Anatomical studies of claw conformation in New Zealand dairy cattle

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Abstract

The aim of the studies reported in this thesis was to extend our current knowledge on estimating and monitoring claw conformation under New Zealand conditions.

Initially post mortem material was used to: (1) evaluate the utility of claw volume estimation with data from cull dairy cows; (2) validate the use of a portable ultrasound machine in the estimation of internal claw dimensions; and (3) apply morphometry to quantify vascular change in histological sections from the collected claw material.

Analysis of the relationship between claw volume and hoof conformation revealed differences between the claws of dairy cattle used for this study and those of beef cattle which had been used previously. This necessitated the development of a different predictive model for dairy cattle which was subsequently used in the live animal studies. Validation of ultrasound estimates for sole and soft tissue thickness against calliper measurements in the sectioned claw, found that the portable machine used accurately estimated mean distance to distal phalanx (DP) and was thus suitable for categorising claws as having thin, marginal or adequate sole thickness.

Morphometrical techniques were successfully applied to sections from frozen claw material; the ratio of vessel lumen area to overall vessel area was found to vary depending on site, claw and overall vessel size. No relationship was found between the ratio and claw horn haemorrhages, but the level of such lesions was low in the animals available for analysis.

Subsequently, selected conformation traits were assessed on-farm in two consecutive cohorts of first lactation heifers. Some variables were identified as being useful to evaluate conformational change within a pasture-based system. These demonstrated dynamic change over the course of lactation. However, fluctuation in these variables at pasture means that any intervention study would need to have a greater impact on the claw than the variations produced by the background environment. Other variables showed stability over lactation and between years of study, and are therefore potentially suited to the investigation of claw size in relation to the development of lesions and lameness.

In Year 1, ultrasound estimates of DP were recorded as a proxy for sole thickness. Values decreased significantly after calving to a nadir at approximately Day 110. The change in DP between Days 10 and 110 was associated with the initial value of DP on Day 10. The study concluded that thin soles increased in depth while thick soles wore to become thinner, indicating that heifers with thinner soles we were able to accommodate to the changes occurring around and after calving and that the response of the claw to encountering tracks, collecting yards and milking parlours is not simply an increase in net wear.

In Year 2, a novel method to capture changes in heel conformation was successfully trialled. Non-weight bearing heel length was found to alter rapidly after calving while other measures responded slowly in comparison and suggested extended monitoring was advisable. The on-farm studies established that, for most conformational variables, there is value in recording information from all four claws of the hind feet in a pasture based system.

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Chapter one: Literature review

Introduction

It has long been recognized that being able to accurately assess conformational traits could be of significant benefit when determining selection policies and gauging the impact of factors such as the environment and nutrition on the hoof and on the risk of lameness (Hahn et al., 1984a). Claw horn quality was defined by Politiek et al. (1986) as the '*product of horn characteristics, claw shape and the anatomy and physiology of inner structure*'. Taking that definition, Vermunt and Greenough (1995) undertook a literature review in order to investigate the conformational characteristics of the bovine claw which impacted on horn quality. The review established, firstly, that the majority of lameness conditions were located in the claw and, secondly, that there was a need to employ accurate and repeatable measurement methods for trait evaluation to avoid the inaccuracies and bias inherent in systems based simply on visual appraisal.

Vermunt and Greenough (1995) highlighted eight traits that were commonly used in the classification of bovine claws: toe angle, length of dorsal border, heel height, diagonal length, claw length, toe height (as a ratio of heel height), claw width and surface area of the sole in ground contact (see Figures 1.1 and 1.2). They also highlighted the use of ratios, such as toe height:heel height and length of dorsal border:heel depth, for classifying claw traits. Vermunt and Greenough (1995) reported that these traits were affected by genetics, age (lactation number), anatomy (claw position and animal size) and animal management.

The aim of the present review was, to look at each of these claw traits in turn and to: (1) give an overview of how they have been measured and the reasons for which they have been measured; and (2) describe the relationships that have been identified between claw traits and other conformational traits, production factors, environment, management, and clinical lameness or claw horn lesions.

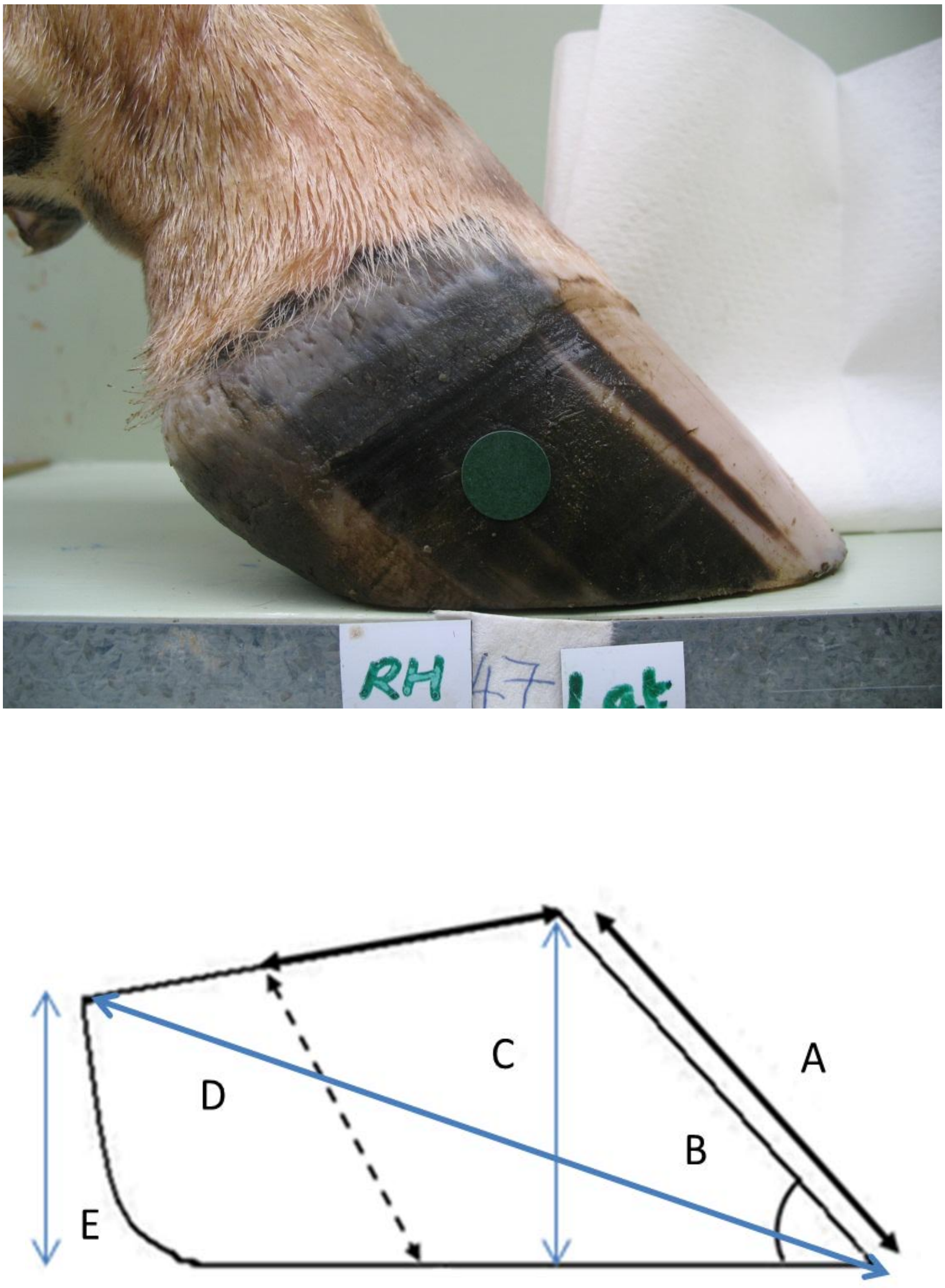

Figure 1.1: Photograph of right hind, lateral claw from the lateral aspect, and a schematic illustration of five of the conformational features identified by Vermunt and Greenough (1995) as being commonly used in the classification of bovine claw conformation: (A) length of dorsal border (B) toe angle (C) toe height (D) diagonal claw length and (E) heel height, dashed arrow represents abaxial groove.

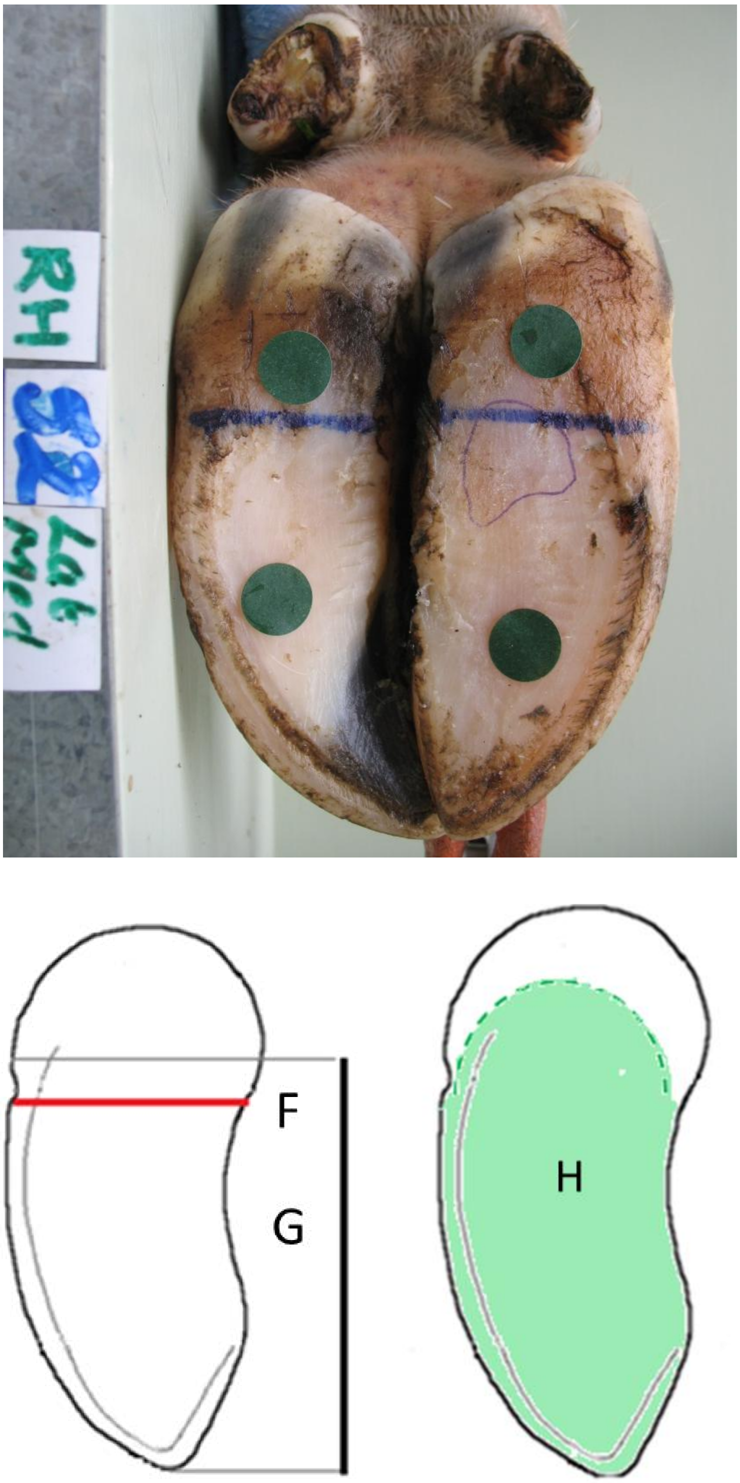

Figure 1.2: Photograph right hind foot from the solar aspect, and a schematic illustration of other conformational features identified by Vermunt and Greenough (1995), as commonly used in the classification of bovine claw conformation: (F) claw width, (G) claw length and (H) surface area in contact with the ground.

Toe Angle

Method of assessment

Toe (hoof or wall) angle has been measured in several different ways. Firstly, the angle can be measured in the weight bearing limb (Distl et al., 1984; Hahn et al, 1984b; Boelling and Pollott, 1998a) or in the lifted foot in either a foot crush (Ahlström et al., 1986; Midla et al., 1998; Telezhenko et al., 2009), or a tilt table (Vokey et al., 2001; Kremer et al., 2007).

Figure 1.3: Photograph of a left front medial claw illustrating the different landmarks for the positioning of equipment to measure toe angle; the hairline (Hl), the perioplic horn (Ph) and at the angle the dorsal border to the ground surface (Gs).

Most commonly, toe angle (Figure 1.3) has been defined as the angle between the dorsal border of the hoof and the ground surface measured at the tip of the toe (e.g. Distl et al., 1990; Wells et al., 1993; Vermunt and Greenough, 1996; Manske et al., 2002). However, other studies have reported the angle of the dorsal border to the horizontal at a more proximal position on the dorsal border – usually at a specified distance from the hair line at the coronary band or at the line demarcating the perioplic horn (Hahn et al., 1984a; Wells et al., 1993; Offer et al., 2001; Manske et al., 2002). Measurement in this more proximal position would assess the angle of more recently produced horn. Another, less commonly used, alternative is to measure the angle between the slope of the dorsal border and a line perpendicular to the ground surface (Midla et al., 1998). Thus, when comparing between studies that have measured toe angle, it is important to pay attention to the method of measurement.

Toe angle is relatively easy to assess in post mortem material; for example, Nuss and Paulus (2006) obtained highly repeatable toe angle measurements using a goniometer. However, measurement in the live animal presents more of a challenge. Much of the literature on toe angle has explored ways of measuring it cheaply and accurately in the live animal without interrupting management procedures (Hahn et al., 1984a). Assessment of toe angle by eye is inexpensive and results in little interference with day-to-day management and so is often employed by breed associations as part of breed standards (Rogers et al., 1989; Distl et al., 1990). However, the accuracy achieved with such methods is often questioned, although there are few studies comparing measured and visually estimated toe angle. Smit et al. (1986a) compared hoof angle in 1472 individuals measured using a protractor and by eye. Mean measured hoof angle was 47.8° (standard deviation (SD): 4.14) whereas the mean estimation by eye was 48.8° (SD: 3.2). Interestingly, hoof angle estimated by eye showed a lower genetic correlation with claw disorders than did measured hoof angle.

Early measurements of toe angle utilised equipment which contacted the claw and the ground at the same time. In a bid to improve on this method, Hahn et al. (1984b) assessed four simple methods of measuring hoof angle and reported that a customized commercial protractor was the most practical and efficient of those (they did not describe the other instruments they tested). Practicality was based on ease of application, ease of reading the measure and minimising disturbance of the cow with measures being taken during milking. The modification of the protractor involved the addition of a wooden block in which two nails had been placed 3 cm apart. The slope of the dorsal wall was measured with the upper of the two nails sited just below the line of the periople. Hahn et al. (1984a) reported that accuracy and ease of application was improved by washing the feet to remove organic matter and to help identification of landmarks. They also found that, once trained, operators could record toe angle accurately over multiple observations. Similar measurements were used by both Smit et al. (1986a) and Phillips et al. (1996) in their studies.

In a matched case control study comparing clinically lame animals with paired controls, Wells et al. (1993) compared angle measurement at the toe with that of a more proximal assessment (in the upper 3cm of the claw dorsal wall) for the rear claws. The two methods were highly correlated ($r=0.78$, $P=0.0001$). However, the results obtained indicated that site of measurement can influence findings; rear lateral toe angle was significantly associated with clinical lameness (a 1° rise in toe angle reduced the odds of lameness by 0.92), but only when the angle was assessed at the tip of the toe. The authors postulated that this difference occurred because the angle measured at the toe tip represented more directly the abnormal toe overgrowth that was occurring in the lame cows. Manske et al. (2002) also assessed both proximal and distal angles and found that mean distal angle was consistently lower than mean proximal angle (Table 1.1).

Table 1.1: Toe angle data (left hind, lateral claws) from Manske et al. (2002) demonstrating the mean values obtained for the two different sites at which toe angle was assessed.

Assessment time	**Toe angle : Mean (SD) degrees**	
	Proximal	**Distal**
Autumn	49 (5.5)	43 (7.7)
Spring (autumn trim)	48 (5.9)	43 (7.6)
Spring (no autumn trim)	47 (6.3)	40 (9.1)

Digital methods of toe angle measurement have also been explored (Vermunt and Greenough, 1996; Browne et al., 2007), with toe angle being measured using a digitised image of the side view of a hoof. Vermunt and Greenough (1996) performed three repeated digital estimations on each image to provide an average value for statistical analysis. However, the process of setting up the hoof image to standardise the picture and ensure the quality of the data means that this is not a rapid technique.

More recently, the combined use of a carpenter's profile gauge and a protractor has been developed, with the gauge recording the shape of the toe, sole and dorsal wall and this record then being measured by protractor (Livesey and Laven, 2007). This method removes the need to read the protractor at close proximity to the animal's claw in the live situation, so reduces the time spent at the cow side.

Another alternative is the use of an engineer's electronic angle finder or commercial angle meter placed on the claw (Manske et al., 2002; Telezhenko et al., 2009). Depending on the actual instrument used, the foot may or may not need to be lifted to obtain measurements. This is a simple technique which allows multiple measurements of each foot to be taken rapidly, but the angle finder must be positioned on the claw in a regular and repeatable fashion to ensure consistency.

Despite the impact of measurement technique on toe angle, some studies have merely reported that toe angle was measured, without detailed methodology (e.g. Somers et al., 2005; Ouweltjes et al., 2009).

The effect of claw position on toe angle

The position of the claw (i.e. front *vs.* hind, lateral *vs.* medial) has a significant effect on its conformation. Several studies have shown that mean toe angle depends on the limb (front or hind) and claw (lateral or medial) from which the measurement is taken. In general, claws from front feet have greater angles than hind feet, while lateral claws have greater toe angles than medial claws from the same foot (Hahn et al., 1984b; Leach et al., 2005; Nuss and Paulus 2006; Nuss et al., 2011), Likewise, both Telezhenko et al. (2009) and Ouweltjes et al. (2009) reported that medial hind claws had higher toe angles than lateral hind claws. However, this is not always the case as Vermunt and Greenough (1996) found no significant differences between the toe angles of front and hind lateral claws over multiple examinations of animals between 12-13 and 24-26 months of age, and Distl et al. (1984) demonstrated no effect of claw position on toe angle for young Simmental bulls at 6, 9 and 12 months of age.

Along with other differences in conformation between front and back feet, perceived differences in toe angle led Andersson and Lundström (1981) to claim that both back and front feet needed to be measured to properly assess hoof conformation, while Hahn et al. (1977) suggested that both medial and lateral claws should be measured in studies characterising the conformation of the bovine hoof. This would imply that four claws (two medial and two lateral) from at least one front limb and one hind limb would be the minimum needed to properly assess toe angle and other hoof conformation measures. However, Andersson and Lundström (1981) stated that measuring one front and one hind claw was sufficient to reliably estimate claw conformation, and this suggestion was taken up by Vermunt and Greenough (1996) who evaluated the impact of two management systems on hoof conformation using digitised images of lateral front and hind claws only.

Nevertheless, the majority of horn lesions that cause lameness, both in housed cows (Murray et al., 1996) and cows at pasture (Lawrence et al., 2011), are in the hind feet, which suggests that hoof conformation of the hind feet is particularly important. Additionally, access to hind limbs is often easier and safer, thus just measuring hind feet can significantly speed the process. Therefore, a large number of studies which have looked at the association between environment, nutrition, time or lameness and hoof conformation have focussed on the conformation of the hind hooves only (e.g. Livesey et al., 1998; Offer et al., 2001; Vokey et al., 2001), with many studies measuring the conformation in only one hind foot (Webster, 2001; Livesey and Laven, 2007; Telezhenko et al., 2009) and some in only one claw of one hind foot (Manske et al., 2002; Loberg et al., 2004).

The impact of only measuring the conformation of one hind foot is unclear. Changes in toe angle can be different between front and hind limbs. For example, Vermunt and Greenough (1996) reported that toe angle tended to increase over time in the front feet of dairy heifers (comparing 12 month old animals with animals at calving) but not in hind feet, resulting in front feet at calving being steeper than hind feet. However, housing (either in a dry lot or indoors or on concrete slats) had no effect on front foot toe angle, but mean hind foot toe angle of dry lot heifers was lower at calving than that of heifers on concrete slats. Thus for toe angle, there were different effects of time and environment dependent on whether the hind foot or front foot was measured.

