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A prospective cohort study of factors associated with the digital cushion thickness in dairy cattle

Bethany E. Griffiths,[1]* Matthew Barden,[1] Alkiviadis Anagnostopoulos,[1] James P. Wilson, Evangelos Lamprou,[2] William Tulley,[1] Cherrill Bedford,[1] Androniki Psifidi,[3] Georgios Banos,[4] and Georgios Oikonomou[1]

[1]Department of Livestock and One Health, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Leahurst Campus, Liverpool CH64 7TE, United Kingdom
[2]Department of Animal Production, School of Animal Bioscience, Agricultural University of Athens, Athens 11855, Greece
[3]Department of Clinical Science and Services, Royal Veterinary College, North Mymms, Hertfordshire AL9 7TA, United Kingdom
[4]Animal & Veterinary Sciences, SRUC, Easter Bush, Midlothian EH25 9RG, United Kingdom

ABSTRACT

Key factors such as stage of lactation, parity, and body fat reserves have been associated with the digital cushion thickness (DCT), however, there are discrepancies between the results of previously published studies. The objective of this study was to examine the association of stage of lactation, body fat reserves, parity, and lesion incidence with DCT in a large cohort of intensively monitored cows. Across 4 UK farms, 2,352 cows were prospectively enrolled and assessed at 4 time points: before calving (T1-Precalving), immediately after calving (T2-Calving), in early lactation (T3-Early), and in late lactation (T4-Late). At each time point, BCS was recorded, the presence of sole lesions (sole ulcers and sole hemorrhage) and white line lesions was assessed by veterinarians, and an ultrasound image was taken to retrospectively measure the backfat thickness (BFT) in the pelvic region and the digital cushion on the hind left lateral claw. Mixed effects multivariable linear regression models, with the cow as a random effect, were fit to examine the association between the explanatory variables and DCT. The explanatory variables tested were farm, parity, stage of lactation, BCS, BFT, height, the presence of a lesion at the time of measurement, the chronicity of a lesion during early lactation, the predicted maximum daily milk yield, and the rate of milk production rise in early lactation. Stage of lactation and farm were both associated with DCT; however, an interaction was present, and this DCT pattern of change was farm-dependent. Two distinct patterns emerged; one indicated the nadir to occur shortly after calving, the other indicated the nadir to occur during early lactation. Neither BFT nor BCS were significantly associated with DCT. Heifers displayed thinner digital cushions compared with multiparous cows; however, this effect was dependent on the stage of lactation, with heifers having a thinner digital cushion up until late lactation, by which time DCT was commensurate with multiparous animals. Sole lesions and white line lesions at the time of measurement were associated with DCT (sole lesion: estimate = −0.07 mm, 95% CI = −0.14–0.00; white line lesion: estimate = 0.28 mm, 95% CI = 0.15–0.42).

Key words: BCS, backfat thickness, lameness, dairy cattle, digital cushion

INTRODUCTION

Lameness is a major challenge to the UK dairy industry. It is a welfare concern (Whay et al., 1997) and also reduces the efficiency of dairy production (Gomez and Cook, 2010; Randall et al., 2016; Charfeddine and Pérez-Cabal, 2017). Lameness is a visible condition, and combined with its high prevalence, has the potential to damage the social license by which dairy farms operate (Griffiths et al., 2018; Randall et al., 2019). Claw horn disruption lesions (**CHDL**), of which sole ulcers, sole hemorrhage (sole lesions) and white line disease are the most prevalent, are the main noninfectious lameness-causing lesions in dairy cattle (Murray et al., 1996).

Although there are several known factors (genetic, management, environmental, and anatomic) associated with the development of CHDL, the inciting cause is currently unknown (Barker et al., 2010; Newsome et al., 2017a; Barden et al., 2022). Compression of the horn-producing keratinocytes within the corium during locomotion and weight bearing is one theory behind their development (Räber et al., 2004).

The cow has a limited suspensory apparatus compared with the horse; instead, a greater reliance is placed upon

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*Corresponding author: hlbgriff@liverpool.ac.uk

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

the supportive apparatus, which includes the digital cushion (Räber et al., 2004, 2006). This cushion comprises 3 spatially arranged pads located between the distal phalanx and the corium and is composed of soft fat and connective tissue (Räber et al., 2004). The function of this structure is thought to protect the corium from compressive and concussive forces associated with locomotion and standing (Räber et al., 2004).

Thin digital cushions have been associated with the development of CHDL (Bicalho et al., 2009; Machado et al., 2011; Newsome et al., 2017a). Several studies identified the digital cushion to be thinnest during the early lactation period (Bicalho et al., 2009; Stambuk et al., 2019; Griffiths et al., 2020). This change in thickness has been hypothesized to be associated with fat mobilization to support the energy demands of lactation (Bicalho et al., 2009). Several studies have found an association between BCS and the digital cushion thickness (**DCT**; Bicalho et al., 2009; Machado et al., 2011; Griffiths et al., 2020). Another study identified an association between backfat thickness (**BFT**) and DCT, with a small effect size (Newsome et al., 2017b). This correlation between fatty structures within the cow suggests that fat mobilization is systemic in nature, with the digital cushion adipose tissue being vulnerable to mobilization.

There are discrepancies between the results of studies investigating DCT, with some having found the nadir in DCT to instead occur shortly after calving (Newsome et al., 2017b; Bach et al., 2021). The timing suggests that periparturient relaxation of the suspensory apparatus and subsequent compression of the digital cushion by the pedal bone (the so-called "calving effect") could be responsible (Tarlton et al., 2002; Knott et al., 2007), rather than fat mobilization from the digital cushion. The proximity of this nadir to calving could account for the differing results reported by Bicalho et al. (2009) and Stambuk et al. (2019), who both measured the digital cushion within 30 d after calving, possibly after the nadir had occurred. This is supported by a small study conducted by Bach et al. (2021), which found no association between a change in BCS and a change in DCT within the periparturient period, and in a larger study conducted by Newsome et al. (2017b), which found the nadir in BFT to occur during early lactation, whereas the digital cushion was at its thinnest shortly after calving. Furthermore, one study found the DCT nadir to be dependent on parity, with primiparous animals displaying thin digital cushions before calving and during early lactation, and multiparous animals displaying a thin digital cushion shortly after calving (Stambuk et al., 2019). Due to the labor-intensive nature of data collection, previous studies enrolled relatively small numbers of cows from single (Bicalho et al., 2009; Stambuk et al., 2019; Bach et al., 2021) or multiple (Newsome et al., 2017b; Griffiths et al., 2020) farms. Although BCS has been measured in all previous studies, objective measures of backfat were only included by Newsome et al. (2017b). Current knowledge gaps still exist regarding the interactions between BCS, BFT, and DCT, with a wide range of effects and interactions present.

Our objective in the present study is to examine the association of key factors including stage of lactation, body fat reserves (BFT and BCS), parity, height, milk yield, and lesion presence with DCT in a larger cohort of intensively monitored UK dairy cows across several farms. The null hypothesis is that these factors are not associated with DCT.

MATERIALS AND METHODS

This study was conducted under the ethical approval of the University of Liverpool Research Ethics Committee (VREC269a, VREC466ab) and reported in accordance with the STROBE guidelines (von Elm et al., 2008).

