Interpretive Summary

Lameness derived from claw horn lesions is a chronic, debilitating condition that affects cattle worldwide. Claw horn lesions present a significant welfare and economic challenge to the future of the dairy industry. To date, there is little understanding of the impact these diseases have on the supportive structures in the hoof capsule. This research has demonstrated that a key supportive structure in the cow's hoof is compromised in animals that have experienced more lameness in their life. We hypothesize that the differences observed are a result of genetic, developmental and disease related inputs.

Running head – Lameness and digital cushion volume in dairy cows

A history of lameness and low body condition score is associated with a reduced digital cushion volume, measured by magnetic resonance imaging, in dairy cattle

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ABSTRACT

Claw horn lesions (CHLs) are the result of a failing of the functional anatomy of the hoof in dairy cows. The digital cushion is understood to be a vital structure in the prevention of CHLs. CHLs have previously been shown to lead to pathological change to the pedal bone, however their impacts on the digital cushion are unknown. The primary aim of this study was to examine associations between the history of CHLs through an animal's life, and the structure of the digital cushion at slaughter utilising magnetic resonance imaging (MRI). The retrospective cohort study resulted in the scanning of 102 pairs of hindfeet, collected from adult Holstein dairy cows culled from a research herd, using a 3-Tesla, research grade MRI scanner. Volume and fat measurements were calculated for each digital cushion within each claw from a modified DIXON quant sequence. Animal level variables were constructed around the animals lactating lifetime, with lameness scores and body condition score (BCS) collected at least fortnightly. The combined volume of digital cushion in the lateral claws was used as the outcome variable in multivariable linear models. The volume of digital cushion was negatively associated with the number of lameness events or CHL recorded. Furthermore, animals of BCS>3, culled later in lactation, or of a greater bodyweight were more likely to have a higher volume of digital cushion in the lateral claws. We propose that the observations made in the current study are the effects of a range of factors broadly associated with genetic, developmental and disease related inputs. Our understanding of how we can select for genetically more robust animals and how we can pre-condition the hoof before first calving need to be improved to reduce the risk of future CHLs in adult dairy cattle. Furthermore, understanding optimal treatment regimens and their impact on hoof anatomy may reduce the recurrence of CHLs in the current lactation and future lactations.

Key words:

Dairy Cow, Digital Cushion, MRI, Lameness, Claw Horn Lesion

INTRODUCTION

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

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

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

at a BCS > 2.5 (out of 5) minimises the risk of CHLs requiring treatment (Green et al., 2014). Additional to this, an eight year study of a single herd showed that cattle with a BCS < 2 were more likely to be lameness scored as either lame or severely lame (Randall et al., 2015). It was hypothesised by both studies that their respective findings were due to a positive association between BCS and the thickness of the digital cushion, meaning that animals with a higher BCS have a thicker digital cushion. Increased digital cushion thickness has been suggested to offer a better ability to dissipate the concussive forces associated with hoof-strike, thereby minimising the risk of CHL development (Bicalho et al., 2009).

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

A study conducted by Newsome et al. (2016) illustrated the associations between lameness and pathological change to the pedal bone. Lameness and CHL occurrence during life were found to be positively associated with the presence of new bone formation on the caudal aspect of the pedal bone (the area where the initial point of hoof-strike occurs) in the hindfeet of cull dairy

cattle. It was suggested that once formed, the presence of this osteoma could predispose animals to future CHL development, leading to a “downwards spiral” of lameness and CHLs.

At present, there are large gaps in the understanding of the aetiology of CHLs with limited knowledge of the interactions between lameness events and the structure of the digital cushion. Newsome et al. (2016) postulated that the inflammatory nature of CHL pathogenesis could lead to the utilisation of the fatty acids in the digital cushion as inflammatory mediators and / or the development of scar tissue in or around the digital cushion. If correct, this could alter the size, structure and functionality of the digital cushion and reduce its ability to dissipate the concussive forces of hoof-strike.