Nonetheless, it remains unclear how such changes are related to the aetiopathogenesis of claw horn lameness – for example, whether heifers which have an increased (or decreased) change in front foot toe angle relative to the mean prior to calving have an altered risk of lameness. Furthermore, the lack of an environmental effect on front foot toe angle in the study by Vermunt and Greenough (1996) suggests that front foot toe angle is less sensitive to environmental effects than hind foot toe angle, and therefore may be a less valuable measurement.

If the conformation of only one hind foot is measured, this leaves the question of whether measuring the toe angle of both claws is necessary. Despite Andersson and Lundström (1981) stating that measurement of both medial and lateral claws was unnecessary, studies which have evaluated the conformation of only one foot have often measured both lateral and medial toe angle (e.g. Webster, 2001; Telezhenko et al., 2009). The data from these studies support measuring only one claw. For example, Telezhenko et al. (2009) reported that although the toe angle of the medial claw was significantly greater than that of the lateral claw there was no significant interaction between environment and claw. Specifically, they noted that the difference in toe angle between the medial and lateral claws was the same across all environments. Similarly, time seems to have little impact on the relationship between the toe angles of the lateral and medial claws; Offer et al. (2000) reported that medial and lateral toe angle responded similarly over lactation in cows which were initially housed and then kept at pasture.

Cow effects on toe angle

Age and toe angle.

There are few studies assessing conformation in calves and fewer addressing toe angle specifically. Nüske et al. (2003) reported that between 4 days and 12.5 weeks of age, toe angle reduced from 60.2° ± 0.2 to 57.8° ± 0.2. They suggested that this change was due to increased body weight and animal activity. Distl et al. (1984) demonstrated that toe angle decreased, with a sigmoid relationship, for young Simmental bulls as body weight increased.

Hahn et al. (1984b) also found a negative association between toe angle and days of age in heifers of average age 20.4 months (Table 1.2). Similarly Vermunt and Greenough (1996) reported that between 12 and 26 months of age, overall mean toe angle decreased, however, from the data presented this appears to be true only for the hind limbs of outdoor managed animals, for forelimbs toe angle increased and for housed heifers toe angle remained the same (Table 1.3).

Table 1.2: Toe angle and results for regression on age in days for Holstein heifers of breeding age (Hahn et al., 1984b).

Claw	**Mean (SE) angle (least squares)**	**Regression of age (days) on toe angle (SE)**
Front medial	44.5 (0.25)	-0.736 (0.190)
Front lateral	46.6 (0.25)	-0.217 (0.228)
Hind medial	44.6 (0.30)	-1.252 (0.210)
Hind lateral	46.9 (0.31)	-1.297 (0.185)

Table 1.3: Toe angles for Holstein heifers, housed indoors and outdoors. Vermunt and Greenough (1996).

Foot	**Management**	**Toe angle degrees (12-13 months)**	**Toe angle degrees at calving (24-26 months)**
Front	**Indoors**	51.4 ± 0.6	54.1 ± 0.6
	Outdoors	52.0 ± 1.0	54.3 ± 0.8
Hind	**Indoors**	52.3 ± 0.8	52.4 ± 0.4
	Outdoors	53.6 ± 1.1	50.4 ± 0.8

Nuss and Paulus (2006), using cadaver material, reported that hind limb toe angle was smaller in animals over 36 months of age compared to those <36 months. For the medial claw, mean (SD) toe angle was 50.7 °(1.9) and 49.1°(2.4) for animals <36 months of age and >36 months of age, respectively, with equivalent figures of 58.1°(2.0) and 48.2°(2.7) for the lateral claw. This is consistent with the results of Hahn et al. (1984b) where lactation number (1 to 4+) was utilised as a proxy for age (Table 1.4). Hahn et al. (1984b) discussed age as a confounder for stage of lactation during first lactation as animals continued to grow after calving (mean age at calving: approximately 25 months). In their study the difference in toe angle for front compared to hind feet reduced as animals aged.

Table1.4: Toe angle (degrees) for Holstein cows in Lactations 1-4+. (Hahn et al., 1984b).

Claw	Lactation			
	1	**2**	**3**	**4+**
Hind medial	43.6	41.7	41.9	41.9
Hind lateral	45.1	42.9	43.3	42.8

In reality the effect of age is hard to separate from the concurrent effects of stage of lactation, season and environment. Livesey and Laven (2007) reported that in their first lactation, the toe angle of heifers decreased immediately after calving but then increased over the next six months. The pattern of change was the same irrespective of whether the heifers were housed in straw or cubicle yards, whilst housing had only a limited impact on mean toe angle. Hahn et al. (1984b) reported that stage of lactation was important in the first lactation, less so in the second and had no effect in the third and subsequent lactations. Both seasonal and year effects on toe angle were demonstrated by Boelling and Pollott (1998a). Offer et al. (2000) also reported that toe angle changed significantly during lactation over a range of parities. They suggested that rather than age, the main driver for these changes appeared to be whether the cows were housed or managed at pasture as claws had steeper toe angles in housed cows compared to those at grass. In contrast, Leach et al. (2005), who measured toe angles in heifers from weaning until calving, reported that toe angle was greatest when the heifers were housed as yearlings after their first summer at grass. Livesey and Laven (2002) reported that, at calving, heifers reared on straw with no access to concrete had significantly lower toe angles than cubicle-reared ones and that, whereas there was no change in toe angle in the cubicle-reared heifers after calving, the toe angle of the straw-reared heifers significantly increased. In contrast, Ahlström et al. (1986) reported no evidence of an age related trend in toe angle over an extended study spanning up to five lactations in four breed groups.

Thus, whilst the relationship of toe angle to time is clearly not simple, the limited published data strongly suggest that the relationship is heavily influenced by factors other than age, and have not identified a simple ‘age effect’ on toe angle.

Genetics and toe angle

Breed appears to have a significant influence on toe angle, with steep angles (>50°) being reported in large beef breeds such as the Simmental (Distl et al., 1984; Nuss et al., 2011) and much shallower angles in dairy breeds such as the Holstein-Friesian (Offer et al., 1997; Offer et al., 2000; Webster, 2001; Browne et al., 2007), and Swedish Jersey (Ahlström et al., 1986). Ahlström et al. (1986) monitored Swedish Friesian, Swedish Red and White and their crosses alongside Swedish Jersey over a five year period, and reported a significant effect of breed on toe angle in both front and back feet and in both heifers and cows ($P \leq 0.001$). However, while Ahlström et al. (1986) reported that the mean toe angle of Swedish Red and White cattle was approximately 54-55°, a later study by Loberg et al. (2004) reported mean toe angles, in the same breed, of between 37.5°± 1.54 and 44.8°± 1.85 depending on exercise regime (Table 1.5). In young calves, Nüske et al. (2003) showed that German Fleckvieh and German Friesian Fleckvieh crosses (Fleckvieh sires) had larger toe angles than German Holstein calves or German Friesian Fleckvieh (Friesian sires). There is clearly significant overlap between breeds across studies, so, as for age, factors other than breed such as environment, season, and management can obscure the association between breed and toe angle.

Table 1.5: Toe angle data LSM (SE) left hind, lateral claw for Swedish Red & White Friesians subjected to four exercise regimens (Loberg et al., 2004).

Exercise regime	Toe angle in degrees prior to Spring Trim	Net change in angle between Autumn and Spring assessments
Exercised daily	42.6 ± 1.58	-8.4 ± 1.43*
Exercised twice a week	40.6 ± 1.79	-9.3 ± 1.65
Exercised once a week	44.8 ± 1.85	-6.6 ± 1.65**
Not exercised	37.5 ± 1.54	-13.4 ± 1.41

* $P<0.05$; ** $P<0.01$ compared to not exercised group

Toe angle is heritable, although estimates of the magnitude of its heritability show significant variation between studies (h^2=0.03-0.85). Hahn et al. (1984b) concluded from their study that selection on claw traits was likely to improve heifer conformation, with both toe angle and a ratio between toe angle and toe length being potential measures for selection. The heritability values returned in this study were affected by claw position (front vs. hind and medial vs. lateral, Table 1.6). Lactation number may also influence the heritability of toe angle but the findings are not consistent between studies. Boelling and Pollott (1998b) obtained only a small variation in heritability with lactation number for Holstein-Friesian cows, but Choi et al. (1993) found toe angle heritability to vary between 0.03 and 0.39 over four lactations with no consistent pattern between lactations. Again, claw position (medial vs. lateral) had an influence on heritability in the first lactation. An effect of sire on toe angle in the medial claw of both front and hind feet was demonstrated for heifers (Hahn et al., 1984b),

When considered alongside traits for milk yield, udder depth and teat placement, toe angle contributed 32-35% of the total variance in "*stayability*" for an animal within the herd (Rogers et al., 1989) and has been associated with increased milk yield (a 5° increase in angle was associated with 225 kg increase in milk yield between animals in their 1st and animals in their 2nd lactation) and improved reproductive performance (McDaniel et al., 1984).

Table 1.6: Toe angle heritability (h^2) and standard error (SE) for Holstein heifers by claw position (Hahn et al., 1984b)

Claw	toe angle h^2 (SE)	toe angle: toe length ratio h^2 (SE)
Front medial	0.38 (0.30)	0.48 (0.32)
Front lateral	0.40 (0.30)	0.19 (0.25)
Hind medial	0.55 (0.33)	0.51 (0.32)
Hind lateral	0.85 (0.39)	0.60 (0.39)

Ratio created using toe angle in degrees and toe length in millimetres

Management effects on toe angle

Environment and toe angle

The influence of the surface environment on toe angle is currently thought to be mediated via a modification of claw horn growth and wear rates. This impact is dependent on the abrasive properties of the environment (Boelling and Pollott, 1998a; Leach et al, 2005; Livesey and Laven, 2007; Telezhenko et al., 2009), such that exposure to abrasive surfaces leads to increased wear and a resultant rise in weight bearing surface area. This increased loading is postulated to have a stimulatory effect on the basal keratinocytes in the horn-producing epidermis of the corium, which results in increased horn production (Greenough, 2007).

Toe angle is related to the ratio between toe length and heel height – as the ratio increases (i.e. as toe length gets longer relative to heel height) toe angle decreases. Similarly, toe angle can also be related to toe height (see line C in Figure 1: Laven and Laven, 2013). Hoof growth and wear are thus key determinants of toe angle. However, results of studies incorporating measures of growth and wear rates with floor material and toe angle have been inconsistent in their findings; with some demonstrating no effect of floor surface on growth and wear or toe angle (Webster, 2001; Somers et al., 2005) and others an effect on growth and wear but not toe angle (Vokey et al., 2001).

An impact of environment on toe angle was clearly present in the study reported by Livesey and Laven (2002). In maiden heifers kept indoors on straw, with no access to concrete, from just after mating until first calving, toe angle was significantly lower than in heifers reared in a cubicle yard with concrete passageways. Six weeks prior to calving, the mean toe angle of the right hind lateral claw of heifers in the straw yard was 27°, while in the cubicle-reared heifers it was 41° (Laven R.A., personal communication). A similar study undertaken by Vermunt and Greenough (1996) followed two groups of heifers from 12-13 months of age through to calving. The heifers were managed in either an outdoor dry-feedlot incorporating a straw-bedded open shelter or, indoors in a cubicle system with rubber mats and concrete slatted passageways. Although Vermunt and Greenough (1996) reported that the toe angles of the hind feet of outdoor-managed animals became shallower during the study period and were significantly lower at calving than those managed indoors, the difference in the means reported (50.4° vs. 52.4°, respectively) were much smaller than those of Livesey and Laven (2002), and no such difference was seen in the front claws (54.3° vs. 54.1°, respectively).

The absence of a simple relationship, in maiden heifers, between a supposedly higher wear environment and higher toe angles is also seen in the report by Leach et al. (2005). The two groups of heifers in that study were kept in similar environments so there was no contemporaneous comparison, but the highest toe angles in that study were recorded when the heifers were housed in cubicle yards after their first summer at pasture; subsequent measures of toe angle in the housed heifers tended to show a decrease in toe angle. The reason for this is that claw conformation is related to both growth and wear – increased wear can lead to (or be associated with) increased growth, as was seen in the lateral claws of the hind feet measured by Leach et al. (2005). Thus, it appears that it is the balance between the two that determines claw conformation. Indeed, Vokey et al. (2001) showed that the increased wear rates associated with alley stall combinations including concrete and sand were in general matched by increased growth rates and no change in toe angle. The lack of an effect on toe angle could also be attributed to an interaction with additional factors which affect claw horn growth and quality such as hormones, disease, season, nutrition, behaviour and exercise (van Amstel and Shearer, 2001; Loberg et al., 2004).

The effect of environment has also been evaluated in lactating cattle. Boelling and Pollott (1998a) measured toe (foot) angle in a herd of 157 cattle over two years. They found that despite a small but significant difference in toe angle between years, the same effect of season on toe angle was seen in each year – higher in the first and last three months of the year (housing period: average toe angle ≈49.5°) and lower in the middle six months (pasture: average toe angle ≈45°). They concluded that it was the difference in wear resulting from the two surfaces that drove the seasonal effect. Nonetheless, as with Leach et al. (2005), this is an assumption as there was no contemporaneous comparison of environments, or any measure of wear.

In heifers in their first lactation, Livesey and Laven (2007) directly compared the impact of housing in covered straw-yards on toe angle with that of housing in a cubicle system (with either rubber mats or mattresses). Both houses had similar concrete passageways. They reported that toe angle tended to be smaller in the heifers housed on straw than in those housed in cubicles, but the difference between groups was only significant when the heifers in the straw yards and heifers in cubicles with mattresses were compared (*P*=0.005). In contrast to the conclusions of Boelling and Pollott (1998a) that reduced wear was responsible for smaller toe angle, Livesey and Laven (2007) found that the smaller increase of toe angle on the lateral wall in heifers housed on straw after calving was associated with greater wear than in the heifers housed in cubicles with mattresses. They therefore suggested that the lower toe angle in straw-housed heifers resulted from lower heel bulb growth rather than toe elongation. This is consistent with their findings in pregnant heifers kept in straw yards or cubicles (Livesey and Laven, 2002), although it remains a conjecture as no measures of hoof conformation other than toe angle were reported by Livesey and Laven (2007).

In contrast, Somers et al. (2005) suggested that the principal reason for the higher toe angle of cattle in cubicles compared to those in straw-yards was that cattle housed in straw yards had less horn abrasion at the toe. Their study compared cattle on different surfaces across herds (each herd having a single surface type – straw yard or concrete surfaces that were solid, slatted or grooved). The cattle to be examined were stratified by parity and their claws were trimmed prior to the study (which started with the housing season and ran for 18 weeks). They measured all the claw traits which Vermunt and Greenough (1995) recommended with the exception of ground surface area, and found that toe angle was the only measure that was significantly affected by housing alone. Mean toe angle in straw-yarded cattle (42.5°) was lower than that of the cattle housed on any of the three concrete systems; additionally mean toe angle of the cattle on the slatted and grooved concrete (46.1° and 46.6°, respectively) were lower than those on the solid concrete (47.8°). Somers et al. (2005) also reported claw (toe) height and heel depth. Interestingly, it was the lack of an effect on the latter two measures that Somers et al. (2005) used to justify their conclusion that the effect of housing on toe angle was mediated via wear at the toe rather than a heel response. However, significantly changing toe angle without a simultaneous change in either dorsal border length or toe height is not possible, as the three measures form a virtual right angled triangle (Figure 1). Laven and Laven (2013) recalculated mean toe height, assuming that the mean toe angle and dorsal border reported by Somers et al. (2005) were correct, and found that mean toe height was lower in the straw-yarded cattle. This is consistent with the hypothesis that the low toe angles are associated with reduced heel depth.

A range of studies have examined the impact of flooring on toe angle with the aim of optimising claw health in housed animals; in particular, mitigating the potential negative impacts of hard / abrasive surfaces such as concrete and asphalt. The use of rubber mats in cubicles or alley-ways leads to reduced toe angle compared to bare concrete (Kremer et al., 2007; Telezhenko et al., 2009). This effect was also seen when rubber walk ways were used in conjunction with slatted-concrete surfaces (Ouweltjes et al., 2009). Asphalt produced particularly steep toe angles when compared to concrete and slatted-concrete surfaces with or without rubber coating (Telezhenko et al., 2009). The abrasive properties of a surface are not the only candidate for an influence on conformational change; in a review, Cook and Nordlund (2009) suggested the compressibility of a surface may also play a role in its impact on toe angle.

The level of activity exhibited on a surface can also play a role. Loberg et al. (2004) showed that for cows housed in tie stalls (rubber mattress with chopped straw bedding) exercise had an influence on toe angle. Exercised cows had a smaller reduction in toe angle between the spring hoof trimming and the autumn assessment than cows which were not exercised (Table 1.5). This would seem to be consistent with the stimulatory mechanism discussed above. However, the biggest impact on toe angle was seen in cows exercised only once per week and the impact of exercising twice a week was not significant. Clearly, factors other than length of exposure altered the response to exercise.

In cattle that are principally pasture-based, exposure to tracks and feed pad surfaces can also alter the growth and wear of claw horn (Mason et al., 2011), and there is anecdotal evidence that extended walking distances can impact on conformation and lameness (Cook and Norlund, 2009). This may occur by, for example, excessive wear, thinning of soles (Jubb and Malmo, 1991) and loss of sole concavity (Tranter and Morris, 1992). However no direct effect of walking distance on toe angle has been reported. Indeed, a recent study (Burow et al., 2014) related track surface rather than the distance cows have to walk along the track to the risk of lameness. Nonetheless, claw overgrowth was associated with increased odds ratio for lameness [1.66 (95 % CI: 1.28-2.16)].

Trimming and toe angle

Routine trimming as a management tool is designed to influence toe angle (Toussaint-Raven et al., 2003). A shallow toe angle can be corrected by reduction of toe length and, where possible, levelling of the ground surface between the claws of a foot. The effect of trimming on claw angle appears to persist; Manske et al. (2002) assessed toe angle in Swedish dairy cattle at a spring trimming intervention. They demonstrated that animals which had not been trimmed in the previous autumn had shallower toe angles than those that had been trimmed in the autumn (Table 1.1).

Nutrition and toe angle

There is little information related specifically to the effect of diet with respect to toe angle. Dietary protein source was investigated by Offer et al. (1997) who compared animal protein in the form of a commercial diet combining fish meal, blood, meat and bone meal) to a vegetable protein source (soya-bean meal), but no effect on toe angle was demonstrated. The influence of the forage base in the rearing diet fed from three months of age through to early pregnancy was examined by Offer et al. (2001), who found no significant effect of diet on toe angle between animals housed under the same conditions and fed either a grass-silage ration or hay with a barley concentrate .

Leach et al. (2005) obtained a similar result when they compared dairy heifers reared on a wet grass-silage (fermented) diet with heifers reared on a dry straw-based (unfermented) diet. Webster (2001) examined the effect of dietary moisture content on the claw conformation of first lactation heifers, post calving, in either straw or cubicle-based housing. The diets fed were forage based with identical values for metabolisable energy (ME) but different dry matter content (DM) (60% [dry] or 25% [wet]). Again no effect on toe angle was discovered in any of the four treatment groups. The average toe angle (lateral, medial claw right hind foot) for each group is reported in Table 1.7.

Table 1.7: Mean toe angle (degrees) and standard deviation (SD) for 40 Holstein/Friesian heifers housed in either straw yards or cubicles, fed either a wet or dry forage ration. (Webster, 2001).

Right Hind	**Average toe angle (degrees) (SD)**			
	Straw yard		**Cubicle housed**	
	Dry diet	**Wet diet**	**Dry diet**	**Wet diet**
medial	44.6 (3.2)	45.4 (3.4)	46.5 (3.6)	45.3 (2.6)
lateral	45.0 (3)	45.1 (2.8)	45.8 (3.1)	44.8 (2.8)

Offer et al. (2000) followed animals for four lactations and found a repeated pattern of change in toe angle with time, which was associated with management changes between pasture and housing. Toe angle was smaller when on pasture than when housed. However, the type of pasture (high clover sward *vs.* traditional rye grass) did not affect angle.