Study Design and Population

Animals were enrolled in this prospective cohort study from 4 UK commercial dairy farms (farms A–D). This study was designed to evaluate the study population at 4 key time points during a lactation cycle. Farms were located in the northwest of England and North Wales and were selected for convenience based on proximity and their willingness to participate, due to the practicalities of frequent visits. Animals registered as Holstein and expected to calve between April and December 2019 were prospectively enrolled with no further inclusion or exclusion criteria. From February 2019 to March 2020, data were collected from each farm weekly or twice weekly. Animals were examined at 4 time points: before parturition (**T1-Precalving**; −90 to −14 d), immediately after parturition (**T2-Calving**; 0 to +14 d), in early lactation (**T3-Early**; +40 to +130 d), and during late lactation (**T4-Late**; >170 d). Due to resource constraints and the practicalities of sampling, once the T4-Late visits commenced, enrollments ceased, resulting in a total of 2,352 animals. A full description of farm characteristics is provided by Griffiths et al. (2024). Briefly, all farms calved cows all year-round. Farms A, B, and C housed lactating cattle year-round, whereas farm D housed high-yielding cattle year-round, and low-yielding cattle were grazed during summer. All farms housed lactating cattle on freestalls. Cows were milked 3 times daily on farms A, B, and C and twice daily on farm D. Farms A, B, and C recorded 305-d milk yields of ~11,000 to 11,500 L, whereas farm D recorded ~9,000 L. In all herds, parous cows were prophylactically foot-trimmed twice a year at drying off and ~60 to 100 d after parturition. All herds

regularly foot-bathed lactating cows after milking. Farm A foot-bathed with either copper sulfate or formalin 3 times a week, farm B foot-bathed with formalin twice daily, farm C foot-bathed cows with either copper sulfate or formalin daily, and farm D foot-bathed cows with formalin 3 times a week.

Data Collection

Backfat thickness, BCS, lesion incidence, and cow height were measured, with the milk yield, parity, and farm being recorded to examine their association with the thickness of the digital cushion. Measurements were taken at all time points. Data were recorded using Microsoft Access 2010 (Microsoft Corp.).

Digital Cushion Thickness. An ultrasound image of the hind left lateral claw was taken. This image was captured on lifted feet after lesion identification and foot trimming had taken place. Due to time constraints, not all claws could have an ultrasonographical examination. The hind left lateral claw was arbitrarily chosen over the hind right lateral claw. Hind-lateral claws were chosen due to the increased frequency of CHDL (Murray et al., 1996; Toholj et al., 2014; Griffiths et al., 2020). B-mode ultrasonography was used to examine the claw as described by Kofler et al. (1999). A 5-cm linear probe inside a gel standoff was used with a Dramiński Vet 4 Mini ultrasound machine (Dramiński S. A.). An image depth of 4 cm was used, and the frequency was set to 6 MHz. The probe was placed on the midline of the sole, and the image was stored when the distal phalanx, digital cushion, tuberculum flexorum, and interface of the sole and soft tissues were observed (Kofler et al., 1999).

Lesion Identification. Before calving (T1-Precalving) and during early lactation (T3-Early), all claws from multiparous animals were routinely trimmed according to a modified Dutch 5-step method (Toussaint-Raven, 1989), which included a wider and deeper modeling of the lateral claws of the hind feet compared with the traditional method (Sadiq et al., 2021). Shortly after calving (T2-Calving) and during late lactation (T4-Late), feet were not prophylactically trimmed, but a thin layer of horn was removed to uncover the presence of any sole or white line lesions. Primiparous animals were only prophylactically trimmed according to the modified Dutch 5-step method at T3-Early; at all other time points, a thin layer of horn was removed. Lesions on the hind left lateral claw were then identified according to the International Committee for Animal Recording claw health atlas (Egger-Danner et al., 2014) and then graded. Supplemental Table S1 (see Notes) describes the system used for grading sole lesions and white line lesions; these scores are, however, roughly comparable to absent (score 0), mild (score 1), moderate (score 2), and severe (score 3). All researchers who undertook the scoring of lesions were qualified veterinarians; the vast majority (over 90%) of lesions were scored by a single researcher, with the remainder scored by a further 3 researchers.

Body Condition Score and Backfat Thickness. At each time point, BCS was assessed before ultrasound measurements using a scale from 1 (under-conditioned) to 5 (over-conditioned) in increments of 0.25 (Ferguson et al., 1994). An ultrasound image of subcutaneous fat in the pelvic region (BFT), as described by van der Drift et al. (2012) was taken. Due to access constraints within the handling systems, the left side was measured on farm C, and the right side on all other farms. B-mode ultrasonography using a 5-cm linear probe and Dramiński Vet 4 Mini ultrasound machine (Dramiński S. A.) was used. The frequency was set to 5 MHz and the image depth set to 6cm. An image was stored for BFT when the interface between skin and probe and the fascia of the underlying gluteus muscle forming a triangle shape was visualized.

Other Explanatory Variables. Height was measured in 5-cm increments from the highest point of the sacral vertebrae, using pre-measured marks placed onto the handling system at the start of the study. Monthly milk production data were obtained from milk recording services. Information regarding parity and calving date were obtained from farm records.

Data Editing

In total 2,352 cows were initially enrolled, with a maximum of 9,408 records possible. This dataset was then filtered to remove missing data. Missing records occurred if the animal did not calve during the study period, if a cow missed a stage visit, if DCT data were missing, or if lesion data at the time of measurement were not present. Several cows were missing a T4-Late time point, as they had left the herd before they could be assessed due to delays associated with the COVID-19 pandemic.

The proportion of missing data was then calculated for each variable. Simple imputation employing group means based on farm, stage, and parity were used due to a low percentage of missing data (<6%; Little and Rubin, 2002).

Dependent Variable Processing: Digital Cushion Thickness

Ultrasonographic images were analyzed by a single assessor blinded to parity, farm, stage of lactation and presence of lesion using ImageJ software (Schneider et al., 2012). A single measurement was taken from the saved images. Digital cushion thickness was measured from the most proximal arch of the distal phalanx to the interface between the sole horn and the soft tissues,

representing the thickest part. This point regularly corresponds to the interconnecting axial and abaxial pads of the digital cushion, primarily the axial pad (Räber et al., 2004). Although this measurement includes the digital cushion, it also encompasses all soft tissues, such as the corium (Räber et al., 2004). Sole soft tissue thickness (Newsome et al., 2017b) is therefore a more precise term; however, for consistency with the majority of research published within this area, DCT will be used throughout this study. Measurements were only taken if the tuberculum flexorum, distal phalanx, and the interface between the sole and the sole soft tissues were identifiable with confidence. The DCT was treated as a continuous variable.

Explanatory Variable Processing

Lesion Identification. For the purposes of this analysis, lesion data were treated as 2 binary explanatory variables: the presence or absence of a sole lesion or a white line lesion (Table 1). A further 2 categorical variables were created based upon the previously described binary lesion variables (Table 1), with 4 levels: the presence (new, recovering, or chronic) or complete absence of a sole lesion or white line lesion at T3-Early (Table 1).

