta, and R. C. Bicalho. 2014. Genetic characterization of digital cushion thickness. J. Dairy Sci. 97:532-536. https://doi.org/10.3168/jds.2013-7212.

Pearson, T. A., and T. A. Manolio. 2008. How to interpret a genome-wide association study. JAMA. 299:1335-44. https://doi.org/10.1001/jama.299.11.1335.

Pérez-Cabal, M. A., and R. Alenda. 2002. Genetic relationships between lifetime profit and type traits in Spanish Holstein cows. J. Dairy Sci. 85:3480-3491. https://doi.org/10.3168/jds.S0022-0302(02)74437-8.

Pritchard, J. K., M. Stephens, N. A. Rosenberg, and P. Donnelly. 2000. Association Mapping in Structured Populations. Am. J. Hum. Genet. 67:170-181. https://doi.org/10.1086/302959.

Räber, M., C. Lischer, H. Geyer, and P. Ossent. 2004. The bovine digital cushion–A descriptive anatomical study. Vet. J. 167:258–264. https://doi.org/10.1016/S1090-0233(03)00053-4.

Räber, M., M. R. Scheeder, P. Ossent, C. 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. https://doi.org/10.1016/j.tvjl.2005.03.009.

Randall, L. V., M. J. Green, M. G. G. Chagunda, C. Mason, S. C. Archer, L. E. Green, 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. https://doi.org/10.3168/jds.2014-8863.

Rastani, R. R., S. M. Andrew, S. A. Zinn, and C. J. Sniffen. 2001. Body composition and estimated tissue energy balance in Jersey and Holstein cows during early lactation. J. Dairy Sci. 84:1201-1209. https://doi.org/10.3168/jds.S0022-0302(01)74581-X.

Schmutz, S. M., J. M. Stookey, D. C. Winkelman-Sim, C. S. Waltz, Y. Plante, and F. C. Buchanan. 2001. A QTL study of cattle behavioral traits in embryo transfer families. J. Hered. 92:290–292. https://doi.org/10.1093/jhered/92.3.290.

Schnabel, R.D., T. S. Sonstegard, J. F. Taylor, and M. S. Ashwell. 2005. Whole-genome scan to detect QTL for milk production, fertility and functional traits in two US Holstein families. Anim. Genet. 36:408-416. https://doi.org/10.1111/j.1365-2052.2005.01337.x.

Scholey, R. A., R. W. Blowey, R. D. Murray, R. F. Smith, J. P. Massey, W. E. Ollier, and S. D. Carter. 2012. Investigating host genetic factors in bovine digital dermatitis. Vet. Rec. 171:624. https://doi.org/10.1136/vr.101251.

Scholey, R. A., N. J. Evans, R. W. Blowey, J. P. Massey, R. D. Murray, R. F. Smith, W. E. Ollier, and S. D. Carter. 2013. Identifying host pathogenic pathways in bovine digital dermatitis by RNA-Seq analysis. Vet. J. 197:699-706. https://doi.org/10.1016/j.tvjl.2013.03.008.

Schrooten, C. S., H. Bovenhuis, W. Coppieters, J. A. M. Van Arendonk. 2000. Whole genome scan to detect quantitative trait loci for conformation and functional traits in dairy cattle. J. Dairy Sci. 83:795-806. https://doi.org/10.3168/jds.S0022-0302(00)74942-3.

Sham, P. C., and S. M. Purcell. 2014. Statistical power and significance testing in large-scale genetic studies. Nat. Rev. Genet. 15:335–346. https://doi.org/10.1038/nrg3706.

Shearer, J. K., and S. R. van Amstel. 2011. Lameness in dairy cattle. Pages 233-253 in: Dairy Production Medicine. C. A. Risco and P. M. Retamal, ed. John Wiley and Sons, UK. https://doi.org/10.1002/9780470960554.ch19.

Shearer, J. K., S. R. Van Amstel, and B. W. Brodersen. 2012. Clinical diagnosis of foot and leg lameness in cattle. Vet. Clin. Food Anim. 28:535-556. https://doi.org/10.1016/j.cvfa.2012.07.003.

Shearer, J. K., P. J. Plummer, and J. A. Schleining. 2015. Perspectives on the treatment of claw lesions in cattle. Veterinary Medicine: Research and Reports. 6:273-292. https://doi.org/10.2147/VMRR.S62071.

Scherer, A., and G. B. Christensen. 2016. Concepts and relevance of genome-wide association studies. Sci Prog. 99:59-67. https://doi.org/10.3184/003685016X14558068452913.

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 47:1179-1187. https://doi.org/10.1016/S0093-691X(97)00098-8.

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. https://doi.org/10.3168/jds.2013-6997.

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. https://doi.org/10.1053/tvjl.2001.0651.

Tellam, R. L., D. G. Lemay, C. P. Van Tassell, H. A. Lewin, K. C. Worley, and C.G. Elsik. 2009. Unlocking the bovine genome. BMC Genomics 10:193. https://doi.org/10.1186/1471-2164-10-193.

The Bovine Genome Sequencing and Analysis Consortium, C. G. Elsik, R. L. Tellam, and K. C. Worley. 2009. The genome sequence of taurine cattle: A window to ruminant biology and evolution. Science 324:522-528. https://doi.org/10.1126/science.1169588.

Toussaint Raven, E. 1989. Cattle Footcare and Claw Trimming. Farming Press, Ipswich, UK.

USDA National Agricultural Statistics Services. 2019. Milk production and milk cows. Available at: https://www.nass.usda.gov/Charts_and_Maps/Milk_Production_and_Milk_Cows/milkcows.php Accessed Apr. 27, 2019.

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. https://doi.org/10.3168/jds.2010-3183.

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. https://doi.org/10.3168/jds.2014-8302.

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. https://doi.org/10.3168/jds.S0022-0302(03)73884-3.

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. https://doi.org/10.3168/jds.S0022-0302(05)73053-8.

Van Dorp, T. E., J. C. M. Dekkers, S. W. Martin, and J. P. T. M. Noordhuizen. 1998. Genetic parameters of health disorders, and relationships with 305-day milk yield and conformation traits of registered Holstein cows. J. Dairy Sci. 81:2264-2270. https://doi.org/10.3168/jds.S0022-0302(98)75806-0.

VanRaden, P. M., J. B. Cole, and K. L. Parker Gaddis. 2019. Net merit as a measure of lifetime profit: 2018 revision. Available at: https://aipl.arsusda.gov/reference/nmcalc-2018.htm Accessed Apr. 10, 2019.

Vergara, C. F., D. Döpfer, N. B. Cook, K. V. Nordlund, J. A. A. McArt, D. V. Nydam, and G. R. Oetzel. 2014. Risk factors for postpartum problems in dairy cows: Explanatory and predictive modeling. J. Dairy Sci. 97:4127-4140. https://doi.org/10.3168/jds.2012-6440.

Vermunt, J. J., and P. R. Greenough. 1994. Predisposing factors of laminitis in cattle. Br. Vet. J. 150:151-164. https://doi.org/10.1016/S0007-1935(05)80223-4.

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. https://doi.org/10.1016/j.tvjl.2006.10.006.

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. https://doi.org/10.3168/jds.2009-2326.

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. https://doi.org/10.3168/jds.2012-6261.

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. https://doi.org/10.3168/jds.2017-12954.

Wetterstrand, K. A. 2018. DNA sequencing costs: Data from the NHGRI Genome Sequencing Program (GSP). Available at: https://www.genome.gov/27541954/dna-sequencing-costs-data/ Accessed Apr. 27, 2019.

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. https://doi.org/10.1016/S1090-0233(98)80058-0.

Whay, H. R., D. C. J. Main, L. E. Green, and A. J. F. Webster. 2003. Assessment of the welfare of dairy cattle using animal-based measurements: Direct observations and investigation of farm records. Vet. Rec. 153:197-202. https://doi.org/10.1136/vr.153.7.197.

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. https://doi.org/10.1146/annurev-animal-021815-111422.

Womack, J. E. 2005. Advances in livestock genomics: opening the barn door. Genome Res. 15:1699-705. https://doi.org/10.1101/gr.3809105.

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. https://doi.org/10.1038/nrg3457.

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. https://doi.org/10.3168/jds.2015-10705.

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. https://doi.org/10.1186/gb-2009-10-4-r42.

CHAPTER 2:

A LONGITUDINAL STUDY OF DIGITAL CUSHION THICKNESS AND ITS FUNCTION AS A PREDICTOR FOR COMPROMISED LOCOMOTION AND HOOF LESIONS IN HOLSTEIN COWS

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Key words: digital cushion, lameness, hoof lesion, claw horn disruption lesion, dairy cow

ABSTRACT

Lameness is a major animal welfare and economic issue for the dairy industry and is a challenge to overcome due to multifaceted causes. Digital cushion thickness (DCT) is a strong predictor of lameness and is phenotypically associated with incidence of claw horn disruption lesions (CHDL; sole ulcers and white line disease). We hypothesized that DCT varies between digits and across lactation within the cow. This variation could be characterized to predict the occurrence of CHDL or compromised locomotion. Body condition score (BCS), visual locomotion score (VLS), DCT, and presence or absence of lesions were collected at 4 time points: < 40 d prepartum (DPP), 1 to 30 d in milk (DIM), 90 to 120 DIM, and ≥ 255 DIM for 183 commercial Holstein cows enrolled in the study. Cows underwent digital sonographic examination for the measurement of DCT evaluated at the typical sole ulcer site beneath the flexor tuberosity for the right front medial and lateral digits and right hind medial and lateral digits. Factors such as parity number and stage in lactation were obtained from farm management software (DairyComp 305; Valley Agricultural Software, Tulare, CA). Cows were grouped by parity: primiparous (parity = 1) or multiparous (parity ≥ 2). The prevalence of CHDL among time points ranged from 0 to 4.2% for primiparous cows versus 2.5 to 25% for multiparous cows, while the prevalence of lameness based on VLS of 3 to 5 ranged from 1.7 to 8.3% for primiparous cows versus 12.7 to 33% for multiparous cows. Digital cushion thickness varied within primiparous and multiparous cows based on stage of lactation and digit ($P < 0.05$) and was thicker for both parity groups prior to dry off (≥255 DIM) and thinnest prior to calving (< 40 DPP) and after peak lactation (90 to 120 DIM). The DCT of the front medial digit was thickest for primiparous heifers, while the hind lateral digit was thickest for multiparous cows. The DCT of the hind medial digit was thinnest for both parity groups. Parity group and DCT of

the hind lateral digit < 40 DPP were important predictors of CHDL ($P < 0.05$), while parity group and DCT of the hind lateral digit and front lateral digit at 1 to 30 DIM were key predictors of VLS lameness ($P < 0.05$). These results may help identify animals with higher odds of developing these diseases by highlighting key time points and specific digits of importance for monitoring. In addition, it improves our biological understanding of the relationship between DCT and lameness.

INTRODUCTION

Improvements in animal welfare are a priority for the animal agricultural industry that benefit both animals and producers. There is a growing concern for the wellbeing of animals in response to the demand of consumers for welfare-certified products (Bicalho et al., 2009). Lameness is a debilitating condition that challenges the sustainability of production systems due to the pain and ensuing animal welfare issues, along with significant economic losses (Warnick et al., 2001; Vermunt, 2007). It is defined as the clinical presentation of impaired locomotion, regardless of cause, and is caused by a variety of diseases, such as noninfectious and infectious hoof lesions, and is influenced by environmental and genetic factors (Archer et al., 2010). Estimated costs for lameness range from $120-500 per case with specific diseases, such as sole ulcers, averaging $220 per case (Cha et al., 2010).

The multifaceted nature of lameness makes it a difficult problem to solve. However, digital cushion thickness (DCT) has been shown to be a strong predictor of lameness and claw horn lesions (Bicalho et al., 2009). The digital cushion is an important structure in dampening the compression in the heel under the distal phalanx (Räber et al., 2004). Prolonged exposure to concrete surfaces causes the solar surface of the hind lateral claw to flatten and increase in width changing the dynamics inside the claw. Instead of confining weight bearing to the wall, part of

the weight load is transferred to the central part of the sole creating abnormal pressure (Mülling and Greenough, 2006). If the digital cushion is not thick enough to act as a barrier, this pressure could cause more problems, most notably, claw horn disruption lesions (CHDL); e.g. sole ulcers and white line disease. Therefore, the objectives of this study were 1) to determine the variation of DCT within the cow across lactation and between digits, and 2) to determine the optimal variables of time point and digit to measure DCT for predicting lameness defined by visual locomotion score (VLS) and CHDL.

MATERIALS AND METHODS

Approval from the Cornell University Institutional Animal Care and Use Committee (Protocol #2014-0121) and signed owner consent was obtained prior to commencement of this study.

Study Herd

Data were collected from a single dairy farm located near Ithaca, New York from October 13, 2015 to September 27, 2016. The farm milked 3,800 Holstein cows 3 times daily in a 100-cow rotary milking parlor. The lactating cows were kept in 10 housing pens that had deep-bedded free-stalls filled with anaerobically digested and separated manure solids with quicklime. The barn alleys had grooved-concrete flooring and were cleaned by manual scrapers. All the walkways to and from the milking parlor and the holding pen were covered with rubber. The walk from a pen to the parlor ranged from 181 to 534 m. The cows stood an average of 1 hour for each milking. Footbaths were located in the exit lanes of the milking parlor. Each cow was scheduled to receive routine hoof trimming twice yearly based on a farm protocol created in DairyComp 305 (Valley Agriculture Software, Tulare, CA) that selected cows for routine trimming once they were past 150 d since last routine trimming. Additionally, all cows received

routine hoof trimming at dry off. Lame cows were identified by visual detection of an asymmetric gait when returning from the milking parlor. A systematic lameness scoring system was not utilized. The lame cows were evaluated and treated that same evening.

Data Collection and Study Design

A total of 183 animals were conveniently enrolled and sampled in a prospective cohort study targeting 245-270 d carried calf (DCC) on 10 enrollment days. The cows were followed throughout their subsequent lactation, with DCT measurements performed at 4 time points (Table 2.1). Time points were chosen in an aim to measure DCT when it was likely thinnest, thickest, and corresponding to physiological changes over lactation that might effect DCT. However, ranges in the targeted time periods were affected as farm hoof trimming schedules were accommodated. The first measurement was taken < 40 d prepartum (DPP) targeting improved body condition prior to calving. Fifty-nine nulliparous cows were evaluated during their regular trimming when they were moved to the close-up pen at 240 to 260 DCC, while 118 multiparous cows were evaluated 248 to 267 DCC. The second measurement occurred post calving at 8 to 34 d in milk (DIM) with the cow in presumed energy deficit. The third measurement occurred mid lactation at 93 to 118 DIM when Bicalho et al. (2009) found DCT to be thinnest. The fourth measurement occurred during late lactation at ≥ 255 DIM with a range of 255 to 335 DIM and a mean of 285 DIM. The large range in sampling time related to when cows became pregnant and therefore when they were going to be dried off. This event aimed at measuring cows as their milk production was decreasing prior to dry off and they were expected to have regained body condition. The third and fourth time points also coincided with routine hoof trimming schedules to minimize impact on the cows and farm management.

Table 2.1 Descriptive statistics of cow height and summary statistics of the number of cows at each measurement time point for digital cushion thickness separated by parity group[1]

		Parity		Descriptive Statistics			
Variable	Time Point	Primiparous[2], n	Multiparous, n	Mean	SD	Min	Max
Cow height[3], cm				146	4	137	156
< 40 DPP[4]	1	59	118				
8 to 34 DIM[5]	2	59	109				
93 to 118 DIM	3	52	97				
≥ 255 DIM	4	48	76				

[1]Cows were from 1 large commercial farm in New York.
[2]Nulliparous animals at < 40 DPP transitioned to primiparous for all time points afterwards.
[3]Assessed as the average of both the distance from the floor to the withers and the floor to the dorsal aspect of the caudal sacral joint.
[4]DPP = d prepartum.
[5]DIM = d in milk.

Hoof trimming was completed by 3 trained farm employees. One trimmer was responsible for cows in lactation while 2 trimmers handled the nulliparous animals prior to calving. The 2 trimmers worked simultaneously, consistently trimming either the front or hind foot, respectively. Cows were restrained for hoof trimming using 2 types of standing hoof trimming chutes. The first measurement was done using a HSeries Chute (Comfort Hoof Care, Baraboo, WI), and the other 3 measurements were done using an Appleton Steel Trimming Chute (Appleton Steel, Appleton, WI).

Measures, including body condition score (BCS), height, and lesion presence, were recorded based on their potential for affecting DCT or lameness. The body condition score ranged from 1 to 5 with a quarter point system as described by Edmonson et al. (1989). The VLS ranged from 1 to 5 with 1 = normal, 2 = presence of a slightly asymmetric gait, 3 = cow moderately favors 1 or more limbs, 4 = severely lame, and 5 = non-weight bearing lame (Sprecher et al., 1997; Bicalho et al., 2007). Both were assessed and collected by the same researcher at each time point to minimize rater variability. A binary lesion score was determined by farm records: 0 = no new lesion, 1 = new lesion. The lesion score was determined based on the DCT measurement intervals. Lesion score for the first measurement was based on the

presence or absence of a new lesion from the time the animal was enrolled until the parturition date. Lesion score for the second, third, and fourth measurements were determined from 1 to 30 DIM, 31 to 120 DIM, and 121 DIM to the date of the last measurement, respectively. Cow height, assessed as both the distance from the floor to the withers and the floor to the dorsal aspect of the caudal sacral joint, was measured at the beginning and end of the study. Parity, DIM at each measurement event, and parturition date were obtained from the farm management software.

The cows underwent digital sonographic B-mode examination with an Aquila Vet ultrasound machine (Esaote Europe BV, Maastricht, the Netherlands) equipped with a curved array dual-frequency probe set at 7.5 MHz. If the time point was coincident with hoof trimming, the digital cushion measurement was taken immediately after being trimmed. The measurement was always performed at the typical sole ulcer site located beneath the flexor tuberosity in the medial aspect of the middle pad evaluating the distance from the inner margin of the sole to the distal edge of the tuberculum flexorum of the third phalanx (Figure 2.1) (Bicalho et al., 2009). Ultrasonography of only the right digits both front and hind were assessed. The ultrasound machine settings (i.e., depth, echo-amplification, persistence, pre- and post-processing) were kept unchanged throughout the study.

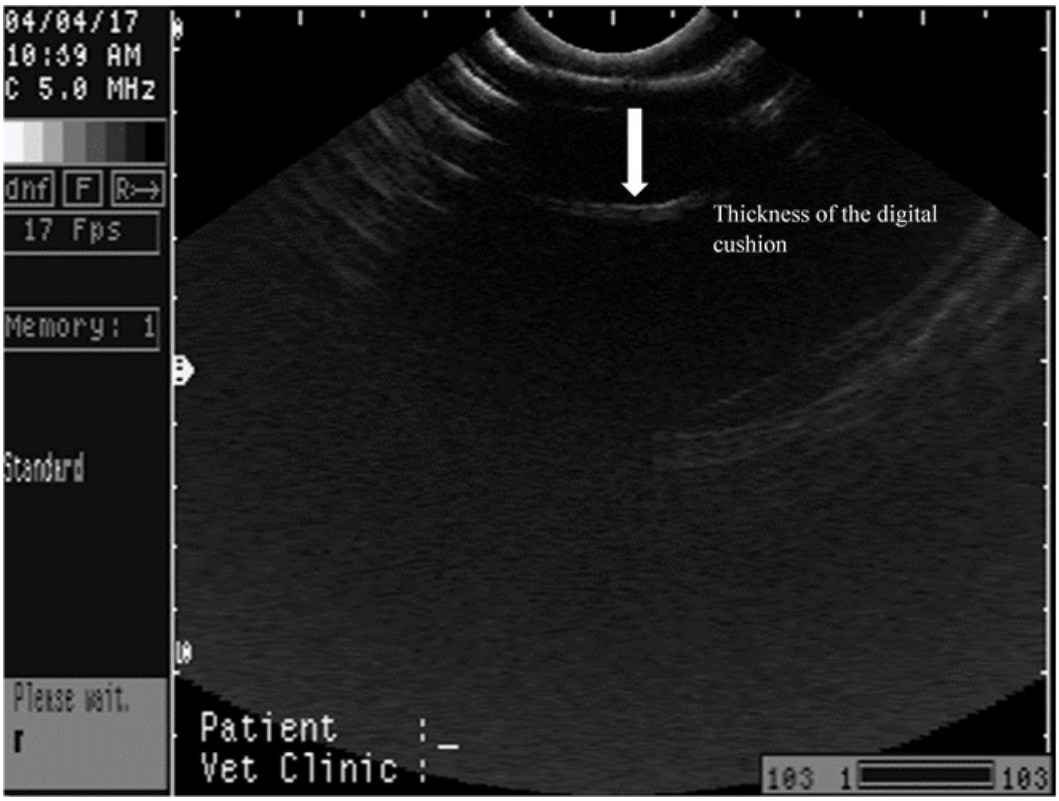

Figure 2.1 Typical ultrasonographic image that was observed in the study at the sole ulcer site beneath the flexor tuberosity in the medial aspect of the middle pad. The thinner and shortest echogenic line at the top of the arrow represents the inner margin of the sole, and the larger, longer, and brighter echogenic line at the bottom of the arrow represents the margins of the distal edge of the third phalanx. The distance between these lines measured represented the thickness of the digital cushion.

Variable Definitions

To facilitate analysis and interpretation, the variables of BCS, average height, VLS, and DCT were categorized into terciles with thresholds based on the given dataset (Oikonomou et al., 2013; Bludau et al., 2014; Mahen et al., 2018). The BCS were categorized as BCSG = 1 if BCS < 3, BCSG = 2 if BCS = 3 or 3.25, BCSG = 3 if BCS > 3.25. Average height of each cow was categorized as short if average height < 144 cm, average if average height ≤ 148 cm and ≥ 144 cm, or tall if average height > 148 cm. The VLS were categorized as LAME = 1 for those with VLS < 3, while LAME = 2 for those with VLS ≥ 3. All variables relating to DCT were

categorized into terciles based on pooling the data from all digits: thin if DCT < 0.96 cm, average if DCT ≤ 1.19 cm and ≥ 0.96 cm, or thick if DCT > 1.19 cm.

Furthermore, the variables of time point, parity, and digit (CLAW) were created. Time point ranged from 1 to 4 representing the 4 measurement time points. The variable parity = 1 represented those in their first lactation and parity = 2 for those with lactation > 1. The variable CLAW represented the specific hoof digits and was categorized as CLAW = FM for front medial digit, CLAW = FL for front lateral digit, CLAW = HM for hind medial digit, and CLAW = HL for hind lateral digit. The variable of employee conducting the hoof trimming was not included in the models as it coincided with time point, distinguishing the one trimmer who did not do time point 1 nulliparous cows, and CLAW, distinguishing the two trimmers who consistently trimmed either the front or hind foot for the nulliparous cows.

Statistical Analysis

To determine whether DCT varied across lactation or differed between digits, 2 linear mixed models (LMM) were fitted to the data using PROC MIXED in SAS (SAS Institute Inc., Cary, NC). The first LMM included random effects of CLAW nested within cow to control for repeated measures and multiple measurements from each cow (4 digits evaluated each time) using a compound symmetry covariance structure. Similarly, the second LMM included the random effect of time point nested within cow to control for repeated measures and the multiple measurements collected from each cow (4 measurement time points per claw). Parity group, time point or CLAW, and their interaction term were included in the models as fixed effects. Pairwise mean comparisons were calculated for the statistically significant effects in both models, adjusting *P*-values for multiple comparisons using Tukey-Kramer method. The assumptions of homogeneity, normality, and independence of the residuals for both mixed models were met.

To predict the occurrence of the outcomes CHDL and lameness (VLS ≥ 3), several multiple logistic regression models were fitted in JMP Pro 12 (SAS Institute Inc., Cary, NC) to see which measurement time point of DCT was most predictive of later development of CHDL and lameness (VLS ≥ 3). Both outcomes were binary (0 = no event, 1 = at least one CHDL or VLS event, respectively, in lactation after the second measurement). Varying days at risk were accounted for in both models by including the number of measurement time points completed as a covariate (TRISK). For predicting CHDL, 11 cows were excluded because they either started with a CHDL or had a CHDL prior to their first measurement in lactation at 1 to 30 DIM. For predicting lameness based on VLS, 34 cows were excluded because they either were lame at enrollment or were lame at their first measurement in lactation at 1 to 30 DIM.