Several studies have investigated dietary supplementation with methionine to try and describe its influence on horn quality and by extension conformation. The availability of sulphur containing amino acids in the diet can alter the quality of horn (Clark and Rakes, 1982; Tomlinson et al., 2004; Galbraith et al., 2006; Livesey and Laven 2007) and thereby influence rates of growth and wear. A link to the modification of conformation has not yet been demonstrated, perhaps because few studies sample conformation variables alongside the feed supplementation regimen. However, toe angle was assessed by Livesey and Laven (2007) in animals supplemented with methionine. Although this resulted in steeper toe angles (mean 46.2° with methionine compared to 43.6° without methionine), the effect was not significant and was not consistent across other treatment groups. The authors pointed out that control cows may not have been methionine deficient, reducing the impact of the intervention.

The water soluble B vitamin, biotin, is another nutrient that is implicated in horn quality, being intimately involved in the keratinisation process. Although biotin has been shown histologically to increase the quality of the horn formed in repair of sole ulcer lesions (Lischer et al., 2002) and reduce the incidence of some claw horn diseases such as white line disease (Tomlinson et al., 2004), Midla et al. (1998) found that biotin supplementation had no significant effect on toe angle.

Links to other conformational parameters, lameness, lesions and locomotion

Toe angle and dorsal border are negatively correlated: many studies that report toe angle have also measured dorsal border length (Vermunt and Greenough, 1996; Offer et al., 1997; Vokey et al., 2001; Telezhenko et al., 2009) probably due to the ease by which these two measures can be obtained. Vokey et al. (2001) reported figures for correlation between toe angle and dorsal border length for all claws of the hind feet (left hind; $r=-0.40$, -0.29; right hind; -0.37, -0.56; for medial and lateral claws respectively; $P<0.001$ for all correlations).

In contrast, the relationship between toe angle and heel depth is positive, although the size of the correlation varies between studies. Vokey et al. (2001) reported that there were correlations of between $r=0.30$ to 0.37 depending on claw, while Gitau et al. (1997) found a correlation of r=0.53. Livesey and Laven (2007) proposed that toe angle was influenced by dynamic changes in the heel bulb and that hypertrophy of this structure, due to environmental modification of horn growth and wear, resulted in a steeper toe angle and the characteristic “*boxy claw*” seen when animals are managed in cubicle systems. Phillips et al. (1998) reported that the relationship between toe angle and foot volume was negative.

Toe angle was positively correlated (r=0.318) to horn wear rates (Offer et al., 2001) and a positive correlation exists between lateral hind claw toe angle and bulb horn hardness (Shore-A-meter) (Offer et al., 1997, 2003). While the latter studies found no relationship between toe angle and lameness, claw lesions or heel horn erosion, Smit et al. (1986a) found that a higher toe angle was associated with less interdigital dermatitis, reduced total lesion score and sole ulcers, while Wells et al. (1993) described an association between lateral claw toe angle and clinical lameness. This association linked overgrowth of the claw in lame animals to reduced toe angle.

Dorsal border length, toe length

Method of assessment

Dorsal border length is easily measured in a repeatable fashion (Andersson and Lundström, 1981; Distl et al., 1984; Vermunt and Greenough, 1995; Boelling and Pollott, 1998a). Although it is defined by most authors as the distance between the distal tip (toe) of the claw and the skin:horn junction at the coronet (Ahlström et al., 1986; Vermunt and Greenough 1996; Somers et al., 2005; Browne et al., 2007), there is some ambiguity in the literature, with, for example, Boelling and Pollott (1998a) measuring dorsal border to a point described as the dorsal hairline. Nuss and Paulus, (2006) further specified the proximal landmark; they measured dorsal border from a position 1 cm abaxial to the interdigital space at the level of the coronet to the most distal point of the dorsal wall.

Probably the most common alternative landmark used to define dorsal border is the use of the line of the perioplic horn as the proximal reference point (Hahn et al., 1984a; Choi and McDaniel, 1993; Phillips and Schofield, 1994; Gitau et al., 1997). However, this distance is variable between individuals (Ahlström et al., 1986) and, clearly, shorter than the more commonly used definition (see figure 1.4). To reduce the error in establishing the proximal landmark Ahlström et al. (1986) recommended palpation in addition to visual assessment.

As for toe angle, dorsal border length has been assessed in the standing animal with the foot on the floor (Distl et al., 1984; Hahn et al, 1984b; Ahlström et al., 1986; Boelling and Pollott, 1998a) and with the foot lifted (Midla et al., 1998; Telezhenko et al., 2009) as well as on a tilt table (Vokey et al., 2001; Kremer et al., 2007).

Measurement of dorsal border has been made using computer digitised image techniques (Vermunt and Greenough, 1996; Browne et al., 2007), visual scoring scales (Smit et al., 1986a), flexible tape measures (Ahlström et al., 1986; Scott et al., 1999) dividers (Hahn et al., 1984b; Phillips and Schofield, 1994) and callipers (Manske et al., 2002; Nuss and Paulus 2006; Kremer et al., 2007; Telezhenko et al., 2009). Digital scoring methods necessitate an estimate of the position of the skin: horn junction below the covering hair unless the hair is clipped (see Figure 1.4).

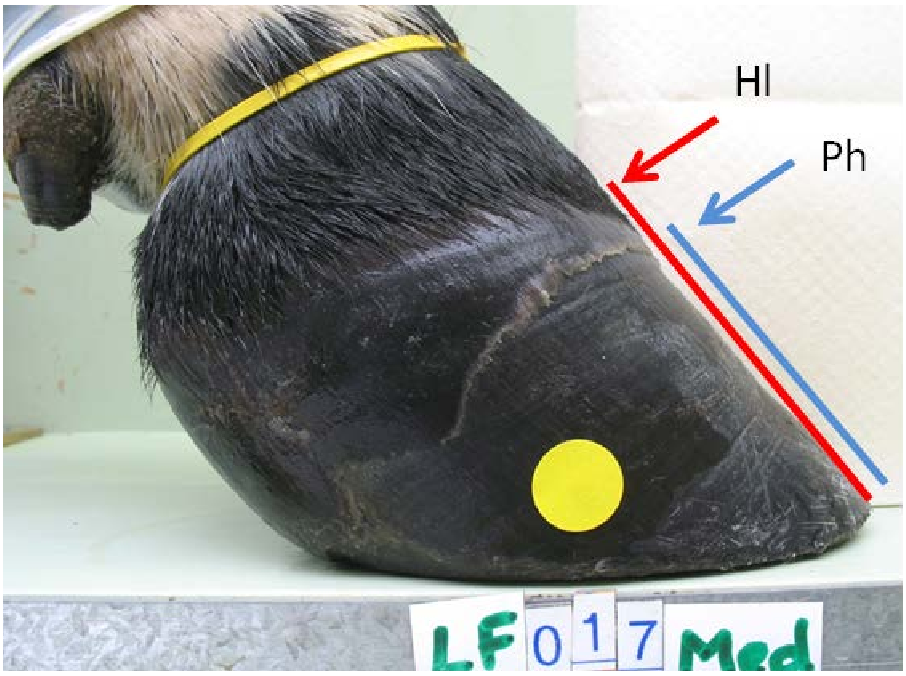

Figure 1.4: Conformational features used as a site of measurement for dorsal border length. Photograph left front claw from the medial aspect illustrating the difference in position of the skin horn junction below the hair at the coronary band (Hl) and the perioplic horn (Ph).

Whilst very suitable for post-mortem studies, the use of callipers requires special care in the live animal due to the potential of the points to inflict a noxious stimulus and damage to the skin (Hahn et al. 1984a; Ahlström et al., 1986). Calliper or straight rule measures of dorsal border length may result in a smaller value than the use of a flexible tape measure if concavity of the dorsal wall is present. However, Capion et al. (2008) reported in a study of hoof shape in five herds (two straw-based and three cubicle-housed) that there was only limited concavity in the feet of lactating heifers. Mean concavity was 0.7 and 1.6 mm for hind and front feet, respectively, with a range of -3 to 5 mm for hind feet and -5 to 7.5 mm for front feet. Thus for a foot with straight measurement of 7.5 mm, even the maximal concavity would not increase true toe length to >7.6 mm (Laven and Laven, 2013). Nevertheless, even though the effect may be small, it should be considered when developing a protocol for hoof measurement.

Perhaps more important than concavity, when comparing which method of dorsal border length measurement to use, is deviation of the toe. If there is significant deviation of the toe, then clearly a flexible measure will reflect dorsal border length more accurately than a straight line measure, and measurement of the actual hoof will be better than measurement from a two-dimensional digital image. However, toes for which deviation significantly affects dorsal border measurement are likely to be grossly abnormal. In such cases, identifying abnormal deviation is more important than measuring dorsal border length, particularly in studies evaluating the factors which affect dorsal border length. Deviation can be scored visually (Murray, 1994) and should be part of all studies of hoof conformation, even if it is just to exclude cows from prospective experiments.

Smit et al. (1986a) used two methods of assessing the dorsal border - calliper measurement (proximal landmark stated as hairline, however periople was illustrated) and scoring by eye on a linear scale (5-64; shorter toes had higher score) to assess dorsal border length. In 1472 individuals, mean measured dorsal border length was 74.4 mm (SD 5.72), mean score for dorsal border length was 34.7 (SD 6.37). The scored values were only moderately correlated (r=0.53) with the measured values, and the genetic correlation between visual and calliper measurements was also only moderate. Smit et al. (1986a) suggested that this was related to not cleaning off feet prior to the visual assessment. Indeed this has been generally advised as an aid to improving the accuracy of such measurements (Hahn et al., 1984b).

A target figure of 75 mm has often been quoted as a target for toe length in adult Friesian cows when undertaking corrective foot trimming (e.g. Toussaint Raven, 2003),as this figure is thought to be linked to an adequate depth of sole horn. However, toe length exceeding 80 mm is not unusual, particularly in larger breeds (Manske et al., 2002; Nuss and Paulus, 2006; Nuss et al., 2011; Telezhenko et al., 2009).

The effect of claw position on dorsal border length

As for toe angle, a range of feet and claw position combinations have been used in conformation assessments from all claws of all feet (Distl et al., 1984); to one claw of one (usually hind) limb (Phillips and Schofield, 1994; Boelling and Pollott, 1998a; Fjeldaas et al., 2011).

Foot has been reported to influence dorsal border length but the findings are not consistent; they range from front limb claws being significantly longer than hind limbs (Vermunt and Greenough 1996) through no effect of foot (Hahn et al.,1984b) to hind limbs having longer claws (Ahlström et al.,1986; Nüske et al. (2003). Very few studies have compared left and right limbs; Boelling and Pollott (1998a) described a pilot study which suggested that mean dorsal border length values for left and right hind feet were not statistically different from each other.

The relationship of claw within foot is generally thought to have a significant impact on dorsal border length, with lateral hind and front medial claws being longer than their foot partners (Andersson and Lundström, 1981; Vermunt and Greenough, 1995; Offer et al., 2000; Nuss et al., 2011). However, such differences are not always seen. This is particularly the case for calves, for example, Nüske et al. (2003) reported that medial claws tended to be bigger in all feet in calves of <70 days of age, while Distl et al. 1984 found no difference between lateral and medial claws in bulls up to 12 months of age, and Hahn et al. (1984b) no difference in heifers of breeding age; even in mature cattle differences in claws within the same foot are not always reported (e.g. Phillips et al., 1996). The relationship between the claws may vary with stage of lactation; in dairy cows kept in cubicles, Offer et al. (1997) reported a gain of 5.5 mm for medial claws and 6.9 mm for lateral claws over the 24 week course of their study.

Fatehi et al. (2003) assessed the evenness of medial and lateral claws on a pseudo-linear scale (0-9) where 0 was obvious difference and 9 was uniform claw length. This value was not influenced by environment (tie versus free stalls or solid versus slatted floors) but differed between herds which used routine foot trimming and those which did not..

Heritability of claw uniformity in this study was modified in a small way by housing system (0.03 vs. 0.04 for tie vs. free stall respectively).

Although some authors suggest that assessment of dorsal border length of one claw in one foot is sufficient for assessment of hoof conformation (Andersson and Lundström 1981; Boelling and Pollott 1998a), measurement of dorsal border length in both claws would seem the best option if time and resources permit, particularly as both Vokey et al. (2001) and Telezhenko et al. (2009) found that medial and lateral claw dorsal border length responded differently over time to a range of environmental conditions.

The reporting of data at the claw level would also prevent the reporting of average dorsal border length either within a single foot (Phillips et al., 1996) or across all eight claws (Andersson and Lundström 1981; Kremer et al., 2007).

Cow effects on dorsal border length

Age and dorsal border length

As cattle age, dorsal border length is observed to increase, although the size of the effect varies with the stage of life (Vermunt and Greenough, 1995). Nüske et al. (2003) found that, in calves, dorsal border length in calves increased from 4.3 cm to 4.6 cm between Weeks 1 and 12. Continued increase in dorsal border length was seen in heifers between 12-13 months and 24-26 months of age from 7.0 cm to 7.2 cm on the front and 7.0 cm to 7.1 cm behind between housing and calving (Vermunt and Greenough, 1996) and as young stock matured through their first lactation and beyond Table 1.8 (Hahn et al., 1984b; Ahlström et al., 1986; Offer et al., 2000).

Table 1.8: Length of dorsal border in hind limb claws of Holstein cows (1st-4th lactations), as reported by Hahn et al. (1984b)

Dorsal border length (mm)	Lactation 1	Lactation 2	Lactation 3	Lactation 4+
Hind medial claw	60.6	66.5	66.0	66.7
Hind lateral claw	60.8	67.6	68.4	69.6

Several published studies suggest that this increase with age seems to disappear around the third or fourth lactation (e.g. Hahn et al., 1984b; Ahlström et al., 1986; Offer et al., 2000). Boelling and Pollott (1998a) reported an increase in dorsal border length between first and third lactations (79.5 mm, to, 81.6 mm), but no significant change thereafter.

However, not all studies show an effect of age on dorsal border length in lactating cows. Leach et al. (2005) reported that although dorsal border length increased throughout the rearing period, a plateau was reached at approximately 1 month prior to first calving and dorsal border length remained fairly static thereafter at around 75 mm. The results of Hahn et al. (1984b) were not dissimilar, inasmuch as size difference between claws increased with advancing lactation number, particularly in the hind feet

Genetics and dorsal border length

Heritability of dorsal border length is moderate to high. Also, shorter claw length (higher toe angle) is linked to longevity within the herd (Vermunt and Greenough, 1995). Choi and McDaniel (1993) reported heritability to be greater for lateral claw dorsal border length than medial claw dorsal border length and found increased heritability with lactational age. Average heritability over all lactations was 0.25 (range 0.08-0.53). Further to this Choi and McDaniel (1993) demonstrated a genetic correlation between the difference in length between claws of a foot at first lactation and the age of animals at culling (survival to fifth lactation).

Whilst significant differences between beef and dairy claws exist (Browne et al., 2007) a breed effect on dorsal border length is not always apparent and differences can be small (Andersson and Lundström, 1981). However, the Jersey breed does appear to be an exception and is often reported to develop long toes compared to other breed groups (e.g. Andersson and Lundström, 1981; Gitau et al., 1997).

Management effects on dorsal border length

Environment and heel height

A more consistent relationship between environment and dorsal border length exists than for toe angle. Increased wear leads to shorter toes (Telezhenko et al., 2009), with the greatest wear (and shortest toes) being seen with the most abrasive surfaces - particularly asphalt (Telezhenko et al., 2009). Concrete is also abrasive (Telezhenko et al., 2009), whilst rubber (Vokey et al., 2001) and straw (Vermunt and Greenough, 1996; Laven and Livesey, 2002) are at the other end of the scale.

The impact of concrete on dorsal border length can be modified by incorporating grooves or slats or by topping with rubber matting; Fjeldaas et al. (2011) reported mean dorsal border length of the right hind lateral claw to be longer (by 4-7.3 mm) when a herd used solid rubber alleyways compared to the use of concrete alleyways or mixed flooring systems. Vokey et al. (2001) postulated that whilst alley surface influenced claw horn growth rate, stall surface had a greater effect on wear rate, supporting the suggestion made by Fjeldaas et al. 2011 that combinations of materials could be used to influence dorsal border length.

The provision of rubber matting to reduce wear in the housed environment is now commonplace and several authors have demonstrated increased dorsal border length associated with its use (Vokey et al., 2001; Ouweltjes et al., 2009; Telezhenko et al., 2009; Fjeldaas et al., 2011). Vokey et al. (2001) concluded that any alley/stall surface combination where claws were exposed to concrete or sand, led to increased growth and wear rates over animals where rubber was included in the environment. The magnitude of the effect of environmental manipulation can be quite large, for example when Telezhenko et al. (2009) compared dorsal border length for the lateral and medial claws in concrete systems with (94.9 mm and 92.2 mm) or without (88.9 mm, 85.0 mm) rubber walk ways or topping.

Exercise regime can also result in conformational change for housed animals (Loberg et al. 2004). That study examined the impact of exercise regime on cows housed in tie stalls with rubber surfaces and chopped straw bedding, both minimally abrasive surfaces associated with longer dorsal border length. Non-exercised animals developed the longest claws compared to those exercised out of doors once or twice a week or on a daily basis. Exercise paddocks were accessed via concrete walk ways and the group exercised daily had significantly less net-growth. From these data the authors concluded that increased wear occurred on exposure to the more abrasive outside surfaces during exercise.

Extremes of wear are not required for an environmental effect to be present. Boelling and Pollott (1998a) reported an effect of year on dorsal border length (Year 1, 80.6 mm vs. Year2, 83.4 mm), and an effect of season within year, such that dorsal border length was longer in the spring and summer (83.4 mm and 83.1 mm) and shorter in autumn and winter, which coincided with housed periods (80.6 mm and 81.5 mm). This fluctuation in dorsal border length with a seasonally managed environment was also seen by Offer et al. (2000). Nevertheless, not all studies have shown an environmental effect on dorsal border length. For example, Somers et al. (2005) found no effect of floor type on dorsal border length and both Phillips and Schofield (1994) and Fregonesi and Leaver (2001) reported that dorsal border length was marginally, but not significantly, greater in cows in straw yards cubicles. Straw-housed animals in all of these studies had some access to concrete which potentially mitigated for the lack of wear associated with straw bedding (Fregonesi and Leaver, 2001). This is consistent with the conclusion that dorsal border length is dependent on the balance between growth and wear in a given management system.

An asymmetrical response between claws of a foot in environmental studies has been observed (Vokey et al., 2001; Telezhenko et al., 2009). In the former study, cows on rubber alleys and mattress beds (RAMB) developed longer medial claws than cows on concrete alleys in combination with either concrete stalls or mattresses. However, lateral claws of the RAMB animals were only longer when compared to the concrete alley and concrete stall combination. The asymmetrical response between claws may be influenced by the uneven loading of claws during locomotion (as described by van Der Tol et al., 2003). Force plate analysis has revealed that lateral hind claws take the greater part of loading on the foot when the ground impact occurs. Over the stance phase, the load shifts more evenly across both claws prior to “push off” into the next stride. This would amplify the impact of exposure to an environmental factor such as abrasive concrete on the lateral claw and stimulate a growth response form horn producing cells in the lateral claw (Greenough, 2007). This may explain why Vokey et al. (2001) found that lateral claws grew faster than medial claws for several alley-stall combinations (i.e. concrete-concrete, concrete-mattress and rubber-concrete). Wear rate was unaffected by claw position across the study (Vokey et al., 2001) which, for the most part, agrees with the results of Telezhenko et al. (2009), who found claw growth rate to be affected by position only on mastic-asphalt floors. Medial hind claws wore faster than their lateral partners on all the surfaces in that study which resulted in reduced asymmetry between claws for the mastic-asphalt floor. The use of foot trimming as a management tool can influence dorsal border length (Toussaint-Raven, 2003) independently of the effects of environment. In Swedish dairy cattle, Manske et al. (2002) found that dorsal borders were shorter in the spring if claws had been trimmed the previous autumn (83 mm) than if they had not (88 mm). The method of trimming (i.e. traditional vs. a modified Dutch system) did not alter dorsal border length outcome (Ouweltjes et al., 2009). Notwithstanding the findings of Manske et al. (2002), routine trimming interventions at 150 and 305 days in milk did not eliminate the effect of floor (rubber topped concrete slats compared to concrete slats) on dorsal border length (Kremer et al., 2007). Rubber surfaces still resulted in increased dorsal border length (150DIM; 9.3 cm vs. 8.6 cm, $P<0.001$; 305DIM; 9.2 cm vs. 8.7 cm, $P<0.001$ for rubber and concrete respectively). From the reverse perspective Telezhenko et al. (2009) concluded that incorporation of exposure to abrasive floors into management systems could not replace routine foot trimming in the control of claw conformation.