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

MATERIALS AND METHODS

Study Design

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

Study Herd and Herd Management

The study population included Holstein cows culled from the SRUC (Scotland's Rural College) research herd, based at the Crichton Royal Farm (Dumfries) between August 2016 and September 2018. Animals were housed at one of two facilities (Langhill or Acrehead) where milking took place three times a day. The Langhill herd is one of the most intensively monitored and studied research herds in the UK. Data collection pertinent to the current study is reported below, please see Pryce et al. (1999), Chagunda et al. (2009) and Randall et al. (2015) for a more fulsome description of herd management. Whilst part of the same enterprise, the Acrehead herd is less intensively monitored and has more of a commercial focus.

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

milk yields were between 8,500 and 12,000Kg per cow per year dependent upon the management system to which the animal belonged.

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

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

Sample Collection – Hindfeet at slaughter

Immediately after slaughter, the distal hindlimbs of all animals were recovered at the slaughterhouse by a single cut at the hock joint and labelled with the animal and limb

identification. Hindlimbs were stored locally at -20 ˚C before being transported to the University of Nottingham at -20˚C, every 4-6 months. To minimise storage volume and facilitate positioning within the MRI scanner, the hoof was removed from the remainder of the limb using a single cut approximately 2cms above the coronary band whilst frozen. Feet were maintained at -20 ˚C within labelled polythene packaging until required for scanning.

Hoof MRI Scanning Protocols

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

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

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

Scans were exported as Digital Imaging and Communications in Medicine (DICOM) files and anonymised for image analysis to calculate digital cushion volume and fat content, to minimise

the potential for observer bias. To calculate digital cushion volume (mls), regions of interest (ROIs) were drawn on individual cushions using MATLAB (The Mathworks Inc.) and volume was computed from the number of voxels within a ROI. Fat Fraction maps were created on the scanner using the mDIXON Quant package (Philips, Best, Netherlands, NL), ROIs were then drawn over the cushion of interest and histogram analysis completed to compute the modal fat fraction with full width half maximum to assess skew. From ROIs on slices of digital cushion that included fat, the volume of fat within each cushion was computed.

Data Handling and Descriptive Analysis

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

Statistical Modelling

Multivariate linear regression models were constructed to investigate the associations between lameness history and digital cushion volume. The outcome variable was the total volume (mls) of digital cushion contained within the lateral claws of both hindfeet at cull, which was calculated by summing the volumes of the axial, middle and abaxial cushion contained within the left and right hindfeet lateral claws.

Three final models were constructed (Models A – C):

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

Explanatory variables to explore the associations between events in the first and last lactations as well as the lactating lifetime of the animal were calculated as follows; number of CHL treatments recorded (either as a continuous or categorical (0-1, 2-6, 7+) variable), proportion of lameness scores sound (i.e. score 0 – 2; proportion was calculated by summing the number of lameness scores recorded as sound and dividing by the total number of recorded scores), proportion of BCS >3 (proportion was calculated by summing the number of BCS recorded >3 and dividing by the total number of recorded scores), the animals average liveweight (in Kgs

as derived by averaging all recorded liveweights for the period described) and daily milk yield (calculated by averaging the daily milk yield in litres for the period described), age at cull (in months), days in milk (DIM) at cull, the genetic line to which the animal belonged (C or S) and the feed trial to which the animal belonged throughout its lifetime (higher or lower plane of nutrition). The DIM at which an animal first became lame, and the average BCS and lameness score in the four weeks before cull were also calculated. Animals could only be included in the models if a complete dataset for all variables was present up to seven days before cull.

Linear regression models were constructed in R Studio taking the format:

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

$$[eO_i] \sim N(O, \sigma_e^2)$$

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

Model fit was assessed by visual assessment of residuals and the removal of outlying datapoints (large influence or high leverage) to check their impact on model parameters; if coefficients remained statistically similar (<5% difference) with the same biological interpretation, then model fit was deemed adequate.