After categorization, potential model covariates, including parity, average height, BCSG, and the 4 digital cushion measurements from < 40 DPP and 1 to 30 DIM, were tested for association with CHDL or lameness (VLS ≥ 3) using univariate analysis using JMP Pro 12, and any variable with Pearson x^2 $P < 0.2$ was offered to the multiple logistic regression model for predicting either CHDL or lameness (VLS ≥ 3). Multicollinearity was checked between the variables being offered to the model such that if a relationship was found (Kappa or Gamma ≥ 0.3), multiple iterations of the same model were run offering a different combination of covariates to determine which to retain. Manual backward stepwise regression was used to refine the model such that variables were discarded 1 by 1 starting with the largest *P*-value until all terms in the model had a $P < 0.05$. All biologically plausible 2-way interactions with > 5 observations per cell were tested. To assess the model fit and overall predictability of the logistic regression models, a receiver operating characteristic curve analysis was performed. The final

model was chosen based on the highest area under the curve (AUC) for model predictability and on-farm application.

RESULTS AND DISCUSSION

A total of 183 animals were enrolled in the study, with 177 included in the final data set. Table 1 depicts the number of primiparous and multiparous cows at each measurement time point. Lactation ranged from 1 to 7 in this study with a median of 3 lactations. Overall, 6 cows were removed from the study due to 1 of the following: they were measured < 245 DCC or > 270 DCC, > 40 DPP, did not have an ear tag, or had dangerous behavioral problems. For the last measurement (≥ 255 DIM), 124 animals remained. Those that left prior to the fourth measurement were either culled by the farm or died. The actual measurement time points were < 40 DPP, 8 to 34 DIM, 93 to 118 DIM, and ≥ 255 DIM.

The prevalence of CHDL among measurement time points ranged from 0 to 4.2% for primiparous cows and from 2.5 to 25% for multiparous cows (Figure 2.2A), while the prevalence of lameness based on VLS of 3 to 5 ranged from 1.7 to 8.3% for primiparous cows and from 12.7 to 33% for multiparous cows (Figure 2.2B). Multiparous cows had the highest prevalence of CHDL ≥ 255 DIM and the highest prevalence of VLS lameness between 90 to 120 DIM. Many studies have reported higher odds of claw horn lesions for older cows in mid and late lactation, as well as a higher prevalence of lameness in general (Bicalho et al., 2007; Barker et al., 2009; Machado et al., 2011; Solano et al., 2016). This could be a downstream effect of the mobilization of adipose tissues, such as the digital cushion, when the animals go through energy deficit during early lactation because sole ulcers and white line disease occur 8 to 12 wk or more after the initial event (Rastani et al., 2001; Räber et al., 2006; Shearer and Van Amstel, 2017). The prevalence of postpartum diseases associated with excessive lipolysis, such as ketosis, displaced

abomasum, and metritis, have been reported higher in multiparous compared to primiparous cows (Markusfeld, 1987; Humer et al., 2016). Primiparous cows are in a different metabolic state than multiparous cows because they need nutrients for their continual growth in addition to producing milk (Wathes et al., 2007). Lameness prevalence of multiparous cows in the dry period (12.7%) was similar to the prevalence described by Vergara et al. (2014) who reported a mean prevalence of 11.2% (ranging from 8.7 to 13.4%) for dry cows in 4 large commercial free-stall herds in New York and Wisconsin, as well as Foditsch et al. (2016) who reported an average prevalence of 14% for dry cows in 23 large, high producing commercial herds in New York. The higher prevalence of lameness and CHDL in multiparous cows could also be due to changes in the integrity of the anatomy of the bovine claw. As a cow gets older and experiences lameness and lesion events, the integrity of her claw is changed. Newsome et al. (2016) demonstrated the presence of increased new bone growth on the flexor tuberosity of the distal phalanx in cows that suffered more lameness and claw horn disruption lesions throughout life. These bone spurs on the bottom of the distal phalanx could be continuously pinching the digital cushion, thus altering the ability of the digital cushion to dissipate the forces acting on the claw structures, changing the adipose tissue to connective scar tissue (Räber et al., 2004). Additionally, prolonged exposure to hard surfaces, trauma, and multiple experiences of metabolic and hormonal changes around calving decreases claw horn quality (Cook and Nordlund, 2009).

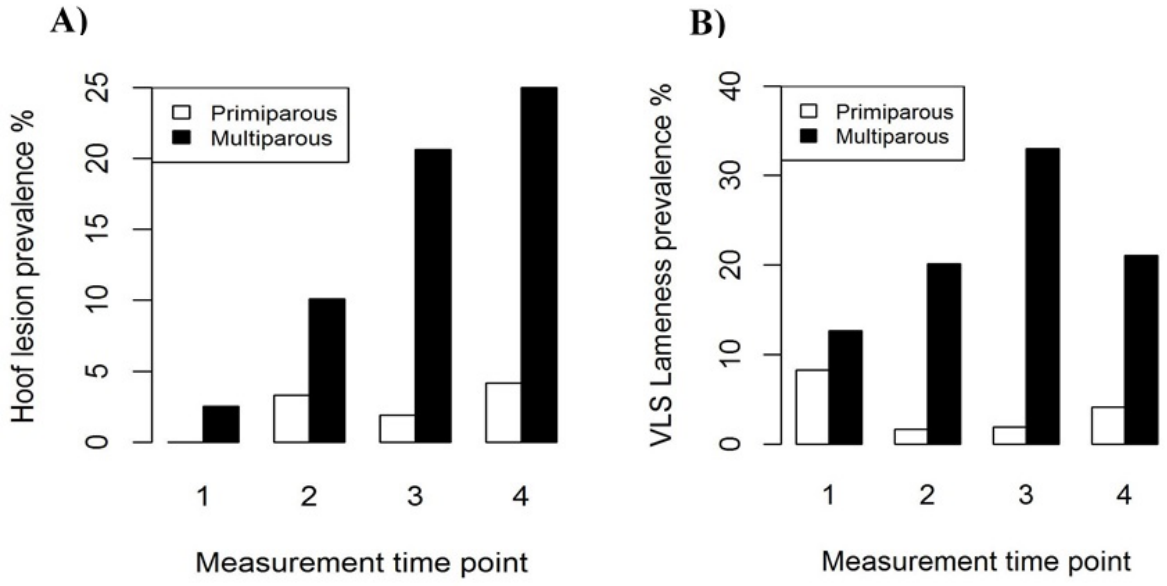

Figure 2.2 Prevalence of (A) hoof lesions and (B) VLS lameness at each measurement time point by parity group. The lesions included were sole ulcers and white line disease, known together as CHDL. Lameness was defined as animals with VLS ≥3. The number of primiparous cows at each measurement time point was 60, 60, 52, and 48, respectively. The number of multiparous cows at each measurement time point was 118, 109, 97, and 76, respectively. Time points correspond to 1) <40 DPP, 2) 1 to 30 DIM, 3) 90 to 120 DIM, and 4) ≥255 DIM.

Least squares means for DCT by measurement time point and parity are summarized in Table 2.2. Digital cushion thickness varied by time point depending on parity group ($P < 0.01$). Digital cushion thickness was found to be thickest for both parity groups at ≥ 255 DIM compared to the other measurement time points ($P < 0.05$). This measurement occurred at the end of lactation right before dry off, a time when cows should be producing less milk and have higher body condition. The measurement time points with thinnest DCT of primiparous animals occurred < 40 DPP and 90 to 120 DIM ($P < 0.05$). The first measurement was close to parturition when there are multiple demands for energy, such as the cow preparing for milk production and heifer growth, which may result in mobilizing fat reserves. Additionally, this measure differed by hoof trimmer with 2 staff trimming either the front or hind foot respectively

for nulliparous cows, while a third trimmer did the cows in lactation which included multiparous cows for time point 1 and all cows for times points 2-4. While the variable of hoof trimmer is noted, we expect that it is of less impact since 2 trimmers were used at this time point. The latter measurement is when energy balance is being restored and cows are beginning to gradually recover BCS until the end of lactation (Machado et al., 2010). Additionally, multiparous cows had thin DCT at 1 to 30 DIM ($P < 0.05$), possibly due to them reaching peak lactation quicker and producing more milk than primiparous cows (Ray et al., 1992; Coffey et al., 2006).

Table 2.2 Least Squares Means (LSM) and SEM for digital cushion thickness per measurement time point separated by parity group[1]

	Primiparous[2]		Multiparous	
Measurement	LSM, cm	SEM	LSM, cm	SEM
< 40 DPP[3]	0.96^{ey}	0.02	1.11^{bcdz}	0.01
1-30 DIM[4]	1.09^{cdy}	0.02	1.07^{dy}	0.01
90-120 DIM	0.94^{ey}	0.02	1.12^{bcz}	0.02
≥ 255 DIM	1.18^{aby}	0.02	1.21^{ay}	0.02

[1]Data was analyzed by linear mixed model that included the random effect of claw nested within cow and the terms parity and time point. Least squares means presented in this table is from the interaction of parity group and time point. A total of 177 cows were included in this analysis.
[2]Nulliparous animals at < 40 DPP transitioned to primiparous for all time points afterwards.
[3]DPP = d prepartum.
[4]DIM = d in milk.
[a-e] = LSM within a column with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$ and if at least one superscript is the same, then the LSM are the same.
[y, z] = LSM within a row with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$.

Least squares means for DCT per CLAW by parity are summarized in Table 2.3. Digital cushion thickness varied by CLAW depending on parity ($P < 0.01$). This variation is likely reflective of the comparison of 2 digits measured from the right front hoof while the other 2 digits were measured from the right hind hoof. It is known that 60% of a cow's body weight is borne by the forelimbs meaning there is an unequal distribution of weight which could cause differences in DCT of the front versus hind digits (Bergsten et al., 2007). Both parity groups had the thinnest DCT in the hind medial digit ($P < 0.05$). This may be explained by the majority of cows being cow-hocked in the hind causing the toes to point out and displace more weight on the

inner claws (Shearer et al., 2012). The hind medial claw lands more underneath the animal when walking or standing, thus dispersing the load of weight and pressure on the outside hoof wall where the suspension of the laminar folds exists (Toussaint Raven, 1989).

Table 2.3 Least Squares Means (LSM) and SEM for digital cushion thickness per claw by parity group[1]

	Primiparous[2]		Multiparous	
Claw	LSM, cm	SEM	LSM, cm	SEM
Front medial	1.14^{aby}	0.02	1.14^{by}	0.01
Front lateral	1.05^{cdy}	0.02	1.11^{bcy}	0.01
Hind medial	0.91^{ey}	0.02	1.05^{dz}	0.01
Hind lateral	1.05^{cdy}	0.02	1.18^{az}	0.01

[1]Data was analyzed by linear mixed model that included the random effect of time point nested within cow and the terms parity and claw. Least squares means presented in this table is from the interaction of parity group and claw. A total of 177 cows were included in this analysis.
[a-e] = LSM within a column with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$ and if at least one superscript is the same, then the LSM are the same.
[2] Nulliparous animals at < 40 DPP transitioned to primiparous for all time points afterwards.
[y, z] = LSM within a row with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$.

Among the 4 digits measured, the hind lateral digit had the thickest DCT for multiparous cows ($P < 0.05$) versus the front medial digit in primiparous cows ($P < 0.05$). The medial claw of the forelimbs bear more weight and incur more lesions than the lateral claws while the reverse is true in the hind limbs (Toussaint Raven, 1989; Murray et al., 1996; Van der Tol et al., 2002). Nuss et al. (2011) discovered the front medial claws resembled the lateral hind claws in claw length and sole length. The inside front claw might be thickest for those in their first parity because they have less weight in their hind limbs since their udders are not as large as the older cows. When the udders are large, they displace the closeness of the hind limbs and cause the surface area of the solar surface of the lateral claw to increase in width (Bergsten et al., 2007).

The final lameness (VLS ≥ 3) logistic regression model included the terms parity, DCT of the front lateral digit and hind lateral digit at 1 to 30 DIM, and TRISK with AUC of 0.83 (Table 2.4) while the final CHDL logistic regression model included the terms parity, DCT of the hind lateral digit at < 40 DPP, and TRISK with AUC of 0.79 (Table 2.5). The DCT of the

hind lateral digit and parity group were important predictors in both models ($P < 0.05$). Multiparous cows had higher odds of a CHDL or lameness (VLS ≥ 3) event in lactation of the study period compared to primiparous cows ($P < 0.01$). Cows with a thin DCT of the hind lateral digit < 40 DPP had 5 times greater odds of a CHDL event compared to cows with an average DCT of the same digit ($P = 0.01$). Cows with a thin DCT of the hind lateral digit within the first month post-calving had 10.7 times greater odds of a lameness event compared to cows with an average DCT of the same digit ($P < 0.01$) and 6.6 times greater odds of a lameness event compared to cows with a thick DCT of the same digit ($P < 0.01$).

Table 2.4 Contrast odds ratios (OR), CI, and P-values reported for the final lameness logistic regression model based on visual locomotion score (VLS)[1]

Variable	Contrast	OR	95% CI	P-value
Parity	multiparous-primiparous	8.9	2.4 – 46.9	< 0.01
FL2[2]	average-thin	11.5	2.4 – 91.8	< 0.01
	thick-thin	5.5	0.8 – 54.7	0.09
	average-thick	2.0	0.6 – 9.1	0.27
HL2[3]	thin-average	10.7	2.6 – 56.5	< 0.01
	thin-thick	6.6	1.8 – 28.7	< 0.01
	thick-average	1.6	0.4 – 7.1	0.48

[1]The outcome variable was the occurrence of a lameness event based on VLS in lactation following the digital cushion thickness (DCT) measurement. The independent variables retained in the final model were parity, DCT of the front lateral digit and hind lateral digit at 1 to 30 d in milk (DIM), and time at risk. A total of 143 cows were included in this analysis.
[2]FL2 = Front lateral DCT measured at the second time point at 1 to 30 DIM.
[3]HL2 = Hind lateral DCT measured at the second time point at 1 to 30 DIM.

Table 2.5 Contrast odds ratios (OR), CI, and P-values reported for the final claw horn disruption lesion (CHDL) logistic regression model[1]

Variable	Contrast	OR	95% CI	*P*-value
Parity	multiparous-primiparous	7.3	2.3 – 28.7	< 0.01
HL1[2]	thin-average	5.0	1.5 – 19.9	0.01
	thin-thick	1.6	0.6 – 4.7	0.36
	thick-average	3.1	0.9 – 12.4	0.07

[1] The outcome variable was the occurrence of CHDL in lactation following the digital cushion thickness (DCT) measurement. The independent variables offered to the final model were parity, DCT of the front lateral digit and hind lateral digit at < 40 d prepartum (DPP), and time at risk. The variables retained in the model were parity, DCT of the hind lateral digit at < 40 DPP, and time at risk. A total of 166 cows were included in this analysis.
[2]HL1 = Hind lateral DCT measured at the first time point < 40 DPP.

As opposed to the inner hind claw, the load on the outside hind claw is greater and abnormal due to conformation shifting the pressure landing on the middle sole rather than the hoof wall. Walking on concrete causes the wall to be worn down and a disproportionate amount of the sole bears weight (Bergsten et al., 2007). This sole contains the digital cushion rather than the suspension needed to bear the weight. Exposure to concrete surfaces for long periods of time causes trauma and inflammation that can create changes to the distal phalanx, such as the bone spurs mentioned earlier, which in turn affects the digital cushion such that the adipose tissue becomes scar tissue and it loses its cushioning capacity (Räber et al., 2004; Mülling and Greenough, 2006; Newsome et al., 2016).

Having thin DCT of the outside hind digit increased the odds of a CHDL or lameness (VLS ≥ 3) event. Due to the unequal distribution of weight and pressure on the lateral hind digit and other reasons mentioned, a thin digital cushion has more trouble acting as a barrier between the distal phalanx and the sole, hence, problems such as lameness or claw horn lesions, develop (Van der Tol et al., 2003). This is especially important right before calving and in the month following calving when the cow has undergone the stressful event of giving birth and has been exposed to periparturient hormones, such as relaxin and estrogen (Tarlton et al., 2002). Relaxin

is known to distend the reproductive tract for parturition and can have an effect on other structures throughout the body involving connective tissue, likely causing the distal phalanx to sit lower within the hoof capsule exerting more pressure on the digital cushion (Tarlton et al., 2002; Bicalho and Oikonomou, 2013; Newsome et al., 2017).

The forelimb has not been studied as greatly as the hind limb because over 90% of hoof lesions causing lameness occur in the hind limbs, mostly in the lateral claw (Bergsten et al., 2007; Shearer et al., 2012). Although the results presented here agree with the importance of the hind lateral claw, they also reveal the importance of claws in the forelimb. Digital cushion thickness of the front lateral digit at 1 to 30 DIM was an influential predictor of a lameness (VLS ≥ 3) event in lactation, with cows having 11.5 times greater odds of lameness if their DCT was average compared to thin ($P < 0.01$). This could be due to CHDL possibly being present when the measurement was taken even though the individual was not demonstrating impaired locomotion. Newsome et al. (2017) discovered the thickness of the digital cushion to be greater when a sole ulcer was present due to increased vascularization, edema, or inflammation in the underlying tissues. Furthermore, the average thickness of the digital cushion could be due to the quality of the tissue and may reflect hard scar tissue rather than soft adipose tissue (Räber et al., 2004).

This study determined DCT of the front lateral digit to be important in predicting subsequent lameness. Newsome et al. (2017) uniquely related digital cushion thickness to back fat thickness. However, they assessed the digital cushion differently in that they measured sole soft tissues at 3 different sites. Both Newsome et al. (2017) and the current study found DCT to be thickest about 8 wk prepartum when the animals are near or starting the dry off period. This

study did find measurements of DCT taken 1 to 30 DIM to be important predictors of subsequent VLS lameness occurrence.

It is important to note that one of the main limiting factors of this study was that it assessed only 177 animals from a single large commercial herd in Upstate New York. Additionally, the current study was unable to investigate the relationship between DCT and specific diseases separately, such as sole ulcer and white line disease, due to the low prevalence of each type of lesion. Similarly, BCS lacked sufficient variation to be informative.

This study determined which claws are more important to measure and at what time in lactation they are most informative to identify animals more prone to developing CHDL or experiencing a lameness event later in lactation despite these limitations. If producers measure the DCT of a hind and front lateral digit within the first month after parturition, then this may help them identify cows with a higher odds of developing CHDL or a VLS lameness event. This could allow producers to monitor those cows more closely and adjust management practices such that CHDL and lameness are prevented or detected early enough to alleviate pain, improve resolution of lameness, avoid possible major changes to the hoof structures, and prevent negative downstream effects like decreased productivity.

An additional longitudinal study targeting dry off (< 140 DPP) and 90 to 120 DIM which correlated to the thickest and thinnest periods of DCT is underway to validate the findings in this study with more animals from multiple herds and for subsequent genomic analysis. Long-term, genomic markers related to DCT will be evaluated for use in genetic selection to reduce lameness.

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REFERENCES

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

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. https://doi.org/10.3168/jds.2012-6017.

Bergsten, C., A. Brizzi, C. K. W. Mülling, and K. Nordlund. 2007. Genetic Selection and Conformation. Pages 141-154 in: Bovine Laminitis and Lameness: A hands-on approach. P. Greenough, ed. Elsevier, Edinburgh, UK. p. 141-154. https://doi.org/10.1016/B978-0-7020-2780-2.50016-1.

Bicalho, R. C., S. H. Cheong, G. Cramer, and C. L. Guard. 2007. Association between a visual and an automated locomotion score in lactating Holstein cows. J. Dairy Sci. 90:3294-3300. https://doi.org/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. https://doi.org/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. https://doi.org/10.1016/j.livsci.2013.06.007.

Bludau, M. J., A. Maeschli, F. Leiber, A. Steiner, and P. Klocke. 2014. Mastitis in dairy heifers: Prevalence and risk factors. Vet. J. 202:566-572. https://doi.org/10.1016/j.tvjl.2014.09.021.

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. Pre. Vet. Med. 97:1-8. https://doi.org/10.1016/j.prevetmed.2010.07.011.

Coffey, M. P., J. Hickey, and S. Brotherstone. 2006. Genetic aspects of growth of Holstein-Friesian dairy cows from birth to maturity. J. Dairy Sci. 89:322-329. https://doi.org/10.3168/jds.S0022-0302(06)72097-5.

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. https://doi.org/10.1016/j.tvjl.2007.09.016.

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. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.

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:e0146718. https://doi.org/10.1371/journal.pone.0146718.

Humer, E., A. Khol-Parisini, B. U. Metzler-Zebeli, L. Gruber, and Q. Zebeli. 2016. Alterations of the lipid metabolome in dairy cows experiencing excessive lipolysis early postpartum. PloS ONE 11:e0158633. https://doi.org/10.1371/journal.pone.0158633.

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. https://doi.org/10.3168/jds.2010-3177.

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. https://doi.org/10.2460/ajvr.72.10.1338

Mahen, P. J., H. J. Williams, R. F. Smith, and D. Grove-White. 2018. Effect of blood ionised calcium concentration at calving on fertility outcomes in dairy cattle. Vet. Rec. Published Online First: 11 July 2018. https://doi.org/10.1136/vr.104932.

Markusfeld, O. 1987. Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationships among traits. J. Dairy Sci. 70:158-166. https://doi.org/10.3168/jds.S0022-0302(87)79990-1.

Mülling, C. K. W., and P. R. Greenough. 2006. Applied physiopathology of the foot. In: XXIV World Buiatrics Congress. Nice, France. World Association for Buiatrics, Paris, France.

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.

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. https://doi.org/10.3168/jds.2015-10202.

Newsome, R. F., M. J. Green, N. J. Bell, N. J. Bollard, C. S. Mason, H. R. Whay, and J. N. Huxley. 2017. 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. https://doi.org/10.3168/jds.2016-12012.

Nuss, K., C. Sauter-Louis, and B. Sigmund. 2011. Measurements of forelimb claw dimensions in cows using a standardised sole thickness: a post-mortem study. Vet. J. 190:84-89. https://doi.org/10.1016/j.tvjl.2010.10.002.

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. https://doi.org/10.3168/jds.2012-6308.

Räber, M., C. Lischer, H. Geyer, and P. Ossent. 2004. The bovine digital cushion–A descriptive anatomical study. Vet. J. 167:258–264. https://doi.org/10.1016/S1090-0233(03)00053-4.

Räber, M., M. R. Scheeder, P. Ossent, C. 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. https://doi.org/10.1016/j.tvjl.2005.03.009.

Rastani, R. R., S. M. Andrew, S. A. Zinn, and C. J. Sniffen. 2001. Body composition and estimated tissue energy balance in Jersey and Holstein cows during early lactation. J. Dairy Sci. 84:1201-1209. https://doi.org/10.3168/jds.S0022-0302(01)74581-X.

Ray, D. E., T. J. Halbach, and D. V. Armstrong. 1992. Season and lactation number effects on milk production and reproduction of dairy cattle in Arizona. J. Dairy Sci. 75:2976-2983. https://doi.org/10.3168/jds.S0022-0302(92)78061-8.

Shearer, J. K., S. R. Van Amstel, and B. W. Brodersen. 2012. Clinical diagnosis of foot and leg lameness in cattle. Vet. Clin. Food Anim. 28:535-556. https://doi.org/10.1016/j.cvfa.2012.07.003.

Shearer, J. K., and S. R. Van Amstel. 2017. Pathogenesis and Treatment of Sole Ulcers and White Line Disease. Vet. Clin. Food Anim. 33:283-300. https://doi.org/10.1016/j.cvfa.2017.03.0031.

Solano, L., H. W. Barkema, S. Mason, E. A. Pajor, S. J. LeBlanc, and K. Orsel. 2016. Prevalence and distribution of foot lesions in dairy cattle in Alberta, Canada. J. Dairy Sci. 99:6828-6841. https://doi.org/10.3168/jds.2016-10941.

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 47:1179-1187. https://doi.org/10.1016/S0093-691X(97)00098-8.

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. https://doi.org/10.1053/tvjl.2001.0651.

Toussaint Raven, E. 1989. Cattle Footcare and Claw Trimming. Farming Press, Ipswich, UK.

Van der Tol, P. P., J. H. 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. https://doi.org/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. https://doi.org/10.3168/jds.S0022-0302(03)73884-3.

Vergara, C. F., D. Döpfer, N. B. Cook, K. V. Nordlund, J. A. A. McArt, D. V. Nydam, and G. R. Oetzel. 2014. Risk factors for postpartum problems in dairy cows: Explanatory and predictive modeling. J. Dairy Sci. 97:4127-4140. https://doi.org/10.3168/jds.2012-6440.