When examined by Smit et al. (1986b) formalin foot bathing was associated with longer dorsal border length (72.8 mm vs. 74.2 mm, $P=0.05$): an effect thought to be mediated via decreased wear due to increased claw horn hardness). Although there is support for improved claw conformation when formalin foot bathing is combined with trimming (Randhawa et al., 2008), direct links between conformation and formalin baths have not been pursued.

Nutrition and dorsal border length

Dietary impact on dorsal border length has not been a primary feature of many studies. As for toe angle, Offer et al. (1997) found no effect of protein source on dorsal border length. However, forage source in the rearing diet (from three months of age through to early pregnancy) did influence dorsal border length; grass-silage reared animals had shorter toes than those on a hay and barley diet (mean dorsal border length 74.6 mm and 77.0 mm for medial claws, and 74.6 mm and 76.5 mm for lateral claws, respectively; $P<0.05$). This appears to be driven by increased growth rate in the hay- and barley-fed animals compared to those on grass-silage, as wear rate was unaffected by diet (Offer et al., 2001).

The nature and composition of the precalving diet of dairy heifers (wet grass-silage, fermented diet versus a dry straw-based, unfermented diet) was found to have no significant effect on dorsal border length (Leach et al., 2005). Sustained feeding of either high clover or a rye grass based pasture did not influence dorsal border length over the course of four lactations (Offer et al., 2001).

Links to other conformational parameters, lameness, lesions and locomotion

Dorsal border length and toe angle tend to be negatively correlated, as discussed earlier. In addition, Boelling and Pollott (1998a) found dorsal border length had a strong correlation with claw diagonal but a weak correlation to heel depth. This was supported by Vokey et al. (2001) who demonstrated a low, positive, position-related association between dorsal border length and heel depth. This association was initially only present for the lateral hind claws. However, by the end of the study the same relationship between dorsal border length and heel height was seen in all hind claws ($r=0.20$ to 0.27). Significant correlation between claw horn wear and dorsal border length was demonstrated by Offer et al. (2001) ($r=-507$) i.e. suggesting that increased wear reduced toe length.

Dorsal border length is sometimes reported as a ratio with other variables such as heel height or toe angle. The ratio of dorsal border length to heel height was considered by Ahlström et al. (1986) and Nuss et al. (2011). Ahlström et al. (1986) identified a breed effect on the ratio, such that Swedish Jerseys had the highest ratio (1st gestation: front 2.7, hind 4.4; 1st- 5th lactation: front 2.6, hind 4.0). This study also demonstrated a positive effect of age on the dorsal border length: heel height ratio. Nuss et al. (2011) reported that dorsal border length: heel bulb length was reduced for lateral claws compared to medial claws and proposed this to reflect higher heel bulbs in the lateral claw. Andersson and Lundström (1981) assessed the ratio of dorsal border length to posterior wall height and found that medial claws had larger ratios than lateral claws.

Long dorsal border length has been related to claw disease severity, presence of sole ulcer and higher total lesion score. However, the effect was described as smaller than that of toe angle (Vermunt and Greenough, 1995). Increased asymmetry between the claws of front feet as dorsal border length increases has also been described (Capion et al., 2008). Gitau et al. (1997) reported that an increase in dorsal border length of 1 cm was associated with increased likelihood of lameness, heel erosion, underrunning and overgrowth. Increased locomotion score was associated with overgrowth of the dorsal border (Ward, 2001), whilst Boelling and Pollott (1998a) associated increased dorsal border length with reduced locomotion scores (LS).

Heel conformation

The ability to easily monitor heel conformation would be welcome, as the bulb of the heel (particularly in the lateral hind claw) is the site of ground strike during locomotion (van der Tol et al., 2003). Thus the bulb of the heel, in conjunction with the lateral portion of the sole, constitutes the main weight bearing structure of the claw (Carvalho et al., 2005). As such, the depth, capacity and shock-absorption properties of the bulb may well influence lesion development and lameness (van der Tol et al., 2003; Somers et al., 2005). Vermunt and Greenough (1995) listed heel height as a trait for assessment of hoof conformation; and several other aspects of the heel bulb such as heel length and heel angle have also been suggested as worth assessing (Phillips et al., 1996; Nüske et al., 2003; Nuss and Paulus, 2006; Kremer et al., 2007), as have the relationship between heel measures and other parameters such as dorsal border length.

Heel height (heel depth)

Method of assessment

The distance from the ground surface to the skin: horn junction at the extreme of the plantar/palmar aspect of the claw bulb (Figure 1.5) is the most commonly measured heel parameter (Vermunt and Greenough, 1996; Vokey et al., 2001; Browne et al., 2007; Nuss et al., 2011, 2013); however, it is notoriously hard to measure accurately in the live animal (Hahn et al., 1984a; Vermunt and Greenough, 1995; Boelling and Pollott, 1998a). Ahlstrom et al. (1986) used a set landmark, the midpoint of the heel bulb, to site the heel measure at the same point on each claw. Other studies have attempted to make measurement easier by starting at the hair line, rather than the skin: horn junction (Hahn et al., 1984b; Choi and McDaniel, 1993; Phillips and Schofield, 1994; Fatehi et al., 2003). Some authors designate this variable “heel depth” (e.g. Gitau et al., 1997; Somers et al., 2005) but the term “heel height” has been used in this review.

Heel height has been measured with callipers (Nuss and Paulus, 2006; Kremer et al., 2007; Nuss et al., 2011, 2013), rules (Hahn et al., 1984a; Phillips and Schofield, 1994) and dividers (Nuss and Paulus, 2006; Nuss et al., 2011). Because it is a measurement from the animal to the ground surface it is usually done in the standing live animal (Hahn et al., 1984a; Phillips and Schofield, 1994; Vermunt and Greenough, 1995) and only rarely in the lifted foot (Ahlstrom et al., 1986). Digital analysis of images has also been used (Vermunt and Greenough, 1996; Browne et al., 2007). This method allowed successful comparison of the trait between two management systems but required post collection processing (Vermunt and Greenough, 1996).

Measurement of heel height in post mortem material is relatively common (Phillips et al., 1996; Phillips et al., 1998; Nuss and Paulus, 2006; Nuss et al., 2011). Such examination also allows heel height at the level of the corium to be measured after the exungulation of the claw (Nuss and Paulus, 2006 and Nuss et al., 2011).

Hahn et al. (1984b) reported that in the live animal measurement of heel height had much lower repeatability than other conformational traits (such as toe angle and dorsal border length). For first lactation animals, heel height repeatability was 0.24 ± 0.05 for front and 0.14 ± 0.05 for hind lateral claws, respectively, compared to ≥0.40 for toe angle and ≥0.50 for dorsal border length. Interestingly, the repeatability of heel height measurements reduced with lactation number, particularly in the lateral hind claw (often the claw chosen for study). In contrast Boelling and Pollott (1998a) found that repetition of heel measures over a relatively short time span (3 weeks) improved repeatability (0.91). Interestingly, however, Boelling and Pollott also identified a systematic measurement error in left vs. right foot which they suggested reflected the inherent difficulty of measuring heel height. They considered that the lack of a physical landmark on the heel and dirt on the feet were responsible for much of this difficulty. This suggestion is supported the higher repeatability for heel height obtained by Andersson and Lundström (1981), whose post mortem study employed a landmark (the proximal end of the abaxial groove) to mark the site at which heel height was measured; (defined as “posterior wall height”). This measure was applied with success in the live animal by Midla et al. (1998). However, although of similar magnitude to heel height, the posterior wall height measure is not the same as heel height (Figure 1.5) and results are not interchangeable.

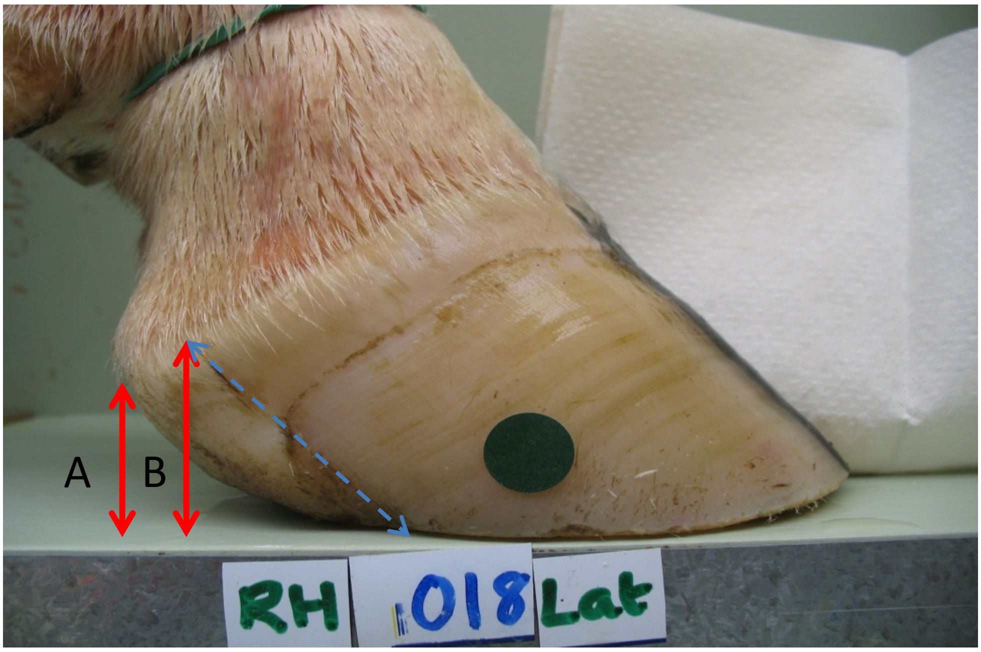

Figure 1.5: Photograph of the right hind claw from the lateral aspect illustrating (A) heel height (as defined by Vermunt and Greenough, 1995) and (B) height of the posterior wall (as defined by Andersson and Lundström, 1981).The blue dashed arrow marks the abaxial groove.

Visual scoring of heel height is also described in the literature. A “pseudo-continuous scale” was used by Fatehi et al. (2003), with a score of 1 being assigned for little or no heel height and a top score of 9 for 4 cm or more. Despite the limitations of such visual systems, Fatehi et al. (2003) were able to demonstrate differences in heel score between different management systems and allowed a measure of heritability to be derived. Other visual scales also exist; for example, a 50-point scale spanning from “low” to “high” heel was used for visual assessment by Dal Zotto et al. (2007).

The effect of claw position on heel height

There is general agreement in the literature that front limb heels are higher than hind limb heels (Andersson and Lundström, 1981; Ahlstrom et al., 1986; Phillips et al., 1996; Nüske et al., 2003). The effect of foot (right or left) is inconsistent across studies (Phillips et al., 1996; Boelling and Pollott, 1998; Nuss et al., 2011). Nuss et al. (2011), who demonstrated a significant effect of limb position on lateral claw heel height, showed that mean heel height for the right and left feet of heifers was 42.5 mm (SD 3.9) and 40.1 mm (3.4) respectively (*P*<0.011). Equivalent figures for cows were 43.9 mm (4.1) and 41.4 mm (4.2), *P*<0.003.

Within a foot, lateral heel height is usually reported as being greater than medial heel height (Smit et al., 1986a; Vermunt and Greenough, 1996; Nüske et al., 2003; Nuss and Paulus, 2006; Ouweltjes et al., 2009). However, Nuss et al. (2011) found that while heifers had a significant difference in heel height between claws in the front limb, the same was not true for cows. The relationship between heel height for claws of the same foot may be altered by foot position; Ahlstrom et al. (1986) found no difference in the mean between medial and lateral claws of the front feet (31 mm and 28 mm for cows and heifers, respectively) but significant difference between claws within hind feet (25 mm vs. 20 mm for lateral and medial claws respectively in cows; 21 mm vs. 17 mm for heifers). Differences in heel height between claws of a foot may result in a difference in the observed ground surface level between claws in the lifted foot: for example Telezhenko et al. (2009) described a 6 mm "*protrusion*" of the lateral claw surface beyond the medial claw surface.

Cow effects on heel height

Age and heel height

In very young calves, there was no increase in heel height between approximately 4 and 70 days of age (Nüske et al., 2003). Vermunt and Greenough (1996) also reported no change in heel height (front or hind) between entry to housing or a feedlot system at 12-13 months and calving at 24-26 months. Ahlstrom et al. (1986) reported a decrease in heel height over first pregnancy in housed animals, but once the heifers had calved the trend was for increasing height with age. Similarly, Midla et al. (1998) reported a significant reduction in front limb heel height over the course of first lactation for both claws, although the hind limb claws did not demonstrate the same pattern of change.

As cattle mature beyond first lactation, increased heel and, by extension, claw size has been a common finding (Andersson and Lundström, 1981; Ahlstrom et al. 1986; Boelling and Pollott, 1998a (see Table 1.9); Somers et al., 2005). As for other variables, stage of lactation has less impact on heel height as lactation number increases, which suggests that much of the change in the first lactation is growth related (Hahn et al, 1984b). Ahlstrom et al., (1986) recorded the greatest change in heel height between the first gestation and first lactation examinations (lateral > medial), but older animals have greater unevenness between hind claws of the same foot (Ahlstrom et al., 1986; Nuss and Paulus, 2006).

Table 1.9: Change in hind limb lateral claw heel height with lactation number, (mean ± SEM.) Boelling and Pollott (1998a).

Lactation	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Heel height (mm)	35.9 ± 0.45	36.2 ± 0.62	39.3 ± 0.70	39.5 ± 1.56	40.2 ± 1.04	43.1 ± 1.09	41.6 ± 1.25

Genetics and heel height

As might be expected heel height was greater in the claws of beef heifers than the claws of dairy heifers (Browne et al., 2007), even in a small scale study (n=8). Heel height is affected by breed (Ahlstrom et al. 1986; Baird et al., 2009), but the influence is small and can be modified by animal age; for example, Ahlstrom et al. (1986) demonstrated an effect of breed once animals had calved but not before calving. In particular, Swedish Jerseys had smaller heel height estimates than Swedish Friesians, Swedish Red and Whites and their crosses. Interestingly, heel height was greater in Swedish Red and White singleton calves than twin-born calves throughout the whole period between the pre-calving heifer and fifth lactation (Ahlstrom et al., 1986). Baird et al. (2009) demonstrated greater heel height for Norwegian dairy cows compared to Holstein-Friesians (35.6 mm vs. 33.6 $P<0.05$). Nüske et al. (2003) also found similar data for singleton calves compared to those from multiple births for several aspects of conformation in German Fleckvieh, German Holstein and their crosses, suggesting this may not be breed related *per se*.

As for toe angle and dorsal border length, moderate to high heritabilities have been calculated for heel height (Vermunt and Greenough, 1995). Values vary across studies, however; Hahn et al. (1984b) calculated heel height heritability to be 0.58 ± 0.34 (lateral claw of the right front), whereas Choi and McDaniel, (1993) suggested that heritability may differ between medial and lateral claws. Some of the variation between studies may be explained by the breed (Smit et al., 1986a) or lactation number (Choi and McDaniel, 1993) of the animals studied. Low heritability estimates (0.07) have been reported for Italian Brown Swiss cows (Dal Zotto et al., 2007) and for the Holstein (0.02-0.16 dependent on lactation number: Choi and McDaniel, 1993). In contrast, Fatehi et al. (2003) reported that heel height was one of the more successful traits (0.90 ± 0.06) used to provide genetic correlation for first lactation Holstein animals.

Management effects on heel height

Environment and heel height

That there is an influence of production system on heel height over time is widely accepted but the relationship is not well defined or understood. No effect on heel height was demonstrated by Vermunt and Greenough (1996) when indoor managed (concrete slats) dairy heifers were compared to those managed outdoors (dry feed lot) from housing to calving over a period of 12 months. However, Gitau et al. (1997) demonstrated that zero-grazed (housed) cattle in Kenya had shallow heels compared to those managed at pasture, in small paddocks or on tethers (Table 1.10).

Table 1.10: Heel height data (mm) from cows in different management systems (Gitau et al., 1997).

Heel height	**Pasture**	**Paddock**	**Tether**	**Zero-grazed**	**Overall**
(mm)	24.9^{a} (08)	25.2^{a} (0.8)	25.2^{a} (0.8)	23.7^{b} (0.8)	25.9 (0.4)

Means with different superscripts are significantly different from each other (P<0.05).

Fatehi et al. (2003) used survey data to demonstrate that heel height was higher in the free housed systems compared to tie stalls, a finding that the authors linked to the ability of animals to exercise.

When soft surfaces such as straw or rubber are compared to abrasive substrates (asphalt or concrete) change can be demonstrated, although results are inconsistent. Webster (2001) did not measure heel height but described a conformational change between management groups of pregnant and first lactation heifers over a six month period (4 weeks pre-calving to 24 weeks post-calving). Heels were thin [shallow] and smooth on concrete-cubicles compared to thick [deep] and pitted on straw.

Phillips and Schofield (1994) demonstrated an increase in heel height estimates for dairy cows housed on straw yards for six months (+42.4 µm/day) and a decrease for animals on concrete-cubicles (-27.2 µm/day). But, when Somers et al. (2005) investigated a variety of surfaces, heel height was reported to increase after trimming on all floor types (concrete floors solid, grooved or slatted and straw yards) over time (Table 1.11). The average increase in heel height was 6 mm over a period of approximately 4 months.

Table 1.11: Mean heel height (mm) of right hind lateral claws after trimming presented by floor type: (Somers et al., 2005).

	Weeks after trimming				
Heel height (mm)	**2**	**6**	**10**	**14**	**18**
Slatted concrete	43 ± 0.7	44 ± 0.7	46 ± 0.7	48 ± 0.8	49 ± 0.8
Solid concrete	42 ± 0.8	46 ± 0.8	47 ± 0.8	47 ± 0.8	46 ± 0.8
Grooved concrete	41 ± 0.7	45 ± 0.7	46 ± 0.8	48 ± 0.8	50 ± 0.8
Straw yard	40 ± 1.0	42 ± 0.8	43 ± 0.8	42 ± 0.9	45 ± 0.9

Similarly, Ouweltjes et al. (2009) described an increase in heel height over a 3 month period on both concrete and rubber-coated concrete floors. The increase in heel height for lateral claws was greater on the softer rubberised surface than the concrete floor (initial ≈46.5 mm, increased to ≈52.5mm [rubberised] and ≈48.5mm [concrete]). The difference was attributed to a lower rate of growth and wear on the rubber coated surface.

No effect of concrete vs. straw has also been reported (Fregonesi and Leaver, 2001), although the trend over the 17 week study was for heels to be lower on the concrete surface (4.3 cm vs. 4.5 cm for straw), which is in alignment with the findings of Phillips and Schofield (1994). However, when Scottish Highland cattle were moved from pasture to loose housing with a concrete floor heel height increased in front lateral claws (≈ 47.5 mm to 53 mm: Nuss et al., 2013). Increased heel height when animals were moved from relatively soft to more abrasive conditions was also discussed by Livesey and Laven (2002). In their study, animals reared on straw had shallow heels compared to those reared on concrete, a change in heel and claw conformation was seen in the straw reared animals when they were housed after calving. Their heels became thicker and more like the heels and general claw conformation of cubicle reared animals. These findings support a link between initial claw conformation and claw response to managed environment.

Boelling and Pollott (1998a) reported that heel height was maximal during the first half of the year (January March and April to June) and then decreased (July to September and October to December). Cows were housed (concrete) from October to April so the pattern of change between April and September did not correspond to periods of housing and pasture management, whereas the changes seen in toe angle and dorsal border length in this study were related to housing. Boelling and Pollott (1998a) felt that environment could only explain the change if there was a 3 month delay in heel height response.