Backfat Thickness and BCS. A single assessor analyzed the ultrasound images. From each saved image, a single measurement was taken using ImageJ software (Schneider et al., 2012). Backfat thickness was measured at the point of the triangle-shaped fascia of the underlying gluteus muscle to the interface between the skin and the probe. This measurement includes all soft tissues, including fat and the dermis (Schröder and Staufenbiel, 2006). Back soft tissue thickness is therefore a more precise term than BFT, throughout this study however, BFT will be used for consistency with the majority of research published within this area. Measurements were only taken if landmarks were identifiable with confidence. These landmarks included the fascia of the gluteus muscle and the interface between the skin and the probe. Backfat thickness was treated as a continuous variable, and BCS was grouped (≤2.5, 2.75–3.25, ≥3.5).

Milk Yield Data. To capture early lactation milk production, lactation curves with the MilkBot model (Ehrlich, 2013) were fit to monthly production records from milk recording. The scale (the overall scale of milk yield across a lactation, in kg/day) and ramp (the rate of milk yield increase postpartum, in days) parameters for this curve were offered to the model as explanatory variables.

Other Explanatory Variables. Farm was included as a 4-level categorical variable. Parity was grouped into 3 levels according to parity at T3-Early: primiparous, second-lactation, and 3 or more lactations. Height was treated as a continuous variable.

Statistical Analyses

To test our hypothesis, explanatory variables were analyzed against DCT at the cow level. Data analysis was undertaken using R Studio (R Core Team, 2020; V4.2.2), with the the Tidyverse (Wickham et al., 2019), lmerTest (Kuznetsova et al., 2017), Broom (Robinson et al., 2023), performance (Lüdecke et al., 2021), emmeans (Lenth, 2023), and ggeffects (Lüdecke, 2018) packages. To initially explore the data, univariable analyses were undertaken with linear regression using the lm() function.

Multivariable Analyses

Mixed effects multivariable linear regression models were fit using the lmer() function from the lmerTest pack-

Table 1. Definition of lesion variables

Variable	Present	Absent
Sole lesion	Hind left lateral claw: • Grade ≥1 sole ulcer • Grade ≥2 sole hemorrhage	Hind left lateral claw: • No sole ulcer • Grade <2 sole hemorrhage
White line lesion	Hind left lateral claw: • Grade ≥2 white line lesion	Hind left lateral claw: • Grade <2 white line lesion
Temporal sole lesion	• New lesion: Sole lesion present at T3-Early but no sole lesion at either T1-Precalving or T2-Calving • Recovering lesion: A sole lesion at either T1-Precalving or T2-Calving but no sole lesion at T3-Early • Chronic lesion: Sole lesion present at T3-Early and sole lesion present at either T1-Precalving or T2-Calving	No sole lesion at T1-Precalving, T2-Calving, or T3-Early
Temporal white line	• New lesion: White line lesion present at T3-Early but no white line lesion at either T1-Precalving or T2-Calving • Recovering lesion: A white line lesion at either T1-Precalving or T2-Calving but no white line lesion at T3-Early • Chronic lesion: White line lesion present at T3-Early and white line lesion present at either T1-Precalving or T2-Calving	No white line lesion at T1-Precalving, T2-Calving, or T3-Early

age (Bates et al., 2015; Kuznetsova et al., 2017), with cow as a random intercept. The continuous dependent variable was DCT. The variance-covariance structure used was unstructured.

The first multivariable model (model 1) examined lesion variables at the time point of measurement and the second (model 2) examined lesion variables during early lactation. Three additional models were fit for each parity group (model 3a for first-parity animals, model 3b for second-parity animals, and model 3c for third or higher parity animals). All explanatory variables (farm, parity, stage of lactation, DIM, MilkBot scale, MilkBot ramp, height, BFT, BCS, sole lesion, and white line lesion) were offered to the models. All variables were removed in a backward stepwise fashion using the Akaike Information Criterion (Akaike, 1973). To account for variation in DIM at each stage, we forced an interaction between DIM and stage, with DIM mean-centered within stage. All biologically plausible 2-way interactions were tested in the model. These interactions were visualized, and a likelihood ratio test ($P \leq 0.05$) was used to compare between models. Linearity and homoscedasticity were assessed by visualizing scatter plots of residuals. Variance inflation factors were calculated for each explanatory variable to assess for multicollinearity. Influential points and outliers were evaluated using Cook's distance (Cook, 1977). The intraclass correlation coefficient and marginal and conditional R^2 were calculated for each model.

RESULTS

Dataset Overview

In total 2,352 cows were initially enrolled, with a maximum of 9,408 records possible. Missing records occurred if the animal did not calve during the study period (n = 148), if a cow missed a stage visit (n = 637), if DCT data were missing (n = 579) or if lesion data at the time of measurement were not present (n = 3). Several cows (n = 477) were missing a T4-Late time point as they had left the herd before they could be assessed due to delays associated with the COVID-19 pandemic. Reasons for the expected calving having not occurred included abortion, euthanasia due to health reasons, or having died. Therefore, the final data set included 2,290 cows and featured 7,564 records with measurements. Descriptive statistics on the study population are provided in Supplemental Tables S1 and S2 (see Notes). Of the study population, 602 (26.3%) were primiparous animals, 721 (31.5%) were second-lactation animals, and 967 (42.2%) were in their third or higher lactation. Farm A recorded 130 cows (5.7%), farm B recorded 416 cows (18.2%), farm C recorded 1,513 cows (66.1%), and farm D recorded 231 cows (10.1%). The mean DCT was 6.78 mm (SD = 0.97 mm). The digital cushion was at its thinnest shortly after calving (T2-Calving: mean = 6.51 mm, SD = 0.93 mm), compared with before calving (T1-Precalving: mean = 6.79 mm, SD = 1.01 mm) and during lactation (T3-Early: mean = 6.80 mm, SD = 0.93 mm; T4-Late: mean = 7.16 mm, SD = 0.87 mm).

Univariable Analyses

Univariable associations between explanatory variables and DCT are detailed in Supplemental Table S3 (see Notes).

Multivariable Analyses: Overview

No violations of the assumptions regarding linearity, residual distributions, and multicollinearity (variance inflation factors <5) were detected.

Multivariable Analyses: Concurrent Lesion Presence

The first model (model 1) investigated explanatory variables and lesions present at the time of measurement on the thickness of the digital cushion. Model 1 included 7,564 records from 2,290 cows. The explanatory variables remaining within the model (Table 2) were farm, parity, stage of lactation, DIM, height, and the presence of a sole lesion and white line lesion at each time point. Of the tested interactions, the farm by stage of lactation interaction (Figure 1a) and parity by stage of lactation interaction (Figure 1b) remained within the model.

Multivariable Analyses: Temporal Lesion Presence

The second model (model 2) examined explanatory variables and lesion presence during early lactation on the thickness of the digital cushion. Model 2 featured 7,118 records from 2,048 cows. The explanatory variables remaining in the model (Table 2) were farm, parity, stage of lactation, DIM, height, MilkBot scale, the temporal sole lesion presence and the temporal white line lesion presence. Of the tested interactions, farm by stage of lactation interaction (Figure 2a) and parity by stage of lactation interaction (Figure 2b) remained within the model.

Multivariable Analyses: Parity Interaction Models

The third model (model 3a, 3b, and 3c) examined explanatory variables and lesions present at the time of measurement on the thickness of the digital cushion, focusing on each parity group in turn.