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. https://doi.org/10.1016/j.tvjl.2006.10.006.

Warnick, L.D., D. Janssen, C. L. Guard, and Y. T. Grohn. 2001. The effect of lameness on milk production in dairy cows. J. Dairy Sci. 84:1988–1997. https://doi.org/10.3168/jds.S0022-0302(01)74642-5.

Wathes, D. C., Z. Cheng, N. Bourne, V. J. Taylor, M. P. Coffey, and S. Brotherstone. 2006. 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. https://doi.org/10.1016/j.domaniend.2006.05.004.

CHAPTER 3:

GENOME-WIDE ASSOCIATION STUDIES OF DIGITAL CUSHION THICKNESS IN HOLSTEIN COWS

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ABSTRACT

The bovine digital cushion is a compression pad between the distal phalanx and sole and has been associated with claw horn disruption lesions. Digital cushion thickness (DCT) is estimated to be moderately heritable, therefore the objectives of our study were to examine influences of management and environment on digital cushion thickness and explore associations of genetic markers with DCT. In a cohort of 502 Holsteins from 5 farms in New York, USA, DCT and body condition score were collected <137 d prepartum and from 86 to 127 d in milk corresponding to periods where the digital cushion is thickest and thinnest based on previous research. Cows underwent digital sonographic examination of the digital cushion evaluated at the typical sole ulcer site for the right front and hind foot. Linear mixed models were conducted on DCT with the fixed effects of time point, digit, farm, wither height, sacral height, back length, body condition score group, and multiple farm system variables, separately, and included random effects to control for the random subset of cows per farm, repeated measures, and multiple measurements from each cow. The phenotypic results indicated DCT varied by sample time point, sacral height, parity, digit, farm, body condition score group, and wither height. For the genotypic study, 447 DNA samples were genotyped on the Illumina BovineHD 777K beadchip. Quality assessment of markers and samples provided a final data set of 431 samples and 579,449 markers. Genome-wide association studies were conducted for DCT testing inheritance models and genetic variation of digit, foot, time point, and average thickness. One marker passed the Bonferroni correction threshold and 26 passed false discovery rate from 4 genome-wide association studies with covariates of sequencing batch plate, parity group, body condition score, wither height, and sacral height. Ten candidate genes were identified in the regions of the associated SNP with 2 genes on *Bos taurus* autosomes 24 and 29 influencing

biological functions potentially effecting the digital cushion. *MC4R* and *DLG2* were related to fat deposition and bone growth, respectively. The genetic markers discovered in this study have the opportunity to be used in breeding programs utilizing genomic selection to select against claw horn disruption lesions and lameness due to associations between the markers and DCT. Further studies on the biologically plausible candidate genes can illuminate genetic variants within the genes and how they relate to DCT through gene regulation and expression.

INTRODUCTION

The digital cushion is an important structure in dampening the compression of the corium tissue that produces sole horn under the distal phalanx (Lischer et al., 2002; Räber et al., 2004; Shearer et al., 2015). It is a complex structure composed mostly of adipose and connective tissue (Räber et al., 2004). Approximately 90% of the digital cushion lies behind the navicular bone in the heel area while only 10% extends forward beneath the distal phalanx (Mülling and Greenough, 2006). Digital cushion thickness (DCT) at the typical sole ulcer site beneath the distal phalanx has been shown to be a strong predictor of lameness and the claw horn disruption lesions (CHDL) of sole ulcers and white line disease (Bicalho et al., 2009; Newsome et al., 2017a,b; Stambuk et al., 2019). These CHDL are the most prevalent claw diseases associated with lameness and pain (Murray et al., 1996; Oikonomou et al., 2013).

Selecting for thicker digital cushion could have a faster rate of genetic gain and greater impact on decreasing the occurrences of CHDL and lameness on farms than selecting against certain lesions separately due to the higher heritability of DCT than CHDL and lameness determined by visual locomotion scores. Digital cushion thickness was previously determined to be moderately heritable (0.33 ± 0.09) and had a negative genetic correlation with CHDL (-0.60 ± 0.29) (Oikonomou et al., 2014). Heringstad et al. (2018) reviewed multiple studies and found

heritability estimates for CHDL to range from 0.01 to 0.12. Sprecher et al. (1997) estimated the heritability of lameness according to visual locomotion scoring to range from 0.07 to 0.10. Thus far, only one other study has investigated the genetics of DCT, specifically investigating the impact of diet on the expression of 27 candidate genes in the digital cushion from a small cohort of non-pregnant non-lactating dairy cows and found lipogenic genes to be consistently upregulated by feeding a higher-energy diet (Iqbal et al., 2016). However, the genetics behind the thickness of the digital cushion beneath the distal phalanx in pregnant and lactating cows from multiple farms has not been studied. Therefore, the objectives of our study were 1) to conduct a study examining influences of management and environment on DCT across multiple farms and 2) to identify genomic regions associated with DCT and how they might differ by foot, digit, or stage in lactation.

MATERIALS AND METHODS

Approval from the Cornell University Institutional Animal Care and Use Committee (Protocol #2014-0121) and signed owner consent was obtained prior to commencement of this study. Data were collected from a convenience sample of 5 commercial dairy farms located near Ithaca, New York, USA from October 13, 2015 to October 26, 2017. The farms were selected based on easy access to cows, hoof trimmers, chutes, and willingness of farm owners and managers to accommodate the study. Cow data and management systems are outlined in Table 3.1. The total number of cows ranged from 150 to 4,400 per farm. The cows walked a distance of 3.5 to 379 m to the milking parlor 2 to 3 times a day, and production ranged from 39 to 42 kg milk/cow/d. All cows were housed in free-stall barns with bedding that included either paper fiber, sawdust, sand, or manure solids. All farms were on a routine schedule to have cows

trimmed by a hoof trimmer. All lame cows were treated when identified as lame by farm employees.

Table 3.1 Farm system data from farm surveys for 5 commercial farms in a prospective cohort study of digital cushion thickness

Variable	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Production data					
Total cows	4,400	150	2,013	420	1,080
Milking frequency/d	3	3	2 or 3	3	3
Type of parlor	100 stall rotary	Double 10 parallel	Double 17 parallel	Double 12 parallel	Double 16 parallel
Average milk yield, kg/cow/d	41	39	42	42	40
Average distance to the parlor	379 m	53.3 m	183 m	3.5 m	183 m
Flooring					
Type of flooring in barn	Concrete and rubber	Concrete	Concrete and rubber	Concrete and rubber	Concrete and rubber
Location of rubber	Feed alley, holding area lanes to the parlor	-	Holding area and parlor	Feed alley, holding area and parlor	Feed alley and holding area
Type of scraping	Manual	Manual	Automatic	Automatic	Manual
Bedding					
Type of bedding	Manure solids	Fresh sand	Recycled sand	Paper fiber and sawdust	Manure solids
Footbath					
Days/wk footbath run	7	2	3	4	7
Treatment solution	Formalin	Copper sulfate	Formalin	Copper sulfate	Copper sulfate
Trimming					
Schedule for trimming	Every 5 months	180 DIM and dry off	Every 6 months	150 DIM and dry off	150 DIM and dry off
Occupation of hoof trimmer	Farm employee	Veterinarian/employee	Contracted and farm employee	Contracted	Contracted
Frequency they trim on farm	Daily	Once/wk	Once/wk	Once every other wk	Once every other wk
Average cows trimmed/d	48	4	20	30	40
Type of trim table	Standing	Standing	Tilt table	Tilt table	Standing
Lesion and lameness					
Types of lesions seen most often	Sole ulcers and white line abscess	Digital dermatitis and white line abscess	Sole ulcers and digital dermatitis	White line abscess and digital dermatitis	White line abscess
Routine locomotion scoring	No	No	Yes	No	Yes

Data Collection and Study Design

A total of 502 Holstein cows were enrolled in a prospective cohort study where DCT measurements were evaluated at 2 time points. Cows were randomly selected based on the availability of cows at or near dryoff on farm, as well as cows that were undergoing hoof trimming on the day of the herd visits. The number of cows per farm depended upon the total number of cows on farm, the schedule of hoof trimming for the cows, the schedule of the hoof trimmer, and the number of cows that could be seen by the hoof trimmer and researcher at the same time per visit. The convenient sample contained more multiparous than primiparous cows. Of the five farms, 2 contributed 64% of the cows almost equally whereas the other 3 farms had similar contribution in the number of cows representing the remaining 36%. The first measurement was <137 d prepartum (DPP) when cows are near or in the dry period prior to calving. This time period corresponds to when the digital cushion has been found to be thicker in primiparious and multiparious cows (Bicalho et al., 2009; Stambuk et al., 2019). This measurement had a mean of 47.6 ± 25.8 DPP and a range of 1 to 136 DPP with 90% of the cows being sampled between 1 to 77 DPP. Figure 3.1 shows the range and percentage of cows for DPP, and a majority of cows were sampled from 10 to 40 and 50 to 90 DPP. The large variation in DPP was due to the farms' trimming schedule and management practices. Multiparous cows that were not in the dry period at the first measurement were >250 DIM. Six cows did not have a DPP for the first time point because they died or were culled prior to calving but were 204 to 226 d carried calf at the first time point. The second measurement occurred early to mid-lactation from 86 to 127 DIM when DCT was found to be thinnest and coincided with routine hoof trimming schedules to minimize impact on the cows and farm management (Bicalho et al., 2009; Stambuk et al., 2019).

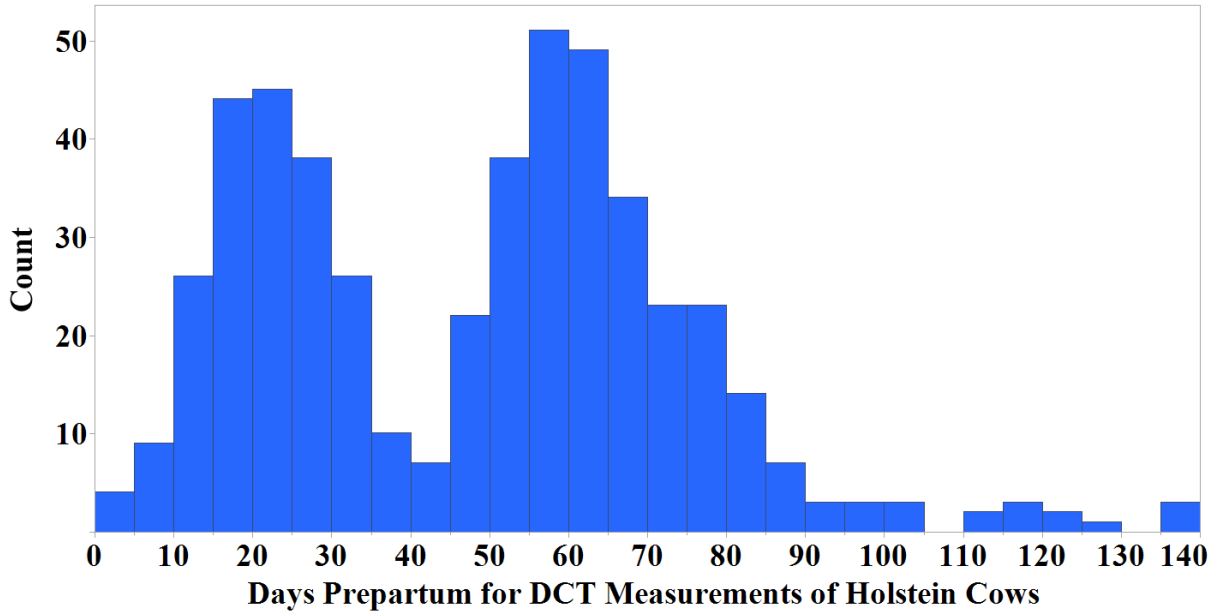

Figure 3.1 Range of DPP for Holsteins cows for DCT measurements.

Hoof trimming was completed by contracted professionals or trained farm employees, and cows were restrained for hoof trimming using either a standing chute or tilt table. Two farms had an Appleton Steel Trimming Chute (Appleton Steel, Appleton, WI), 1 farm had a Comfort Chute H-series (Comfort Hoof Care, Baraboo, WI), and 2 farms trimmed cows on a layover Riley Built trailer model hoof trimming chute (Riley Built, Lubbock, TX).

At each measurement, a single trained researcher collected BCS, which ranged from 1 to 5, with a quarter point system as described by Edmonson et al. (1989). Cow height was assessed at both wither and sacral height, determined by the distance from the floor to the withers and the distance from the floor to the dorsal aspect of the caudal sacral joint, respectively. Back length was assessed as the distance between the withers and caudal sacral joint. Wither height, sacral height, and back length were measured at the beginning of the study. Parity, DIM at each measurement event, and calving date were obtained from the farm management software

(DairyComp 305; Valley Agricultural Software, Tulare, CA). The cows underwent digital sonographic B-mode examination with an Aquila Vet ultrasound machine (Esaote Europe BV, Maastricht, the Netherlands) of the 4 right digits immediately after trimming. The measurement was collected at the typical sole ulcer site located beneath the flexor tuberosity in the medial aspect of the middle pad evaluating the distance from the inner margin of the sole to the distal edge of the tuberculum flexorum of the third phalanx (Bicalho et al., 2009; Stambuk et al., 2019).

Variable Definitions

The variables of BCS, cow height, and back length were categorized into terciles based on the given dataset for interpretation in the statistical analysis. The BCS were categorized into BCS groups (BCSG): 1 if BCS <2.75, 2 if BCS was ≤2.75 and ≥3.25, and 3 if BCS >3.25. Both wither height and sacral height were categorized as short if height <145 cm, average if height ≤150 cm and ≥145 cm, or tall if height >150 cm. The length between height measurements across the back was categorized as short if length <104 cm, average if length ≤110 cm and ≥104 cm, or long if length >110 cm. Time point was either 1 for <137 DPP or 2 for 86 to 127 DIM. Parity was 1 for the cows in their first lactation and 2 for the cows with lactation >1. The variable digit (CLAW) represented the specific hoof digits and was categorized as FM for front medial digit, FL for front lateral digit, HM for hind medial digit, and HL for hind lateral digit. All variables for statistical analysis of DCT were cow level variables. Management characteristics for statistical analysis were based on survey of farm level variables.

Statistical Analysis

Prior to any statistical analysis, 6 cows were excluded based on sampling outside the targeted time points. A total of 496 cows were used in the statistical analysis with 104

primiparous cows and 392 multiparous cows at the first measurement time point, while the second measurement time point had 97 primiparous and 349 multiparous cows. The difference in sample size between the two time points was due to farm culling or death. Within this project, primiparous cows were those nulliparous at the first time point who transitioned to primiparous before the second time point. As 35% of the cows in this study were identical to the cows in Stambuk et al. (2019), we performed our analysis with and without these cows included. Given that there were no biological or statistical differences in our outcomes between the 2 analyses (data not shown), we opted to include all cows in the final analysis. For the sample size calculation, a sample size of 496 cows was determined to show an expected difference of 0.05 (power: 0.97, α: 0.05, one-way ANOVA).

Stage of Lactation. For statistical analysis of stage of lactation across parity, independent variables were the fixed effects of time point (<137 DPP or 86 to 127 DIM), parity group (primiparous or multiparous), and the interaction of time point and parity group, along with cow nested within farm and CLAW nested within cow as random effects with repeated measures of time points for cows and claw (4 digits evaluated at each time period) within cows. The autoregressive covariance structure was used because it resulted in the lowest Akaike information criterion for repeated measures (Littell et al., 1998).

Claw Digit. For statistical analysis of CLAW, independent variables were the fixed effects of CLAW (FM, FL, HM, or HL), parity group (primiparous or multiparous), and the interaction of CLAW and parity group, along with cow nested within farm and time point nested within cow as random effects with repeated measures of time points for cows. The autoregressive covariance structure was used because it resulted in the lowest Akaike information criterion for repeated measures (Littell et al., 1998).

Body Measurements. Independent variables for the statistical analysis of sacral height, wither height, and BCSG were the fixed effects of treatment group (short, average, and tall for both sacral and wither height, and thin, average, fat for BCSG) and parity group (primiparous or multiparous) and the interaction of treatment group and parity group. Cow nested within farm, time point nested within cow, and CLAW nested within cow were random effects with repeated measures. The autoregressive covariance structure was used because it resulted in the lowest Akaike information criterion for repeated measures (Littell et al., 1998).

Herd Management Characteristics. For analysis of herd management characteristics, all farm level variables were analyzed separately. Independent variables for the statistical analysis of milking parlor type (parallel or rotary), milk production (≤41 kg or > 41 kg), bedding type (manure solids, sand, or sawdust), rubber flooring (yes or no), manure scraping system (manual or automatic), footbath length (< 4 or ≥ 4 m), visual locomotion score (yes or no), trimming chute type (standing or layover) and hoof trimmer (contractor, farm employee, or veterinarian/employee) were the fixed effects of treatment group and parity group (primiparous or multiparous) and the interaction of treatment group and parity group. Cow nested within farm, time point nested within cow, and CLAW nested within cow were random effects with repeated measures. The autoregressive covariance structure was used because it resulted in the lowest Akaike information criterion for repeated measures (Littell et al., 1998).

For all DCT analyses, the MIXED procedure of SAS University Edition (SAS Institute Inc., Cary, NC) was used to obtain solutions and conduct the ANOVA. Pairwise mean comparisons evaluated significant effects in all models and *P*-values were adjusted for multiple comparisons using the Tukey-Kramer method. All treatment results were reported as least squares means, with significance declared at $P < 0.05$.

Genotyping and Quality Control

Whole blood (10 mL) was obtained via the coccygeal vessels into vacutainer tubes with the anticoagulant K_2EDTA for subsequent DNA extraction. Genomic DNA was extracted following the Gentra Puregene Blood Kit extraction protocol (Qiagen, Valencia, CA). A total of 447 samples were genotyped on the Illumina BovineHD 777K beadchip (Illumina, Inc., San Diego, CA) at GeneSeek (Neogen Genomics, Lincoln, NE). One sample was dropped for breed misidentification. The initial 777,962 SNP were assessed for quality using Golden Helix SVS version 8.8.3 (Golden Helix, Bozeman, MT). A total of 198,513 SNP were removed if they had a call rate < 0.90 (n = 9,341), a minor allele frequency < 0.05 (n = 188,415), and Hardy-Weinberg Equilibrium *P*-value < 0.0001 (n = 65,140). Twelve samples were subsequently removed with a genotyping call rate < 0.90. To evaluate population structure and relatedness, an identity-by-state similarity matrix was used to calculate genome-wide identity-by-descent estimates (Purcell et al., 2007). Three animals were removed with an estimated identity-by-state score greater than 0.85 denoting substantial relatedness. The final dataset included 431 samples and 579,449 SNP.

Genome-Wide Association Studies

Digital cushion thickness was defined as the measurements of each claw and time point (n = 8; 4 claws at 2 times points), the averages of claw (n = 4), time point (n = 2), foot (n = 2; front and hind), and all measurements combined (n = 1) to test whether genomic regions associated with averaging the measurements captured more genetic variation underlying DCT and to explore relationships between genomic regions related to digit, foot, or time point. Digital cushion thickness was evaluated as a quantitative measure. Genetic heritability of DCT and the power to predict an association were determined for the 17 dataset definitions of DCT including the covariates of sequencing batch plate, parity group, BCS, wither height, and sacral height with

the use of GCTA (Yang et al., 2011). If the power was > 0.80, then the trait was evaluated in a genome-wide association study (GWAS). Associations were calculated using EMMAX algorithms embedded in the Golden Helix SVS software to correct for population structure and relatedness by including a genomic relationship matrix as a random effect in the models (Kang et al., 2010).

Association studies with the covariates of sequencing batch plate, parity group, BCS, wither height, and sacral height were considered in additive, dominant, and recessive inheritance models. The covariate of farm was investigated but could not be included with sequencing batch plate because they were highly correlated. Sequencing batch plate accounts for the differences based on sequencing batch as well as farm because 1 batch only contained samples from 1 farm while the other batch included samples from the other 4 farms. Likewise, individual farm variables (production, bedding, etc.) were highly correlated with farm and therefore captured within the sequencing batch plate variable. Digital cushion thickness and composition are greatly influenced by parity group, BCS, wither height, and sacral height thus they were included as covariates. Body condition score was an average between the 2 time points while wither and sacral heights were continuous measurements.

Quantile–quantile plots were used to determine the model of best fit for each trait and inheritance model. Quantile-quantile plots graphically assessed the number and magnitude of observed associations between genotyped SNP and DCT compared to the expected *P*-values from the null hypothesis. Pseudo lambda values were evaluated as a measure of inflation in the model, with values around 1 showing no inflation and values greater than 1.1 showing inflation. Bonferroni adjusted *P*-value or False Discovery Rate (FDR) adjusted *P*-value < 0.05 distinguished SNP significantly associated with DCT. Candidate regions were examined for

putative candidate genes based on the linkage disequilibrium (LD) structure or, if LD was not present, a 1 Mb window centered on the significantly associated SNP using the *Bos taurus* ARS-UCD1.2 assembly in the NCBI Genome Data Viewer (https://www.ncbi.nlm.nih.gov/genome/82).

RESULTS

Descriptive Statistics

Table 3.2 has means and standard deviations of Holstein cow measurements for the 5 commercial dairy farms. Cow measurements include DCT, parity, BCS, wither and sacral heights.

Table 3.2 Descriptive statistics (mean and standard deviation) of Holstein cow measurements for the 5 commercial dairy farms.

Variable	Farm 1 (n = 149)	Farm 2 (n = 40)	Farm 3 (n = 169)	Farm 4 (n = 73)	Farm 5 (n = 65)	Overall Mean	Min	Max
DCT[1]	1.05 (0.25)	1.40 (0.27)	1.32 (0.28)	1.13 (0.26)	1.01 (0.28)	1.18 (0.30)	0.30	2.33
BCS	3.22 (0.20)	3.12 (0.50)	3.24 (0.50)	2.89 (0.50)	3.75 (0.60)	3.24 (0.50)	1.75	4.75
Wither height, cm	143 (6.1)	149 (4.5)	146 (5.7)	145 (5.5)	143 (4.9)	145 (5.9)	129	160
Sacral height, cm	149 (4.3)	151 (5.5)	150 (4.0)	149 (4.5)	148 (5.0)	150 (4.6)	135	165
Parity	2.4 (1.3)	3.5 (1.3)	2.7 (1.5)	3.2 (1.4)	2.5 (1.3)	2.7 (1.4)	1	9

[1]DCT = digital cushion thickness.

Linear Mixed Models of Digital Cushion Thickness

Variables Without Parity Interactions Associated with DCT. The interactions of measurement time point or sacral height with parity group did not exceed the threshold of $P < 0.05$ ($P = 0.12$ and 0.67, respectively). Digital cushion thickness varied by time point and sacral height separately ($P < 0.05$). Digital cushion thickness was thicker at <137 DPP compared to 86 to 127 DIM ($P < 0.001$). Cows that had greater sacral height had greater DCT compared to those with short or average sacral height ($P < 0.05$) (Table 3.3).

Table 3.3 LSM and SEM for digital cushion thickness per measurement time point and sacral height

Variable	Categories	Number of observations	LSM, cm (SEM)	*P*-value
Time point				< 0.001
	<137 DPP[1]	496	1.21[a] (0.07)	
	86 to 127 DIM	446	1.16[b] (0.07)	
Sacral height[2], cm				< 0.001
	Short (<145)[3]	78	1.13[b] (0.07)	
	Average (145 to 150)	190	1.17[b] (0.07)	
	Tall (>150)	221	1.21[a] (0.07)	

[1]DPP = days prepartum.
[2]Assessed as the distance from the floor to the dorsal aspect of the caudal sacral joint.
[3]Category thresholds are in parentheses.
[a-b] within a variable, category LSM with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$.

Variables Interacting with Parity Associated with DCT. Least squares means for DCT by CLAW, BCSG, and wither height by parity are summarized in Table 3.4. Digital cushion thickness varied separately by CLAW, BCSG, and wither height depending on parity ($P < 0.05$). The DCT of the average BCSG cows and cows with short withers height varied by parity group with multiparous cows having thicker DCT ($P < 0.05$). Both parity groups had the thinnest DCT in the hind medial digit and the thickest DCT in the front medial digit ($P < 0.05$). Front lateral, hind medial, and hind lateral digits were different when compared between parity groups, with multiparous cows having thicker DCT than primiparous cows ($P < 0.05$). Only the front medial

digit was not different between parity groups. For primiparous cows, cows of average BCSG had thinner DCT than cows of heavier BCSG ($P < 0.05$). For multiparous cows, cows of thin BCSG had thinner DCT than cows of average and heavier BCSG ($P < 0.05$).