The extended time required for heel conformation change within a system to be measureable was reported by Kremer et al. (2007). Between calving and 150 DIM, mean heel height increased in cows housed on both rubber-matted slatted concrete and slatted concrete surfaces with straw bedding, but the difference between surfaces was not significant (0.74 cm and 0.69 cm, respectively; *P*=0.56). However, from 150 to 305 DIM the increase was significant (1.05 cm and 0.73 cm, respectively; *P*<0.001).

Trimming and heel height

Reduced heel height post trimming was seen by several authors (Smit et al., 1986a; Somers et al., 2005; Carvalho et al., 2005). In the survey by Fatehi et al. (2003) routine trimming had little effect on heel height scores (trimmed 4.93 [SD 1.28] not trimmed 4.76 [1.32]) and the expected reduction in variation for heel height associated with recent trimming (<3 months prior) was not seen. A short-lived effect of trimming method was seen by Ouweltjes et al. (2009).

The conformational response of the heel to management change appears to follow on from an initial alteration in toe angle or dorsal border length (Boelling and Pollott, 1998a). Heel change occurs slowly and depends on the conformation at entry to a particular system; it is modified by the nature of the underfoot surface (such as abrasive properties) and freedom of animal movement. This further indicates that load bearing and resultant stimulation of horn growth versus the rate on horn wear play an important role in shaping the claw.

Nutrition and heel height

Animals on a more intensive plane of nutrition developed greater heel height (approximately 1 mm more on front feet $P \leq 0.05$, and 1-2 mm more on hind lateral and medial claws $P \leq 0.001$) than those in a lower intensity nutrition group (Ahlstrom et al., 1986). This finding was supported by Smit et al. (1986b), who reported an increase of 0.36 mm in heel height for a 1 kg rise in concentrates fed. Baird et al. (2009) compared animals on a high concentrate diet to those on a low concentrate diet or in a grazed production system. Greater right hind lateral heel height (36.2 mm) was seen for high concentrate diet animals than those on the low concentrate diet or in a grazed production system (33.8 mm, $P<0.05$). The nature of protein source in the diet (animal or soya) had no significant effect on heel height over a 24 week period, although pooled data confirmed an increase in height with time (Offer et al., 1997).

A reduction in front limb heel height was seen in biotin-supplemented dairy cows (LF: lateral claw -4.9 mm, $P=0.05$; medial claw -4.3 mm, $P=0.01$) compared to a non-supplemented control group (left front: lateral claw -2.5 mm, $P=0.05$; medial claw -1.0 mm, $P=0.01$). However, there was no significant difference between groups in the hind limb Midla et al. (1998). The reasons for this difference were not apparent. Lischer et al., (2002) concluded that biotin supplementation resulted in better horn quality (at the microscopic level) rather than a faster rate of repair / growth of horn.

Links to other conformational parameters, lameness, lesions and locomotion

Correlation between heel height and other conformational measures such as foot angle, dorsal border length and the diagonal length of the claw are described as being "*weak and erratic*" by Boelling and Pollott (1998a); although significant correlations have been reported. For example, Gitau et al. (1997) reported that toe angle and heel height were positively correlated (r=0.53). Correlation coefficients obtained by Vokey et al. (2001) for the hind claws were smaller (r=0.23-0.37). Gitau et al. (1997) reported a negative association between dorsal border length and heel height (r=-0.40), but Vokey et al. (2001) returned a positive relationship (r=0. 20-0.27). After 105 DIM there were positive correlations between dorsal border length and heel height for both lateral and medial claws, but before 105 DIM the correlations was only significant for lateral claws.

Relationships between heel height and production traits such as milk yield are also variable. For example, Smit et al. (1986b) and Vermunt and Greenough (1995) linked increase in heel height to improved milk yield. Both related the response to improved nutrition, with Vermunt and Greenough (1995) suggesting that the impact of nutrition on heel height was mediated via an elevated horn growth rate. In contrast, Choi and McDaniel (1993) found small negative correlation between milk yield and heel height (Lactation 1, -0.06; 2, -0.05; 3, -0.35; 4, -0.03). Choi and McDaniel (1993) also found a positive genetic correlation with days open after calving. Higher heels were associated with more days open and the size of the genetic correlation varied with lactation number (Lactation 1, 0.99; 2, 0.32; 3, 0.47; 4, 0.53). Differences between claws may also be associated with productivity; Smit et al. (1986b) reported that both increased heel height and greater asymmetry in heel height between claws of a foot had a positive association with rolling herd average production.

Positive associations have been reported between measures of body condition score or heart girth and heel height. Gitau et al. (1997) calculated a rise of one unit in body condition score or 10 cm in heart girth were associated with an increase in heel height of 1.2 mm and 2 mm, respectively while a moderate genetic correlation (0.35) with body condition score was reported by Dal Zotto et al. (2007).

Several studies have suggested a relationship between heel height and clinical lameness. Shallow heels (concurrent with long toes) were implicated in increased clinical lameness (Gitau et al., 1997), increased locomotion score (Ward, 2001), heel lesions, under-running and overgrowth of the sole (Gitau et al., 1997). Webster (2001) postulated that thin heels had a reduced protective effect and contributed to increased claw horn lesions.

High heels have been associated with increased digital lesions (Andersson and Lundström 1981), in particular sole ulcer (Smit et al., 1986b; Vermunt and Greenough, 1995) and increased locomotion score (Anon 1993 cited by Boelling and Pollott, 1998a; Ward, 2001). A significant association between claw lesion score and greater heel height in the lateral claw for both left and right hind feet (Spearman's $\rho=0.26$, $P=0.004$; $\rho=0.37$, $P=0.001$, respectively) was demonstrated by Vokey et al. (2001). Moreover, cows with moderate to severe interdigital dermatitis had significantly higher left lateral and medial claw heel height than cows with no interdigital dermatitis lesions ($P\leq0.02$).

Additionally, differences between claws in heel height have been linked to lameness, with equally sized claws being associated with a reduced risk of claw horn lesions and lameness (Vermunt and Greenough, 1995); perhaps because such claws are associated with more even weight bearing (Toussaint Raven, 2003; Bryan et al., 2012).

Asymmetrical heel height between claws in the same foot has been associated with disease. Disparity between claw surfaces was reported as a significant risk factor for lameness by Bryan et al. (2012). Andersson and Lundström (1981) related asymmetry in posterior wall height between claws of the same foot to increased severity of claw lesions (e.g. no haemorrhage or sole ulcer (mean disparity ± SEM) =8.1 mm ± 0.2, degree 3 haemorrhage (mean disparity ± SEM) =12.2 mm ± 1.4, $P<0.001$). In agreement with these findings, Smit et al. (1986b) reported that heel height difference was correlated to sole ulcer ($r=0.23$, $P<0.05$).

In summary, it is evident that heel height is much harder to measure accurately in a repeatable fashion than, for example, toe angle or dorsal border length. This notion is supported by reports of several other features of heel conformation which have been assessed in the literature such as heel angle, heel bulb length and heel bulb width. The existence of these additional variables suggests that no one heel variable is a definitive indicator of change in heel conformation.

Heel Angle

Heel angle was defined by Phillips et al. (1996) as the angle between the tangent of the curve of the heel bulb and the horizontal (Figure 1.6A). The subjective landmark for placing the tangent makes this angle hard to measure with accuracy and repeatability. An obtuse angle (Figure 1.6B) between the heel bulb and the sole surface has also been used (Nüske et al., 2003; Kremer et al., 2007).

In young calves between 4 and 70 days of age obtuse bulb angle increased 2.0° from a mean of 119.2°± 0.26 to 121.2°± 0.28, the angle was greater in hind than front claws (Nüske et al., 2003). Phillips et al. (1996) also demonstrated a difference in (acute) heel angle between front and hind feet in older animals (left front 46.5°, right front 47.4°, left hind 40.6° and right hind 40.8°; note: the angle reported here is an average of both claws in the foot). The post mortem study of Phillips et al. (1996) did not demonstrate a significant difference between heel angle in right and left feet either in front or hind limbs. Nuss and Paulus (2006) describe the heel angle to be greater in the lateral claw of animals in their study, by visual assessment.

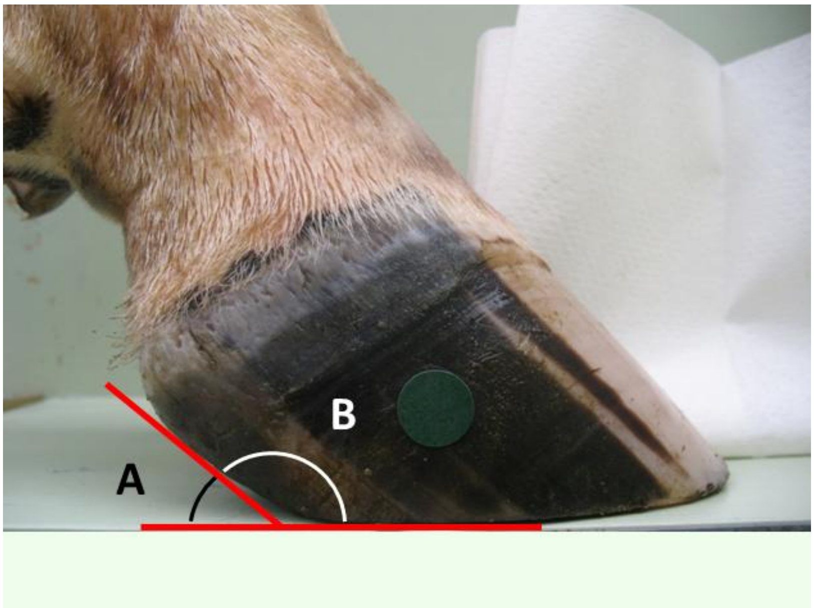

Figure 1.6: Photograph right hind lateral claw illustrating the two different heel angles reported in the literature; (**A**) angle between a line at a tangent to the curve of the heel bulb and the horizontal, (**B**) the opposite obtuse angle.

Having measured an increase in both toe angle and dorsal border length over time, Offer et al. (1997) extrapolated that heel angle with the ground must also have increased by default. The obtuse heel angle (B) reported by Kremer et al. (2007) increased with time on both concrete slatted and to a greater extent on rubber-matted concrete slatted floors. Claw trimming reduced the angle reported at 150 DIM but the change after trimming was much greater in the rubber - matted group (Table 1.12).

Table 1.12: Obtuse heel angle data in degrees (mean ± SEM) at 21 days precalving, 150 DIM pre-trim, 150 DIM post-trim and 305 DIM. From Kremer et al. (2007).

Surface	Heel angle 21 days precalving	Heel angle 150 DIM pre-trim	Heel angle 150 DIM post-trim	Heel angle 305 DIM
concrete slats	120.86 ± 1.01	131.78 ± 0.50	130.55 ± 0.37	134.37 ± 0.36
rubber-mats over concrete slats	123.1 ± 1.04	138.9 ± 0.51	131.6 ± 0.46	141.4 ± 0.37

Bulb or heel length

This parameter is most often measured in post-mortem material rather than the live animal. Definitions of this dimension vary between authors. Distl et al. (1984) used dividers to measure heel length: from the hairline at the heel *"that part of the "torus corneus" which does not belong to the "solea cornea" limited by the "margo coronalis" and the "basis tori"* to the ground surface. The distance was then measured with a ruler. The definition given was also illustrated in the paper (p591) and appears to correspond with the bulb length measure of Nuss and Paulus (2006). The variable and its ratio to dorsal border length had low repeatability in the live animal (Simmental bulls) compared to toe angle, dorsal border length, surface area and circumference measures (Distl et al., 1984). Bulb length was longer on front limbs (Distl et al., 1984; Nuss and Paulus, 2006) and greater for lateral than medial claws in both heifers and cows (Nuss and Paulus, 2006; Nuss et al., 2011).

Ahlstrom et al. (1986) started to use this variable but rejected it when it became clear that the measure covered a wide variety of heel conformation with respect to bulb shape and the height of the coronary band above the weight bearing surface. In a single trait these different conformations could not be teased apart for investigation, so heel height was used in its place.

Nuss and Paulus (2006) reported a heel bulb length in addition to heel bulb height. These measures differed subtly in their definition (Figure 1.7). Mean heel bulb length was longer than height and significantly greater in the lateral compared to the medial claw.

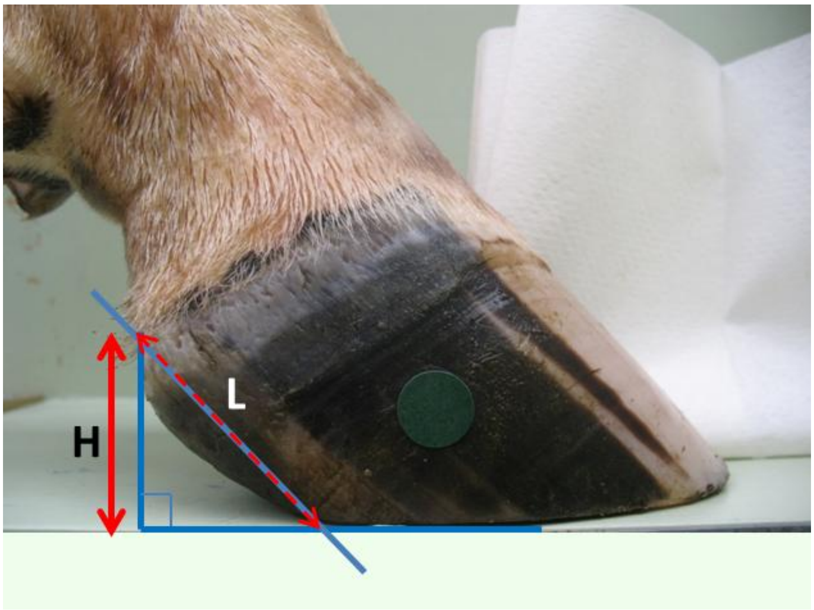

Figure 1.7: Photograph of right hind lateral claw illustrating the difference between heel height (H) and bulb length (L). As defined by Nuss and Paulus (2006).

A link between heel bulb length (heel height and heel angle) and the length of the sole was described by Nuss and Paulus (2006), with bulb length being longer in the medial claw and being associated with a reduced length of sole. Kremer et al. (2007) used an undefined heel length variable to assess all eight claws of German Holstein x German Fleckvieh F1 crosses using a sliding calliper. Heel length increased with time, but the study found no effect of flooring on heel length from 21d pre calving until 150 DIM. However, the increase from 150-305 DIM was significantly greater for animals on rubber-matted slatted concrete floors compared to those on a concrete slatted surface (305 DIM-150 DIM post-trim 1.04 cm for rubber and 0.61 cm for concrete, $P<0.001$). Kremer et al. (2007) suggested that the change in heel conformation was a response to the managed environment; with decreased wear on the rubber surface leading to a shift in the load-bearing region of the claw necessitating conformational change at the heel to compensate. As was the situation with heel height, there appears to be a delayed heel response which lags behind changes in more dynamic variables such as the toe angle of dorsal border length.

Claw Diagonal

The diagonal length of the claw runs from the apex (tip) of the toe to the skin-horn junction (Vermunt and Greenough, 1995) or hairline (Boelling and Pollott, 1998a), at the rear of the claw (Figure 1.8). This point was described by Loberg et al. (2004) as the proximal end of the heel (bulb). Digital assessment of claw diagonal was described by Vermunt and Greenough (1995), but was not assessed in their later conformation study (Vermunt and Greenough, 1996). Indeed, claw diagonal has only been used in a few live animal studies (Nüske et al., 2003; Somers et al., 2005; Kremer et al., 2007; Ouweltjes et al., 2009).

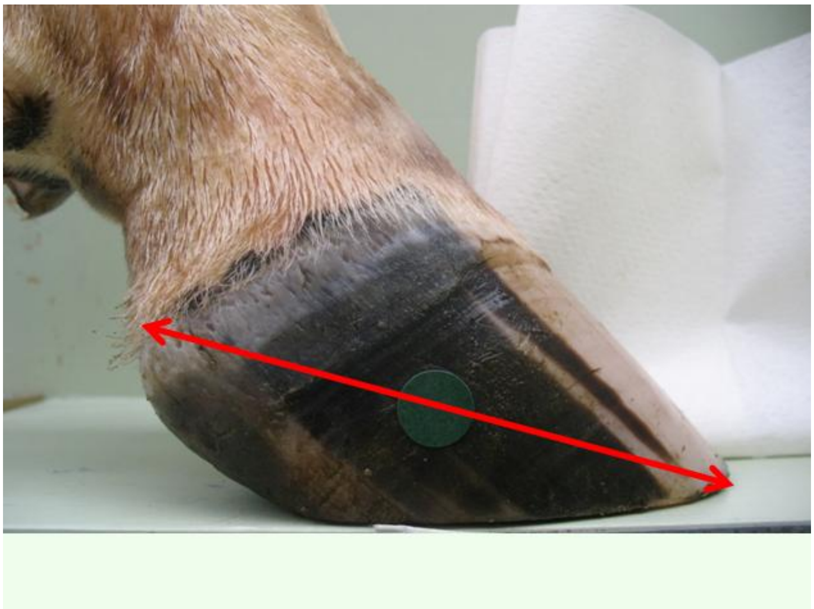

Figure 1.8: Photograph of the right hind claw from the lateral aspect illustrating; claw diagonal as defined by Vermunt and Greenough (1995).

Boelling and Pollott (1998a) reported that measurement of the claw diagonal was accurate and had a high repeatability (0.87). They also found that the size of the claw diagonal was unaffected by whether the right or left lateral hind claw was assessed.

The length of the claw diagonal is affected by claw and age. For calves, claw diagonal was greater in medial than lateral claws (Nüske et al., 2003), while in older cattle Ouweltjes et al. (2009) reported claw diagonal was greater for lateral than medial claws.

Nüske et al. (2003) reported that in the young calf mean claw diagonal rose from 6.0 mm to 6.6 mm over the first 12 weeks of life. This increase is relatively large compared to that seen for other variables such as dorsal border length. They also found a breed effect, with

German Fleckvieh and German Fleckvieh sire crosses having greater values than German Holstein or German Holstein sire crosses.

In adult cattle, Boelling and Pollott (1998a) reported that claw diagonal tended to rise with lactation number. Those authors also found a significant effect of year and season. They concluded that the seasonal effect was related to the management cycle with longer claw diagonals reported in cattle at pasture compared to housed cows.

Somers et al. (2005) also reported that claw diagonal increased with time (2 weeks post calving (WPC): 13.5 cm, 6 and 10 WPC: 13.7 cm, 14 WPC: 13.8 cm, 18 WPC: 13.7 cm; *P*=0.03), but described the changes as "*marginal*". They found no effect of floor surface on claw diagonal (mean claw diagonal: solid concrete 137 mm, slatted concrete 136 mm, grooved concrete 138 mm, straw yard 137 mm) or interaction between floor type and time. In contrast, Kremer et al. (2007) demonstrated modification of claw diagonal with floor surface when rubber was compared to concrete. Increase in claw diagonal between calving and 150 DIM was greater for rubber topped flooring than concrete (1.49 cm vs. 0.92 cm, *P*=0.001). Changes were more even between surfaces later in lactation after foot trimming at 150 DIM (1.23 cm versus 1.03 cm between 150 DIM and 305 DIM, *P*=0.013Similar findings for concrete and rubber surfaces are reported by Ouweltjes et al. (2009) (claw diagonal 129 mm vs. 125 mm; *P*<0.001 respectively).

An influence of access to exercise on claw diagonal was described by Loberg et al. (2004). Cows allotted daily or twice weekly exercise regimes had significantly shorter claw diagonals than those on once a week or no exercise. Cows housed on rubber mats assigned to the no paddock exercise regime had significantly longer claw diagonals than groups exercised more than once a week (Table 1.13). This agrees with Boelling and Pollott (1998a) who reported increased claw diagonal with decreased animal mobility.

In relation to other conformational parameters claw diagonal was moderately to highly correlated to toe angle and dorsal border length (r=- 0.58 and 0.80, respectively) but association with heel depth was limited(r=0.12) (Boelling and Pollott, 1998a). In an attempt to explain high rates of heel horn erosion in not-exercised cows Loberg et al. (2004) equated long claw diagonal to lower heel height and therefore increased exposure to slurry / dirt in the housed environment.