Table 2. Results from the mixed effect multivariable linear regression model estimating the association between explanatory variables, including concurrent lesions and DCT (mm; model 1)[1]

Fixed	Term	Estimate	95% CI	*P*-value
Intercept		2.59	1.77, 3.42	<0.001
Farm	A	Referent		
	B	0.47	0.29, 0.65	<0.001
	C	0.39	0.22, 0.55	<0.001
	D	0.22	0.03, 0.42	0.026
Parity	First	Referent		
	Second	0.94	0.84 – 1.05	<0.001
	≥3	1.16	1.05, 1.26	<0.001
Stage	T1-Precalving	Referent		
	T2-Calving	0.13	−0.05, 0.31	0.156
	T3-Early	0.09	−0.11, 0.28	0.374
	T4-Late	0.64	0.45, 0.83	<0.001
Sole lesion	Absent	Referent		
	Present	−0.07	−0.14, 0.00	0.039
White line lesion	Absent	Referent		
	Present	0.28	0.15, 0.42	<0.001
Height (cm)		0.02	0.02, 0.03	<0.001
DIM		0.00	0.00, 0.00	<0.001
Stage × DIM	T1-Precalving × DIM	Referent		
	T2-Calving × DIM	−0.01	−0.02, 0.00	0.014
	T3-Early × DIM	0.00	−0.01, 0.00	0.206
	T4-Late × DIM	0.00	0.00, 0.00	0.893
Farm × stage	A × T1-Precalving	Referent		
	B × T1-Precalving	Referent		
	C × T1-Precalving	Referent		
	D × T1-Precalving	Referent		
	A × T2-Calving	Referent		
	B × T2-Calving	0.10	−0.09,0.30	0.288
	C × T2-Calving	−0.28	−0.46, −0.11	0.002
	D × T2-Calving	−0.16	−0.37, 0.05	0.130
	A × T3-Early	Referent		
	B × T3-Early	0.08	−0.11, 0.27	0.412
	C × T3-Early	0.25	0.07, 0.43	0.006
	D × T3-Early	0.12	−0.09, 0.33	0.256
	A × T4-Late	Referent		
	B × T4-Late	0.10	−0.11, 0.30	0.344
	C × T4-Late	0.44	0.25, 0.62	<0.001
	D × T4-Late	0.40	0.17, 0.62	0.001
Parity × stage	First × T1-Precalving	Referent		
	Second × T1-Precalving	Referent		
	≥3 × T1-Precalving	Referent		
	First × T2-Calving	Referent		
	Second × T2-Calving	−0.30	−0.41, −0.20	<0.001
	≥3 × T2-Calving	−0.40	−0.50, −0.29	<0.001
	First × T3-Early	Referent		
	Second × T3-Early	−0.31	−0.44, −0.19	<0.001
	≥3 × T3-Early	−0.43	−0.56, −0.31	<0.001
	First × T4-Late	Referent		
	Second × T4-Late	−0.79	−0.90, −0.67	<0.001
	≥3 × T4-Late	−0.84	−0.96, −0.72	<0.001
Random		SD	95% CI	
Cow	Intercept	0.55	0.53, 0.58	

[1]The marginal R^2 and conditional R^2 were 0.24 and 0.57, respectively. The proportion of total variance explained by the random effect (intraclass correlation coefficient) was 0.43.

Model 3a. This first model focused on first-lactation animals and featured 2,131 records from 602 primiparous animals. The explanatory variables remaining in the model (Table 3) were farm, stage of lactation, DIM, height, the presence of a sole lesion, and the presence of a white line lesion. Two of the tested interactions remained: DIM by stage of lactation, and farm by stage of lactation (Figure 3a).

Model 3b. The second model focused on second-lactation animals, with 2,449 records from 721 second-lactation animals. The variables retained within model 3b (Table 4) were farm, stage of lactation, height, DIM,

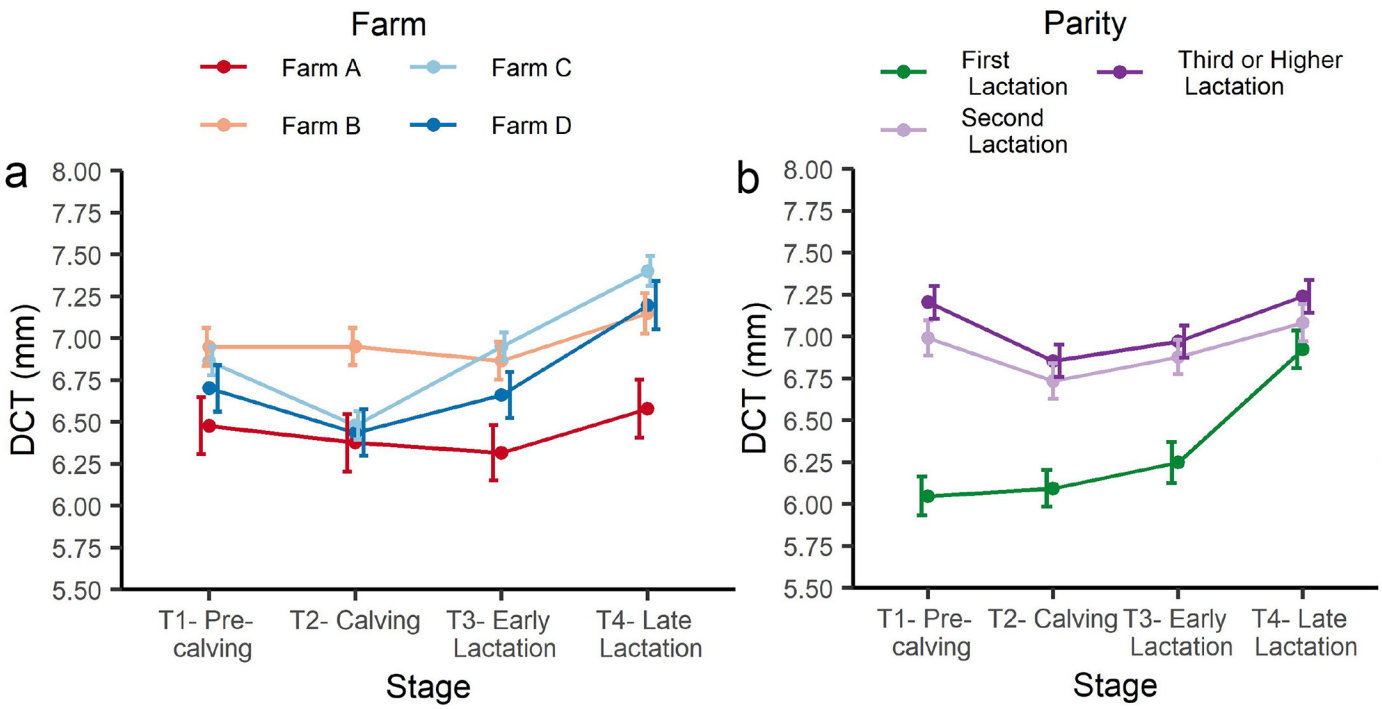

Figure 1. Interactions presented from the mixed effect linear regression model (model 1; estimates and 95% CI, Supplemental Table S4, see Notes) examining the association between explanatory variables, lesion presence at the time of measurement, and DCT. (a) The interaction between farm and stage of lactation on DCT (mm). (b) The interaction between parity and stage of lactation on DCT (mm).