Table 3.4 LSM and SEM for digital cushion thickness by CLAW, BCSG, and wither height for primiparous and multiparous cows

			Primiparous	Multiparous	
Variable	Categories	Number of cows	LSM, cm (SEM)	LSM, cm (SEM)	*P*-value
CLAW[1]					0.006
	Front Medial	496	1.19^{ay} (0.07)	1.26^{ay} (0.07)	
	Front Lateral	496	1.13^{by} (0.07)	1.21^{bz} (0.07)	
	Hind Medial	493	0.99^{cy} (0.07)	1.13^{cz} (0.07)	
	Hind Lateral	494	1.12^{by} (0.07)	1.19^{bz} (0.07)	
BCSG[2]					0.002
	Thin (<2.75)[3]	166	1.17^{aby} (0.10)	1.14^{by} (0.08)	
	Average (2.75 to 3.25)	179	1.08^{by} (0.08)	1.21^{az} (0.08)	
	Fat (>3.25)	101	1.21^{ay} (0.08)	1.24^{ay} (0.08)	
Wither height[4], cm					0.049
	Short (<145)	238	1.10^{ay} (0.07)	1.19^{az} (0.07)	
	Average (145 to 150)	160	1.22^{ay} (0.09)	1.19^{ay} (0.07)	
	Tall (>150)	91	-	1.22^{a} (0.07)	

[1]CLAW = digits.
[2]BCSG = BCS group.
[3]Category thresholds are in parentheses.
[4]Assessed as the distance from withers to the dorsal aspect of the caudal sacral joint measured across the back.
[a-c] within a variable, category LSM with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$.
[y-z] within a variable, category across parity groups, LSM with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$.

Farm Variables by Parity Associated with DCT. Least squares means for DCT by farm management characteristics and parity are summarized in Table 3.5. Multiparous cows had thicker DCT than primiparous cows for 2 factors related to production, which were when the farm milked cows on a rotary parlor and the farm averaged ≤41 kg/cow/d ($P < 0.05$). Furthermore, multiparous cows had thicker DCT than primiparous cows for elements related to

cow comfort, such as when the free stalls were bedded with manure solids, rubber flooring was in the feeding alley, and farm employees had to manually scrape manure out of alleyways with skid loaders ($P < 0.05$). Lastly, multiparous cows had thicker DCT than primiparous cows for factors related to the prevention of lameness, including when the hoof trimmer used a standing chute, the footbath length was <4 m, the farm employees visually assess and score locomotion as a tool for detecting lameness, and the hoof trimmer was a trained farm employee ($P < 0.05$). In regard to both parity groups and bedding type, the cows with stalls bedded with sand had thicker DCT than the cows bedded with manure solids ($P < 0.05$). The digital cushion was thinner for both primiparous and multiparous cows when there was rubber present in the feed alley of the barn ($P < 0.05$). Multiparous cows had thicker DCT when a veterinarian/employee was the hoof trimmer than with a contracted trimmer ($P < 0.05$).

Table 3.5 LSM and SEM for digital cushion thickness by farm management characteristics for primiparous and multiparous cows

Variable	Categories	N[1]	Primiparous LSM, cm (SEM)	Multiparous LSM, cm (SEM)	*P*-value
Parlor type					< 0.001
	Parallel	4	1.20^{ay} (0.09)	1.22^{ay} (0.09)	
	Rotary	1	0.95^{ay} (0.18)	1.11^{az} (0.18)	
Milk yield, kg/day					< 0.001
	≤41	3	1.04^{ay} (0.10)	1.18^{az} (0.10)	
	>41	2	1.22^{ay} (0.12)	1.23^{ay} (0.12)	
Bedding type					< 0.001
	Manure solids	2	0.94^{by} (0.04)	1.07^{bz} (0.03)	
	Sand	2	1.35^{ay} (0.04)	1.36^{ay} (0.03)	
	Sawdust	1	-	1.13^{b} (0.05)	
Rubber flooring					< 0.001
	Yes	3	0.96^{by} (0.03)	1.09^{bz} (0.03)	
	No	2	1.35^{ay} (0.04)	1.36^{ay} (0.03)	
Scrape					< 0.001
	Manual	3	1.04^{ay} (0.10)	1.18^{az} (0.10)	
	Automatic	2	1.22^{ay} (0.12)	1.23^{ay} (0.12)	
Footbath length, m					< 0.001
	<4	3	1.04^{ay} (0.11)	1.21^{az} (0.11)	
	≥4	2	1.15^{ay} (0.13)	1.17^{ay} (0.13)	
VLS[2]					< 0.001
	Yes	3	1.04^{ay} (0.11)	1.21^{az} (0.11)	
	No	2	1.15^{ay} (0.13)	1.17^{ay} (0.13)	
Chute type					< 0.001
	Standing	3	1.04^{ay} (0.10)	1.18^{az} (0.10)	
	Layover	2	1.22^{ay} (0.12)	1.23^{ay} (0.12)	
Hoof trimmer					0.009
	Contracted	2	1.03^{ay} (0.06)	1.07^{by} (0.05)	
	Farm employee	1	0.95^{ay} (0.07)	1.11^{abz} (0.07)	
	Veterinarian	1	-	1.40^{a} (0.08)	

[1]Number of farms per variable category.
[2]Farm assessed visual locomotion score (VLS) for lameness.
[a-b] within a variable, category LSM with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$.
[y-z] within a variable, category across parity groups, LSM with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$.

Genome-Wide Association Studies

Seventeen GWAS models were evaluated and 13 models did not provide significant results after correction for multiple testing. Two GWAS identified the same SNP passing Bonferroni correction for multiple testing on BTA24 related to the quantitative measure of DCT.

Another GWAS identified 22 SNP on BTA14 while a fourth GWAS identified four SNP on BTA29, with all SNP passing FDR. The models were the average DCT of the front medial digit, average DCT of the digits in the front foot, average DCT of the digits from the first time point, and average DCT of the digits from the second time point, respectively (Figure 3.2 and 3.3). BovineHD2400017222 on BTA24 was the only SNP in common amongst 2 of the 4 models, including the quantitative GWAS of the average front medial digit (Figure 3.2a) which is 1 of the 2 digits comprising the quantitative GWAS of the average DCT of the digits in the front foot (Figure 3.2b), with Bonferroni adjusted *P*-values of 0.01 and 0.02. Overall, the 26 SNP that passed FDR had an adjusted *P*-value range of 0.003 to 0.04 (Table 3.6).

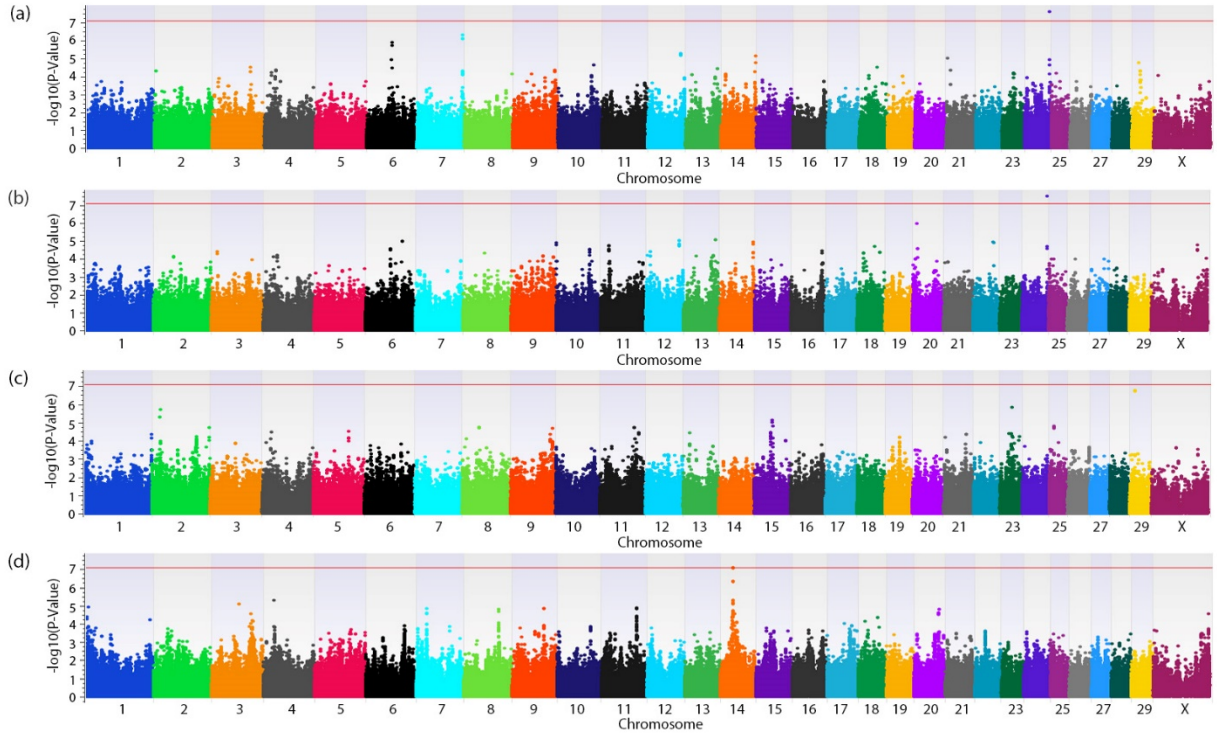

Figure 3.2 Manhattan plots for digital cushion thickness by (a) average front medial digit, (b) average front foot, (c) average of time point 1 measurements at <137 DPP, and (d) average of time point 2 measurements at 86 to 127 DIM. The red horizontal line indicates the Bonferroni adjusted *P*-value threshold of < 0.05.

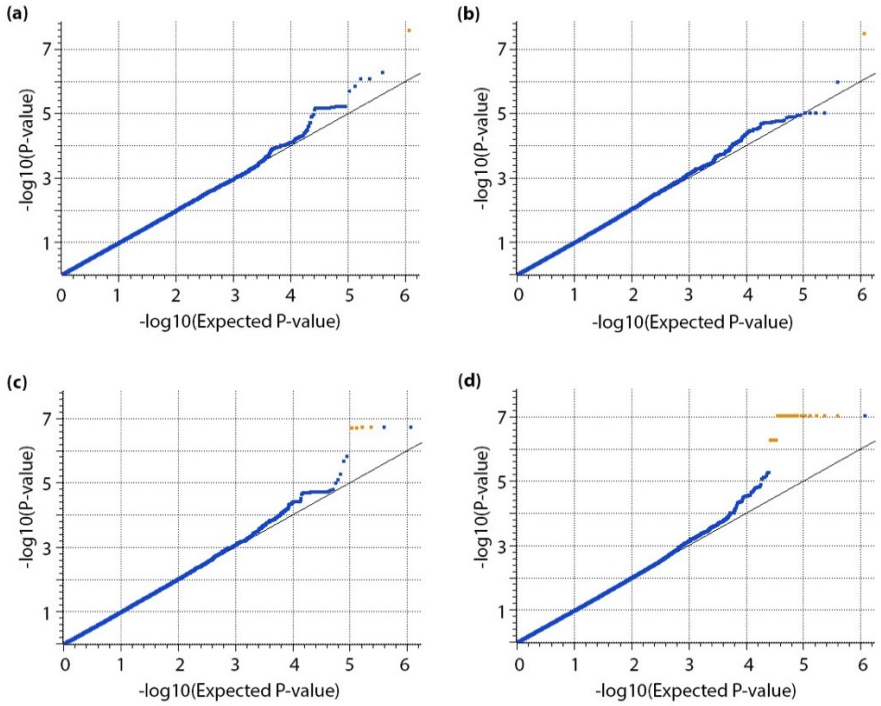

Figure 3.3 Quantile-quantile plots of digital cushion thickness by (a) average front medial digit, (b) average front foot, (c) average of time point 1 measurements at <137 DPP, and (d) average of time point 2 measurements at 86 to 127 DIM. The blue squares are markers that had a False Discovery Rate *P*-value ≥ 0.05 and the orange squares are markers that had a False Discovery Rate *P*-value < 0.05.

Table 3.6 Markers associated with digital cushion thickness (DCT) that surpassed the Bonferroni or False Discovery Rate (FDR) < 0.05

Marker	BTA	Position	Model[1]	Inheritance	Bonf *P*-value	FDR	Call Rate	Prop Var Explained[2]	Major Allele	Minor Allele	Minor Allele Frequency
BovineHD1400009245	14	30386060	Avg S2	Dominant	0.29	0.02	1	0.06	A	C	0.19
BovineHD1400009251	14	30393531	Avg S2	Dominant	0.29	0.02	1	0.06	A	C	0.19
BovineHD1400009258	14	30421600	Avg S2	Dominant	0.29	0.01	1	0.06	C	T	0.19
BovineHD1400009259	14	30430816	Avg S2	Dominant	0.29	0.01	1	0.06	C	T	0.19
BovineHD1400009260	14	30433464	Avg S2	Dominant	0.29	0.01	1	0.06	C	T	0.19
BovineHD1400009261	14	30434126	Avg S2	Dominant	0.29	0.01	1	0.06	G	A	0.19
BovineHD1400009276	14	30466855	Avg S2	Dominant	0.053	0.03	1	0.07	A	G	0.19
BovineHD1400009277	14	30469316	Avg S2	Dominant	0.053	0.02	1	0.07	A	G	0.19
BovineHD1400009278	14	30470355	Avg S2	Dominant	0.053	0.01	1	0.07	C	T	0.19
BovineHD1400009279	14	30471227	Avg S2	Dominant	0.053	0.01	1	0.07	G	T	0.19
BovineHD1400009280	14	30472270	Avg S2	Dominant	0.053	0.009	1	0.07	G	A	0.19
BovineHD1400009282	14	30478301	Avg S2	Dominant	0.053	0.008	1	0.07	A	G	0.19
BovineHD1400009283	14	30478817	Avg S2	Dominant	0.053	0.007	1	0.07	A	G	0.19
ARS-BFGL-NGS-70865	14	30479967	Avg S2	Dominant	0.053	0.006	1	0.07	T	C	0.19
BovineHD1400009284	14	30480434	Avg S2	Dominant	0.053	0.005	1	0.07	C	T	0.19
BovineHD1400009285	14	30481089	Avg S2	Dominant	0.053	0.005	1	0.07	C	T	0.19
BovineHD1400009286	14	30482025	Avg S2	Dominant	0.053	0.004	1	0.07	A	G	0.19
BovineHD1400009287	14	30482840	Avg S2	Dominant	0.053	0.004	1	0.07	A	G	0.19
BovineHD1400009288	14	30483789	Avg S2	Dominant	0.053	0.004	1	0.07	A	G	0.19
BovineHD1400009289	14	30485005	Avg S2	Dominant	0.053	0.004	1	0.07	T	C	0.19
BovineHD1400009290	14	30485892	Avg S2	Dominant	0.053	0.003	1	0.07	C	T	0.19
BovineHD1400009291	14	30486374	Avg S2	Dominant	0.053	0.003	1	0.07	T	C	0.19
BovineHD2400017222	24	59111990	Avg FM Avg Front	Recessive	0.01 0.02	0.01 0.02	1 1	0.08 0.07	A	G	0.37 0.37
BovineHD2900003473	29	11827051	Avg S1	Recessive	0.11	0.02	0.97	0.07	C	A	0.2
BovineHD2900003474	29	11827872	Avg S1	Recessive	0.11	0.02	1	0.07	A	G	0.2
BovineHD2900003475	29	11828561	Avg S1	Recessive	0.11	0.04	1	0.07	T	C	0.2
BovineHD2900003477	29	11832405	Avg S1	Recessive	0.11	0.03	1	0.07	T	C	0.2

[1]For the GWAS models, Avg S2 = average DCT of all digits at second time point measurement. Avg FM = average front medial digit, Avg Front = average DCT measurements of the digits in the front foot, and Avg S1 = average DCT of all digits at second time point measurement.
[2]Proportion of variance in the trait explained by the marker.

The range of pseudo-lambda values for the 4 models with significant SNP was between 0.99 and 1.0 indicating limited stratification due to substructure. Genetic heritability estimates including the covariates of sequencing batch plate, parity group, BCS, wither height, and sacral height for the 4 models with significant SNP ranged from 0.27 to 0.33 with an average of 0.31 ± 0.13.

Linkage disequilibrium structure was evaluated for 27 markers that surpassed the Bonferroni adjusted cutoff or FDR. Twenty-six of the 27 markers were in blocks of LD (Figure 3.4). Ten candidate genes were identified in the NCBI Genome Data Viewer either within the block of LD if R^2 was > 0.80 or within a 1 Mb window centered on the significantly associated SNP (Table 3.7). Twenty-five of the 27 markers resided within genes. The putative candidate genes were Discs Large MAGUK Scaffold Protein 2 (*DLG2*) and Melanocortin 4 Receptor (*MC4R*), which function in bone resorption and fat deposition, respectively.

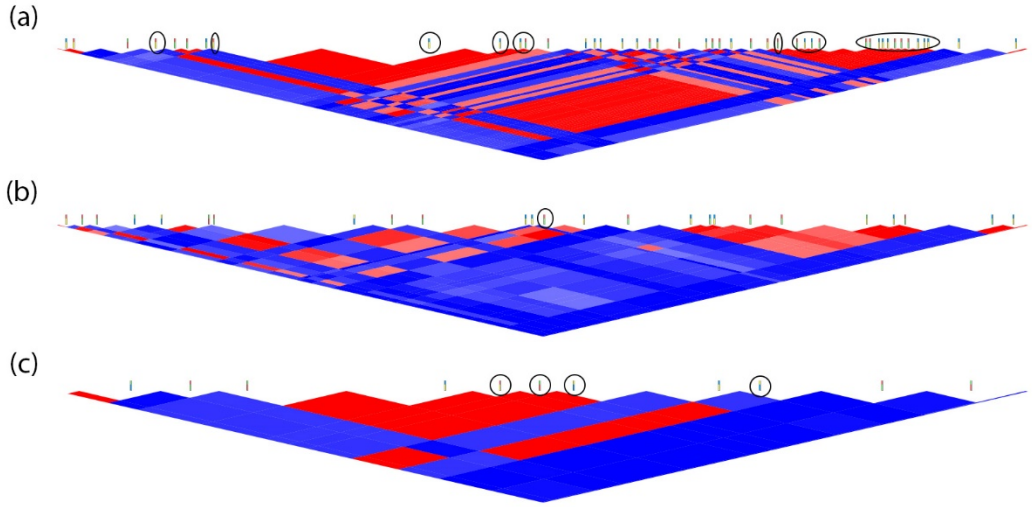

Figure 3.4 Linkage disequilibrium plots for markers (a) BovineHD1400009245, BovineHD1400009251, BovineHD1400009258, BovineHD1400009259, BovineHD1400009260, BovineHD1400009261, BovineHD1400009276, BovineHD1400009277, BovineHD1400009278, BovineHD1400009279, BovineHD1400009280, BovineHD1400009282, BovineHD1400009283, ARS-BFGL-NGS-70865, BovineHD1400009284, BovineHD1400009285, BovineHD1400009286, BovineHD1400009287, BovineHD1400009288, BovineHD1400009289, BovineHD1400009290, BovineHD1400009291, (b) BovineHD2400017222, and (c) BovineHD2900003473, BovineHD2900003474, BovineHD2900003475, and BovineHD2900003477 that passed Bonferroni adjusted significance threshold or False Discovery Rate. The circles correspond to the location of the 27 significant markers evaluated for candidate genes. The colors represent correlation between two alleles with blue shading for $R^2 < 0.50$ and red shading for $R^2 > 0.50$ with the shading darkening as the value reaches the extremes of 0 and 1.

Table 3.7 Candidate genes determined from the markers associated with digital cushion thickness that surpassed the Bonferroni significance cutoff or False Discovery Rate

BTA	Position Range (bp)[1]	Markers[2]	Candidate Genes[3]
14	30386060-30486374	**BovineHD1400009245, BovineHD1400009251, BovineHD1400009258, BovineHD1400009259, BovineHD1400009260, BovineHD1400009261,** BovineHD1400009276, **BovineHD1400009277, BovineHD1400009278, BovineHD1400009279, BovineHD1400009280, BovineHD1400009282, BovineHD1400009283, ARS-BFGL-NGS-70865, BovineHD1400009284, BovineHD1400009285, BovineHD1400009286, BovineHD1400009287, BovineHD1400009288, BovineHD1400009289, BovineHD1400009290, BovineHD1400009291**	***DNAJC5B,*** ***TRIM55****, TRNAY-GUA, TRNAA-AGC*
24	59611990-58611990	BovineHD2400017222	*PMAIP1, MC4R, LOC613321, LOC529488, LOC782054*
29	11821859-11832405	**BovineHD2900003477, BovineHD2900003475, BovineHD2900003474, BovineHD2900003473**	***DLG2***

[1]Range determined by linkage disequilibrium or within 500,000 bp upstream and 500,000 bp downstream of marker.
[2]The markers are those genotyped within the position range that were determined to be significantly associated with DCT. Markers are bolded if they resided within a gene.
[3]Genes are bolded if a marker is annotated within them.

DISCUSSION

In our initial epidemiologic study of DCT, we characterized the variation in DCT across lactation in 177 cows and determined digital cushion risk factors associated with lameness and key time points when digital cushion is thickest or thinnest (Stambuk et al., 2019). The current analysis evaluated 496 cows from 5 farms, more than tripling the number of individuals in our analysis by focusing on these 2 time points. This allowed us to corroborate our phenotypic results in a larger cohort across multiple farms and build a dataset more appropriate for genetic analysis. A genome-wide investigation of DCT provides an opportunity to understand the underlying biological mechanisms influencing the digital cushion.

Further investigation into the functions of the candidate genes for DCT revealed 2 biologically plausible genes related to fat tissue deposition and bone growth. *MC4R* located on BTA24, is a major controller of feed intake and energy expenditure, and mutations in this gene have been related to back fat thickness, marbling, carcass and live weight in cattle (Huang et al. 2010; Liu et al. 2010; Seong et al. 2012; Switonski et al., 2013). *MC4R* was identified as a candidate gene for the average front medial digit and average DCT of the digits in the front foot, which includes the front medial digit, GWAS models. The digital cushion consists of mostly adipose tissue, and this study determined the front medial digit to be thickest for both parity groups (Räber et al., 2004). Furthermore, the digits of the front feet have been shown to contain more fat than the digits in the hind feet (Räber et al., 2004). It is biologically plausible *MC4R* is affecting DCT through fat tissue accumulation.

All 4 markers that passed FDR for the average DCT of time point 1 measurements when the digital cushion is thickest, were annotated in *DLG2*, located on BTA29. Structural variant mutations in this gene are involved with osteosarcoma, with *DLG2* deletions accelerating bone tumor growth in canine and human cell lines (Chen et al., 2014; Shao et al., 2019). A study by Newsome et al. (2016) suggested that bone development on the caudal aspect of the distal phalanx impacts CHDL which is genetically correlated with DCT (Oikonomou et al., 2014). Potentially there could be mutations in *DLG2* influencing bone development in the distal phalanx, thereby impacting the thickness of the digital cushion. On average, time point 1 was < 2 mo prior to calving when cows are of greater body mass than in lactation due to the growing fetus as the cow gets closer to parturition. Therefore, it is biologically plausible *DLG2* has a greater influence on DCT at time point 1 due to the increase in pressure exerted on the digital cushion during that period.

The heritability of DCT based on the 4 GWAS models with significant SNP was found to be similar to previous research. The average heritability in the current study was 0.31 ± 0.13 when accounting for the covariates of sequencing batch plate, parity group, BCS, wither height, and sacral height used in the GWAS models which is slightly less than the 0.33 ± 0.09 estimated by Oikonomou et al. (2014). The difference could be due to the methods of determining heritability, population differences, and covariates included in the model. The current study determined heritability based on genetic markers while Oikonomou et al. (2014) determined heritability based on the estimates of variance components from a univariate analysis. Additionally, the current study defined DCT multiple ways and included measurements from the front foot while Oikonomou et al. (2014) only defined DCT as an average of all measurements with some cows only having the 4 hind digits.