Table 1.13: Claw diagonal (tip of toe to proximal end of heel bulb: mm) of Swedish Red and white cows, matched by lactation within groups. Loberg et al. (2004), (mean ± SEM of left hind lateral claw).

Exercise regime	Claw diagonal mm ± SEM (left hind, lateral claw)
Exercise daily	143.4 ± 1.64 **
Exercised twice a week	142.9 ± 2.03 *
Exercised once a week	146.1 ± 1.89
Not exercised	150.6 ± 1.64

(**P*<0.05, ***P*<0.01, when compared to cows on no exercise regime)

Although claw diagonal was proposed as the most useful single measure of claw conformation and size by Boelling and Pollott (1998a), reports of its use are not common. This may be because measuring claw diagonal instead of toe angle and dorsal border length results in a loss of detail in the conformational information gathered, whilst not providing reliable new information regarding the impact of management changes. In addition, a conformational measure that is rarely used is less likely to be used simply because most studies do not measure it.

Claw length (sole length, claw diagonal length, sole diagonal length)

Claw length is described in multiple studies (Vermunt and Greenough, 1996; Kremer et al., 2007; Baird et al., 2009; Nuss et al., 2011), but nomenclature, definitions and methodology (callipers or rules versus computer analysis) are inconsistent (indeed they are often not given), significantly hindering direct comparison between studies.

Vermunt and Greenough (1995) defined claw length to be the length of the abaxial claw wall and bulb in contact with the ground surface (Figure 1.9). This definition appears to fit with the schematic for sole length provided by Andersson and Lundström (1981) and to be similar to the “bottom hoof length” described by Philips et al. (1996, 1998). Both Vermunt and Greenough (1996) and Browne et al. (2007) used digital image analysis to obtain this measure of claw length from an abaxial image of the lateral claw.

Nuss and Paulus (2006) obtained a diagonal claw length, which included the heel bulb, and also a diagonal sole length, along the same projected line but which included only the sole surface (Figure 1.10): The precise calliper measures described in this study are illustrated schematically (Nuss and Paulus, 2006) and photographically (Nuss et al., 2011) and lend themselves to measurement of post mortem material, particularly as diagonal sole measures can also be made after exungulation of the claw. The same diagonal claw length measure was used in calves by Nüske et al. (2003).

In calves, diagonal claw length (Table 1.14) is longer in medial claws than lateral claws for all feet (Nüske et al., 2003); whereas in adult cattle, the longest claw length is reported in the lateral claws of the hind limb and medial claws of the front limb (Andersson and Lundström, 1981; Nuss and Paulus, 2006; Nuss et al., 2011). Multiple studies report hind lateral claws to be longer than front lateral claws (Andersson and Lundström, 1981; Vermunt and Greenough, 1996; Nuss et al., 2011). For example, Vermunt and Greenough (1996) reported means of 95 mm (lateral) and 97 mm (medial respectively. In the forelimb, claws are more evenly matched for length, but the medial claw tends to be longer; both for diagonal sole length (before and after exungulation of the claw) and diagonal claw length (Nuss et al., 2011).

In calves, a 1.6 cm increase in claw length has been demonstrated over the first 12 weeks of life from 4.0 cm to 5.5 cm (Nüske et al., 2003). Hind claws are longer in cows than heifers (Nuss and Paulus, 2006) and claw length of hind lateral claws increased between housing at 12-13 months and calving at 24 - 26 months of age (Vermunt and Greenough, 1996). Kremer et al. (2007) demonstrated a rise in claw length in first and second lactation German Holstein and German Fleckvieh F1 crosses, irrespective of housed surface. A similar difference between heifers and cows was found for front limb diagonal claw length, but not front limb diagonal sole (Nuss et al., 2011: Table 1.14).

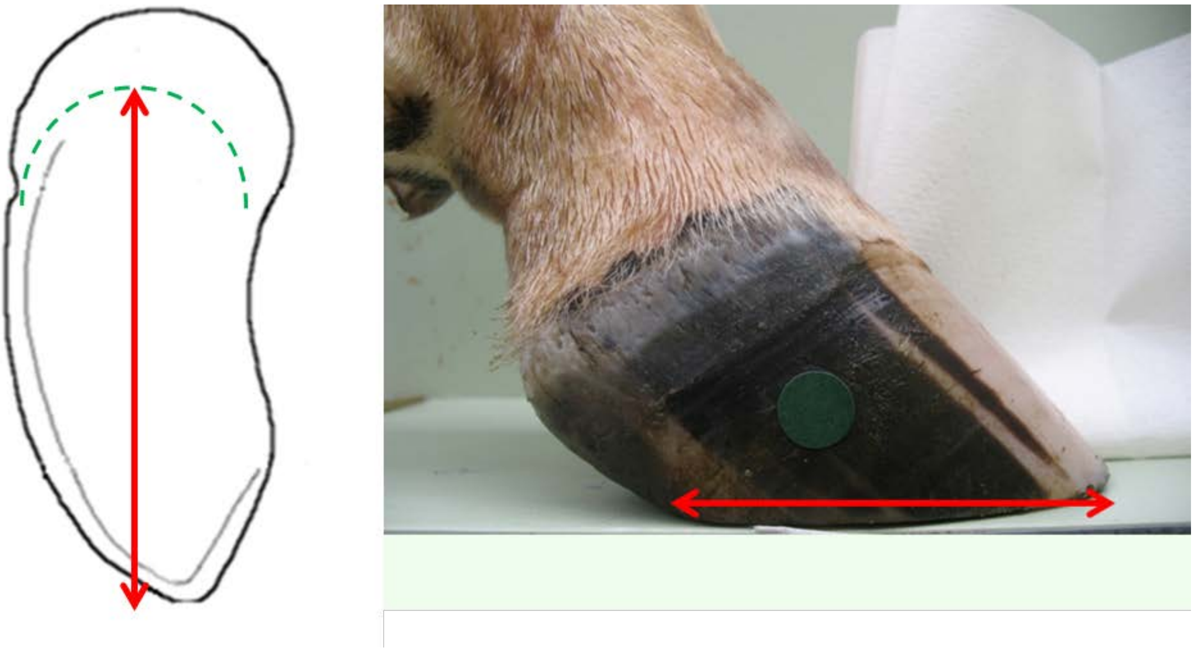

Figure 1.9: Length of abaxial claw wall and bulb in contact with ground surface. Left: Schematic diagram showing claw length assessed from line of lost ground contact in region of the sole-heel junction. Right: Photograph right hind claw from the lateral aspect illustrating claw length (as defined by Vermunt and Greenough, 1995).

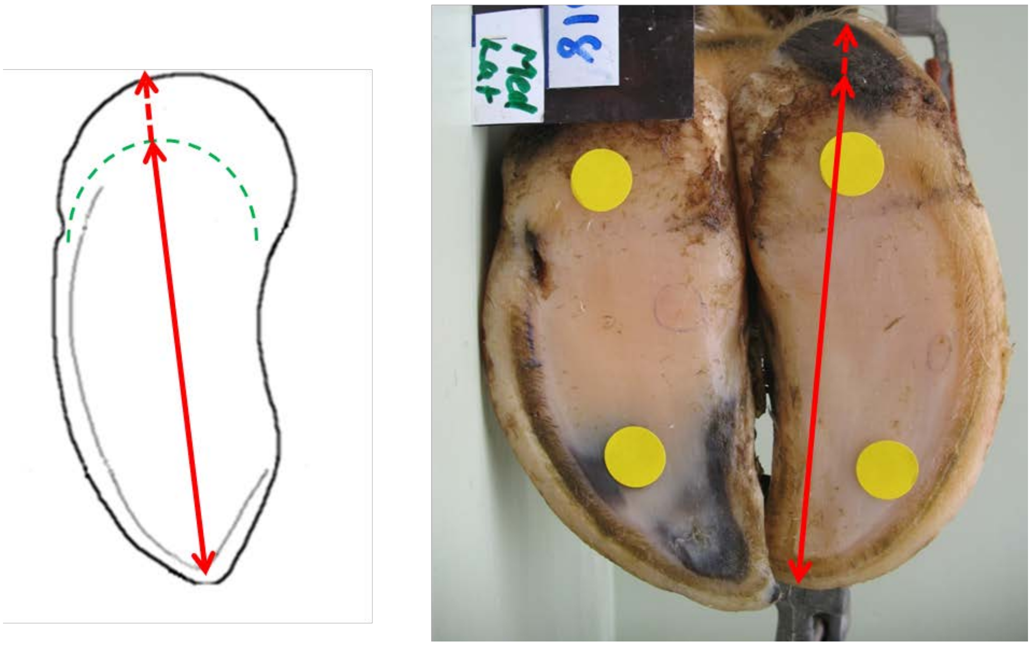

Figure 1.10: Diagonal variables. Left: Schematic diagram showing line at sole heel junction where ground contact is lost (green dash); claw length (solid plus dashed arrow), and sole length (solid arrow only), as defined by Nuss and Paulus (2006). Right: Photograph of right front claw palmar aspect showing the same variables.

Table 1.14: Claw length and sole length in heifers and cows measured post mortem in mm (SD). Note that the landmarks on the foot differ between papers.

Source	**Foot**	**Medial claw length (mm) (mean, SD)**	**Lateral claw length (mm) (mean, SD)**
Andersson and Lundström (1981)	**Front**	108.7 (9.5)	102.4 (9.5)
	Hind	103.7 (11.7)	110.3 (12.2)
Nuss and Paulus (2006)	**Hind <36mo**	127.4 (6.5)	130.7 (6.0)
	Hind >36mo	136.2 (6.5)	142.8 (9.3)
Nuss et al. (2011)	**Front heifer**	132.3 (7.1)	129.0 (7.2)
	Front cow	140.2 (9.0)	136.4 (10.2)
Source	**Foot**	**Medial sole length (mm) (mean, SD)**	**Lateral sole length (mm) (mean, SD)**
Nuss and Paulus (2006)	**Hind <36mo**	109.8 (8.0)	114.2 (7.4)
	Hind >36mo	115.5 (7.1)	126.1 (10.0)
Browne et al. (2007)	**Hind dairy**	109 (7.9)	-
	Hind beef	90 (9.8)	-
Nuss et al. (2011)	**Front heifer**	113.7 (6.2)	106.1 (6.5)
	Front cow	119.7 (9.6)	112.5 (11.4)

Breed may influence claw length. Andersson and Lundström (1981) compared Swedish Friesian and Swedish Red and White animals (105.0 mm and 106.5 mm, respectively, $P \leq 0.05$). Norwegian cattle had longer claws despite a lower body weight than Holstein Friesian animals (138.6 mm and 135.7 mm, respectively $P=0.05$). This size difference was linked to reduced lesion scores when the two sets of cattle were compared (Baird et al., 2009). Nüske et al. (2003) found that German Holsteins had longer claws that did German Fleckvieh; they also recorded an effect of line within breed. The heritability of claw length was reported as 0.37 for the Danish Friesian by Nielsen and Smedegaard (1984); they also reported a genetic correlation (0.4) between claw length and interdigital dermatitis. A gender effect on claw length was seen in young calves; male calves in the study of Nüske et al. (2003) had longer claws than female calves.

Kremer et al. (2007) demonstrated an effect of floor surface on claw length but the floor effect was not evident until the later stages of the study. During the first trial period (21 days pre-calving to150 DIM), animals on the rubber-matted slatted surface the increase in claw length tended be greater on the rubber than the slatted concrete surface (0.9 cm vs. 0.7 cm; $P=0.055$). In the second part of the trial (150-305 DIM), the increase was greater

(0.87 cm vs. 0.48 cm; *P*<0.001) on the rubber-matted slatted surface. Dairy system also has a significant effect on claw length; grazed animals and housed animals on a high concentrate ration had longer claws than housed animals on a low concentrate diet (139 mm, 139 mm and 133 mm, *P*<0.001, respectively: Baird et al., 2009).

Few studies have investigated links between claw length and other parameters, perhaps because claw length is hard to consistently define and measure in the live cow. However, Andersson and Lundström (1981) suggested that increased sole length was associated with claw disease based, on the basis of comparing older cows affected with claw diseases to younger animals with claw disease. In a more recent study (Baird et al., 2009), no link to digital dermatitis was demonstrated for claw length.

Toe height (claw height)

Vermunt and Greenough (1996) obtained this variable by measuring digital photographs, and the image produced for that paper has subsequently been used as a reference for how claw height could be estimated (Somers et al., 2005; Browne et al., 2007). Somers et al. (2005) collected toe height data directly from the cleaned claw, whilst Browne et al. (2007) used a digital method. The dearth of literature on this variable suggests that it is not particularly easy to obtain in the live animal. Even with a digitally obtained estimate the hair at the coronet may interfere with the landmarks used.

Vermunt and Greenough (1996) reported toe:heel ratio rather than a direct toe height. The ratio was 1.5 ± 0.1 in the front feet and 1.8 ± 0.2 for hind feet (*P*<0.001). The toe: heel ratio remained higher in the hind feet between housing and calving (12 months), and did not vary significantly over that time.

Some direct reports of toe height do exist. In a small post mortem study, Browne et al. (2007) reported that toe height was greater for feet from beef cattle than those of dairy animals (99 mm vs. 89 mm; *P*<0.05), despite the younger age of the beef animals (19-20 compared to 28 months of age). In the live animal, Somers et al. (2005) reported that claw height increased after calving, but only by an average of 0.5 cm (2 weeks post calving (WPC): 6.4 cm, 6 WPC: 6.6 cm, 10 WPC: 6.7 cm, 14 WPC: 6.9, 18 WPC: 6.9 cm; *P*=0.01) the study found no effect of floor surface (mean claw height; solid concrete: 6.7 cm , slatted concrete:

6.8 cm , grooved concrete: 6.8 cm , straw yard: 6.6 cm) or interaction between floor type and time.

Heel width (sole breadth, claw width)

The width of the claw is described as the widest dimension of the claw on the sole surface, at the sole to bulb junction, the position of which is often determined subjectively (Vermunt and Greenough, 1995; Nüske et al., 2003). Subjective selection of the site may explain the low repeatability recorded by Andersson and Lundström (1981) compared to other conformational parameters.

A wide variety of width parameters have been assessed throughout the literature and care should be taken when comparing studies. Phillips et al. (1996) described ‘bottom’ and ‘top’ hoof widths corresponding to the widest region of the entire foot (including both claws and the interdigital cleft) at the level of the ground surface (bottom) and the coronary band (top). The top width would appear to correspond to the dorsal width of Hahn et al. (1984b). Hahn et al. (1984b) also described both lateral width and heel width the three measures were used to calculate a for hoof base area (see Figure 1.11).

Nuss and Paulus (2006) used callipers to record a distinct measure of bulb width on the plantar aspect of the heel bulb, and also recorded a measure of sole width (which corresponded to the claw width of other authors). However, in a later study (Nuss et al., 2011), more robust landmarks were used to site the claw width measure (i.e. a point 25% of the way along a line from the tip of the claw to the heel bulb:diagonal sole length measure) was utilised as a marker to site a measure of claw width perpendicular to this line (see Figure 1.12).

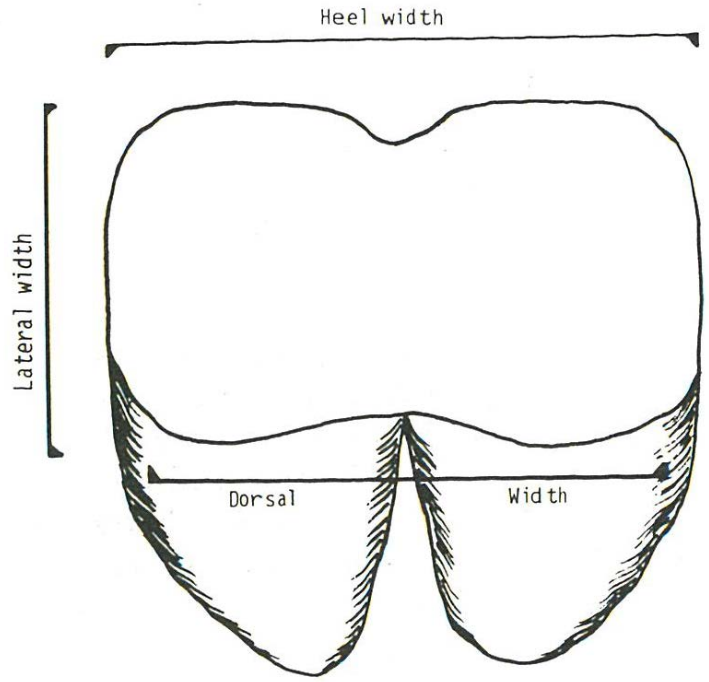

Figure 2. Cross section of the hoof showing the measurements used to obtain the base area.

Figure 1.11: Diagram[1] to illustrate the heel, dorsal and lateral widths measured at the level of the coronet.

Analysis of a digital image was used to estimate the widest distance between axial and abaxial surfaces of the claw at the sole-bulb junction (Vermunt and Greenough, 1996; Browne et al., 2007). The same definition of the widest point was used by Somers et al. (2005) and Ouweltjes et al. (2009) to obtain measures from the live animal. Livesey and Laven (2007) utilised a different, more distal, site to assess the width of the weight bearing surface of the claw at the same level (approximately mid-claw) on both medial and lateral claws using a transparent ruler. Telezhenko et al. (2009) used a contour gauge to transfer the profile of the sole at the widest point of the foot and calculated claw widths (medial and lateral) from these tracings. Baird et al. (2009) recorded claw width in the right hind lateral and medial claws but a definition of the method use is not given.

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[1] Figure 2, page 2988, reprinted from, Hahn, M.V., McDaniel, B.T., Wilk, J.C., 1984. Genetic and environmental variation of hoof characteristics of Holstein cattle. Journal of Dairy Science 67, 2986-2998, with permission from Elsevier. http://www.sciencedirect.com/science/journal/00220302

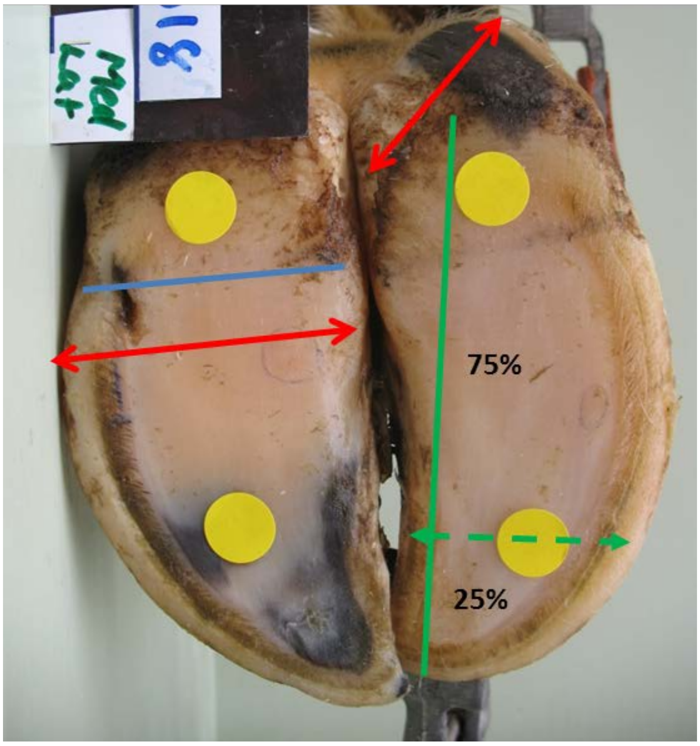

Figure 1.12: Measures of claw width. On the left claw, the red arrow illustrates claw width at the subjectively-assessed widest point. On the right claw, the red arrow illustrates heel bulb width as described by Nuss and Paulus (2006). The dashed green line is the sole width measured at a point 25% of the distance along the sole length (solid green line) from the tip of the sole as described by Nuss et al. (2011).Blue line denotes width sited at the level of the abaxial groove.