Validation of Scanning Protocols

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

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

RESULTS

Validation of Scanning Protocols

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

Animal Dataset

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

283 ***Descriptive Statistics***

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

293 ***Statistical Modelling***

294 Data from 57 animals with a complete history of lameness and BCS data until a maximum of
295 seven days before cull was available to construct all models. Of these 57 animals, 23 had at
296 least one treatment for a CHL recorded during their lactating lifetime. All animals within the
297 final models were culled only from the Langhill herd. Table 2 describes the explanatory
298 variables constructed for animals retained within the models, with the models themselves being
299 described in Table 3. No explanatory variables from the first lactation remained in the final
300 models. Correlations and interactions between variables were examined with no substantive
301 inflation of coefficients observed.

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

304 Increased mean lameness score in the 4-week period before cull was associated with a reduced
305 volume of digital cushion in the lateral claws at slaughter. For every point increase in mean
306 lameness score a decrease of 2.8mls in digital cushion volume was observed (*P = 0.02, 95%*

CI: -4.1 - -1.5mls). Animals with a higher mean BCS in the 4-week period before cull had a significantly increased volume of digital cushion at cull. For every point increase in the mean BCS before cull, an associated increase in digital cushion volume of 2.56ml was observed (*P = 0.02, 95% CI: 1.0- 4.1mls*). In addition to the lameness status (measured by mean lameness score) of the animal in the 4 weeks before cull, the treatment of a CHL at any point during the animals lactating lifetime was significantly associated with digital cushion volume; for every CHL treatment recorded there was an associated decrease in digital cushion volume of 0.62ml at the end of life (*P < 0.001, 95% CI: -1.0 - -0.2mls*). Body weight was significant in the final model; for every additional Kg of liveweight an associated increase in digital cushion volume of 0.05ml was observed (*P<0.001, 95% CI: 0.02 – 0.07mls).*

Fifty three percent of the variation observed in digital cushion volume within the dataset was explained by the model.

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

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

0.02, 95% CI: 0.000 – 0.006). Finally, DIM at cull was significant in the final model. For every day later in lactation at which an animal was culled, an associated increase in digital cushion volume of 0.01ml was observed (*P = 0.02, 95% CI: 0.00 – 0.02mls).*

Forty percent of the variation in digital cushion volume observed within the dataset was explained by the model.

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

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

Fifty four percent of the variation in digital cushion volume observed within the dataset was explained by the model.

DISCUSSION

This is the first study to describe the volume and fat fraction of the digital cushion, using MRI, in a large sample of adult dairy cattle. We identified a substantial range in the volume of digital cushion present within the lateral claws of animals at cull (0 – 29.86ml). Animals

experiencing a history of lameness (either described as CHL treatments, or by proportion of lameness scores submitted as lame) in their lactating life, or an increased mean lameness score in the four weeks before slaughter were more likely to have a reduced volume of digital cushion in the lateral claws at cull. Additionally, animals with a higher proportion of BCS>3 submitted throughout their lactating life or having an increased mean BCS in the four weeks before cull were more likely to have an increased volume of digital cushion in the lateral claws at cull.

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

Genetic foundations linked to the structure of the digital cushion have been described previously. Oikonomou et al. (2014) described how BCS and digital cushion thickness have a moderate heritability, with animals that are genetically predisposed to maintaining BCS at a more stable level are also predisposed to a lower risk of becoming lame. Further to this, Stambuk et al. (2020) isolated genetic markers associated with digital cushion thickness, with those linked to fat deposition, bone growth and keratinocyte function being highlighted as genes of importance. These studies lead to the conclusion that some animals are genetically predisposed to having a thinner/impaired digital cushion. If thinner digital cushions are, as

previously identified, a risk factor for disease, these animals may experience more CHLs and lameness during life. This could lead to the end of life associations we identified.

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

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

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

No significant association between plane of nutrition and digital cushion volume was identified in this study. The primary mechanism through which nutrition is understood to impact the risk of CHLs is through the maintenance of BCS (Bicalho et al., 2009; Newsome et al., 2017a; b), although it is biologically plausible to postulate that other interactions between diet and digital cushion size / composition are possible. As the current study was not specifically designed, or powered, to investigate the associations between dietary management and digital cushion structure, lack of association does not necessarily mean that one does not exist. Further prospective research is warranted to investigate the impacts of diet on digital cushion structure and function, and the subsequent risk of CHLs in dairy cattle.