BFT, and the presence of a white line lesion. Two of the tested interactions remained: DIM by stage of lactation, and farm by stage of lactation interaction (Figure 3b).

Model 3c. The third model focused on third or higher lactation animals, with 2,984 records from 967 multiparous animals. The variables retained within model 3c (Table 5) were farm, stage of lactation, DIM, MilkBot scale, BCS, height, and the presence of a white line lesion. Two of the tested interactions remained: DIM by stage of lactation and farm by stage of lactation interaction (Figure 3c).

DISCUSSION

In our study, farm, stage of lactation, parity, height, and lesion incidence were all significantly associated with DCT, however, no association was found between either body condition score or BFT and DCT. The significant association between the stage of lactation and the thickness of the digital cushion agrees with several previous studies, which found DCT to change through the course of a lactation cycle (Bicalho et al., 2009; Newsome et al., 2017b; Stambuk et al., 2019; Griffiths et al., 2020). However, discrepancies exist between studies with the nadir found to be during early lactation (Bicalho et al., 2009; Stambuk et al., 2019; Griffiths et al., 2020) or at calving (Newsome et al., 2017b; Stambuk et al., 2019). We found a novel interaction between farm and stage of lactation, with 2 distinct patterns emerging: the first found little change over the course of the lactation, with a slight depression in thickness during early lactation. The second pattern found the nadir to occur shortly after calving. Fat mobilization (Bicalho et al., 2009) and periparturient relaxation of the suspensory apparatus within the foot have both been implicated in the change in DCT throughout the course of the lactation (Tarlton et al., 2002; Knott et al., 2007; Newsome et al., 2017b). Furthermore, these differing patterns suggest that farm management has an important role in how the digital cushion changes over the course of a lactation. The inconsistencies between studies may therefore reflect study farm choice. Our study farms all used conventional milking systems with relatively similar housing; however, variations in diet, transition cow management, cubicle quality, standing times, inflammatory management, and walking distances could be the differences that affect the changes in DCT (Table 6).

No association was found between measures of body fat reserves (BFT or BCS) and DCT. This finding disagrees with those from Bicalho et al. (2009) and Newsome et al. (2017b), who both found an association; however, the effect sizes reported were significantly different. This could be explained by each study targeting a different fat pad. The study conducted by Bicalho et al. (2009)

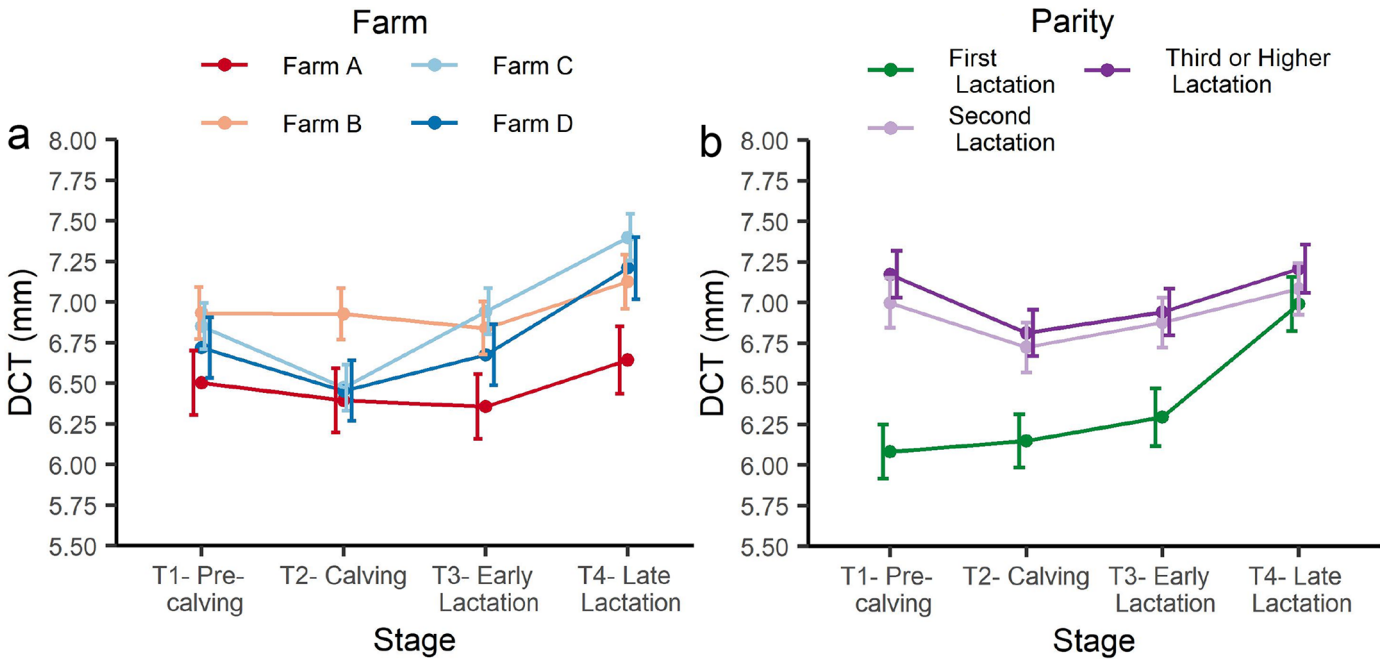

Figure 2. Interactions presented from the mixed effect linear regression model (model 2; estimates and 95% CI, Supplemental Table S5, see Notes) examining the association between explanatory variables, lesion presence during early lactation, and DCT. (a) The interaction between farm and stage of lactation on DCT (mm). (b) The interaction between parity and stage of lactation on DCT (mm).

targeted the middle fat pad, whereas the axial fat pad and middle fat pad were targeted by (Newsome et al., 2017b), and in the present study, the transverse projections between the axial and abaxial fat pads were measured. The axial and abaxial fat pads have higher lipid content compared with the middle fat pad, which underlies the flexor process of the distal phalanx and has a greater proportion of connective tissue (Räber et al., 2006; Baird et al., 2010). Alternatively, this could reflect a farm effect, with farm management, diet, and genetics potentially affecting whether the digital cushion responds to loss of body condition. Body condition score was found to be associated with DCT by Griffiths et al. (2020) in a smaller study, but BCS was treated as a continuous variable in that study, whereas in the present study, it was grouped to accommodate low representation at the extreme ends of the scoring system. An increased bodyweight or BCS before culling in late lactation, a higher proportion of a cow's lifetime spent over a BCS of 3, or an increased mean BCS before culling was associated with an overall increased digital cushion volume, although a larger adipocyte size was noted in the axial and middle fat pads in cows with a higher BCS (Hiss-Pesch et al., 2019; Newsome et al., 2021; Wilson et al., 2021).