Bearing these different definitions of claw width in mind, front claws in calves and young stock 12-26 months of age appear to be wider than hind claws (Nüske et al., 2003; Vermunt and Greenough, 1996). Comparing two studies Nuss et al. (2011) came to the same conclusion; however, the difference between medial and lateral widths was smaller for the front limb than the hind limb. The top and bottom claw widths recorded by Phillips et al. (1996) also followed this pattern; there was no significant difference in width between right and left feet. Lateral claws were wider than medial claws (Nüske et al., 2003; Schwarzmann et al., 2007; Ouweltjes et al., 2009; Telezhenko et al., 2009; Nuss et al., 2011), irrespective of whether on the front or hind foot (Vermunt and Greenough, 1996; Nüske et al., 2003).

Nuss et al. (2011) reported an age effect, whereby cows had significantly wider claws than heifers. Width of both lateral and medial claws was also greater in older than younger cows (Nuss et al., 2011). Browne et al. (2007) proposed a significant difference in the width of the medial claw related to production type of the animal (i.e. beef > dairy animals), which implies a breed effect (beef mean: 54 mm, dairy mean 49 mm ± 2.1 n=4, $P<0.05$) although numbers in this study were low (n=4 for each group). A significant breed difference between Norwegian cattle and Holstein Friesian (107 mm vs. 105 mm; $P<0.05$) was demonstrated whereby the latter had narrower width (Baird et al., 2009). Again this increased width (and claw length) translated to a reduced weight load on claws and fewer claw problems were seen in the Norwegian animals.

Claw width increased over time (Vermunt and Greenough, 1996; Somers et al., 2005; Ouweltjes et al., 2009), with the lateral claw showing a greater increase in width than the medial claw. Ouweltjes et al. (2009) reported that, on rubber flooring, medial claws increased in width from 45.5 mm to 47 mm vs. 50 mm to 54 mm for lateral claws). This said, claw width increase is described as "marginal" by Somers et al. (2005) as values only changed from 5.6 cm ± 0.02 to 5.7 cm ± 0.02.

Claw width does not appear to be influenced by floor surface when housed groups are compared. Somers et al. (2005) found no effect of floor or interaction between floor and time for surfaces which included concrete (solid: 5.8 cm, slatted: 5.7 cm, grooved: 5.7 cm) and straw yards (5.5 cm).Similar results were obtained by both Ouweltjes et al. (2009) and Telezhenko et al. (2009) when a variety of surfaces were assessed (concrete slats versus rubber coated concrete slats: Ouweltjes et al. (2009); and slatted concrete, solid or slatted rubber and asphalt surfaces with or without feed stalls: Telezhenko et al. (2009).

The study of Baird et al. (2009) looked at relationships between disease, production system and conformation. Diet influenced heel width with greater heel width in animals fed a high concentrate diet than those on low concentrate or at pasture (108.2 mm, 104.1 mm and 105.6 mm respectively; $P<0.001$). Cattle with digital dermatitis lesions in this study were found to have significantly wider heels (106.7 mm, 105.3 mm; $P<0.001$) (Baird et al., 2009). A genetic correlation to interdigital dermatitis had been previously established at 0.32 by Nielsen and Smedegaard (1984).

Capion et al. (2008) assessed claw width as part of a "claw symmetry" variable by obtained digitally from a photograph, claws were asymmetric if width or length differed between claws (right front and right hind), and normal, symmetric if they did not. The variable was associated with dorsal border length of the right medial claw and an increase in white line disease but is not directly comparable with claw width.

Hoof base area

Hoof base area has been calculated by (Hahn et al., 1984b) as:

([heel width + dorsal width] /2)* lateral width

This calculated hoof base area was greater for front feet than hind feet (Hahn et al., 1984b; Gitau et al., 1997) and increased as heifers aged. Between first and second lactation the area of the hoof base increased by approximately 5%, despite body weight increasing by around 10% over the same period (Hahn et al., 1984b). Gitau et al. (1997) also found this area to be positively associated with body condition score and heart girth length, and negatively associated with clinical lameness. Hoof base area was linked to flat soles and under running but not overgrowth of claws by Gitau et al. (1997).

Abaxial groove height

This measure was described by Scott et al. (1999) as the distance along the abaxial groove of the claw from skin-horn junction at the coronary band to the distal weight bearing edge of the wall horn.

The variable was collected and used by Scott et al.(1999) to create a model to estimate claw volume and does not appear elsewhere in the literature other than in use as a landmark for posterior wall height at its proximal end (Andersson and Lundström, 1981).

Ground surface area of the claw

Ground surface area is the area included within the outline of the claws. It is therefore not the same as the weight bearing or contact surface area of the claws. Ground surface area has been studied with respect to: selection criteria for young bulls (Distl et al., 1984), claw conformation (Nuss and Paulus, 2006; Nuss et al., 2011), changes over time (Ahlström et al., 1986) and age (Schwarzmann et al., 2007). Although radiographs of calves’ feet have been utilised (Schwarzmann et al., 2007) most studies lift an outline of both claws from a prepared surface e.g. sawdust or tracing the lifted foot, and then apply planometry. Ground surface area is a challenge to obtain with accuracy and repeatability (Distl et al., 1984; Ahlström et al., 1986).

Front claws have a greater ground surface area than hind claws (Distl et al., 1984; Ahlström et al., 1986), but the effect of claw position, medial or lateral is not consistent. Distl et al. (1984) reported that medial and lateral claws did not differ significantly in area, whilst Ahlström et al. (1986) reported means for ground surface area with a distinct order to the size for each claw position (i.e. front medial > hind lateral > front lateral > hind medial) which was maintained as heifers matured to cows. In separate post mortem studies, Nuss and Paulus (2006) and Nuss et al. (2011) also found that, in cows (but not heifers) lateral hind claw area was greater than medial hind claw area, and that front medial claws have a greater area than lateral front claws.

Surface area increased with age in both young bulls and dairy cattle (Distl et al., 1984; Ahlström et al., 1986), particularly between estimates from heifers in their first gestation and the subsequent examination in first lactation Ahlström et al. (1986). Claw symmetry (defined as the ratio between medial and lateral claws) increased as heifers matured to cows (Ahlström et al., 1986). In heifers, the lateral front claws have a greater ground surface area than do the medial claws, while in cows the opposite was true (Nuss et al., 2011). Ahlström et al., 1986 reported that medial front claw area increased significantly with heifer age (with a concurrent reduction in weight bearing per unit area).The authors proposed that this change was a response to mechanical challenge on the medial claw. Work on calves' feet has shown that there is some degree of asymmetry present between medial and lateral claws even at a young age and suggest that this is a natural phenomenon rather than the result of environmental or management impact (Schwarzmann et al., 2007)

A significant breed effect on ground surface area exists, with Swedish Jerseys having the smallest and most symmetrical claw surface area of the breeds studied (Ahlström et al., 1986). Ahlström et al. (1986) found that the front feet of Jersey animals did not increase in symmetry as heifers matured due to their being symmetrical from an early stage. The greatest difference between medial and lateral claw surface area (asymmetry) was seen in Swedish Friesian dairy cattle and their crosses.

More recent studies have utilised modern force plate analysis methods to monitor the surface area of the claws (medial and lateral) that is actually in contact with the ground surface during loading and locomotion rather than the total ground surface area. This is perhaps a more useful measure with respect to the functional response of claws to interventions in the managed environment such as different floor surfaces. For example, Telezhenko et al. (2008) used pressure sensor plates to monitor the distribution of static load across and between the claws, and demonstrated that contact area/pressure distribution are indeed altered by floor surface. Greatest contact area was recorded for animals housed on asphalt floors with no feed stalls.

Study of claw surface area and loading dynamics has led to the conclusion that the lateral hind claw is at increased risk of damage compared to the medial claw, as it experiences higher loads across a smaller contact area. This may explain the increased incidence of lesions and lameness associated with this claw in particular (van der Tol et al., 2003). Carvalho et al. (2005) compared dynamic loading of trimmed with untrimmed feet (concrete housed animals) and concluded that routine trimming can relieve loading in areas such as the heel bulb and lateral sole. However, this relief is achieved by increasing loading of the medial sole, an area where sole ulcer is commonly found. Nuss and Paulus (2006) highlighted that trimming hind feet to level claw surfaces increased the lateral claw surface area and reduced sole thickness, perhaps increasing the vulnerability of the hind lateral claw to injury compared to its medial partner.

Introduction to experimental chapters

From the literature review covering the common variables used to assess claw conformation as listed by Vermunt and Greenough (1995) it is clear that over the last two decades parameters such as toe angle and dorsal border length have featured regularly in on-farm and post mortem-based studies, while others (such as claw diagonal, width and length) have proved less useful in the field situation. Even for those conformational features in widespread use, findings from the literature are inconsistent and sometimes, contradictory, just in the context of the housed conditions in which they have been undertaken. However, most of the literature has been based upon management interventions in the Northern Hemisphere and therefore relate to the impact of housing system on claw conformation. Only a few studies detail pasture-based systems and those peer reviewed studies which have been performed in New Zealand have focussed on the development of claw lesions and lameness, claw wall horn growth and wear, and sole horn wear with loss of sole concavity (Tranter et al, 1991; Tranter and Morris, 1992), rather than describing the impact of pasture management on more widely described variables such as toe angle or dorsal border length.

With the exception of sole concavity (Tranter and Morris, 1992), current peer reviewed literature does not contribute a wide baseline resource for information on claw conformation in the New Zealand dairy cow. Neither does it recommend a good “tool kit” of techniques to explore and understand what is happening to conformation under New Zealand conditions. This toolkit and baseline data need to be established before it is possible to monitor the impact of management interventions with respect to claw conformation; for example, response to the introduction of animal housing, use of rubber matting or new stand-off surfaces into farms.

The literature does link poor conformation to lesion development and lameness (Andersson and Lundström, 1981; Smit et al., 1986a, 1986b; Wells et al., 1993; Vermunt and Greenough, 1995; Gitau et al., 1997; Bryan et al., 2012) and also demonstrates that claw conformation is modified by the environment in which animals are managed (Vokey et al., 2001; Leach et al, 2005; Livesey and Laven, 2002, 2007; Telezhenko et al., 2009: Ouweltjes et al., 2009; Fjeldaas et al., 2011).

In addition, it is clearly recognised that first lactation is a critical period in the development of claw horn haemorrhage and lameness. First lactation heifers experience the double insult of physiological periparturient hormonal change (Tarlton et al., 2002; Knott et al., 2007) and entering the milking herd. Entry to the milking herd brings environmental challenges; for example, exposure to concrete and track surfaces, as well as social interaction with older dairy cows. Damage incurred during the first lactation has an influence on subsequent lactations (Offer et al., 2000). Both claw horn lesions and, anecdotally, poor conformations are seen in New Zealand cull dairy cows. Norton and Stalker (2005) detailed claw asymmetry in 67% of hind feet assessed with a significant (*P*<0.001) association to white line disease).

Therefore this thesis has two sections. The first in preparation for the live animal studies, examines some of the reported but underutilised instruments available to look at claw conformation; such as claw volume prediction and estimation of sole thickness by ultrasonography, as well as including an assessment of histological and morphometrical techniques. The second section, bearing in mind the importance of optimising claw health in first lactation animals, aims to assess the impact of managing such animals within a pasture-based system on conformational features that the literature has shown to be reasonably accurate and reliable, particularly toe angle and dorsal border length, with a view to creating data related to conformational change over time for New Zealand's unique conditions i.e. animals managed in a pasture based system without housing or routine foot trimming, and focussed on the vulnerable first lactation. Additionally in Year 2 there was a focus on mitigating the inherent difficulties in the assessment and monitoring of heel conformation in the live animal.

Overarching Hypothesis

The hypothesis underlying this thesis is that the conformational response of the claw to its environment occurs along a continuum, and that the changes documented for animals at pasture will fall between those extremes described in the literature for animals housed on concrete or asphalt versus those housed on straw or with rubber flooring.

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Chapter two: Predicting the volume of the bovine claw – method validation and development

Introduction

Cattle with claws which are small relative to their bodyweight have an increased risk of claw damage and lameness (Vermunt, 1999). This is, at least partly, because small claws have increased loading forces on the hoof and reduced shock absorption properties (Phillips et al., 1996). However, it is likely that claw size is also acting as a proxy for other measures. This could be measures related to physical protection, such as the capacity and depth of the digital cushion, and sole horn thickness, or features related to ground/claw contact surface area and the stability of the animal in locomotion. None of these measures are amenable to measurement without specialised equipment, whereas claw size could be much easier to determine and could therefore be used to determine lameness risk and in the development of breeding programmes to reduce lameness incidence (Phillips et al., 1996). Unfortunately, simple visual assessments of hoof size are not useful for determining the risk of hoof disease (Smit et al., 1986a) and also have significant between-observer variation (Murray, 1994). Estimation of claw size, with sufficient precision for the measure to be used in lameness evaluation, therefore requires more objective measures than simple visual observation.

Claw volume is a measure of claw size which itself encapsulates data from all dimensions of the hoof. Phillips et al. (1998) showed, in an *in vitro* model, that hoof volume was positively correlated (R^2=0.498, P=0.09) with the coefficient of static friction (i.e. between the claw and floor surfaces) and concluded that young animals with steep toe angles, small hoof volumes and smooth sole surfaces were more likely to slip on a smooth concrete surface. Clark et al. (2004) demonstrated, using cadaver material, that claws with a volume greater than 390cm^3 had 7.8 times greater odds of having vertical fissure in the horn than claws with a volume below this value.

Measurement of claw volume could therefore be a useful method of monitoring claw conformation, if it were possible to do so in the live cow. This has not proved feasible to date, so research on claw volume has focussed on predicting claw volume using linear measurements that can be made in the live animal (Phillips et al., 1996; Scott et al., 1999).

Both studies estimated foot volume in cadaver material obtained from cattle at slaughter using water displacement. Phillips et al. (1996) used limbs from beef cross Friesian cattle (both heifers and steers), whilst Scott et al. (1999) used young (<18 months) beef animals (gender not stated). Preparation of the limbs in these studies had pertinent differences. Both clipped hair away from the coronary band. Phillips et al. (1996) then marked the level of the coronary band and immersed the whole foot (both claws) to this level and measured the displaced volume. In contrast, Scott et al. (1999) sparingly trimmed the claw horn and then separated the medial and lateral claws using a band saw. They then disarticulated the distal interphalangeal joint and removed the skin from the coronary band region before immersing the individual claws in water.

The initial model developed by Phillips et al. (1996) inserted conformation measurements into a geometrically-derived formula for foot volume, evolved from the estimation of the volume of "*known shapes*" starting by approximating the claws to cylinders. However, this formula over-predicted volume and produced low correlations (R^2=0.382 and 0.481 for front foot and hind foot respectively). It was concluded that these low correlations reflected in-built assumptions with respect to heel and toe angles, sole shape and the relationship between dorsal border and rear heel wall within the model. Phillips et al. (1996) thereafter proceeded to statistical modelling (stepwise linear regression) of conformational measurements (digit and heel angles, heel height, dorsal border and the top and bottom hoof widths (across both claws) and top and bottom claw lengths, at the coronary band and floor level respectively) and returned considerably improved correlations (R^2=0.946 and 0.846 for front and hind feet, respectively; P<0.001).

However, some of the parameters were technically difficult to measure, particularly in the live animal (e.g. heel angle; Hahn, 1984a; Phillips et al., 1996). To circumvent the use of these parameters, other variables were added to the model. Insertion of age and carcass weight allowed heel angle to be dropped, and maintained high correlations (R^2=0.92 and 0.93 for front and hind feet, respectively; P<0.001). Phillips et al. (1996) also showed that the best models for the front and hind limbs were different. However, they obtained an acceptable correlations (R^2=0.901, *P*<0.001) when modelling all feet, irrespective of position.

Scott et al. (1999) concentrated upon easily-measured parameters which could be transferred smoothly to the live animal in field applications. They created a model using stepwise regression of five parameters (Figure 2.1a): toe length (length of dorsal border); toe angle; distance around the proximal border of coronary band from the abaxial groove to the flexure of the dorsal surface; distance from the abaxial groove to the toe along the distal weight bearing region; and the length of the abaxial groove from the coronary band to the base of the claw. Toe length and angle were discarded, but the remaining parameters allowed the generation of a predicted volume for whole foot that was highly correlated ($R^2=0.88$, $P <0.001$) with measured volumes.

Predicted volume (Scott et al., 1999) = 17.192*sum of distal +7.467*sum of abaxial groove + 45.27*sum of proximal -798.5

No foot effect (hind vs. front) was identified in this study. As the model they developed was for the whole foot, there was no ability to compare medial and lateral claw volumes.

The volumetric assessment of individual claws could be of considerable value in clinical investigations and research into lameness investigation. It would allow the investigation of claw position and volume (both total and relative between claws) in relation to lesion development, sole thickness and soft tissue depth. Volume could also prove a useful tool in the evaluation and validation of potential genetic markers allowing the monitoring of phenotype in the live animal (Phillips et al., 1996).

The present study was needed to test the equation generated by Scott et al. (1999) with data derived from the feet of New Zealand dairy animals. Pastoral dairy production systems used in New Zealand are thought to influence claw conformation as a result of long distance walking to and from the shed for milking. The New Zealand dairy cow is a much smaller animal than the beef cow breeds (Charolais, Angus, Hereford, Limousin) described by Scott et al. (1999). Furthermore, routine trimming of claws is not a common feature of dairy cow management in New Zealand. Consequently, there are obvious visual differences between the claws of New Zealand dairy cows and the beef breed claws described by Scott et al. (1999) and Nuss et al. (2006). Visually, the claws of dairy cows are smaller, less symmetrical between medial and lateral claws with longer toes, shallower toe angles, and lower heels than those of the beef animals described by Scott et al. (1999). There is only limited data on the hoof conformation of New Zealand dairy cattle (Vermunt and Parkinson, 2002) but referring to the breed differences and heritability reported in the literature (Hahn et al., 1984b; Ahlström et al., 1986) it is likely that there are significant differences between the hooves of such cattle and the beef animals used by Scott et al. (1999).

Hypothesis: Owing to the differences between the management practices and animal breeds in New Zealand dairy systems and those described by Scott et al. (1999) for Canadian beef animals, it is expected that the equation generated by these workers may not predict foot volume with sufficient accuracy when applied to New Zealand data. Furthermore it is felt that the ability to predict individual claw volume would add to the utility of this technique.

The aim of the present study was to test the equation generated by Scott et al. (1999) with data derived from the feet of New Zealand dairy animals and ensure the fit of the equation for the New Zealand situation before application to assess claw volume in the live animal.

Materials and methods

Experiment 1: Volumetric modelling

The distal limbs of 17 dairy animals were collected, individually identified and bagged. The age, as determined by ear tag or dentition (Dyce et al., 2002), breed and cause of death, if applicable, was recorded for each individual. The limbs were washed and stored at 3-5 ° C until measurements were made. The majority of measurements (12/17) were made within 96 hours, but for four animals measurements were made within 8-9 days of death. Sources included a local abattoir (1/17); Massey University post mortem room (animals with a cause of death unrelated to lameness) (9/17) and non-lame cull dairy cows euthanased on farm for reasons unrelated to this study (7/17).

Seven measurements were made on both claws of all feet (Figure 2.1a). All measurements were made using a flexible tape measure (to the nearest millimetre, mm), except for toe angle which was measured using an engineer’s angle finder. All measurements were taken twice and the mean of the two values used for modelling.

After measurement of the claw variables, the right front and left hind limbs were then processed for volume estimation using water displacement. The process and the apparatus used initially were based on the description by Scott et al. (1999); i.e. the volume of water that was displaced when the claws were immersed to the level of the coronary band was collected and recorded (Figure 2.1b). The only difference was that for time efficiency, the claws were not clipped or disarticulated but the limb simply sectioned longitudinally from between the claws with a band saw, so that the medial and lateral claws could be separately assessed for volume. An elastic band was used to restrain bulging fat from the sectioned digital cushion and prevent it entering the water.

However, although Scott et al. (1999) reported an R^2 of 0.999 for repeated volume estimates on the same claws using their hydrometer, the process proved difficult to replicate. The key problems encountered were firstly: deciding when the water had equilibrated. Clark et al. (2004), using a similar method to measure claw volume, allowed a five minute equilibration period, whereas Scott et al. (1999) did not report an equilibration time. Secondly siphoning of water into the collection vessel. Consequently this method was abandoned in favour of measuring water displacement by removing the displaced water by suction with a syringe back to a reference line.