Only 1 other study has focused on the genetics of digital cushion. Iqbal et al. (2016) investigated key genes associated with insulin signaling, carbohydrate metabolism, adipogenic and lipogenic transcription regulators, and regulation of lipolysis and their expression in the digital cushion of 14 non-pregnant non-lactating Holstein cows fed either high or low energy diets. Their goal was to relate target gene expression to the amount of energy in the diets. They found lipogenic genes to be consistently upregulated when feeding the higher energy diet. None of the 27 genes investigated by Iqbal et al. (2016) were candidate genes in the current study. However, Iqbal's study was fundamentally different from ours in which we used GWAS to identify candidate genes associated with digital cushion thickness as opposed to gene expression in the digital cushion related to diet.

This was the first study to evaluate DCT with different farm characteristics. Knowing the association of management practices to DCT allows producers to make better informed decisions

related to hoof health and lameness prevention given the relationship of DCT to lameness (Bicalho et al., 2009; Stambuk et al., 2019). For instance, the digital cushion was thicker when the free stalls were deep bedded with sand or when rubber was not in the feeding alley. However, these 2 management practices cannot be differentiated from one another as the farms that did not have rubber in the feeding alley of the pens were the farms that bedded with sand. Deep bedding with sand reduces the likeliness of a lame event whereas there have been inconsistent results for rubber usage (Andreasen and Forkman, 2012). Studies have reported beneficial effects while others have reported adverse effects like increased incidence of sole ulcers (Vanegas et al., 2006; Kremer et al., 2007). Instead of increasing the time spent feeding, rubber in the feed area significantly increased the time spent standing in the feed area and reduced time spent lying down (Fregonesi et al., 2004; Tucker et al., 2006).

Similar to Stambuk et al. (2019), DCT varied by claw and parity group with the hind medial digit being the thinnest in both parity groups. As noted before, we performed our analyses with and without the 35% of cows represented in Stambuk et al. (2019) that were also within this dataset and found the same biological and statistical differences in our outcomes and therefore included these cows in our final analysis. While this study determined the front medial digit to be thickest for both parity groups, Stambuk et al. (2019) only found the front medial to be thickest for primiparous cows. Conformational similarity of claw and sole length between the front medial claw and hind lateral claw may provide some insight towards our previous findings that the hind lateral claw was thickest for the multiparous cows (Nuss et al., 2011; Stambuk et al., 2019). Furthermore, the current study was able to evaluate variation in DCT within a larger number of Holstein cows and multiple farms. The previous study was conducted on 177 cows

from one farm while the current study was evaluated on 496 cows from 5 farms (Stambuk et al., 2019).

Cows that were tall at the sacral height had thicker DCT than those short or average at the sacral height. Newsome et al. (2017a) found a similar correlation between DCT and wither height on 179 cows in the United Kingdom that were mostly Holsteins in their first through fourth lactations. The current study found wither height to be different between parity groups potentially reflecting differences in DCT based on the relationship of height, age, and geography. Multiparous cows of short stature had thicker DCT than primiparous cows of the same stature. The correlation in the current of sacral height and wither height was 0.63, and therefore, results for DCT were expected to be similar for sacral height and wither height for Holstein cows.

Additionally, this study found a relationship between BCSG and DCT. This agrees with Bicalho et al. (2009) and Machado et al. (2011) who reported DCT to be positively associated with BCS, increasing as BCS increased due to the added fat reserves. However, our previous study and Newsome et al. (2017a) did not find a relationship between BCS and DCT (Stambuk et al., 2019). The contradicting results could be due to sample size and differences in populations. The current study, Bicalho et al. (2009), and Machado et al. (2011) have a comparable number of cows with 496, 501, and 574, respectively, but vary in the number of farms assessed with 5, 1, and 1, respectively.

CONCLUSIONS

This study evaluated 27 genetic markers on BTA14, BTA24, and BTA29 for candidate genes associated with DCT and explored the influence of particular farm characteristics on DCT. The 2 candidate genes highlighted were related to fat accumulation and bone development. A long-term goal of this study was to identify markers for use in genomic selection to reduce

lameness. Unfortunately, only results evaluated on traits with additive inheritance are able to be included in traditional genomic evaluations whereas our markers were identified in either a dominant or recessive inheritance pattern (Cole et al., 2009; Aliloo et al., 2016). However, these markers still have the opportunity to be used in breeding programs utilizing genomic selection against CHDL and lameness determined by visual locomotion score by genotyping the specific markers and knowing the associations between particular genotypes and DCT.

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REFERENCES

Aliloo, H., J. E. Pryce, O. González-Recio, B. G. Cocks, and B. J. Hayes. 2016. Accounting for dominance to improve genomic evaluations of dairy cows for fertility and milk production traits. Genet. Select. Evol. 48:8. https://doi.org/10.1186/s12711-016-0186-0.

Andreasen, S. N., and B. Forkman. 2012. The welfare of dairy cows is improved in relation to cleanliness and integument alterations on the hocks and lameness when sand is used as stall surface. J. Dairy Sci. 95:4961-4967. https://doi.org/10.3168/jds.2011-5169.

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. https://doi.org/10.3168/jds.2008-1827.

Chen, X., A. Bahrami, A. Pappo, J. Easton, J. Dalton, E. Hedlund, D. Ellison, S. Shurtleff, G. Wu, L. Wei, M. Parker, M. Rusch, P. Nagahawatte, J. Wu, S. Mao, K. Boggs, H. Mulder, D. Yergeau, C. Lu, L. Ding, M. Edmonson, C. Qu, J. Wang, Y. Li, F. Navid, N. C. Daw, E. R. Mardis, R. K. Wilson, J. R. Downing, J. Zhang, and M. A. Dyer. 2014. Recurrent somatic structural variations contribute to tumorigenesis in pediatric osteosarcoma. Cell Rep. 7:104-112. https://doi.org/10.1016/j.celrep.2014.03.003.

Cole, J. B., P. M. Van Raden, J. R. O'Connell, C. P. Van Tassell, T. S. Sonstegard, R. D. Schnabel, J. F. Taylor, and G. R. Wiggans. 2009. Distribution and location of genetic effects of dairy traits. J. Dairy Sci. 92:2931-2946. https://doi.org/10.3168/jds.2008-1762.

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. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.

Fregonesi, J. A., C. B. Tucker, D. M. Weary, F. C. Flower, and T. Vittie. 2004. Effect of rubber flooring in front of the feed bunk on the time budgets of dairy cattle. J. Dairy Sci. 94:1243-1255. https://doi.org/10.3168/jds.S0022-0302(04)73270-1.

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. https://doi.org/10.3168/jds.2017-13531.

Huang, M., X. Gao, J. Y. Li, H. Y. Ren, J. B. Chen, and S. Z. Xu. 2010. Polymorphisms in MC4R gene and correlations with economic traits in cattle. Mol. Biol. Rep. 37:3941-3944. https://doi.org/10.1007/s11033-010-0051-0.

Iqbal, Z. M., H. Akbar, A. Hosseini, E. Bichi Ruspoli Forteguerri, J. S. Osorio, and J. J. Loor. 2016. Digital cushion fatty acid composition and lipid metabolism gene network expression in Holstein dairy cows fed a high-energy diet. PLoS One. 11:e0159536. https://doi.org/10.1371/journal.pone.0159536.

Kang, H. M., J. H. Sul, S. K. Service, N. A. Zaitlen, S. Y. Kong, and N. B. Freimer. 2010. Variance component model to account for sample structure in genome-wide association studies. Nat. Genet. 42:348-354. https://doi.org/10.1038/ng.548.

Kremer, P. V., S. Nueske, A. M. Scholz, and M. Foerster. 2007. Comparison of claw health and milk yield in dairy cows on elastic or concrete flooring. J. Dairy Sci. 90:4603-4611. https://doi.org/10.3168/jds.2006-549.

Lischer, C. J., P. Ossent, M. Räber, and H. Geyer. 2002. 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. https://doi.org/10.1136/vr.151.23.694.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216–1231. https://doi.org/10.2527/1998.7641216x.

Liu, H., W. Tian, L. Zan, H. Wang, and H. Cui. 2010. Mutations of MC4R gene and its association with economic traits in Qinchuan cattle. Mol. Biol. Rep. 37:535-540. https://doi.org/10.1007/s11033-009-9706-0.

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. https://doi.org/10.2460/ajvr.72.10.1338.

Mülling, C. K. W., and P. R. Greenough. 2006. Applied physiopathology of the foot. In XXIV World Buiatrics Congress. Nice, France. World Association for Buiatrics, Paris, France.

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.

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. https://doi.org/10.3168/jds.2015-10202.

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. https://doi.org/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. 100:4745-4758. https://doi.org/10.3168/jds.2016-12013.

Nuss, K., C. Sauter-Louis, and B. Sigmund. 2011. Measurements of forelimb claw dimensions in cows using a standardised sole thickness: A post-mortem study. Vet. J. 190:84-89. https://doi.org/10.1016/j.tvjl.2010.10.002.

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. https://doi.org/10.3168/jds.2012-6308.

Oikonomou, G., G. Banos, V. Machado, L. Caixeta, and R. C. Bicalho. 2014. Genetic characterization of digital cushion thickness. J. Dairy Sci. 97:532-536. https://doi.org/10.3168/jds.2013-7212.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A., Ferreira, D. Bender, J. Maller, P. Sklar, P. I. 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. https://doi.org/10.1086/519795.

Räber, M., C. Lischer, H. Geyer, and P. Ossent. 2004. The bovine digital cushion–A descriptive anatomical study. Vet. J. 167:258–264. https://doi.org/10.1016/S1090-0233(03)00053-4.

Seong, J., D. S. Suh, K. D. Park, H. K. Lee, and H. S. Kong. 2012. Identification and analysis of MC4R polymorphisms and their association with economic traits of Korean cattle (Hanwoo). Mol. Biol. Rep. 39:3597-3601. https://doi.org/10.1007/s11033-011-1133-3.

Shao, Y. W., G. A. Wood, J. Lu, Q. Tang, J. Liu, S. Molyneux, Y. Chen, H. Fang, H. Adissu, T. McKee, P. Waterhouse, and R. Khokha. 2019. Cross-species genomics identifies DLG2 as a tumor suppressor in osteosarcoma. Oncogene 38:291-298. https://doi.org/10.1038/s41388-018-0444-4.

Shearer, J. K., P. J. Plummer, and J. A. Schleining. 2015. Perspectives on the treatment of claw lesions in cattle. VMRR. 6:273-292. https://doi.org/10.2147/VMRR.S62071.

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. 47:1179-1187. https://doi.org/10.1016/S0093-691X(97)00098-8.

Stambuk, C. R., J. A. A. McArt, B. 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. Trans. Anim. Sci. 3:9-18. https://doi.org/10.1093/tas/txy107.

Switonski, M., M. Mankowska, and S. Salamon. 2013. Family of melanocortin receptor (MCR) genes in mammals-mutations, polymorphisms and phenotypic effects. J. Appl. Genet. 54:461-472. https://doi.org/10.1007/s13353-013-0163-z.

Tucker, C. B., D. M. Weary, A. M. de Passille, B. Campbell, and J. Rushen. 2006. Flooring in front of the feed bunk affects feeding behavior and use of freestalls by dairy cows. J. Dairy Sci. 89:2065-2071. https://doi.org/10.3168/jds.S0022-0302(06)72274-3.

Vanegas, J., M. Overton, S. L. Berry, and W. M. Sischo. 2006. Effect of rubber flooring on claw health in lactating dairy cows housed in free-stall barns. J. Dairy Sci. 89:4251-4258. https://doi.org/10.3168/jds.S0022-0302(06)72471-7.

Yang, J., S. Hong Lee, M. L. Goddard, and P. M. Visscher. 2011. GCTA: A tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88:76-82. https://doi.org/10.1016/j.ajhg.2010.11.011.

CHAPTER 4:

EXPLORING PHYSIOLOGICAL AND GENETIC VARIATION OF DIGITAL CUSHION THICKNESS OF HOLSTEIN AND JERSEY COWS AND BULLS

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ABSTRACT

The bovine digital cushion is an essential fat pad located in the horn capsule between the distal phalanx and sole horn that has been associated with claw horn disruption lesions. The study of digital cushion thickness (DCT) has been largely restricted to Holstein cows, therefore the objectives of our study were to phenotypically compare DCT and to explore associations of genetic markers with DCT for Holstein and Jersey cows and bulls. In a cohort of 698 cows and 85 bulls (Holstein and Jersey) from 8 farms in New York, USA, data were collected on DCT, body condition score (BCS), wither height, and sacral height. All animals underwent digital sonographic examination of the digital cushion evaluated at the sole ulcer site for the right front and hind foot. Linear mixed models were conducted on DCT separately by cows and bulls with the fixed effects of breed, age, digit, and body condition score group. The models included the repeated statement of claw with animal and the random effect of animal nested within farm to account for the random subset of animals per farm and multiple measurements from each animal. The phenotypic results indicated DCT varied by breed, age, and digit for the cows and varied by breed, age, digit, and body condition score group for the bulls. For the genotypic study, 616 cow DNA samples were genotyped on the Illumina BovineHD 777K beadchip, while 76 bull DNA samples were genotyped on a variety of platforms ranging from 5K to 150K. Data were separated into 8 datasets either separating or combining breeds and sexes. Each dataset was assessed for quality of markers and samples prior to conducting genome-wide association studies for DCT testing the inheritance models and genetic variation of digit, foot, and average thickness. Ten markers passed the Bonferroni correction threshold and 9 passed false discovery rate from 10 genome-wide association studies using a combination of the covariates breed, cows or bulls, sequencing batch plate, age, BCS, wither height, and sacral height. Out of the 43

candidate genes, 8 novel biologically plausible genes were identified on *Bos taurus* autosome 3, 4, 7, and 9. *SFRS18,* and *LRRFIP1* function in fat deposition, whereas *AHR*, *BZW2*, *EFNA5*, *USP45*, and *VAV3* effect bone growth, and *SOSTDC1* is related to epidermal keratinocyte function. The genetic markers associated with DCT in this study were explored for relevance between cows and bulls and within and across breeds for their potential use in marker assisted selection.

INTRODUCTION

The digital cushion is a complex structure composed mostly of adipose and connective tissue that is crucial in dampening the compression of the corium tissue that produces sole horn under the distal phalanx (Lischer et al., 2002; Räber et al., 2004; Shearer et al., 2015). The majority of the digital cushion lies behind the navicular bone in the heel area while only 10% extends forward beneath the distal phalanx (Mülling and Greenough, 2006). Digital cushion thickness (DCT) at the typical sole ulcer site beneath the flexor tuberosity of the distal phalanx has been determined to be a strong predictor of lameness and claw horn disruption lesions (CHDL; sole ulcers and white line disease; Bicalho et al., 2009; Newsome et al., 2017a,b; Stambuk et al., 2019). Claw horn disruption lesions are the most prevalent noninfectious claw disorders associated with lameness and pain (Murray et al., 1996; Oikonomou et al., 2013).

Identifying quantitative trait loci (QTL) associated with digital cushion thickness provides an indirect means to select animals for reduced presence of CHDL and lameness. Digital cushion thickness was previously determined to be moderately heritable (0.32) and had a negative genetic correlation with CHDL (-0.60 ± 0.29) (Oikonomou et al., 2014; Stambuk et al., manuscript in preparation). Heritability estimates for CHDL and lameness according to visual locomotion were low and ranged from 0.01 to 0.12 and 0.07 to 0.10, respectively (Sprecher et

al., 1997; Heringstad et al., 2018). The presence of CHDL and lameness according to visual locomotion had a moderate phenotypic correlation of 0.49 (Oikonomou et al., 2014).

Thus far, DCT has been characterized in Holstein cows, Brown Swiss cows, Simmental-Red Holstein crossbred cows, and Brown Swiss-Ayrshire crossbred cows. However, the DCT was not compared across breeds in the studies with purebred and crossbred cows (Räber et al., 2004; Bicalho et al., 2009; Iqbal et al., 2016; Newsome et al., 2017a,b). Gard et al. (2015) distinguished the total digital cushion volume and surface area in Jersey and Holstein bull calves but did not evaluate bulls older than 6 mo or Jersey cows. Therefore, the objectives of our study were 1) to examine influences of breed on DCT in cows and bulls and 2) to identify QTL associated with DCT in Holstein and Jersey cows and bulls.

MATERIALS AND METHODS

Cows

Cow data were collected from 7 commercial dairy farms (5 Holstein farms and 2 Jersey farms) from October 13, 2015 to October 26, 2017 in New York, USA. The farms were selected based on the willingness of managers to accommodate the study and for easy access to cows, hoof trimmers, and cattle chutes. The total number of cows on farm ranged from 150 to 4,400 cows. The cows were milked 2 to 3 times a day and walked a distance of 3.5 to 379 m to the milking parlor every milking. Cow milk production ranged 28 to 42 kg of milk per cow per day. All cows were group housed in free-stall pens with bedding that included paper fiber, sand, sawdust on mattresses, or dried manure solids. All cows were trimmed by a hoof trimmer on a routine schedule and all lame cows were treated when identified as lame by employees. Approval from the Cornell University Institutional Animal Care and Use Committee (Protocol #2014-0121) and signed owner consent were obtained prior to commencement of the study.

A total of 502 Holstein cows and 196 Jersey cows were enrolled in a prospective cohort study where DCT measurements were evaluated at 2 time points based on a combination of time periods of thickest and thinnest DCT and the trimming schedule of the farm (Stambuk et al., 2019). An average of DCT for each digit and BCS was used in the statistical analysis in order to have 1 measurement per digit similar to the bulls yet captured variation in DCT across lactation. The first measurement was <137 d prepartum (DPP) when cows were near or in the dry period prior to calving. The measurement had a mean of 46 DPP. The large variation in DPP was due to differences in management practices and trimming schedules. The second measurement occurred mid lactation from 86 to 130 d in milk (DIM), with a mean of 107 DIM, when DCT was reported to be thinnest and coincided with routine hoof trimming schedules to minimize impact on the cows and farm management.

Hoof trimming was completed by contracted professionals or trained farm employees, and cows were restrained for hoof trimming. Cows on 4 farms were trimmed on a standing chute; 3 farms had an Appleton Steel Trimming Chute (Appleton Steel, Appleton, WI) and 1 farm had a Comfort Chute H-series (Comfort Hoof Care, Baraboo, WI). Three farms trimmed cows on a layover Riley Built trailer model hoof trimming chute (Riley Built, Lubbock, TX). At each measurement, a single trained researcher collected a body condition score (BCS) from 1 to 5 with a quarter point system as described by Edmonson et al. (1989). Wither height and sacral height were measured at the beginning of the study. Wither height was defined as the distance from the floor to the withers and sacral height was defined as the distance from the floor to the dorsal aspect of the caudal sacral joint. Age, DIM at each measurement event, and parturition date for the cows were obtained from the farm management software (DairyComp 305; Valley Agricultural Software, Tulare, CA). All cows underwent digital sonographic B-mode

examination with an Aquila Vet ultrasound machine (Esaote Europe BV, Maastricht, the Netherlands) following the protocol described in Stambuk et al. (2019). Immediately after trimming, sonograms of the 4 right digits were evaluated. This study measured DCT at the typical sole ulcer cite beneath the flexor tuberosity of the distal phalanx.

Prior to any statistical analysis, 10 cows were excluded based on sampling outside the targeted time points. A total of 688 cows consisting of 496 Holstein cows and 192 Jersey cows were used in the statistical analysis. The variables of age and BCS were categorized into terciles based on the given cow dataset for interpretation of the statistical results. Age of cows were categorized into <3 years old, 3 to 4 years old, and >4 years old. Body condition scores were categorized into BCS groups (BCSG), where cows were in the thin group if BCS <2.8, average group if BCS was 2.8 to 3.2, and fat group if BCS >3.2. The variable digit (CLAW) represented the specific hoof digits and was categorized as FM for front medial digit, FL for front lateral digit, HM for hind medial digit, and HL for hind lateral digit.

For statistical analysis of cows, independent variables were the fixed effects of breed (Holstein or Jersey), age, time point (86 to 127 DIM or <137 DPP), BCSG and CLAW and the random effects of farm and cow nested within farm with repeated measures. The compound symmetry covariance structure was used because it resulted in the lowest Akaike information criterion for repeated measures (Littell et al., 1998). The MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) was used to obtain solutions and conduct the ANOVA. The assumptions of homogeneity, normality, and independence of the residuals for the mixed model was obtained. Pairwise mean comparisons evaluated significant effects in all models and *P*-values were adjusted for multiple comparisons using the Tukey-Kramer method. All treatment results were reported as least squares means, with significance declared at $P < 0.05$.

Bulls

Bull data was collected from 1 artificial insemination corporative facility from March 14, 2018 to July 19, 2018 located in Ithaca, New York, USA. The bull facility had 189 bulls in 2 buildings. The bulls were housed indoors in separate stalls with rubber mats and sawdust; their stalls were cleaned with fresh sawdust added daily. On average, bulls walked 55 m and 82 m to the semen collection area in the first and second building, respectively. Bull collection depended on the priority of the bull and averaged 4 times a week.

A total of 85 bulls were enrolled in a cross-sectional study where DCT was measured 1 time when the bulls were due for their routine hoof trimming. For the bull data, 73 were Holstein bulls and 12 were Jersey bulls. Data collected included DCT measurements of the 4 right digits, BCS, wither height and sacral height. The manager provided the birth dates for the bulls. Hoof trimming was completed by 2 trained employees where 1 trimmed the front feet and the other trimmed the hind feet. Each bull was hand walked to the trim chute separately. Bulls were restrained for hoof trimming using an Appleton Steel Trimming Chute (Appleton Steel, Appleton, WI). All bulls underwent digital sonographic B-mode examination with an Aquila Vet ultrasound machine (Esaote Europe BV, Maastricht, the Netherlands) following the protocol described in Stambuk et al. (2019). Immediately after trimming, DCT of the 4 right digits was measured at the typical sole ulcer cite beneath the flexor tuberosity of the distal phalanx from the sonogram.

Prior to any statistical analysis, the variables age and BCS were categorized into terciles based on the given bull dataset. The variable age was categorized into <2 years old, 2 to 3 years old, and >3 years old. Bulls were thin if BCS <2.75, average if BCS was 2.75 to 3.25, and fat if

BCS >3.25. The variable digit (CLAW) represented the specific hoof digits as denoted in the cows and was categorized as FM, FL, HM, and HL.

For statistical analysis of bulls, independent variables were the fixed effects of breed (Holstein or Jersey), age, BCSG and CLAW and the random effects of with repeated measures of CLAW. The compound symmetry covariance structure was used because it resulted in the lowest Akaike information criterion for repeated measures (Littell et al., 1998). The MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) was used to obtain solutions and conduct the ANOVA. Pairwise mean comparisons evaluated significant effects in all models and *P*-values were adjusted for multiple comparisons using the Tukey-Kramer method. All treatment results were reported as least squares means, with significance declared at $P < 0.05$.

Genotyping and Quality Control

The cows had whole blood (10 mL) obtained via the coccygeal vessels into vacutainer tubes with the anticoagulant K_2EDTA for subsequent DNA extraction. Genomic DNA was extracted following the Gentra Puregene Blood Kit extraction protocol (Qiagen, Valencia, CA). A total of 616 cow samples were genotyped on the Illumina BovineHD 777K beadchip (Illumina, Inc., San Diego, CA) at GeneSeek (Neogen Genomics, Lincoln, NE).

A total of 76 bull genotypes (64 Holstein and 12 Jersey bulls) were provided by the artificial insemination company. The bulls were genotyped on a variety of single-nucleotide polymorphism (SNP) panel sizes, ranging from 5K to 150K. The bulls genotyped on the lower-density SNP panels restricted the total number of SNP available for genome-wide interrogation in the bull datasets and within breed datasets,

The data were separated into 8 datasets based on combinations of breed and cows and bulls. All datasets (Table 4.1) evaluated the same quality assessment of the markers and samples

using Golden Helix SVS version 8.8.3 (Golden Helix, Bozeman, MT). The SNP were removed if they had a call rate < 0.90, a minor allele frequency < 0.03, > 2 alleles, and Hardy-Weinberg Equilibrium *P*-value < 0.0001. To evaluate population structure and relatedness, an identity-by-state similarity matrix was used to calculate genome-wide identity-by-descent estimates (Purcell et al., 2007). Samples with a call rate < 0.90 were removed. One of the sample pairs with an identity-by-decent estimate greater than 0.85 denoting substantial relatedness was removed.