In our study, primiparous animals displayed a significantly thinner digital cushion throughout their first lactation, with the digital cushion only reaching a commensurate size to multiparous animals during late lactation. In a smaller study conducted by Stambuk et al. (2019) primiparous animals had significantly thinner digital cushions than multiparous animals before calving but were not significantly different at calving, before becoming significantly thinner again during early lactation. Similar studies have reported that primiparous animals have thinner digital cushions (Bicalho et al., 2009; Newsome et al., 2017b; Griffiths et al., 2020). Heifers have also been shown to display significantly sparser quantities of lipids and a higher content of loose connective tissue compared with multiparous animals when descriptive anatomical studies have been performed (Räber et al., 2004). Furthermore, we present different patterns of DCT change over the course of each lactation for each farm and parity; to the authors' knowledge, this is the first time interactions between farm and stage of lactation on the digital cushion thickness for each parity group have been observed. This novel finding could have several explanatory mechanisms. Different trends across the lactations may be originally derived from the rearing period, which has already been described to have an effect on the structure (and hypothetically the function) of the digital cushion (Gard et al., 2015). The different outcomes observed across the different farms may also be due to the breeding strategies used on farm, with genetic selection having a role to play in lameness management and foot structure (Barden et al., 2022). Another potential source of the different trends may be

Table 3. Results from the mixed effect multivariable linear regression model estimating the association between explanatory variables, including temporal lesion presence and DCT (mm; model 2)[1]

Fixed	Term	Estimate	95% CI	*P*-value
Intercept		2.58	1.73, 3.43	<0.001
Farm	A	Referent		
	B	0.43	0.24, 0.62	<0.001
	C	0.35	0.18, 0.52	<0.001
	D	0.22	0.01, 0.42	0.039
Parity	First	Referent		
	Second	0.92	0.80, 1.03	<0.001
	≥3	1.09	0.97, 1.21	<0.001
Stage	T1-Precalving	Referent		
	T2-Calving	0.15	−0.04, 0.33	0.119
	T3-Early	0.11	−0.09, 0.31	0.266
	T4-Late	0.71	0.51, 0.90	<0.001
Temporal sole lesion	Absent	Referent		
	New	−0.11	−0.20, −0.02	0.013
	Chronic	−0.26	−0.46, −0.06	0.013
	Recovered	−0.03	−0.17, 0.12	0.719
Temporal white line lesion	Absent	Referent		
	New	0.16	−0.07, 0.39	0.181
	Chronic	0.54	0.18, 0.91	0.003
	Recovered	0.05	−0.18, 0.27	0.685
Height (cm)		0.02	0.01, 0.03	<0.001
MilkBot scale (kg/day)		0.00	0.00, 0.01	0.013
DIM		0.00	0.00, 0.01	<0.001
Stage × DIM	T1-Precalving × DIM	Referent		
	T2-Calving × DIM	−0.02	−0.03, 0.00	0.007
	T3-Early × DIM	0.00	−0.01, 0.00	0.039
	T4-Late × DIM	0.00	−0.01, 0.00	0.283
Farm × stage	A × T1-Precalving	Referent		
	B × T1-Precalving	Referent		
	C × T1-Precalving	Referent		
	D × T1-Precalving	Referent		
	A × T2-Calving	Referent		
	B × T2-Calving	0.10	−0.09, 0.30	0.296
	C × T2-Calving	−0.27	−0.45, −0.09	0.003
	D × T2-Calving	−0.16	−0.37, 0.06	0.153
	A × T3-Early	Referent		
	B × T3-Early	0.06	−0.14, 0.25	0.574
	C × T3-Early	0.24	0.06, 0.42	0.010
	D × T3-Early	0.10	−0.11, 0.32	0.342
	A × T4-Late	Referent		
	B × T4-Late	0.05	−0.16, 0.26	0.616
	C × T4-Late	0.41	0.21, 0.60	<0.001
	D × T4-Late	0.35	0.12, 0.58	0.003
Parity × stage	First × T1-Precalving	Referent		
	Second × T1-Precalving	Referent		
	≥3 × T1-Precalving	Referent		
	First × T2-Calving	Referent		
	Second × T2-Calving	−0.34	−0.45, −0.23	<0.001
	≥3 × T2-Calving	−0.43	−0.54, −0.32	<0.001
	First × T3-Early	Referent		
	Second × T3-Early	−0.33	−0.47, −0.20	<0.001
	≥3 × T3-Early	−0.44	−0.58, −0.31	<0.001
	First × T4-Late	Referent		
	Second × T4-Late	−0.82	−0.94, −0.70	<0.001
	≥3 × T4-Late	−0.88	−1.00, −0.75	<0.001
Random		SD	95% CI	
Cow	Intercept	0.54	0.52, 0.57	

[1]The marginal R^2 and conditional R^2 were 0.25 and 0.57, respectively. The intraclass correlation coefficient was 0.43.

the management of inflammation after calving, which is hypothesized to have an effect on the functionality of the digital cushion, and indeed the management of inflammation around other events such as mastitis (Watson et al., 2022), and lameness (Wilson et al., 2022). This novel finding highlights the importance of multifarm studies in future observational studies of DCT.

The digital cushion was significantly thinner in cows with a sole lesion present at the time of measurement, but it was thicker in cows with a white line lesion. In a

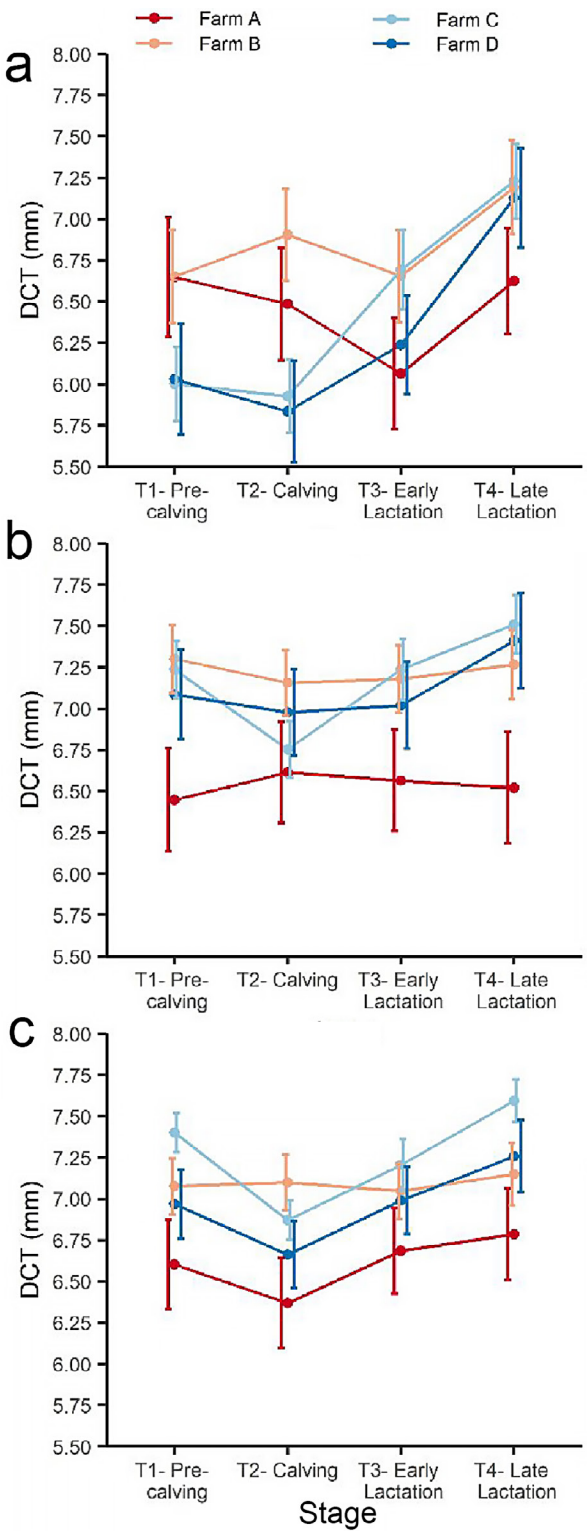

Figure 3. Interactions presented from the mixed effect linear regression models (model 3a, 3b, and 3c; estimates and 95% CI, Supplemental Table S6, see Notes) examining explanatory variables and lesions present at the time of measurement on the thickness of the digital cushion, focusing on each parity group in turn. The interaction between farm and stage of lactation on DCT (mm) for the (a) first parity, (b) second parity, and (c) third or higher parity.