The remodelling of adipose tissue and release of inflammatory mediators can also occur during adipose metabolism, which has been shown to be associated with animals losing body condition (Contreras et al., 2017a; b). Furthermore, the transition period has also been associated with an increase in systemic inflammation and adipose metabolism (Bradford et al., 2015; Contreras et al., 2017b; a). This inflammation and adipose metabolism could result in the digital cushion decreasing in volume, thereby reducing its ability to function normally, and predisposing the animal to future CHLs. The time around the transition period is a potential intervention point to minimise the risk of the digital cushion being adversely influenced, thereby preventing subsequent CHL development. It is possible that the development of CHLs and reduction in BCS could impede the digital cushion’s future ability to function from the earliest stage of the process of inflammation, through the previously

described self-perpetuating cycle. This reduction in digital cushion volume could present as the earliest stage of pathological change in the hoof of the dairy cow. Even mild lameness could lead to a reduction in digital cushion volume, potentially explaining the predisposition of heifers to future lameness if they suffer from CHLs in their first lactation (Randall et al., 2016). The hypothesis that inflammation degrades the structure of the digital cushion presents an opportunity to investigate the ability for anti-inflammatory drugs to minimise an animal's risk for CHL development through administration at times of lameness or systemic inflammation.

Furthermore, we postulate that an animal may never truly recover from a CHL due to the permanent deterioration of the digital cushions structure. Within Model A, when accounting for lameness status of the animal in the four weeks before cull, the historic treatment of a CHL during the animals lactating life was negatively associated with digital cushion volume. This suggests that the differences in digital cushion volume observed in this study are chronic in nature, with historic CHLs presenting a long-term challenge to the structure of the digital cushion. Upon inspection of diseased claws post mortem, Lischer et al. (2002) found that the adipose tissue contained within the digital cushion of animals with sole ulcers was replaced largely by connective tissue indicative of scarring. It has been demonstrated in various species that inflammation and trauma can lead to the remodelling and deposition of collagen fibres (Bonnans et al., 2014). The remodelling and deposition of these fibres may permanently inhibit the digital cushions ability to dissipate force, further predisposing the animal to future CHLs. The study by Lischer et al. (2002) in combination with the present research could be taken to suggest that CHLs have a lasting impact on the structure of the digital cushion. This long-term change in structure could lead to the impediment of the digital cushions ability to function optimally. The authors hypothesise that the digital cushion may never truly recover from the inflammatory process associated with CHLs, thereby

453 predisposing animals to future CHL onset. Further research investigating optimal treatment
454 regimens in “newly lame” animals could prove essential for reducing the global lameness
455 prevalence by breaking of the chronicity cycle we hypothesise.

456 Animals suffering from lameness previously may be predisposed to future lameness through
457 the reduction in digital cushion volume observed in the current study, and the development of
458 bone on the most caudal aspect of the pedal bone observed by Newsome et al. (2016). These
459 pathological changes in the hoof will predispose an animal to CHLs, meaning that intervention
460 or observational studies investigating CHL presence should focus on the use of animals naïve
461 to CHLs. This will ensure the true effects of an intervention or risk factor can be accounted for,
462 without the unknown effects of previous lameness creating “background noise” in terms of
463 lameness prevalence and recurrence. The use of trans-sole ultrasonography is common in
464 research investigating the structure of the digital cushion. However, based on our results, the
465 authors question the accuracy (the degree to which the result of a measurement, conforms to
466 the correct value) and precision (how close the repeated measured values are to each other) of
467 trans-sole ultrasonography. Many animals had little digital cushion in their lateral claws at
468 slaughter, with the middle cushion commonly the smallest cushion by volume or not present at
469 all. Räber et al.(2004) described how the middle cushion does not overlap the most caudal
470 aspect of the pedal bone. When we consider the method of trans-sole ultrasonography
471 commonly described in research publications (Bicalho et al., 2009; Newsome et al., 2017a; b;
472 Fabbri et al., 2020), the “typical ulcer site” is commonly visualised by using the flexor
473 tuberosity along the midline of the plantar aspect of the claw as a landmark. This would capture
474 an image of the middle fat pad of the digital cushion. However, when the lack of middle cushion
475 we identified and the irregularity of the middle cushion overlapping with the pedal bone is
476 considered, it becomes apparent that trans sole ultrasonography at this location may result in
477 the capturing of the thickness of the corium situated under the flexor tuberosity alone. This