Table 4.1 Quality assessment of the markers and samples for the 8 genotype datasets

Dataset	Initial samples	Initial SNP	Total SNP removed	SNP removal parameters[6]				Sample removal parameters[7]		Total SNP	Total samples
				Call rate	MAF	> 2 alleles	HWE	Call rate	IBD		
Cow[1]	615	777,962	240,793	8,558	147,248	0	141,286	11	4	537,169	600
Jersey cow	169	777,962	233,811	7,744	226,073	0	3,083	0	1	544,151	168
Bull[2]	76	154,691	149,548	147,417	14,637	22,209	5,002	0	0	5,143	76
Holstein bull	64	154,691	149,460	147,437	14,637	19,569	4,526	0	0	5,231	64
Jersey bull	12	154,691	139,464	136,910	34,932	5,013	0	2	0	15,227	10
Holstein breed[3]	510	145,530	43,806	18,000	11,061	19,094	4,382	53	3	101,724	454
Jersey breed[4]	181	145,530	31,660	1,593	24,948	5,496	967	4	1	113,870	176
Combined[5]	691	145,530	51,893	7,420	9,286	21,763	19,807	57	4	93,637	630

[1]The final Cow dataset included 432 Holstein and 168 Jersey.
[2]The final Bull dataset included 64 Holstein and 12 Jersey.
[3]The final Holstein breed dataset included 431 cows and 23 bulls.
[4]The final Jersey breed dataset included 168 cows and 8 bulls.
[5]The final Combined dataset included 431 Holstein cows, 168 Jersey cows, 23 Holstein bulls, and 8 Jersey bulls.
[6]The single-nucleotide polymorphisms (SNP) were removed if they had a call rate < 0.90, a minor allele frequency (MAF) < 0.03, > 2 alleles, and Hardy-Weinberg Equilibrium (HWE) *P*-value < 0.0001.
[7]Samples with a call rate < 0.90 and an identity-by-decent estimate > 0.85 were removed.

Genome-Wide Association Studies

Digital cushion thickness was defined as the quantitative measurements of each claw (n = 4), the averages of foot (n = 2), and all measurements combined (n = 1) to explore relationships between genomic regions related to digit, foot, breed, and cows or bulls. Genetic heritability of DCT and power to predict associations were determined for the 7 definitions of DCT per dataset including covariates combination of breed, sex, sequencing batch plate, age, BCS, wither height, and sacral height with the use of GCTA (Yang et al., 2011). If the power was > 0.80, then the trait was evaluated in a genome-wide association studies (GWAS). Associations were assessed using EMMAX (Kang et al., 2010) to correct for population structure and relatedness by including a genomic relationship matrix as a random effect in the models in Golden Helix SVS version 8.8.3 (Golden Helix, Bozeman, MT).

Association studies with the appropriate combination of the covariates breed, sex, sequencing batch plate, age, BCS, wither height, and sacral height were considered in additive, dominant, and recessive inheritance models. The covariate of farm was investigated but could not be included in the cow datasets with sequencing batch plate because they were highly correlated. Sequencing batch plate accounts for the differences based on sequencing batch as well as farm because 1 batch contained samples from 1 farm while the other batch included samples from the other 4 farms where samples on a plate were mostly from the same farm. Farm could not be included with the covariates sex or breed because all bulls were from the same farm. Body condition score was an average between the 2 time points while wither and sacral heights were continuous measurements.

Quantile–quantile plots and pseudo-lambda values were used to determine the model of best fit for each trait and inheritance model. Quantile-quantile plots graphically represented the

deviation of the observed *P*-values from the null hypothesis to assess the number and magnitude of observed associations between genotyped SNP and the DCT. Pseudo lambda values measured inflation in the models, with values around 1 showing no inflation and values greater than 1.1 showing inflation. Either a Bonferroni adjusted *P*-value or False Discovery Rate (FDR) *P*-value < 0.05 distinguished SNP significantly associated with DCT. Candidate regions were examined for putative candidate genes based on the linkage disequilibrium (LD) structure or a 1 Mb window centered on the significantly associated SNP if LD was not present using the *Bos taurus* ARS-UCD1.2 assembly in the NCBI Genome Data Viewer (https://www.ncbi.nlm.nih.gov/genome/82).

RESULTS

Linear Mixed Models of Digital Cushion Thickness

Table 4.2 has descriptive statistics separated by cows and bulls for all DCT measurements, age, BCS, wither height, and sacral height. Holstein cows had a mean DCT 1.34 times thicker than Holstein bulls with a higher standard deviation.

Table 4.2 Descriptive statistics for continuous variables separated by breed and sex

		Holstein			Jersey		
Variable		Mean (SD)	Min	Max	Mean (SD)	Min	Max
Cows							
	DCT[1], cm	1.18 (0.26)	0.57	2.15	1.02 (0.19)	0.48	1.85
	Age, yr	3.71 (1.44)	2	10	3.99 (1.28)	2	8
	BCS[2]	3.00 (0.45)	1.75	4.5	3.02 (0.49)	1.25	4.25
	Wither height[3], cm	145 (5.92)	129	160	126 (3.26)	117	135
	Sacral height[4], cm	149 (4.55)	135	165	129 (3.61)	121	140
Bulls							
	DCT, cm	0.87 (0.20)	0.42	1.49	0.75 (0.21)	0.42	1.61
	Age, yr	3.03 (1.67)	1	7	2.17 (1.15)	1	5
	BCS	2.74 (0.63)	1.5	3.75	3.33 (0.43)	2.75	4
	Wither height, cm	156 (10.46)	128	177	133 (6.87)	122	148
	Sacral height, cm	157 (7.50)	139	175	133 (5.45)	124	141

[1]DCT, digital cushion thickness.
[2]BCS, body condition score.
[3]Assessed as the distance from the floor to the withers.
[4]Assessed as the distance from the floor to the dorsal aspect of the caudal sacral joint.

For the cow dataset, DCT varied by breed, age, and CLAW (Table 4.3). Holstein cows had thicker DCT than Jersey cows ($P = 0.002$). Cows ≥ 3 years old had thicker DCT than those younger ($P < 0.001$). The front medial digit had the thickest DCT while the hind medial digit was the thinnest ($P < 0.05$).

Table 4.3 Least Squares Means (LSM) and SEM for digital cushion thickness for cows

Variable	Categories	LSM, cm (SEM)	*P*-value
Breed			0.002
	Holstein	1.17[a] (0.05)	
	Jersey	1.01[b] (0.06)	
Age, yr			<0.001
	< 3	1.03[b] (0.05)	
	3 to 4	1.12[a] (0.05)	
	> 4	1.12[a] (0.05)	
CLAW[1]			<0.001
	Front Medial	1.16[a] (0.05)	
	Front Lateral	1.10[b] (0.05)	
	Hind Medial	1.02[c] (0.05)	
	Hind Lateral	1.08[b] (0.05)	

[1]CLAW = digits.
[a-c] within a variable, category LSM with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$.

For the bull dataset, DCT varied by breed, age, CLAW, and BCSG (Table 4.4). Holstein bulls had thicker DCT than Jersey bulls ($P = 0.003$). Young bulls ≤ 3 years old had thinner DCT than older bulls ($P < 0.02$). The front digits were thicker than either hind digit with the hind medial being the thinnest ($P < 0.05$). Digital cushion thickness differed based on BCSG such that bulls in the thin group had thinner DCT than those in the thick group ($P = 0.03$).

Table 4.4 Least Squares Means (LSM) and SEM for digital cushion thickness for bulls

Variable	Categories	LSM, cm (SEM)	*P*-value
Breed			0.003
	Holstein	0.87[a] (0.02)	
	Jersey	0.75[b] (0.04)	
Age, yr			0.002
	< 2	0.73[b] (0.03)	
	2 to 3	0.79[b] (0.03)	
	> 3	0.91[a] (0.03)	
CLAW[1]			<0.001
	Front Medial	0.92[a] (0.02)	
	Front Lateral	0.86[a] (0.02)	
	Hind Medial	0.67[c] (0.02)	
	Hind Lateral	0.78[b] (0.02)	
BCSG[2]			0.036
	Thin (< 2.75)[3]	0.75[c] (0.04)	
	Average (2.75 – 3.25)	0.81[bc] (0.03)	
	Thick (> 3.25)	0.87[ab] (0.03)	

[1]BCSG = body condition score group.
[2]CLAW = digits.
[3]Category thresholds are denoted in the parentheses.
[a-c] within a variable, category LSM with different superscripts are different at Tukey-Kramer adjusted $P < 0.05$.

Genome-wide association studies

Genome-wide association studies were not evaluated on the combined bull dataset nor the Holstein bull, or Jersey bull datasets because either they did not have enough animals to calculate heritability or the power to predict an association was < 0.80.

Holstein and Jersey cows. All 7 definitions of DCT evaluating the digit, foot, and average DCT of all measures, had enough heritability and power to be evaluated in a GWAS combining Holstein and Jersey cows. Six of the 7 GWAS models did not provide significant results. The dominant GWAS of the average DCT of all measurements identified 2 SNP on BTA3 (Figure 4.1).

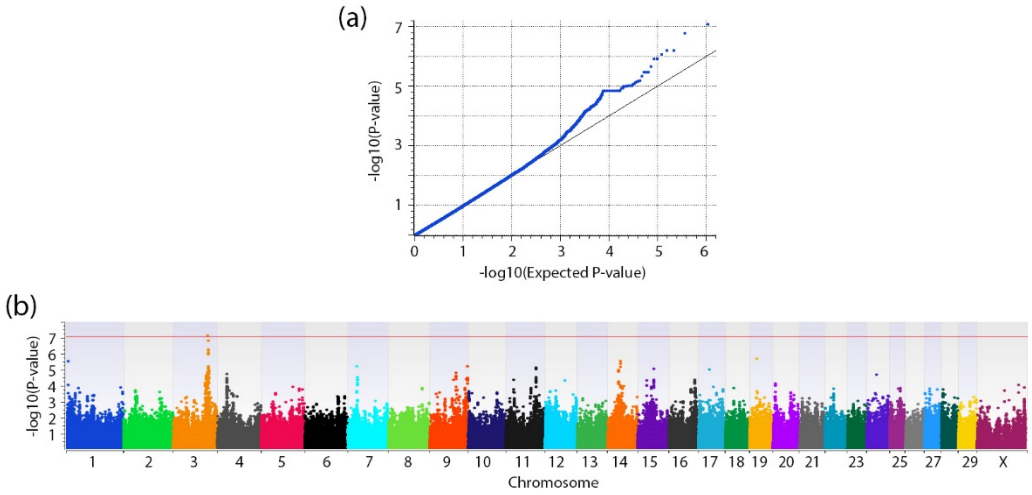

Figure 4.1 (a) Quantile-quantile plot and (b) Manhattan plot for average digital cushion thickness of all measurements for the cow dataset including Holstein (n = 432) and Jersey (n = 168) breeds. The red horizontal line indicates the Bonferroni adjusted *P*-value threshold of 0.05.

Holstein cows. The Holstein cow dataset is the same as published in Stambuk et al. (manuscript in preparation). Stambuk et al. (manuscript in preparation) identified 27 genetic markers on BTA14, BTA24, and BTA29 with respective candidate genes associated with DCT.

Jersey cows. Five definitions of DCT for the Jersey cow dataset did not have enough power to conduct a GWAS. The recessive GWAS for DCT of the hind lateral digit identified 274 markers associated with DCT based on FDR. However, these markers were not investigated for candidate genes due to early deviation and later migration back towards the null on the quantile-quantile plot and the possibility of false positives (Figure 4.2). This model could be improved upon with a larger sample size that captures greater genetic variation of DCT among the Jerseys.

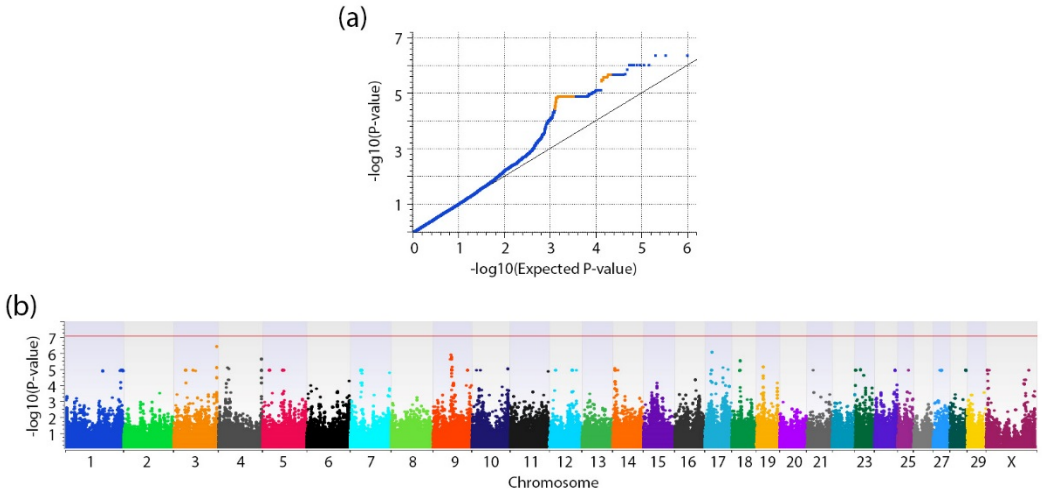

Figure 4.2 (a) Quantile-quantile plot and (b) Manhattan plot for digital cushion thickness of the hind lateral digit for the Jersey cow dataset. The blue squares are markers that had a False Discovery Rate *P*-value ≥ 0.05 and the orange squares are markers that had a False Discovery Rate *P*-value < 0.05. The red horizontal line indicates the Bonferroni adjusted *P*-value threshold of 0.05.

Holstein cows and bulls. All 7 definitions of DCT had sufficient estimated heritability and power to predict an association to be evaluated in GWAS combining Holstein cows and bulls. Three of the 7 GWAS models did not provide significant results. The recessive GWAS for DCT of the front medial digit identified BovineHD0700031927 on BTA7. The recessive GWAS of the average DCT of the front foot identified BovineHD0400007408 on BTA4 and BovineHD1400023369 on BTA14. The additive GWAS of the average DCT of the hind foot identified BovineHD2700001206, BovineHD2700001209, and BovineHD2700001220 on BTA27. The additive GWAS of the average DCT of all measurements identified BovineHD2700001198 on BTA27 (Figure 4.3 and 4.4).

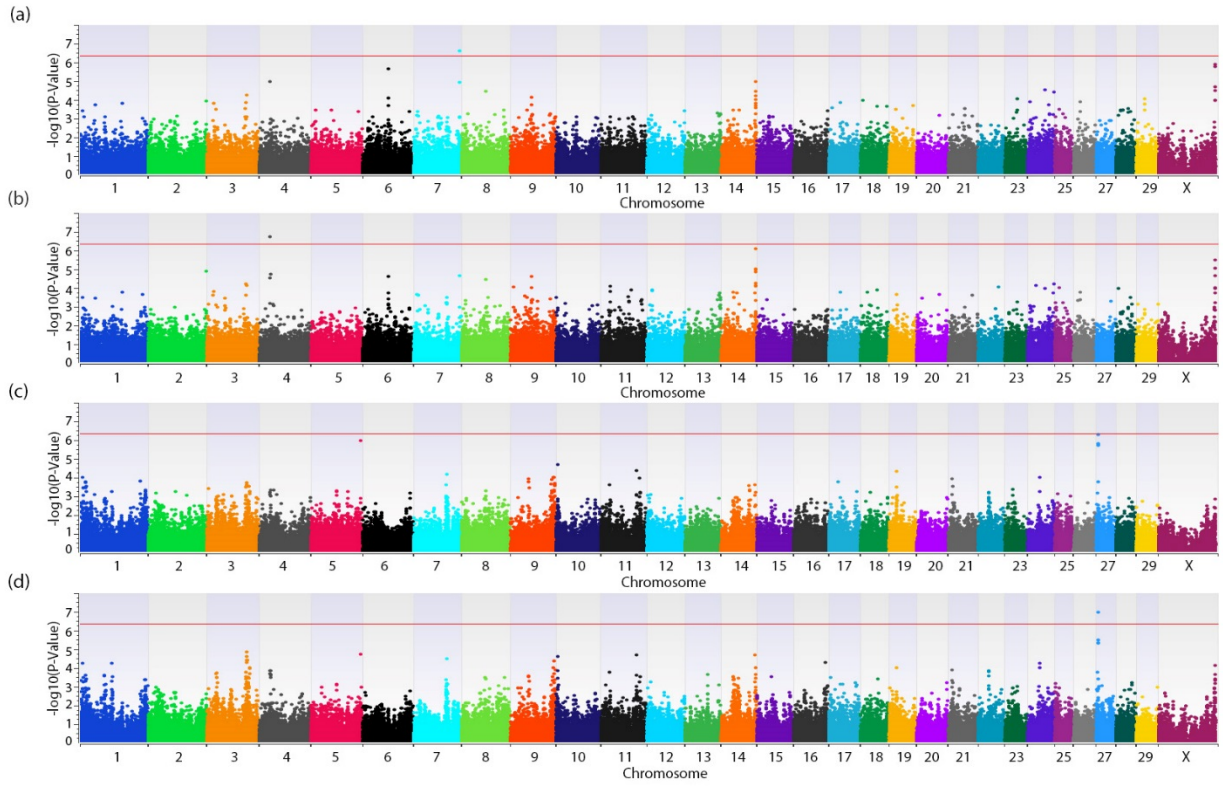

Figure 4.3 Manhattan plots for digital cushion thickness by (a) front medial digit, (b) average of the digits of the front foot, (c) average of the digits of the hind foot, and (d) average of all measurements for the Holstein dataset including cows (n = 431) and bulls (n = 23). The red horizontal line indicates the Bonferroni adjusted *P*-value threshold of 0.05.

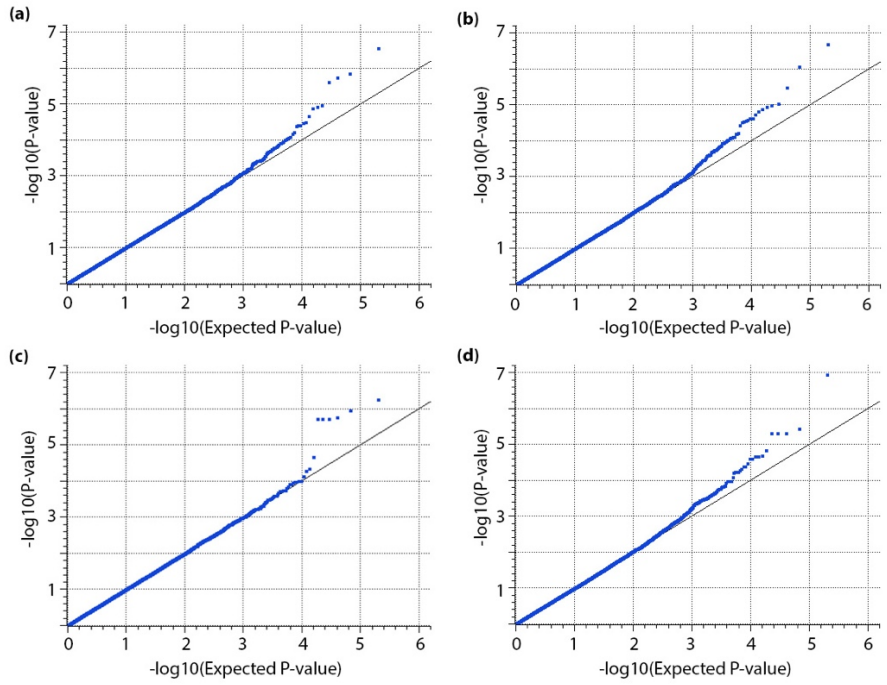

Figure 4.4 Quantile-quantile plots for digital cushion thickness by (a) front medial digit, (b) average of the digits of the front foot, (c) average of the digits of the hind foot, and (d) average of all measurements for the Holstein dataset including cows (n = 431) and bulls (n = 23).

Jersey cows and bulls. Two definitions of DCT for the Jersey dataset including cows and bulls did not have enough power to conduct a GWAS. Two of the 5 remaining GWAS models did not provide significant results. The recessive GWAS of DCT of the hind lateral digit identified BovineHD0300034341, ARS-BFGL-NGS-58312, BTA-69789-no-rs on BTA3, BovineHD0700031919 on BTA7, and BovineHD0900014057 and BovineHD0900014062 on BTA9. The additive GWAS of the average DCT of the hind foot identified Hapmap41614-BTA-67626 on BTA3. The recessive GWAS of the average DCT of all measurements identified BovineHD0300034341 on BTA3 (Figure 4.5 and 4.6). BovineHD0300034341 passed Bonferroni cutoff in the GWAS of DCT of the hind lateral digit and passed FDR in the GWAS of the average DCT of all measurements.

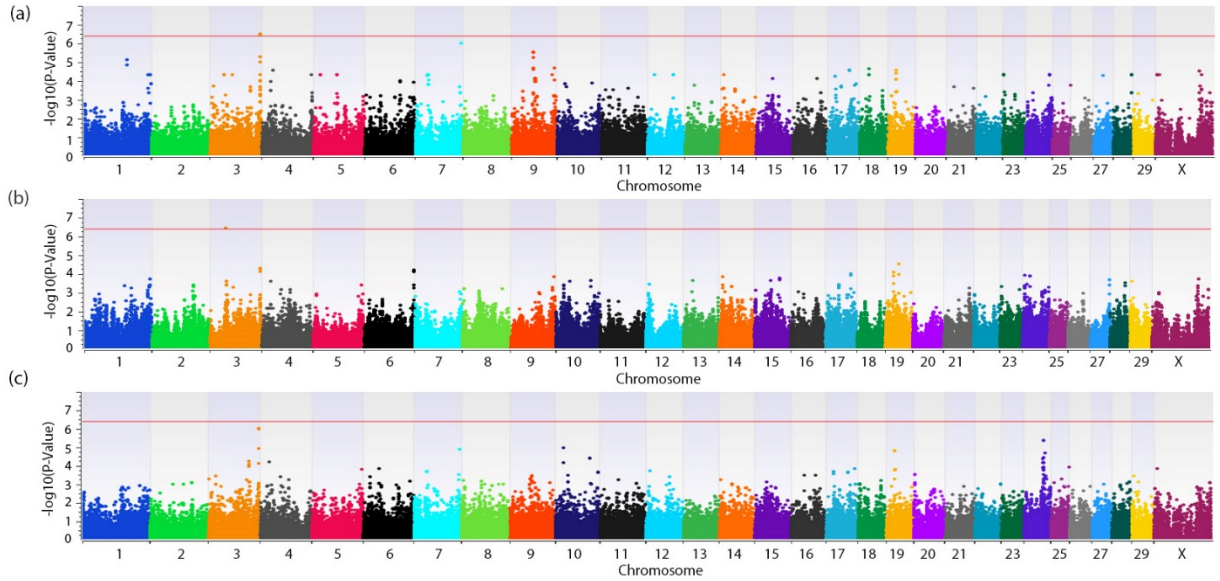

Figure 4.5 Manhattan plots for digital cushion thickness by (a) hind lateral digit, (b) average of the digits of the hind foot, and (c) average of all measurements for the Jersey dataset including cows (n = 168) and bulls (n = 8). The red horizontal line indicates the Bonferroni adjusted *P*-value threshold of 0.05.

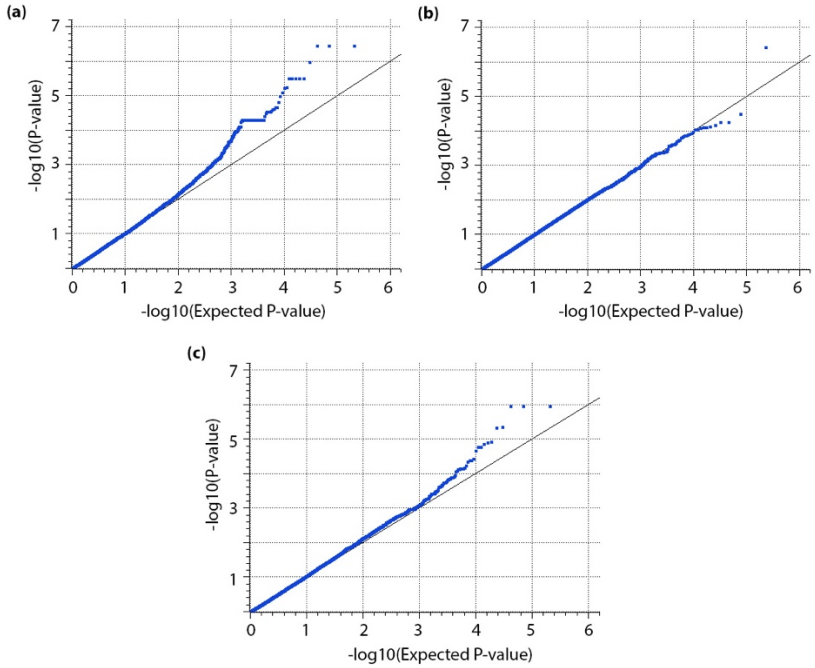

Figure 4.6 Quantile-quantile plots for digital cushion thickness by (a) hind lateral digit, (b) average of the digits of the hind foot, and (c) average of all measurements for the Jersey dataset including cows (n = 168) and bulls (n = 8).