study conducted by Griffiths et al. (2020), a thinner DCT during early lactation was significantly associated with increased odds of displaying a sole hemorrhage lesion at the same time point, and Bicalho et al. (2009) reported that cows in the thinnest 2 quartiles of DCT were at greater odds of a painful foot lesion being present. However, the presence of a sole ulcer on the claw at the time of measurement was shown to be associated with a thicker digital cushion by Newsome et al. (2017b). Our analysis include sole hemorrhage, which is considered to be either the precursor to sole ulcers or the result of a less severe insult (Bergsten, 1994; Lischer and Ossent, 2001; Newsome et al., 2016). The differences we have observed in this study may be accounted for through the following process. The formation of sole hemorrhage may require or cause thinning of the digital cushion. This thinning may happen in tandem with or before the inflammatory process required for sole hemorrhage formation, which thickens the appearance of the digital cushion. However, at the point at which hemorrhage is observed, this thickening may not be great enough to outweigh the initial thinning associated with sole hemorrhage onset. This will therefore present itself as a thinner digital cushion until sole ulcer development, where the nature of the inflammatory event is more severe, leading to further thickening of the digital cushion. Furthermore, the fat pad measurements in our study and that of Newsome et al. (2017b) were different, and this discrepancy may be a result of the occurrence of localized inflammation. Thin digital cushions have been associated with the future development of CHDL (Newsome et al., 2017a; Stambuk et al., 2019; Griffiths et al., 2024), with a history of CHDL predisposing cattle to exostosis and repeated bouts of lameness and CHDL (Foditsch et al., 2016; Newsome et al., 2016; Randall et al., 2016). Furthermore, a history of lameness is associated with a reduced volume of digital cushion (Wilson et al., 2021).

To the authors' knowledge, no other studies have reported the association between white line lesions and thicker digital cushions. However, Griffiths et al. (2024) reported that a thin digital cushion before or shortly after calving was not significantly associated with the development of a white line lesion during early lactation. It is possible that local inflammation associated with a white line lesion could lead to an increased DCT. Inflammation could affect the functional ability of the digital cushion to protect the corium, affecting the future development of sole lesions. This could be true even if the size of the digital cushion is not itself associated with the development of white line lesions later on.

A small but statistically significant association was found with taller cows displaying thicker digital cushions. This is in agreement with Newsome et al. (2017b), who found very similar effect sizes.

Table 4. Results from the mixed effect multivariable linear regression model estimating the association between explanatory variables, including concurrent lesion presence and DCT (mm), for first lactation animals (model 3a)[1]

Fixed	Term	Estimate	95% CI	*P*-value
Intercept		4.07	2.60, 5.55	<0.001
Farm	A	Referent		
	B	0.01	−0.33, 0.34	0.974
	C	−0.65	−0.94, −0.35	<0.001
	D	−0.62	−0.98, −0.26	0.001
Stage	T1-Precalving	Referent		
	T2-Calving	−0.15	−0.47, 0.17	0.362
	T3-Early	−0.59	−0.91, −0.26	<0.001
	T4-Late	−0.02	−0.34, 0.29	0.885
Sole lesion	Absent	Referent		
	Present	−0.16	−0.28, −0.05	0.005
White line lesion	Absent	Referent		
	Present	0.50	0.09, 0.90	0.016
Height (cm)		0.02	0.01, 0.03	0.001
DIM		0.01	0.00, 0.01	0.002
Stage × DIM	T1-Precalving × DIM	Referent		
	T2-Calving × DIM	−0.03	−0.05, −0.01	0.004
	T3-Early × DIM	0.01	0.00, 0.01	0.091
	T4-Late × DIM	0.00	−0.01, 0.01	0.973
Farm × stage	A × T1-Precalving	Referent		
	B × T1-Precalving	Referent		
	C × T1-Precalving	Referent		
	D × T1-Precalving	Referent		
	A × T2-Calving	Referent		
	B × T2-Calving	0.41	0.05, 0.78	0.025
	C × T2-Calving	0.09	−0.23, 0.41	0.597
	D × T2-Calving	−0.03	−0.42, 0.35	0.865
	A × T3-Early	Referent		
	B × T3-Early	0.58	0.23, 0.94	0.001
	C × T3-Early	1.27	0.95, 1.60	<0.001
	D × T3-Early	0.79	0.41, 1.17	<0.001
	A × T4-Late	Referent		
	B × T4-Late	0.56	0.20, 0.92	0.002
	C × T4-Late	1.25	0.93, 1.57	<0.001
	D × T4-Late	1.12	0.74, 1.50	<0.001
Random		SD	95% CI	
Cow	Intercept	0.48	0.44, 0.52	

[1]The marginal R^2 and conditional R^2 were 0.30 and 0.58, respectively. The intraclass correlation coefficient was 0.40.

Our study has limitations which need to be taken into consideration when interpreting our findings. We present results from cows enrolled from 4 farms, 3 of which were fully housed herds. Although these farms feature common operating practices on many UK commercial dairy farms, this study could not be considered representative of the full range of dairy farms. Due to the study design, ultrasound measurements of DCT were only taken of the lateral claw of the hind left foot. Our data were analyzed at the cow level, but lesion and DCT information were only collected from the hind left lateral claw and therefore claw specific. Hindlimbs were chosen because they have the highest incidence of CHDL, and the digital cushions of the feet most at risk of developing CHDL were therefore measured (Murray et al., 1996). Wilson et al. (2021) found no significant differences in the volume of the digital cushion between the hind feet and therefore we believe that the hind left claw is an adequate proxy for the lateral right hind claw. Although every effort was made to ensure consistency in positioning of the probe by using the midline and only capturing an image once all required anatomical locations were visualized simultaneously, we cannot confirm that the probe was positioned in exactly the same location. However, because we used both external and internal anatomical landmarks, we expect this variation to be minimal. No formal measure of agreement between researchers undertaking lesion assessments was calculated; however, >90% of these lesion assessments were conducted by a single person, with all assessments conducted by trained veterinary professionals. Only a small amount of horn was removed to detect lesions in primiparous animals at T1-Precalving and T2-Calving, so there is a small risk that small deep lesions or very early lesions may have been missed. This may have affected whether a small deep lesion was noted at T1-Precalving or T2-Calving for heifers. In multiparous animals, the wider modeling was still apparent at T2-Calving, and the removal of a thin layer of horn would