may result in the over-estimation of digital cushion thickness if no digital cushion is present whatsoever. This is pertinent when we consider that trans-sole ultrasonography cannot distinguish between the soft tissues located within the hoof. Kofler et al. (1999) validated the method of trans-sole ultrasonography in acquiring sole soft tissue thickness measurements, however in this study only healthy digits were imaged (meaning that they may have had intact and healthy digital cushions) and the thickness of the combined corium and digital cushion were measured together (i.e., there was no differentiation between the two).Given the concerns we have identified and its increasing use in the research literature, we believe further work is urgently required to robustly validate trans-sole ultrasonography as an accurate and precise measure of digital cushion thickness.

There are several limitations which should be considered when interpreting the findings of our study. Firstly, and as previously stated, the direction of causality cannot be attributed from a retrospective study such as this. The associations we describe should be interpreted with care and we recommend that they are used to inform hypotheses for future prospective intervention studies. Secondly, the study population examined was based on a convenience sample of 102 animals from a single UK dairy research centre. The 57 animals selected for further analysis were those with complete lameness data up to the point of cull, which may have created a bias in the population studied. It is not possible to comment on the extent of any potential bias or the impact of that potential bias on the results, however, we have not identified any factors which would suggest the animals studied are not representative of intensively managed dairy cattle. The use of cadaver material should also be considered when interpreting our results. From the validation work carried out initially, however, it appears that the influence of the freeze-thaw cycle on digital cushion volume was minimal which suggests our results accurately describe the structure of the digital cushion post-mortem. That said, the ability of the digital cushion to adequately dissipate the forces associated with standing and foot strike are still to

be understood and we were not able to validate whether our findings in non-weight bearing cadaver feet reflect what would be found in the live weight bearing animal. Further work using standing MRI may help shed light on this important deficit in our current understanding.

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

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

626 **Table 1 Descriptive data for the volume of lateral and medial digital cushions and their respective**
627 **fat fractions from a sample of 196 hind claws from the hindfeet of 98 cull dairy cows. Animals**
628 **were culled from the Langhill and Acrehead Herds at the SRUC's (Scotland's Rural College)**
629 **Crichton Royal Farm (Dumfries). Measurements were ascertained using a mDIXON Quant**
630 **sequence with a 3-Tesla MRI scanner based at the Sir Peter Mansfield Imaging Centre**
631 **(University of Nottingham).**