Holstein and Jersey cows and bulls. All 7 definitions of DCT had enough heritability and power to be evaluated in a GWAS combining bulls and cows of both breeds. However, 5 of the 7 GWAS models did not provide significant results. The additive GWAS of DCT of the hind lateral digit identified BovineHD4100007739 on the BTA9 associated with DCT. The additive GWAS of the average DCT of the front foot identified BovineHD3000041411 and BovineHD3000041398 on the X chromosome associated with DCT (Figure 4.7).

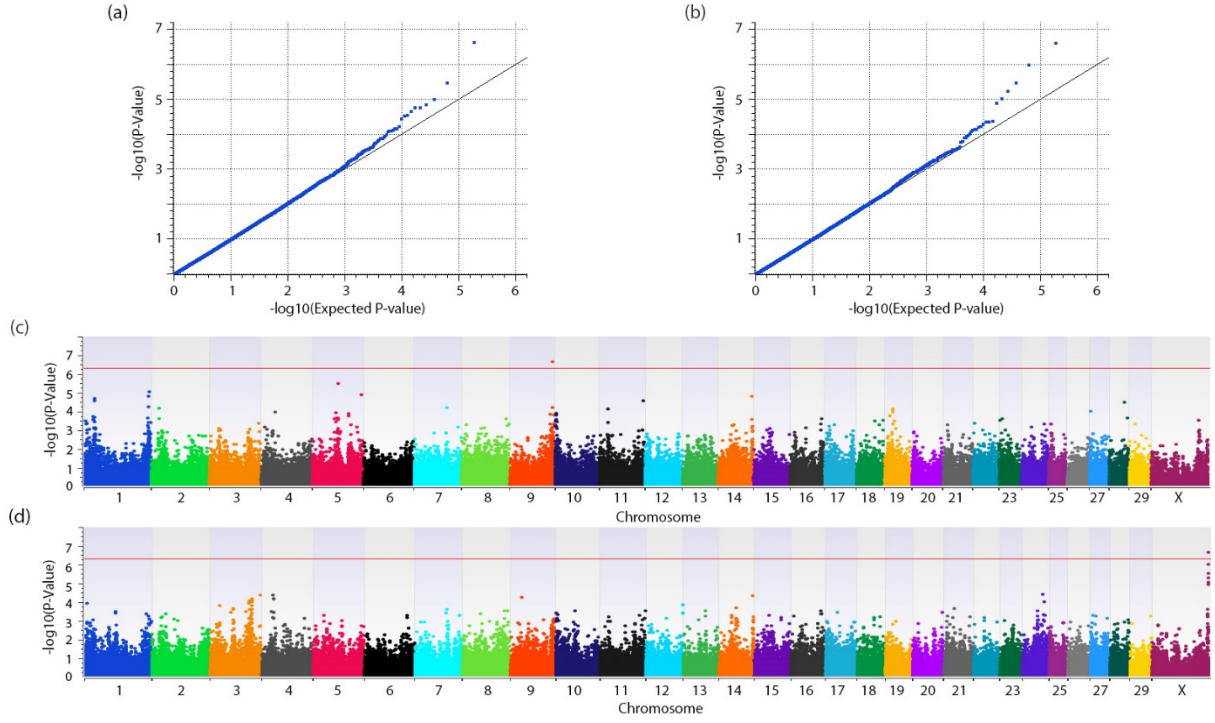

Figure 4.7 Quantile-quantile plot and Manhattan plot for digital cushion thickness by (a, c) hind lateral digit and (b, d) average of the digits of the front foot for the combined dataset including Holstein cows (n = 431), Jersey cows (n = 168), Holstein bulls (n = 23), and Jersey bulls (n = 8). The red horizontal line indicates the Bonferroni adjusted *P*-value threshold of 0.05.

The range of pseudo-lambda values for the 10 models with significant SNP was between 1.0 and 1.02 indicating limited stratification due to substructure. The 10 SNP that passed the Bonferroni cutoff had adjusted *P*-values that ranged from 0.01 to 0.04. The 10 additional SNP that only passed FDR had an adjusted *P*-value range of 0.03 to 0.05 (Table 4.5). BovineHD0300034341 on BTA3 passed the Bonferroni cutoff for the hind lateral digit but also passed FDR for the average of all DCT measurements in the Jersey dataset. Genetic heritability estimates for the 28 DCT models with sufficient power ranged from 0.20 to 0.76 with an average of 0.52 ± 0.15, while the heritability of DCT from the 10 models with significant SNP ranged from 0.33 to 0.76 with an average of 0.58 ± 0.17.

Table 4.5 Markers associated with digital cushion thickness (DCT) that surpassed the Bonferroni or False Discovery Rate (FDR) < 0.05

Marker	BTA	Position	Dataset[1]	Model[2]	Inheritance	Bonf *P*-value[3]	FDR	Prop Var Explained[4]	Major Allele	Minor Allele	Minor Allele Freq[5]
Hapmap41614-BTA-67626	3	36159480	Jersey	Avg Hind	Additive	0.04	0.04	0.16	C	T	0.05
BovineHD0300027656	3	95849910	Cows	Avg DCT	Dominant	0.04	0.04	0.05	G	A	0.46
BovineHD0300027684	3	95930841	Cows	Avg DCT	Dominant	0.09	0.04	0.05	C	T	0.43
ARS-BFGL-NGS-58312	3	117091875	Jersey	HL	Recessive	0.04	0.02	0.16	G	A	0.16
BTA-69789-no-rs	3	117126905	Jersey	HL	Recessive	0.04	0.01	0.16	A	G	0.16
BovineHD0300034341	3	117135023	Jersey	HL Avg DCT	Recessive Recessive	0.04 0.12	0.04 0.04	0.16 0.15	C	T	0.18 0.18
BovineHD0400007408	4	25367830	Holstein	Avg Front	Recessive	0.02	0.02	0.06	T	C	0.25
BovineHD0700031919	7	106853053	Jersey	HL	Recessive	0.12	0.03	0.15	C	T	0.49
BovineHD0700031927	7	106870956	Holstein	FM	Recessive	0.03	0.03	0.06	G	A	0.46
BovineHD0900014057	9	50349353	Jersey	HL	Recessive	0.36	0.04	0.13	C	T	0.20
BovineHD0900014062	9	50370897	Jersey	HL	Recessive	0.36	0.04	0.13	C	A	0.20
BovineHD4100007739	9	99886339	Combined	HL	Additive	0.02	0.02	0.04	G	A	0.44
BovineHD1400023369	14	80447215	Holstein	Avg Front	Recessive	0.09	0.04	0.06	T	C	0.16
BovineHD2700001198	27	4672201	Holstein	Avg Hind	Additive	0.20	0.05	0.05	G	A	0.18
BovineHD2700001206	27	4698475	Holstein	Avg Hind	Additive	0.20	0.04	0.05	C	T	0.18
BovineHD2700001209	27	4709134	Holstein	Avg Hind	Additive	0.20	0.03	0.05	G	A	0.18
BovineHD2700001220	27	4737202	Holstein	Avg DCT	Additive	0.01	0.01	0.06	T	C	0.16
BovineHD3000041411	X	132799322	Combined	Avg Front	Additive	0.02	0.02	0.04	G	A	0.66
BovineHD3000041398	X	132859284	Combined	Avg Front	Additive	0.09	0.05	0.039	T	C	0.65

[1]The Jersey dataset included 168 cows and 8 bulls, the cow dataset included 432 Holstein and 168 Jersey, the Holstein dataset included 431 cows and 23 bulls, and the combined dataset included 431 Holstein cows, 168 Jersey cows, 23 Holstein bulls, and 8 Jersey bulls.

[2]For the GWAS models, Avg Hind = average DCT measurements of all digits in the hind foot, Avg DCT = average of all DCT measurements, HL = DCT of the hind lateral digit, Avg Front = average DCT measurements of the digits in the front foot, and FM = DCT of the front medial digit.

[3]Bonferroni adjusted *P*-values for multiple comparisons.

[4]Proportion of variance in the trait explained by the marker.

[5]Freq = frequency.

Linkage disequilibrium structure was evaluated for 19 markers that surpassed the Bonferroni adjusted cutoff or FDR. Fourteen of the 19 markers were in blocks of LD (Figure 4.8 and 4.9). Forty-three candidate genes were identified in the NCBI Genome Data Viewer either within the block of LD if R^2 was > 0.80 or within a 1 Mb window centered on the significantly associated SNP (Table 4.6). Seven of the 19 markers resided within genes: Hapmap41614-BTA-67626 within Netrin G1 (*NTNG1*) gene; BovineHD0300034341, ARS-BFGL-NGS-58312, and BTA-69789-no-rs within LRR Binding FLII Interacting Protein 1 (*LRRFIP1*) gene; BovineHD0900014057 within Arginine/serine-rich protein PNISR (*SFRS18*) gene; BovineHD0900014062 within PNN Interacting Serine And Arginine Rich Protein (*PNISR*) gene; BovineHD3000041411 and BovineHD3000041398 within Histone-lysine N-methyltransferase PRDM9-like (*LOC100851938*) gene. Eight of the 43 candidate genes had functional annotation more likely related to the digital cushion. *SFRS18* and *LRRFIP1* function in fat tissue deposition. Aryl Hydrocarbon Receptor (*AHR*), Basic Leucine Zipper and W2 Domains 2 (*BZW2*), Ephrin A5 (*EFNA5*), Ubiquitin Specific Peptidase 45 (*USP45*), Vav Guanine Nucleotide Exchange Factor 3 (*VAV3*) have functions related to bone growth, whereas Sclerostin Domain Containing 1 (*SOSTDC1*) is associated with epidermal keratinocyte function.

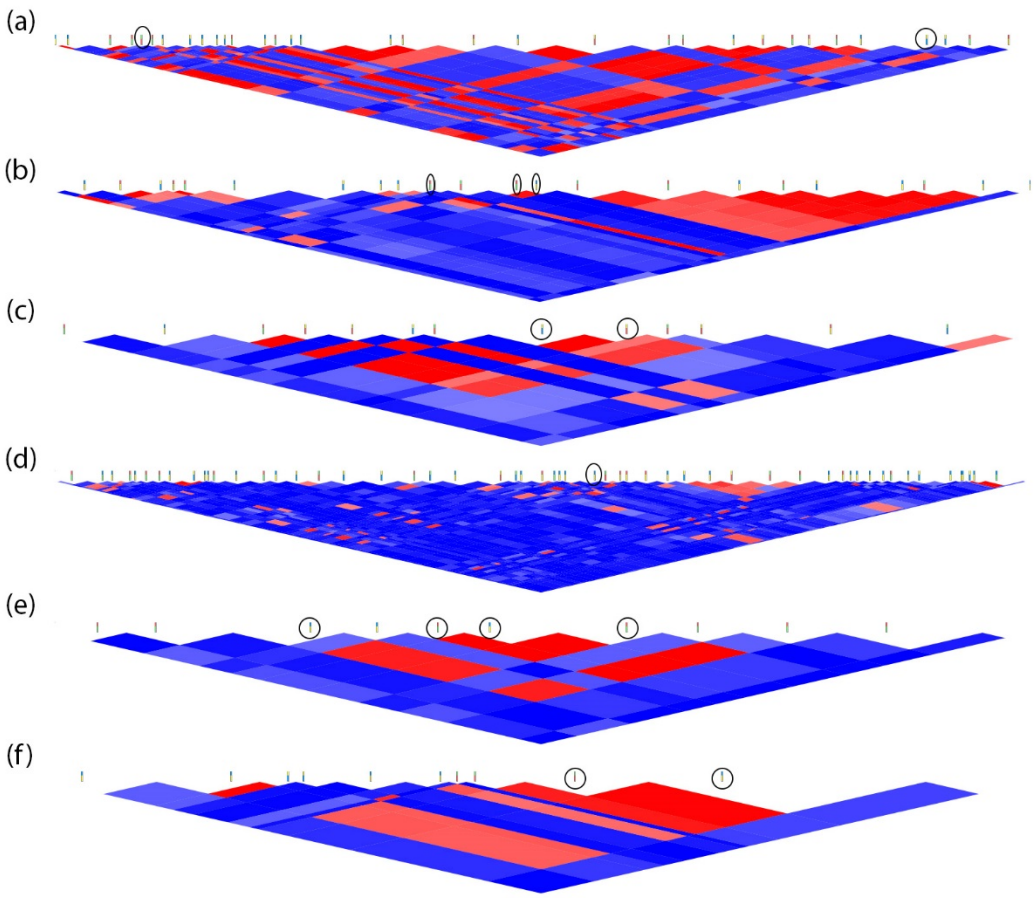

Figure 4.8 Linkage disequilibrium plots for markers (a) BovineHD0300027656 and BovineHD0300027684, (b) ARS-BFGL-NGS-58312, BTA-69789-no-rs, and BovineHD0300034341, (c) BovineHD0900014057 and BovineHD0900014062, (d) BovineHD1400023369, (e) BovineHD2700001198, BovineHD2700001206, BovineHD2700001209, and BovineHD2700001220, and (f) BovineHD3000041411 and BovineHD3000041398 that passed Bonferroni adjusted significance threshold or False Discovery Rate and were in blocks with $R^2 > 0.80$. The circles correspond to the location of the 14 significant markers. The colors represent correlation between two alleles with blue shading for $R^2 < 0.50$ and red shading for $R^2 > 0.50$ with the shading darkening as the value reaches the extremes of 0 and 1.

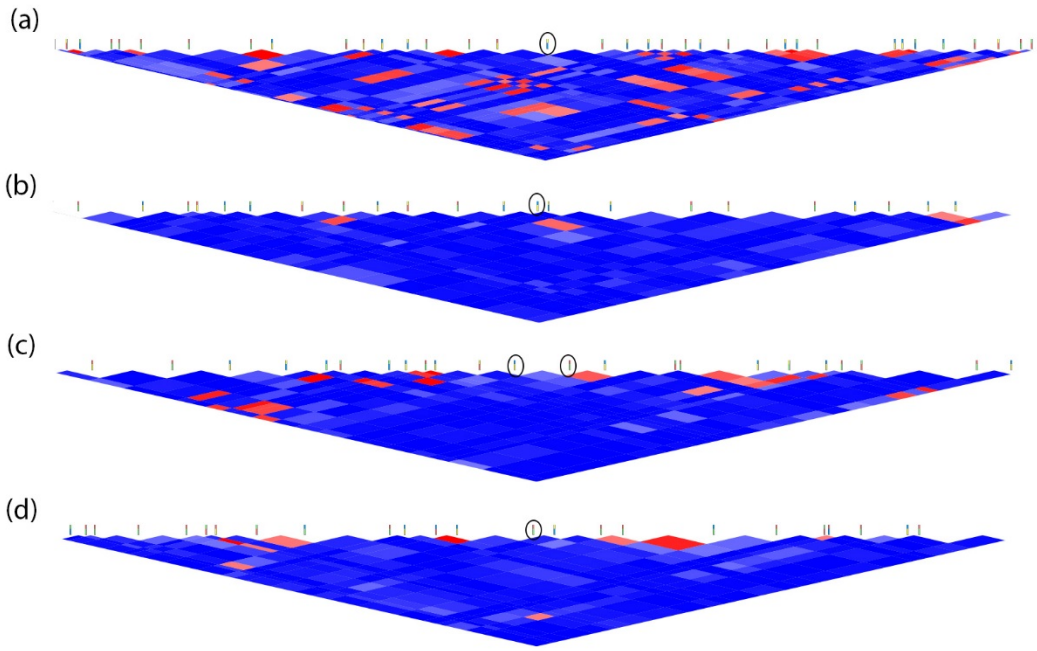

Figure 4.9 Linkage disequilibrium plots for markers (a) Hapmap41614-BTA-67626, (b) BovineHD0400007408, (c) BovineHD0700031919 and BovineHD0700031927, and (d) BovineHD4100007739 that passed Bonferroni adjusted significance threshold or False Discovery Rate and were not in blocks with $R^2 > 0.80$. The circles correspond to the location of the 5 significant markers. The colors represent correlation between two alleles with blue shading for $R^2 < 0.50$ and red shading for $R^2 > 0.50$ with the shading darkening as the value reaches the extremes of 0 and 1.

Table 4.6 Candidate genes determined from the markers associated with digital cushion thickness that surpassed the Bonferroni or False Discovery Rate significance of < 0.05

BTA	Position Range (bp)[1]	Markers[2]	Candidate Genes[3]
3	35659480-36659480	**Hapmap41614-BTA-67626**	***NTNG1****, VAV3, LOC101903424, PRMT6, LOC112445980, TRNAC-ACA, LOC539546*
3	95849910-95930841	BovineHD0300027656, BovineHD0300027684	*DMRTA2*
3	117091875-117135023	**ARS-BFGL-NGS-58312, BTA-69789-no-rs, BovineHD0300034341**	***LRRFIP1***
4	24867830-25867830	BovineHD0400007408	*ISPD, LRRC72, SOSTDC1, LOC112446359, BZW2, ANKMY2, AGR3, AGR2, TSPAN13, LOC101907877, LOC781774, AHR, TRNASTOP-UCA, LOC112446489, LOC112446510*
7	106353053-107370956	BovineHD0700031919, BovineHD0700031927	*EFNA5, FBXL17*
9	50277681-50370897	**BovineHD0900014057, BovineHD0900014062**	***SFRS18, PNISR****, USP45, TSTD3*
9	99386339-100386339	BovineHD4100007739	*LOC112448090, LOC112448089, TRNAC-GCA, LOC100848504, LOC112448110, LOC112448195, LOC112448146*
14	80044488-80662041	BovineHD1400023369	*LOC784458, LOC107133137*
27	4672201-4770314	BovineHD2700001198, BovineHD2700001206, BovineHD2700001209, BovineHD2700001220	-
X	132758690-132859284	**BovineHD3000041411, BovineHD3000041398**	***LOC100851938****, LOC112445167, LOC112445026, LOC783100*

[1]Position determined by blocks of linkage disequilibrium with an $R^2 > 0.80$ or within 500,000 bp upstream and 500,000 bp downstream of marker.
[2]The markers are those genotyped within the position range that were determined to be significantly associated with DCT. Markers are bolded if they resided within a gene.
[3]Genes are bolded if a marker is annotated within them.

DISCUSSION

This is the first study to characterize DCT in Jersey cows and bulls older than 6 mo. Digital cushion measurements of Holsteins were thicker than DCT measurements of Jerseys for both cows and bulls. Holsteins are the largest U. S. dairy breed with mature cows weighing 680 kg (Holstein Association USA, 2019). Hence, thicker digital cushion in Holsteins may support hoof integrity for their larger body size. Age influenced DCT for both cows and bulls. The youngest bulls included were 1 yr old because they had reached sexual maturity and started semen collection whereas the youngest cows were 2 yr old when they were due to calve the first time. Furthermore, it has been established DCT and its composition changes with age (Räber et al., 2004; Bicalho et al., 2009; Stambuk et al., 2019). The hind medial digit was the thinnest for either sex and the front medial digit was thickest for cows consistent with previous cow results (Stambuk et al., 2019). For bulls, both front digits were thicker than either hind digit. Cattle have to bear 60% of their body weight on their forelimbs meaning there is an unequal distribution of weight which could cause differences in DCT of the front versus hind digits (Bergsten et al., 2007). Additionally, the bulls were trimmed by 2 trained employees where 1 would always trim the front and the other would always trim the hind. Variation between the digits could be due to the different trimmers, however their effect cannot be separated from the effect of the front foot versus the hind foot. It is important to note the techniques were similar because the employee who trimmed the front taught the employee who trimmed the hind. Furthermore, Stambuk et al. (2019) reported a similar result for multiparous cows with a dataset of 177 cows. Potentially the difference in DCT between front digits could be better defined if there was a larger sample size of bulls.

Body condition score group influenced DCT in bulls but not cows. For the cows, BCSG was determined from the average BCS from the 2 time points, possibly masking the influence of BCSG on DCT in cows. Bulls with BCS >3.25 had thicker DCT than bulls with <2.75 BCS. Differences in BCSG for the bulls could be due to management practices. Thinner bulls tended to be the older bulls who were handled more often for semen collection, potentially utilizing more body reserves for energy. Younger bulls were handled less often and stocking their energy reserves for growth. Previous research has conflicting results on the relationship between DCT and BCS. Bicalho et al. (2009) and Machado et al. (2011) reported DCT to be positively associated with BCS, increasing as BCS increased due to the added fat reserves. However, Stambuk et al. (2019) and Newsome et al. (2017a) did not find a relationship between BCS and DCT. The contradicting results could be due to sample size and differences in populations, particularly inclusion of multiple breeds and cows and bulls for the current study.

The bulls sampled for this study lived in a facility with different management practices than the cows. All cows were group housed in free-stall barns while all bulls were housed in separate stalls. Cow pens were cleaned whenever the cows went to the parlor to get milked. The bulls had their stalls cleaned with fresh sawdust added daily. Manure accumulated quicker and in greater volume in the free-stall barns due to the larger number of animals per pen. Prior to trimming, the bulls had claws that were drier and showed more natural wear as opposed to wet overgrown claws of the cows. Walking on concrete causes the wall to be worn down and a disproportionate amount of the sole bears weight (Bergsten et al., 2007). The wall horn of the bull claws was often longer than the sole horn, illustrating the weight was being sustained on the hoof wall rather than the sole (Stambuk, personal observation). Previous research and study observation of facilities and management variation suggests environmental factors potentially

influencing hoof wear which may be contributing to the trend seen in the mean DCT measurements by sex with cows having a thicker mean DCT and more variation in the measurements than bulls.

Further investigation into the functions of the candidate genes for DCT revealed 8 novel biologically plausible genes related to fat tissue deposition, bone growth, and epidermal keratinocyte function. These are in addition to the 2 promising genes of *DLG2,* related to bone growth, and *MC4R,* related to fat deposition, recently published in relationship to the Holstein cow dataset (Stambuk et al., manuscript in preparation). *DLG2* was identified as a candidate gene in the GWAS models for the average DCT of time point 1 measurements when the digital cushion is thickest, while *MC4R* was identified for the average front medial digit and average DCT of the digits in the front foot, which includes the front medial digit that was determined to be thickest for Holstein cows in Stambuk et al. (manuscript in preparation). The current study identified 2 candidate genes related to fat tissue deposition, *SFRS18* and *LRRFIP1,* in the GWAS on the hind lateral digit for Jersey. *LRRFIP1* was also a candidate gene for the average DCT of all measurements for Jersey. Previous research associated *SFRS18* in the regulation of intramuscular fat deposition in pigs while *LRRFIP1* was associated with obesity and inflammation in humans (Wang et al., 2008; Plourde et al., 2013). Because the digital cushion consists of mostly adipose tissue, fat tissue accumulation identifies *SFRS18* and *LRRFIP1* as promising candidate genes (Räber et al., 2004). Inflammation effects of *LRRFIP1* could also be affecting the thickness of the digital cushion in relationship to lameness. Newsome et al. (2017a) discovered the thickness of the digital cushion to be greater when a sole ulcer was present due to increased inflammation in the underlying tissues. *LRRFIP1* might have more influence on DCT of the hind lateral digit because the lateral claw of the hindlimb bears more weight and incurs

more lesions than the medial claws (Toussaint Raven, 1989; Murray et al., 1996; Van der Tol et al., 2002).