Table 5. Results from the mixed effect multivariable linear regression model estimating the association between explanatory variables, including concurrent lesion presence and DCT (mm) for second-lactation animals (model 3b)[1]

Fixed	Term	Estimate	95% CI	*P*-value
Intercept		3.03	1.59, 4.48	<0.001
Farm	A	Referent		
	B	0.85	0.55, 1.16	<0.001
	C	0.79	0.51, 1.07	<0.001
	D	0.64	0.28, 0.99	<0.001
Stage	T1-Precalving	Referent		
	T2-Calving	0.17	−0.12, 0.46	0.242
	T3-Early	0.12	−0.17, 0.41	0.425
	T4-Late	0.08	−0.24, 0.39	0.644
White line lesion	Absent	Referent		
	Present	0.40	0.10, 0.70	0.010
Height (cm)		0.02	0.01, 0.03	<0.001
DIM		0.00	0.00, 0.00	0.420
BFT (mm)		0.01	0.00, 0.02	0.134
Stage × DIM	T1-Precalving × DIM	Referent		
	T2-Calving × DIM	0.00	−0.02, 0.01	0.693
	T3-Early × DIM	0.00	−0.01, 0.01	0.904
	T4-Late × DIM	0.00	−0.01, 0.00	0.747
Farm × stage	A × T1-Precalving	Referent		
	B × T1-Precalving	Referent		
	C × T1-Precalving	Referent		
	D × T1-Precalving	Referent		
	A × T2-Calving	Referent		
	B × T2-Calving	−0.31	−0.62, 0.00	0.052
	C × T2-Calving	−0.65	−0.95, −0.35	<0.001
	D × T2-Calving	−0.27	−0.64, 0.09	0.136
	A × T3-Early	Referent		
	B × T3-Early	−0.24	−0.55, 0.08	0.139
	C × T3-Early	−0.12	−0.44, 0.20	0.471
	D × T3-Early	−0.18	−0.55, 0.18	0.321
	A × T4-Late	Referent		
	B × T4-Late	−0.11	−0.46, 0.25	0.549
	C × T4-Late	0.20	−0.13, 0.53	0.243
	D × T4-Late	0.25	−0.15, 0.66	0.223
Random		SD	95% CI	
Cow	Intercept	0.56	0.52, 0.60	

[1]The marginal R^2 and conditional R^2 were 0.10 and 0.53, respectively. The intraclass correlation coefficient was 0.47.

therefore have revealed the presence of any lesions. Although a single assessor was used for the BFT measurements, they were not blinded to stage, farm, and parity. Lastly, feet were lifted to obtain ultrasound images of the digital cushion. A novel method of scanning the digital cushion while weightbearing was developed by Bach et al. (2019); although the sample size was small, their results suggest that weightbearing produces different measurements of the digital cushion to lifted feet.

This study has several strengths; it is the largest study of DCT measurements, conducted across 4 farms with a large number of detailed foot lesion records collected by veterinarians, primarily by a single assessor. The DCT and BFT variables were measured objectively using ultrasound, and those ultrasound images were assessed after data collection had ceased by a single assessor blinded to stage of lactation, parity, farm, and the presence of lesions for DCT. The assessor was also blinded to each of the other measurements obtained via ultrasound and to BCS.

CONCLUSIONS

Our prospective cohort study confirms the association between parity and the thickness of the digital cushion. In a novel finding, farm was shown to interact with stage of lactation in its association with DCT, with 2 distinct patterns observed. Backfat thickness and BCS were not significantly associated with the thickness of the digital cushion. The presence of sole lesions and white line lesions at the time of measure were associated with the thickness of the digital cushion, whereas those animals having a chronic sole lesion during early lactation had thinner digital cushions before calving.

NOTES

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Table 6. Results from the mixed effect multivariable linear regression model estimating the association between explanatory variables, concurrent lesion presence and DCT (mm) for third or higher lactation animals (model 3c)[1]

Fixed	Term	Estimate	95% CI	*P*-value
Intercept		4.08	2.63, 5.52	<0.001
Farm	A	Referent		
	B	0.47	0.18, 0.77	0.002
	C	0.80	0.54, 1.06	<0.001
	D	0.37	0.06, 0.68	0.021
Stage	T1-Precalving	Referent		
	T2-Calving	−0.25	−0.53, 0.04	0.091
	T3-Early	0.09	−0.19, 0.37	0.522
	T4-Late	0.19	−0.11, 0.49	0.220
White line lesion	Absent	Referent		
	Present	0.22	0.05, 0.39	0.012
Height (cm)		0.01	0.01, 0.02	0.002
MilkBot scale (kg/d)		0.00	0.00, 0.01	0.075
BCS	≤2.5	−0.7	−0.18, 0.05	0.261
	2.75–3.25	Referent		
	≥3.5	0.07	0.00, 0.14	0.063
DIM		0.00	0.00, 0.00	0.105
Stage × DIM	T1-Precalving × DIM	Referent		
	T2-Calving × DIM	−0.01	−0.03, 0.01	0.314
	T3-Early × DIM	0.01	0.00, 0.01	0.087
	T4-Late × DIM	0.00	0.00, 0.01	0.227
Farm × stage	A × T1-Precalving	Referent		
	B × T1-Precalving	Referent		
	C × T1-Precalving	Referent		
	D × T1-Precalving	Referent		
	A × T2-Calving	Referent		
	B × T2-Calving	0.26	−0.06, 0.57	0.115
	C × T2-Calving	−0.30	−0.60, 0.00	0.050
	D × T2-Calving	−0.07	−0.41, 0.27	0.678
	A × T3-Early	Referent		
	B × T3-Early	−0.11	−0.42, 0.19	0.469
	C × T3-Early	−0.28	−0.60, 0.05	0.091
	D × T3-Early	−0.06	−0.40, 0.27	0.720
	A × T4-Late	Referent		
	B × T4-Late	−0.11	−0.45, 0.23	0.532
	C × T4-Late	0.01	−0.31, 0.33	0.947
	D × T4-Late	0.11	−0.26, 0.47	0.565
Random		SD	95% CI	
Cow	Intercept	0.58	0.54, 0.62	

[1] The marginal R^2 and conditional R^2 were 0.11 and 0.48, respectively. The intraclass correlation coefficient was 0.42.

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Nonstandard abbreviations used: BFT = back fat thickness; CHDL = claw horn disruption lesion; DCT = digital cushion thickness; T1-Precalving = before calving; T2-Calving = immediately after calving; T3-Early = early lactation; T4-Late = late lactation.

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ORCIDS

Bethany E. Griffiths https://orcid.org/0000-0002-2698-9561
Matthew Barden https://orcid.org/0000-0003-0141-3037
Alkiviadis Anagnostopoulos https://orcid.org/0000-0002-5193-858X
James P. Wilson https://orcid.org/0000-0001-7096-8007
William Tulley https://orcid.org/0000-0003-3550-4754
Cherrill Bedford https://orcid.org/0009-0004-3103-0920
Georgios Banos https://orcid.org/0000-0002-7674-858X
Georgios Oikonomou https://orcid.org/0000-0002-4451-4199