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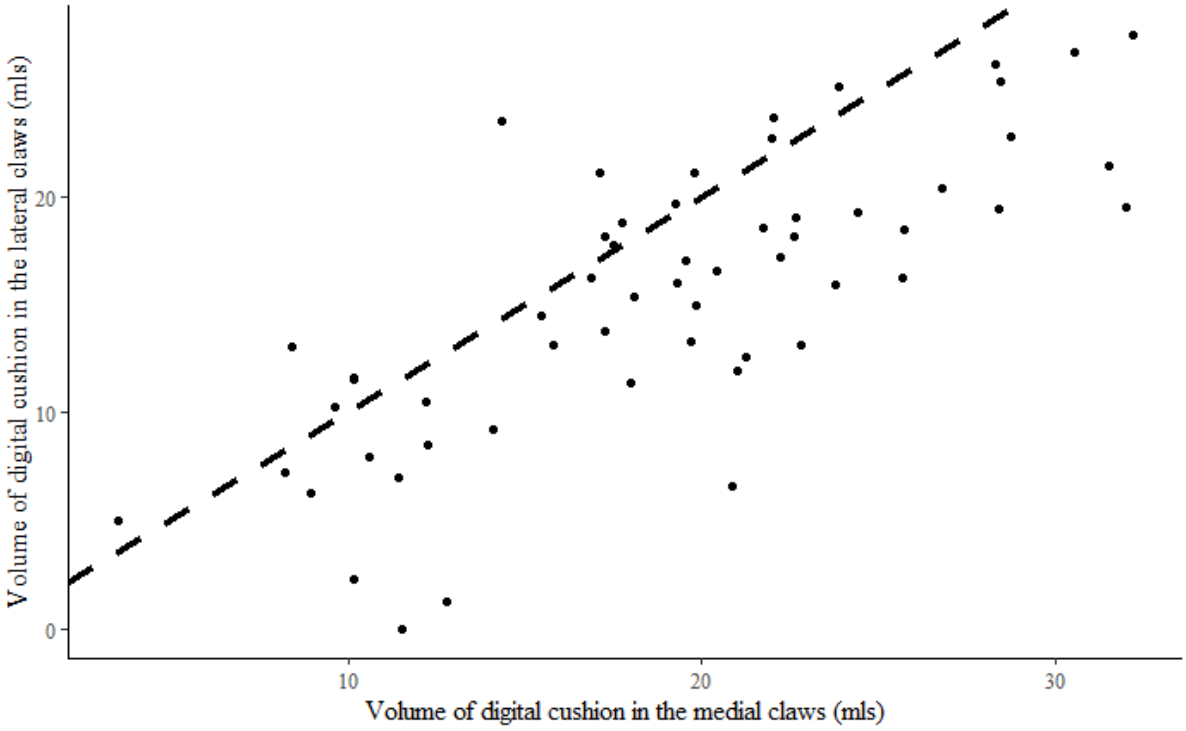

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639 **Figure 1 A scatterplot of the volume of lateral and medial digital cushions from a sample of 228**
640 **hind claws from the hindfeet of 57 cull dairy cows. Animals were culled from the Langhill Herd**
641 **at the SRUC's (Scotland's Rural College)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