Five candidate genes are related to bone formation or resorption depending on expression: *AHR*, *BZW2*, *EFNA5*, *USP45*, and *VAV3*. *AHR* was involved in osteoblast and osteoclast differentiation and maturation in bone marrow stem cells from rats and mice (Korkalainen et al., 2009). *BZW2* affected osteosarcoma in human cell lines by accelerating bone tumor growth (Cheng et al., 2017). *EFNA5* was highly expressed in osteolytic human prostate or breast cancer cells that were inoculated into the bone marrow cavity of the tibial bones of immunocompromised mice (Hensel et al., 2018). Overexpression of *USP45* inhibited osteogenesis or formation of new bone in human patients with glucocorticoid-induced osteonecrosis of the femoral head (Kuang et al., 2019). *VAV3* deficiency lead to dysfunctional osteoclasts and osteosclerosis or abnormal hardening of bone with increased bone density in mice (Faccio et al., 2005). Even though much of these genes' research is based upon disease association, it is biologically plausible that mutations in any of these genes could be influencing bone development in the distal phalanx, thereby impacting the thickness of the digital cushion. For instance, Newsome et al. (2016) suggested that bone development on the caudal aspect of the distal phalanx impacts CHDL which is genetically correlated with DCT (Oikonomou et al., 2014). *AHR* and *BZW2* were candidate genes for the average DCT of the digits in the front foot for Holstein, which includes the front medial digit that was determined to be thickest for Holstein cows in a previous study and both cows and bulls in the current study (Stambuk et al., manuscript in preparation). *USP45* was a candidate gene for the hind lateral digit while *VAV3* was a candidate gene for the average DCT of the digits in the hind for Jersey, which includes the hind medial digit that was determined to be thinnest for both cows and bulls. *EFNA5* was a

candidate gene for the front medial digit for Holstein, which was thickest for both cows and bulls, and the hind lateral digit for Jersey. Overall, candidate genes potentially related to bone development were determined in multiple GWAS models for both breed datasets.

SOSTDC1 was identified as a candidate gene for the GWAS on the average DCT of the digits in the front foot for Holstein, which includes the front medial digit that was determined to be thickest for both cows and bulls. It was previously associated with epidermal keratinocyte function (Hsieh et al., 2014). The claw horn capsule, including the sole horn that lies beneath the digital cushion, is created by the keratinization of living epidermal cells (Mülling et al., 1999). Potentially, there could be mutations in *SOSTDC1* affecting the quality and density of the sole horn of the front digits, thus influencing the quality of the sonogram from which the measurement of DCT was determined. The front digits were among the thickest for both cows and bulls. *SOSTDC1* could be contributing to a thicker digital cushion due to healthier horn quality.

Two other studies have focused on the genetics of digital cushion. Iqbal et al. (2016) targeted specific genes and reported lipogenic genes were consistently upregulated when cows were fed a higher energy diet. None of the 27 genes investigated by Iqbal et al. (2016) were candidate genes in the current study. Stambuk et al. (manuscript in preparation) discovered 27 SNP associated with DCT in Holstein cows across BTA14, BTA24, and BTA29 while the current study identified 19 new SNP across BTA3, BTA4, BTA7, BTA9, BTA14, BTA27, and the X chromosome associated with DCT in multiple breeds and cows and bulls. Both studies determined putative candidate genes related to bone remodeling and fat deposition. The gene that affected fat accumulation in Stambuk et al. (manuscript in preparation) was from the models on DCT of the average front medial digit and average front foot while the 2 genes related to fat in

the current study were from the models on DCT of the hind lateral digit and average DCT of all measurements in Jersey. The genes that functioned in bone growth were from the models on DCT of the front medial digit and average front foot in Holstein and the hind lateral digit and average hind foot in Jersey. Interestingly, this study was the only 1 to identify a gene related to epidermal keratinocyte function from the model on DCT of the average front foot in Holstein.

The average heritability in the current study of DCT based on the 10 GWAS models with significant SNP was 0.58 ± 0.17. This is greater than the 0.33 ± 0.09 and 0.31 ± 0.13 estimated by Oikonomou et al. (2014) and Stambuk et al. (manuscript in preparation), respectively. The difference could be due to the methods of determining heritability, population differences, and covariates included in the model. Previous research determined heritability of Holstein cows, while this study calculated heritability in datasets including both cows and bulls from Holstein and Jersey breeds. Additionally, the datasets that included both sexes had higher heritabilities potentially due to the smaller number of genetic markers capturing more genetic variation across the genome. The average genetic variation in the 9 GWAS models with significant SNP and both sexes was 0.024 while the 1 GWAS model with significant SNP from the cow dataset had a genetic variation of 0.009.

Only results evaluated on traits with additive inheritance are able to be included in traditional genomic evaluations (Cole et al., 2009; Aliloo et al., 2016). Five out of the 10 GWAS models with significant SNP were evaluated with additive inheritance, meaning the 8 markers from these models could be incorporated in genomic evaluations. BovineHD4100007739, BovineHD3000041411, and BovineHD3000041398 were determined to be associated with DCT in the combined dataset, thus these markers are universal and can be applied across Holstein, Jersey, and both sexes. BovineHD2700001198, BovineHD2700001206, BovineHD2700001209,

and BovineHD2700001220 can be incorporated in genomic evaluations for Holstein while Hapmap41614-BTA-67626 can be utilized in Jersey genomic evaluations. The other 11 markers still have the opportunity to be used in breeding programs for marker assisted selection against CHDL and lameness determined by visual locomotion score.

Results indicate DCT is a polygenic complex trait influenced by genetic and environmental factors. Markers associated with DCT change depending on breed, sex, digit, and foot. It is important to note that genomes are not fully understood. Genes are still being discovered and annotated in all species. There could be genes in the QTL regions from this study influencing variation in DCT that are not characterized.

CONCLUSIONS

This study explored the influence of breed and sex on DCT both phenotypically and genotypically and evaluated 19 novel genetic markers on BTA3, BTA4, BTA7, BTA9, BTA14, BTA27, and the X chromosome for candidate genes associated with DCT. The 8 candidate genes highlighted were related to fat accumulation, bone development, and epidermal keratinocyte function. Eight markers on BTA3, BTA9, BTA27, and the X chromosome from additive GWAS models can be applied to genomic evaluations to reduce CHDL and lameness. Further studies on the biologically plausible candidate genes are needed to determine genetic variants within the genes and how they relate to DCT within Holstein or Jersey through gene regulation and expression.

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REFERENCES

Aliloo, H., J. E. Pryce, O. González-Recio, B. G. Cocks, and B. J. Hayes. 2016. Accounting for dominance to improve genomic evaluations of dairy cows for fertility and milk production traits. Genet. Select. Evol. 48:8. https://doi.org/10.1186/s12711-016-0186-0.

Bergsten, C., A. Brizzi, C. K. W. Mülling, and K. Nordlund. 2007. Genetic Selection and Conformation. Pages 141-154 in: Bovine Laminitis and Lameness: A hands-on approach. P. Greenough, ed. Elsevier, Edinburgh, UK. https://doi.org/10.1016/B978-0-7020-2780-2.50016-1.

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. https://doi.org/10.3168/jds.2008-1827.

Cheng, D., S. Li, B. Zhu, T. Yuan, Q. Yang, and C. Fan. 2017. Downregulation of BZW2 inhibits osteosarcoma cell growth by inactivating the Akt/mTOR signaling pathway. Oncol. Rep. 38:2116-2122. https://doi.org/10.3892/or.2017.5890.

Cole, J. B., P. M. Van Raden, J. R. O'Connell, C. P. Van Tassell, T. S. Sonstegard, R. D. Schnabel, J. F. Taylor, and G. R. Wiggans. 2009. Distribution and location of genetic effects of dairy traits. J. Dairy Sci. 92:2931-2946. https://doi.org/10.3168/jds.2008-1762.

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. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.

Faccio, R., S. L. Teitelbaum, K. Fujikawa, J. Chappel, A. Zallone, V. L. Tybulewicz, F. P. Ross, and W. Swat. 2005. Vav3 regulates osteoclast function and bone mass. Nat. Med. 11:284-290. https://doi.org/10.1038/nm1194.

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. https://doi.org/10.2460/ajvr.76.3.246.

Hensel, J., A. Wetterwald, R. Temanni, I. Keller, C. Riether, G. van der Pluijm, M. G. Cecchini, and G. N. Thalmann. 2018. Osteolytic cancer cells induce vascular/axon guidance processes in the bone/bone marrow stroma. Oncotarget. 9:28877-28896. https://doi.org/10.18632/oncotarget.25608.

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. https://doi.org/10.3168/jds.2017-13531.

Holstein Association USA. 2019. Facts about Holstein cattle. Available at: http://www.holsteinusa.com/holstein_breed/holstein101.html?tab=2#TabbedPanels1 Accessed May 3, 2019.

Hsieh, J. C., R. C. Estess, I. Kaneko, G. K. Whitfield, P. W. Jurutka, and M. R. Haussler. 2014. Vitamin D receptor-mediated control of Soggy, Wise, and Hairless gene expression in keratinocytes. J. Endocrinol. 220:165-178. https://doi.org/10.1530/JOE-13-0212.

Iqbal, Z. M., H. Akbar, A. Hosseini, E. Bichi Ruspoli Forteguerri, J. S. Osorio, and J. J. Loor. 2016. Digital cushion fatty acid composition and lipid metabolism gene network expression in Holstein dairy cows fed a high-energy diet. PLoS One. 11:e0159536. https://doi.org/10.1371/journal.pone.0159536.

Kang, H. M., J. H. Sul, S. K. Service, N. A. Zaitlen, S. Y. Kong, and N. B. Freimer. 2010. Variance component model to account for sample structure in genome-wide association studies. Nat. Genet. 42:348-354. https://doi.org/10.1038/ng.548.

Korkalainen, M., E. Kallio, A. Olkku, K. Nelo, J. Ilvesaro, J. Tuukkanen, A. Mahonen, and M. Viluksela. 2009. Dioxins interfere with differentiation of osteoblasts and osteoclasts. Bone. 44:1134-1142. https://doi.org/10.1016/j.bone.2009.02.019.

Kuang, M., F. Xing, D. Wang, L. Sun, J. Ma, and X. Ma. 2019. CircUSP45 inhibited osteogenesis in glucocorticoid-induced osteonecrosis of femoral head by sponging miR-127-5p through PTEN/AKT signal pathway: Experimental studies. Biochem. Biophys. Res. Commun. 509:255-261. https://doi.org/10.1016/j.bbrc.2018.12.116,

Lischer, C. J., P. Ossent, M. Räber, and H. Geyer. 2002. 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. https://doi.org/10.1136/vr.151.23.694.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216–1231. https://doi.org/10.2527/1998.7641216x.

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. https://doi.org/10.2460/ajvr.72.10.1338.

Mülling, C. K., H. H. Bragulla, S. Reese, K. D. Budras, and W. Steinberg. 1999. How structures in bovine hoof epidermis are influenced by nutritional factors. Anat. Histol. Embryol. 28:103-108. https://doi.org/10.1046/j.1439-0264.1999.00180.x.

Mülling, C. K. W., and P. R. Greenough. 2006. Applied physiopathology of the foot. In XXIV World Buiatrics Congress. Nice, France. World Association for Buiatrics, Paris, France.

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.

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. https://doi.org/10.3168/jds.2015-10202.

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. https://doi.org/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. 100:4745-4758. https://doi.org/10.3168/jds.2016-12013.

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. https://doi.org/10.3168/jds.2012-6308.

Oikonomou, G., G. Banos, V. Machado, L. Caixeta, and R. C. Bicalho. 2014. Genetic characterization of digital cushion thickness. J. Dairy Sci. 97:532-536. https://doi.org/10.3168/jds.2013-7212.

Plourde, M., M. Vohl, C. Bellis, M. Carless, T. Dyer, G. Dolley, A. Marette, J. Després, C. Bouchard, J. Blangero, and L. Pérusse. 2013. A variant in the LRRFIP1 gene is associated with adiposity and inflammation. Obesity. 21:185-192. https://doi.org/10.1002/oby.20242.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A., Ferreira, D. Bender, J. Maller, P. Sklar, P. I. 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. https://doi.org/10.1086/519795.

Räber, M., C. Lischer, H. Geyer, and P. Ossent. 2004. The bovine digital cushion–A descriptive anatomical study. Vet. J. 167:258–264. https://doi.org/10.1016/S1090-0233(03)00053-4.

Shearer, J. K., P. J. Plummer, and J. A. Schleining. 2015. Perspectives on the treatment of claw lesions in cattle. VMRR. 6:273-292. https://doi.org/10.2147/VMRR.S62071.

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. 47:1179-1187. https://doi.org/10.1016/S0093-691X(97)00098-8.

Stambuk, C. R., J. A. A. McArt, B. 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. Trans. Anim. Sci. 3:9-18. https://doi.org/10.1093/tas/txy107.

Stambuk, C. R., E. A. Staiger, B. J. Heins, and H. J. Huson. 201_. Genome-wide association studies and relationships of management practices with digital cushion thickness in Holstein cows. J. Dairy Sci.

Toussaint Raven, E. 1989. Cattle Footcare and Claw Trimming. Farming Press, Ipswich, UK.

Van der Tol, P. P., J. H. 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. https://doi.org/10.3168/jds.S0022-0302(02)74216-1.

Wang, X., C. Xue, X. Wang, H. Liu, Y. Xu, R. Zhao, Z. Jiang, M. V. Dodson, and J. Chen. 2008. Differential display of expressed genes reveals a novel function of SFRS18 in regulation of intramuscular fat deposition. Int. J. Biol. Sci. 5:28-33. https://doi.org/10.7150/ijbs.5.28.

Yang, J., S. Hong Lee, M. L. Goddard, and P. M. Visscher. 2011. GCTA: A tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88:76-82. https://doi.org/10.1016/j.ajhg.2010.11.011.

CHAPTER 5:

CONCLUSIONS AND IMPLICATIONS

The overall goal of this research was to improve upon the current knowledge of digital cushion thickness (DCT) and generate novel diagnostic markers of DCT to address dairy cattle lameness on farms through improved accuracy of genetic selection. Studies were conducted with the objectives: (1) to determine the variation in DCT within the dairy cow across lactation and between digits, (2) to determine the optimal variables of stage in lactation and digit to measure DCT for predicting lameness and CHDL in the subsequent lactation, (3) to examine influences of management, breed, and sex on DCT, and (4) to identify genomic regions associated with DCT and how they might differ by breed, sex, foot, or digit.

A longitudinal study assessing variation of DCT across lactation was the first step in meeting these objectives. Through this, we determined that DCT in Holstein cows varied by stage in lactation and digit. Additionally, we determined which claws were essential to measure and at what time in lactation to identify animals more prone to developing CHDL or experiencing a lameness event later in lactation. Specifically, we reported parity group and the hind lateral digit < 40 days prepartum can be used to predict CHDL in the subsequent lactation. Parity group and the front and hind lateral digits within the first month in lactation can be used to predict a lameness event later in lactation.

To explore the genetic regulation of DCT, a larger cohort of cattle were sampled based upon results of our phenotypic analysis identifying two key time points for when the digital cushion is thickest and thinnest. We concluded DCT varied by sample time point, parity, digit, farm, body condition score group, sacral height, and wither height in Holstein cows. We discovered 27 novel genomic markers associated with DCT and 10 candidate genes. The 2

biologically plausible genes, *MC4R* and *DLG2*, functioned in fat tissue accumulation and bone development.

Our genetic investigation was then broadened to compare variation between Holstein and Jersey breeds and both sexes. We determined DCT in cows varied by breed, age, and digit while DCT in bulls varied by breed, age, digit, and body condition score group. Furthermore, we identified 19 unique genomic markers associated with DCT and 43 candidate genes. Of the 8 biologically plausible genes, *SFRS18* and *LRRFIP1* were new genes related to fat deposition as compared to our initial evaluation of just Holstein cows which highlighted *MC4R* and *DLG2*. In addition, *AHR*, *BZW2*, *EFNA5*, *USP45*, and *VAV3* were related to bone remodeling, and *SOSTDC1* influenced hoof quality.

Research results provided key insights into the physiology of DCT based on stage in lactation and digit. Three previous cross-sectional studies conducted by the same research group measured DCT in all 8 digits but did not compare across digits and only used an average of the hind digits in their analysis (Bicalho et al., 2009; Machado et al., 2011; Oikonomou et al., 2014). A more recent U. K. study published during our data collection period also used a longitudinal study design but limited their measure of DCT to the hind digits (Newsome et al., 2017a,b). Overall, the forelimb has not been studied as greatly as the hind limb because over 90% of hoof lesions causing lameness occur in the hind limbs (Bergsten et al., 2007; Shearer et al., 2012). For all of our research, DCT was evaluated in a front foot and hind foot, providing meaningful information on the variation in DCT by digit and foot. Furthermore, DCT beneath the flexor tuberosity of the distal phalanx has not been characterized in Jersey cows or bulls of any breed prior to our research.

Chapter 2 expanded upon the prediction models for CHDL in Bicalho et al. (2009) and Machado et al. (2011) by determining which claws were more critical to measure and at what time in lactation they are most useful to identify animals more prone to developing CHDL or experiencing a lameness event later in lactation. A producer can choose which model to apply depending on the challenges of their farm. However, CHDL account for over 65% of all lesions diagnosed in lame cows and can be very painful (Murray et al., 1996; Bicalho et al., 2007; Bruijnis et al., 2012). Decreasing the CHDL events will also decrease the lameness events. Additionally, only the hind lateral digit would need to be measured within 40 days prior to the parturition date (Stambuk et al., 2019). This could allow producers to monitor those cows more closely, possibly housing them in a pen closer to the milking parlor with a lower stocking density so they have less distance to travel and more room to rest and recuperate, such that CHDL and lameness are either prevented or detected early enough to alleviate pain, avoiding possible major changes to the hoof structures, and preventing negative downstream effects like decreased productivity.

Unfortunately, the on-farm application of measuring DCT will most likely be limited due to the cost of an ultrasound machine, the added labor of learning the technique and performing the evaluation, and the added stress to the animal. However, it could be applied during routine hoof trimming if the timing happens to occur either prior to or right after calving. The other option is to tackle CHDL and lameness genetically. Currently, U. S. genomic evaluations include feet and leg conformation traits to indirectly select against lameness (Wiggans et al., 2017; VanRaden et al., 2019). It would be advantageous for U. S. genetic evaluations to incorporate DCT as an indicator trait against lameness and CHDL due to the higher heritability of DCT and high negative genetic correlation between DCT and CHDL (Oikonomou et al., 2014). Chapters 3

and 4 were the only studies to evaluate the association of markers across the genome with the thickness of the digital cushion under the flexor tuberosity of the distal phalanx. All 46 markers have the opportunity to be applied in marker assisted selection of DCT against CHDL and lameness. Eight markers have the potential to be included in genomic evaluations because they were determined in models of additive inheritance (Cole et al., 2009; Aliloo et al., 2016). Three of the 8 markers were determined in the combined dataset and can be applied across Holstein, Jersey, and both sexes.

Further research is warranted on DCT, particularly tissue differentiation and gene expression in the digital cushion. Bicalho et al. (2009) reported the mean grey value reflecting total brightness of the sonogram image to have a negative linear association with DCT. However, the value was not compared to the amount of adipose or connective tissue in the digital cushion. The majority of studies on live animals were not able to differentiate the composition of digital cushion (Bicalho et al., 2009; Newsome et al., 2017a,b). Newsome et al. (2016) discovered bone development on the caudal aspect of the distal phalanx in the hind claws to increase with age, CHDL occurrence in a cow's life, and a higher proportion of lame locomotion scores. These bone spurs could be continuously pinching the digital cushion, thus altering its ability to dissipate the forces acting on the claw structures, changing the adipose tissue to connective scar tissue (Lischer et al., 2002; Räber et al., 2004). Although Räber et al. (2004) reported the composition of digital cushion changes due to age with digital cushions containing more adipose tissue until after 3 parities when it contains more collagenous connective tissue, the composition could vary per cow due to their experiences of CHDL or lameness. Being able to differentiate between soft adipose tissue and hard scar tissue in live animals would be advantageous in measuring DCT because the quality and quantity of the tissue types could affect the thickness

and cushioning capacity of the digital cushion. For example, a thick digital cushion measurement might be due to containing a larger amount of scar tissue. Therefore, that digital cushion would not have the same compression capacity as a thick digital cushion with mostly adipose tissue.

Discovering markers associated with DCT was the first step in understanding the genetics affecting the digital cushion. Candidate genes were determined from genomic regions associated with DCT, which is different than Iqbal et al. (2016) where they evaluated specific genes involved in lipid metabolism. The results from the studies in this dissertation will hopefully lead to future studies evaluating the putative genes to identify the causal variants affecting DCT and how they relate to DCT within Holstein or Jersey cattle through gene regulation and expression. Identification of the causal variants influencing DCT in cattle will provide more accurate markers to incorporate into genomic prediction such that CHDL and lameness prevalence substantially decreases on farms world-wide.

Lastly, the results from this research could initiate studies on DCT in other artiodactyl animals as a way to combat lameness. Swine, sheep, and goats are major production animals other than cattle that experience lameness and lesions. Anil et al. (2007) reported approximately 60% of sows had white line lesions and determined a relationship between white line lesions and lameness. Sheep and goats experience white line lesions, but less often than infectious lesions such as footrot (Winter, 2011). Because white line disease is one of the lesions associated with DCT, studies evaluating the thickness of the digital cushion could be beneficial to tackle lameness in swine, sheep, and goats (Bicalho et al., 2009; Machado et al., 2011; Stambuk et al., 2019).

REFERENCES

Aliloo, H., J. E. Pryce, O. González-Recio, B. G. Cocks, and B. J. Hayes. 2016. Accounting for dominance to improve genomic evaluations of dairy cows for fertility and milk production traits. Genet. Select. Evol. 48:8. https://doi.org/10.1186/s12711-016-0186-0.

Anil, S. S., L. Anil, J. Deen, S. K. Baidoo, and R. D. Walker. 2007. Factors associated with claw lesions in gestating sows. J. Swine Health Prod. 15:78-83.

Bergsten, C., A. Brizzi, C. K. W. Mülling, and K. Nordlund. 2007. Genetic Selection and Conformation. Pages 141-154 in: Bovine Laminitis and Lameness: A hands-on approach. P. Greenough, ed. Elsevier, Edinburgh, UK. https://doi.org/10.1016/B978-0-7020-2780-2.50016-1.

Bicalho, R. C., S. H. Cheong, G. Cramer, and C. L. Guard. 2007. Association between a visual and an automated locomotion score in lactating Holstein cows. J. Dairy Sci. 90:3294-3300. https://doi.org/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. https://doi.org/10.3168/jds.2008-1827.

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. Anim. 6:962-970. https://doi.org/10.1017/S1751731111002606.

Cole, J. B., P. M. Van Raden, J. R. O'Connell, C. P. Van Tassell, T. S. Sonstegard, R. D. Schnabel, J. F. Taylor, and G. R. Wiggans. 2009. Distribution and location of genetic effects of dairy traits. J. Dairy Sci. 92:2931-2946. https://doi.org/10.3168/jds.2008-1762.

Iqbal, Z. M., H. Akbar, A. Hosseini, E. Bichi Ruspoli Forteguerri, J. S. Osorio, and J. J. Loor. 2016. Digital cushion fatty acid composition and lipid metabolism gene network expression in Holstein dairy cows fed a high-energy diet. PLoS One. 11:e0159536. https://doi.org/10.1371/journal.pone.0159536.

Lischer, C. J., P. Ossent, M. Räber, and H. Geyer. 2002. 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. https://doi.org/10.1136/vr.151.23.694.

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. https://doi.org/10.2460/ajvr.72.10.1338.

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.

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. https://doi.org/10.3168/jds.2015-10202.

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. https://doi.org/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. 100:4745-4758. https://doi.org/10.3168/jds.2016-12013.

Oikonomou, G., G. Banos, V. Machado, L. Caixeta, and R. C. Bicalho. 2014. Genetic characterization of digital cushion thickness. J. Dairy Sci. 97:532-536. https://doi.org/10.3168/jds.2013-7212.

Räber, M., C. Lischer, H. Geyer, and P. Ossent. 2004. The bovine digital cushion–A descriptive anatomical study. Vet. J. 167:258–264. https://doi.org/10.1016/S1090-0233(03)000534.

Shearer, J. K., S. R. Van Amstel, and B. W. Brodersen. 2012. Clinical diagnosis of foot and leg lameness in cattle. Vet. Clin. Food Anim. 28:535-556. https://doi.org/10.1016/j.cvfa.2012.07.003.

Stambuk, C. R., J. A. A. McArt, B. 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. Trans. Anim. Sci. 3:9-18. https://doi.org/10.1093/tas/txy107.

VanRaden, P. M., J. B. Cole, and K. L. Parker Gaddis. 2019. Net merit as a measure of lifetime profit: 2018 revision. Available at: https://aipl.arsusda.gov/reference/nmcalc-2018.htm Accessed Apr. 10, 2019.

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. https://doi.org/10.1146/annurev-animal-021815-111422.

Winter, A. C. 2011. Treatment and control of hoof disorders in sheep and goats. Vet. Clin. Food Anim. 27:187-192. https://doi.org/10.1016/j.cvfa.2010.10.018.