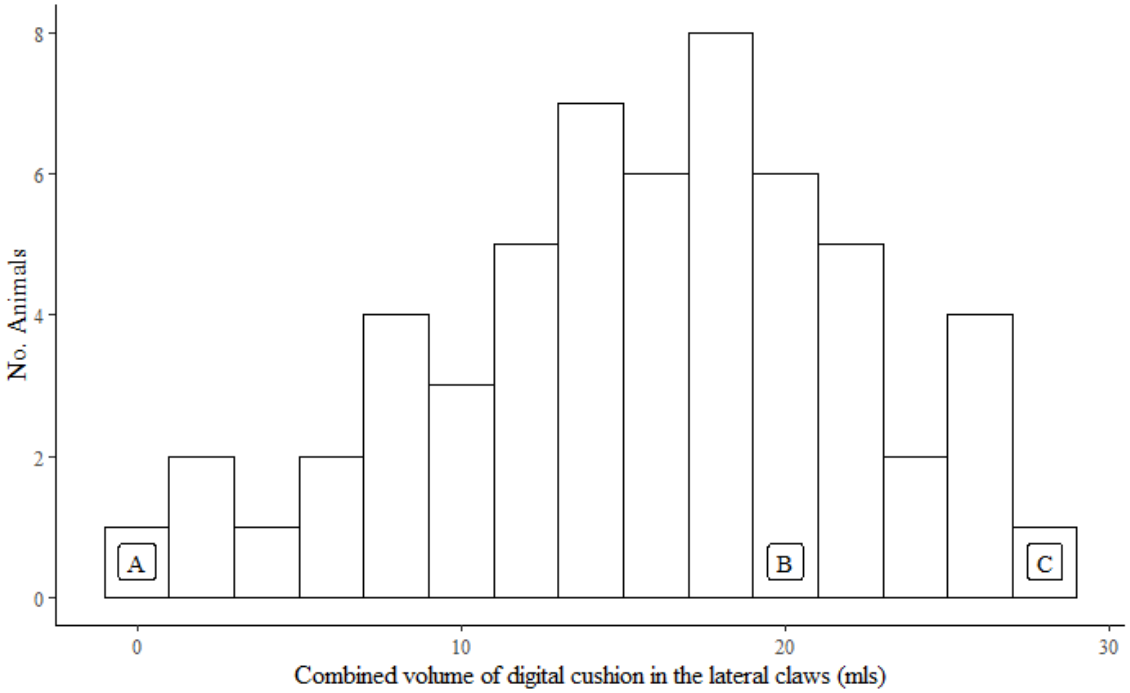

Figure 2a A histogram of the combined volume of digital cushions in 104 lateral claws from the hindfeet of 57 cull dairy cows. Animals were culled from the Langhill Herd at the SRUC's (Scotland's Rural College) Crichton Royal Farm (Dumfries). and selected for analysis if a complete mobility history was present. Measurements were ascertained using a mDIXON Quant se
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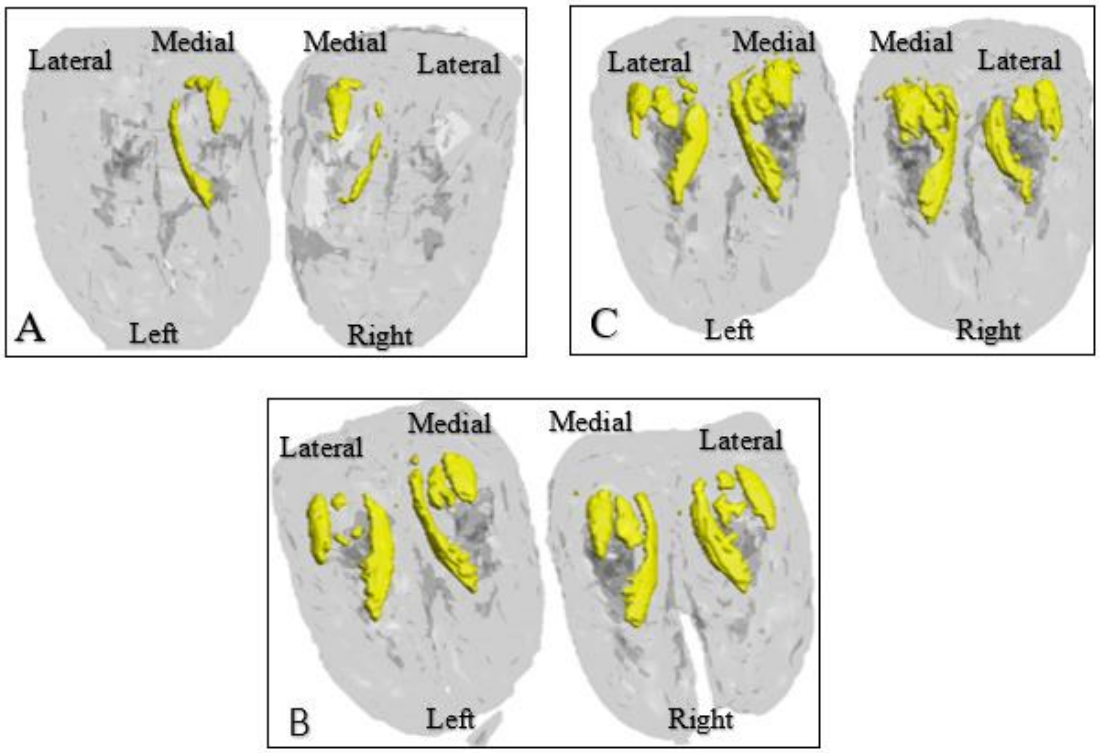

647**Figure 2b Reconstructions of the mDIXON Quant sequence MRI scans for animals represented on Figure 2a. Animals were culled from the Langhill Herd at the SRUC's (Scotland's Rural College) Crichton Royal Farm (Dumfries) and selected for analysis if a complete mobility history was present. Scans were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

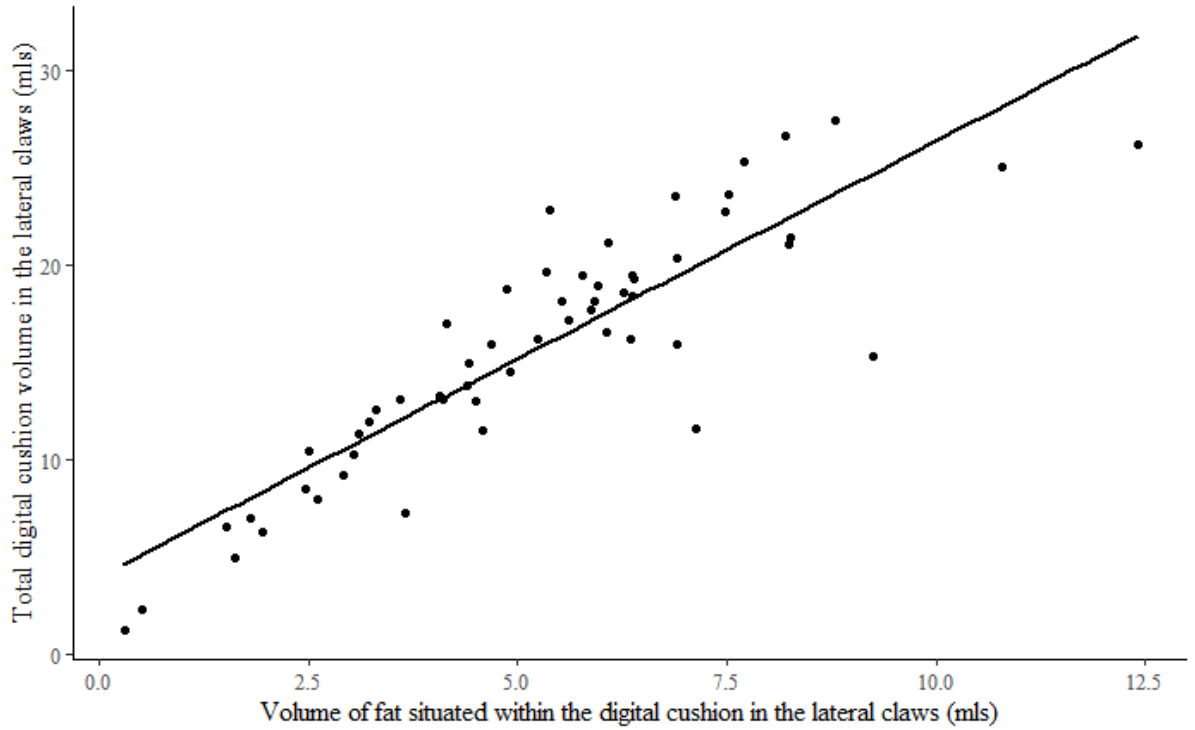

Figure 3. A scatterplot of the combined volume of digital cushions contained within the lateral claws and the respective volume of fat within those cushions from a sample of 104 lateral claws from the hindfeet of 57 cull dairy cows. Animals were culled from the Langhill Herd at the SRUC's (Scotland's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

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

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

669 **Table 3 Outputs from linear regression models investigating the association between lameness**
670 **and the volume of digital cushion in the lateral claws of the hind limbs at slaughter. The outcome**
671 **variable was the combined volume of digital cushion in the lateral claws. The volume of digital**
672 **cushion was determined from a sample of 104 lateral claws from the hindfeet of 57 cull dairy**
673 **cows. Animals were culled from the Langhill Herd at the SRUC's (Scotland's Rural College)**
674 **Crichton Royal Farm (Dumfries) and selected for analysis if a complete mobility history was**
675 **present. Explanatory variables were constructed based on animal data made available from the**
676 **farm database and encapsulated the time spent within the milking herd since first calving. Model**
677 **A explores the associations between lameness in the four weeks before cull and the volume of the**
678 **digital cushion at cull. Models B and C explore the association between the proportion of lameness**
679 **scores submitted as sound, and the number of CHLs recorded, throughout the animals lactating**
680 **life on the volume of digital cushion at cull, respectively.**

681

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

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

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

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

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

	Coefficient	CI (95%)	P-Value
Intercept	13.3		
No. CHL treatments	-0.9	-1.2 - -0.6	<0.001

Mean liveweight	0.04	0.02 – 0.07	0.001
DIM at cull	0.01	0.01-0.02	<0.001
R^2	0.54		

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