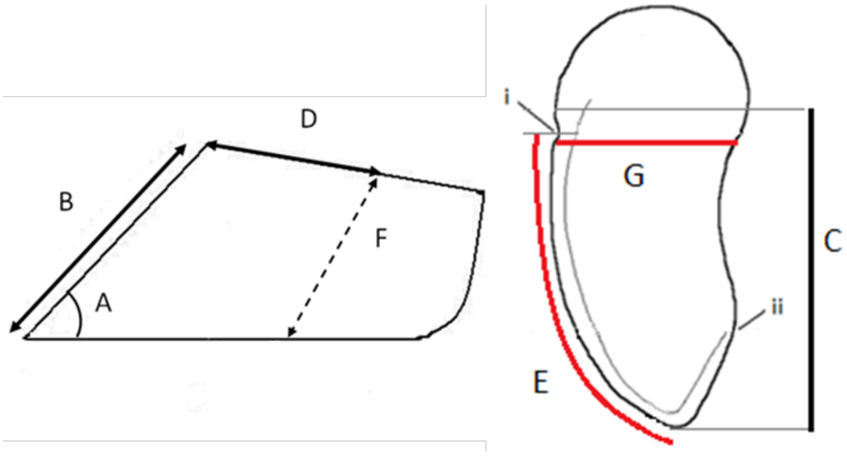

Figure 2.1a: Illustration of the seven variables measured on both claws of the hind feet of 17 dairy cows. Modified from Scott et al. (1999) i, abaxial groove; ii end of the axial white line.

A. **Toe angle:** Angle of dorsal border to weight-bearing surface;
B. Length of **dorsal border** from skin: horn junction at coronary band to apex of toe;
C. **Claw length** on the palmar/plantar surface from apex of toe to point where ground contact is lost (cm);
D. Distance from the point where the coronary band meets the flexure of the dorsal border to the abaxial groove along the coronary band (**proximal**);
E. Distance from the apex of the toe to the abaxial groove along the wall horn (**distal**);
F. Length of the **abaxial groove** from the coronary band to base of claw;
G. **Claw width** on the palmar/plantar surface, at widest point of claw in area of sole/bulb junction.

Figure 2.1b: water displacement equipment after Scott et al. (1999). Water displaced by the claw overflows to collection vessel via rubber tubing.

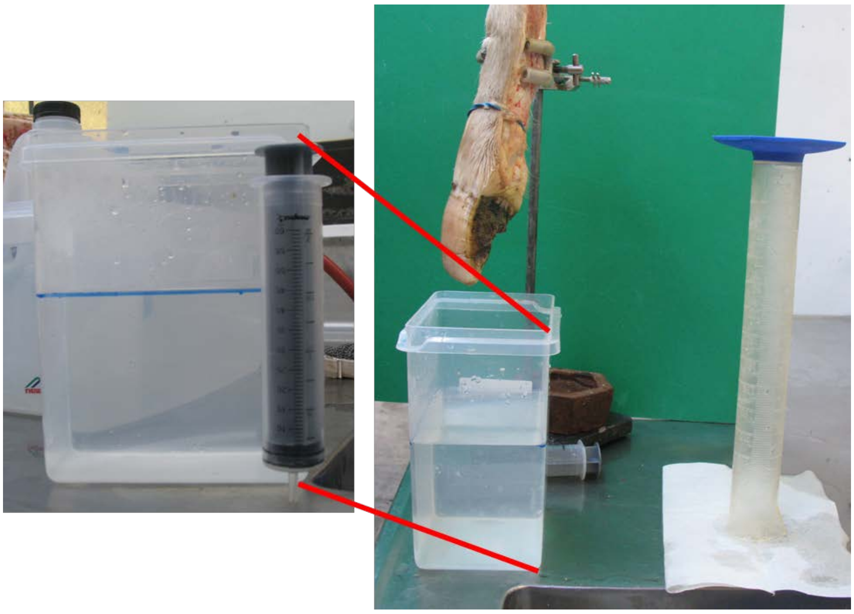

Figure 2.1c: Modified water displacement equipment: Water level was set to a predetermined mark until there was no parallax with the lines marked on either side of the container; a 60mL syringe was used. The claw was immersed to the level of the skin: horn junction at the coronary band and held in place with a retort stand as shown. Water line was returned to preset level by suction and volume measured in the calibrated measuring cylinder shown (cm^3).

A transparent container, with straight sides, just big enough to accept the claws and allow adjustments without the claws contacting the sides, was used (Figure 2.1c). A water level line was marked on both sides of a container so that the two lines could be visualised through the container. At eye level with no parallax, the two lines superimposed into one.

Water level was set to this line using a 60 mL syringe. The clean, dried, claws were individually immersed to the skin: horn junction of the coronary band and held in place with a retort stand. The position of the claw was adjusted, using the retort clamp, until the water line was at the skin: horn junction at the dorsal flexure and at the heel bulb, in a dorso-palmar/plantar plane, and also in an abaxial-axial plane (the axial landmark being the skin/ horn junction in the interdigital region). Water was removed with a 60 mL syringe until the meniscus fell back to the set water level line. The volume removed was recorded in cm^3 to the nearest 0.5 cm^3 using a calibrated measuring cylinder. The procedure was performed twice (from scratch) for each claw to obtain an average value for modelling. The difference between the measured and predicted values (Scott et al., 1999) was investigated.

Experiment 2: Investigation of the relationship between predicted volume and calliper measurements of sole thickness and soft tissue depth.

Feet from a total of 41 individual animals were used (distal limbs sourced and processed as above (abattoir [18/41]; post mortem [12/41]; cull cows [11/41]) to investigate the relationship between predicted volume and calliper measurements of sole thickness and soft tissue depth. The majority (32/41) were measured within 96 hours, but for five animals measurements were made within 5-6 days, and for four animals within 8-9 days of death.

Feet were measured as described for experiment 1. After these measurements had been made, the left front and right hind limbs were frozen for at least 24 hours before being sectioned sagittal plane on a band saw to allow calliper measurement of the sole thickness and soft tissue depth in the frozen claws to be recorded at two sites: Site 1 - the tip of the distal phalanx; Site 2 - 25 mm back along the sole towards the heel (the same sites were used in the ultrasound validation study Chapter 3).

Statistical analysis.

Experiment 1: Volumetric modelling

The results obtained for each parameter were first inserted into the equation of Scott et al. (1999) to predict whole foot volume, using data from both medial and lateral claws (Procedure A; Table 2.1). Linear regression was then used to assess the association between the predicted volumes for this model and those obtained by water displacement. Concordance was assessed with both Lin’s concordance coefficient (CCC) and Laio’s improved concordance coefficient (mCCC).

Lin’s concordance correlation coefficient uses the correlation coefficient between the two methods, in conjunction with the mean and variance of the two measurements, to produce a numerical value reflecting how close the fitted regression line is to the line of identity for the two methods, thereby including both precision and accuracy (Liao, 2003).

$**$**Lin’s CCC**= $p * 2\ \sigma_1\sigma_2 / (\sigma_1^2 + \sigma_2^2 + [\mu_1 - \mu_2]^2)$

Where: σ is the variance, μ the mean and *p* the Pearson’s correlation coefficient.

This calculation is most reliable when the mean and variance of the two measurements are equal or very similar, but is not so robust when these properties diverge. Where divergence is present the accuracy of the position of the regression line in relation to the line of identity is not tied down by the equation (Liao, 2003). For such a scenario, Liao (2003) proposed an improved concordance correlation coefficient, providing a way to fix both precision and accuracy within the calculation. The modification uses two points on the regression line to examine the area between the two lines. The slope of the regression line is thereby fixed in relation to the line of identity and the outcome is more reliable (Liao, 2003).

mCCC = $p * 4\sigma_1\sigma_2 - p(\sigma_1^2 + \sigma_2^2) / (2-p)\ (\sigma_1^2 + \sigma_2^2) + (\mu_2 - \mu_1)^2$

Where: σ is the variance, μ the mean and *p* the Pearson’s correlation coefficient.

Corresponding mCCC values are usually lower, but more robust (Laio, 2003).

Limits-of-agreement plotting was then used to evaluate agreement between the measured volume and that predicted by the model of Scott et al. (1999) (Bland and Altman, 1999).

To allow comparison with the findings of Scott et al. (1999) the values obtained from the medial and lateral claw of an individual foot were added together for each variable under consideration: length of dorsal border, proximal, distal and abaxial groove length (see Figure 1) generating a foot total for the variable.

Additional procedures were then performed to identify whether a whole-foot volume model could be developed which achieved better agreement with the data from this study than that resulting from the model of Scott et al. (1999) (Procedures B, C, and D; Table 2.4) and, in addition, to develop a model for the prediction of individual claw volume (Procedures E, F and G; Table 2.8).

All analyses were undertaken using SPSS version 18 (SPSS Inc. Chicago).

Each procedure resulted in a number of potential models, the best of which was selected using the Akaike Information Criterion (AIC) (for which the smallest value is the most desirable), and Mallow's criterion (Cp) (for which a Cp value equal to or just smaller than the number of parameters in the model (including the constant) is desirable). These criteria were defined as follows (Hwang et al., 2002).

Akaike Information Criterion:

$AIC= n^* \log (SSE/n) +2p$

Where n is the number of observations and p the number of variables in the model, SSE is the sum of squares for the residuals (*error).

Mallow's C*p* index:

$^{**}Cp = \underline{\text{SS Error of the smaller model}} - (n-2p) / \text{Mean square error for full regression}$

Where p is the number of variables in the model equation including the constant.

To investigate which procedure created the best model overall, the concordance between predicted and measured volume was assessed using CCC, mCCC and limits-of-agreement plotting.

Table 2.1: Procedures used to create models to predict volume for concordance and agreement testing against volume measured using water displacement

Whole foot volume

Procedure A: This procedure tested the model developed by Scott et al. (1999)

Procedure B*:* All parameters (foot (right Front or left hind), claw category (lateral or medial claw) and age (30 months and under, over 30 months); toe angle, dorsal border, claw length, claw width, and proximal, distal and height of the abaxial groove) in a backwards linear regression.

Procedure C: This procedure entered all the parameters used by Scott et al. (1999) i.e. proximal, distal, and height of the abaxial groove.

Procedure D: Backwards linear regression starting with the variables entered into Procedure C.

Individual claw volume

Procedure E: Stepwise linear regression using all variables (value for inclusion P≤0.05 and exclusion P≥0.1)

Procedure F: Backwards linear regression using all variables.

Procedure G: Backwards linear regression using those variables that would

Experiment 2: Investigation of the relationship between predicted volume and calliper measurements of sole thickness and soft tissue depth.

The conformational measurements from the left front and right hind limbs were used to predict claw volume using the formula developed in this study. The relationship between predicted claw volume, sole thickness and soft tissue depth in the frozen claw was investigated using linear regression.

Results

Water displacement

The average difference between the two measured volumes obtained by water displacement, for each claw, was 6.16 cm^3 (range 0-20 cm^3). The correlation between the two estimates was highly significant (r=0.966; P<0.001) Figure 2.2.

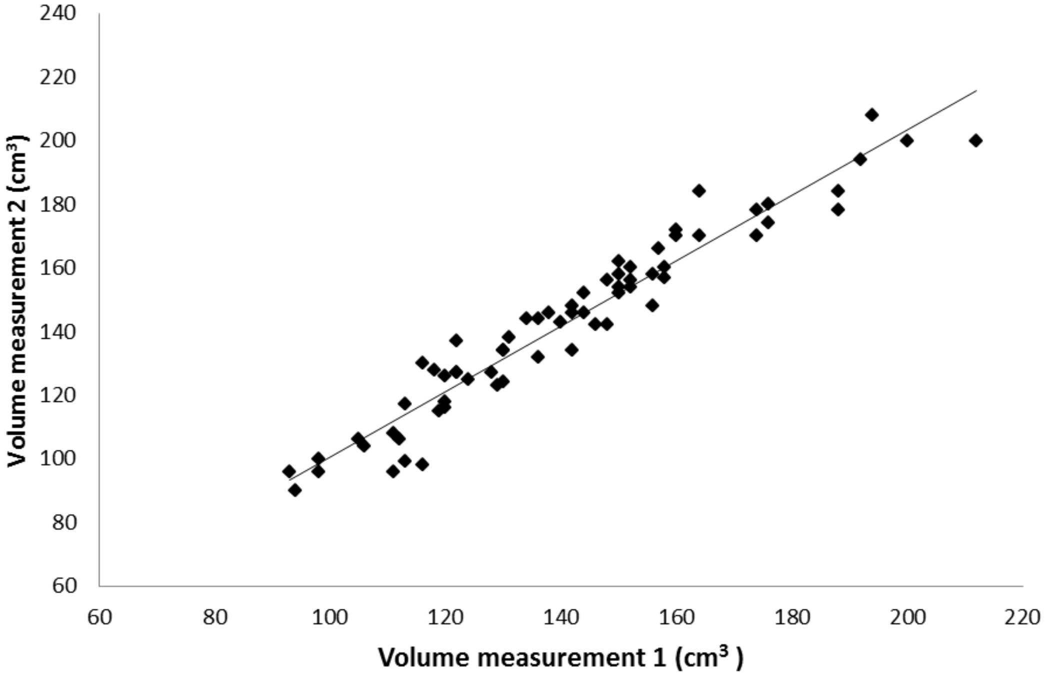

Figure 2.2: First estimate of claw volume plotted against second estimate of claw volume (cm^3), both measured by water displacement. The data are from each claw of the right front and left hind of 17 dairy cattle. (One claw had only one estimate recorded and was excluded). Interpretation: Solid line of best fit

Experiment 1: Volumetric modelling - Whole foot volume modelling procedures

Procedure A

Model A: predicted volume = 17.192*total[a] distal + 7.467*total abaxial groove + 45.27*total proximal - 798.5 (Scott et al. , 1999)

[a] total figures include sum of measurements from medial and lateral claws

There was a significant relationship (R^2=0.877, *P*<0.001) between the volume predicted by the model developed by Scott et al. (1999) (predicted volume) and the sum of the volumes of the medial and lateral claws as measured by water displacement (measured volume). However, predicted volumes were much greater than measured volumes (Figure 2.3), with a mean predicted volume of 510 cm^3 compared to mean measured volume of 286 cm^3.

Calculated values for CCC and mCCC were 0.17 and 0.13 respectively, denoting poor concordance between the volume predicted from the model developed by Scott et al. (1999) and the measured volume obtained by water displacement. In addition, as Figure 2.3 illustrates the proportional error between the two methods increases with volume.

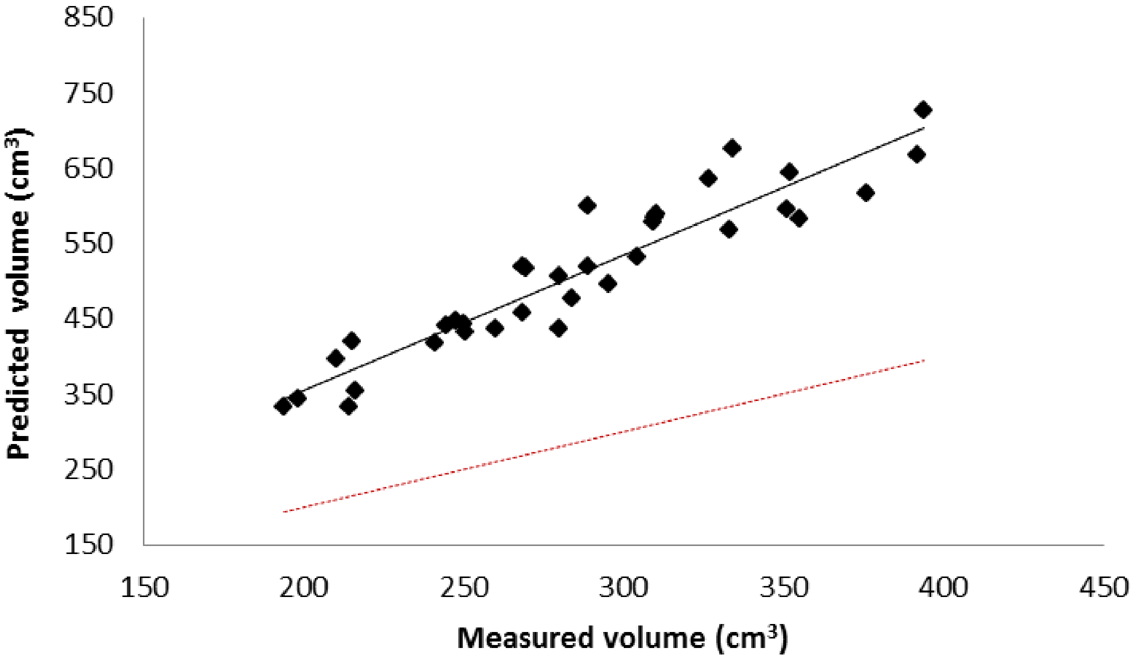

Figure 2.3: Scatter plot of predicted volume obtained using the model of Scott et al. (1999) against measured volume, by water displacement, obtained for 34 whole feet (medial plus lateral claw), right hind and left front, from 17 dairy cows.

Interpretation: Solid line is line of best fit for the model (R2=0.877, P<0.001), dashed line is the line of identity.

Plotting mean difference between predicted volume and measured volume, against the mean of the two methods (Figure 2.4) confirmed this trend (R^2=0.797, P<0.001). The ratio of the two methods was therefore used to create the 95% limits-of-agreement for these two methods (Figure 2.5) (Bland and Altman, 1999).There was no significant association between the mean of the two methods and their ratio (R^2=0.081, *P*=0.103). On average, predicted volume was 1.78 times measured volume. The 95% limits-of-agreement were 1.53 to 2.04, i.e. for 95% of measurements the predicted volume will be between 1.53 and 2.04 times measured volume.

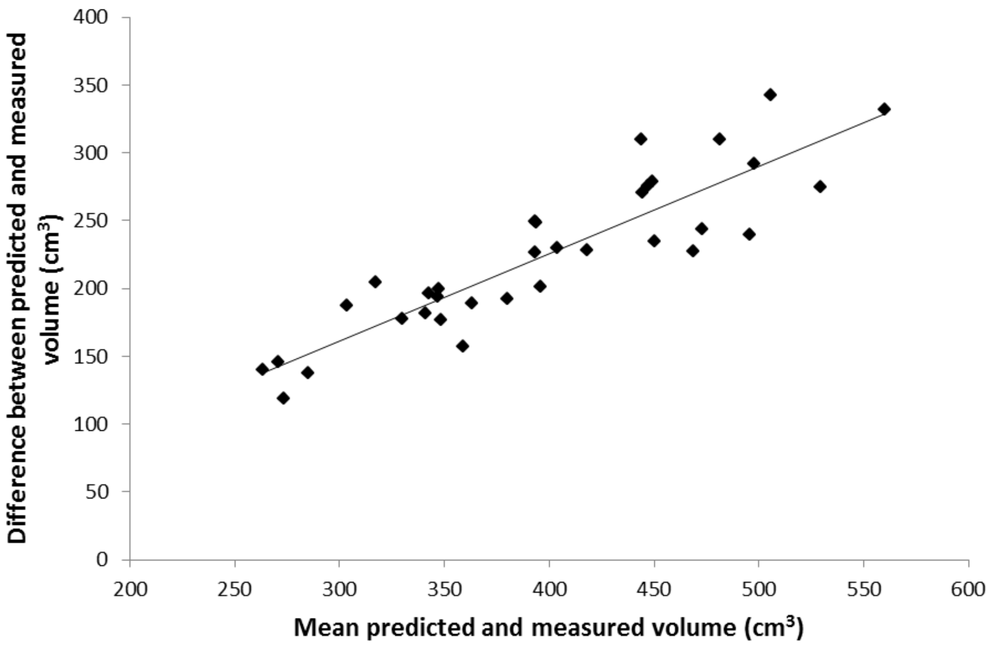

Figure 2.4: Mean difference plot for 34 whole feet (medial plus lateral claw), right hind and left front, from 17 dairy cows. Showing the difference between predicted volume and measured volume against the mean of the two methods. Predicted volume obtained using the model of Scott et al. (1999) measured volume obtained by water displacement.

Interpretation: Solid line is line of best fit, (R^2=0.797, P<0.001).

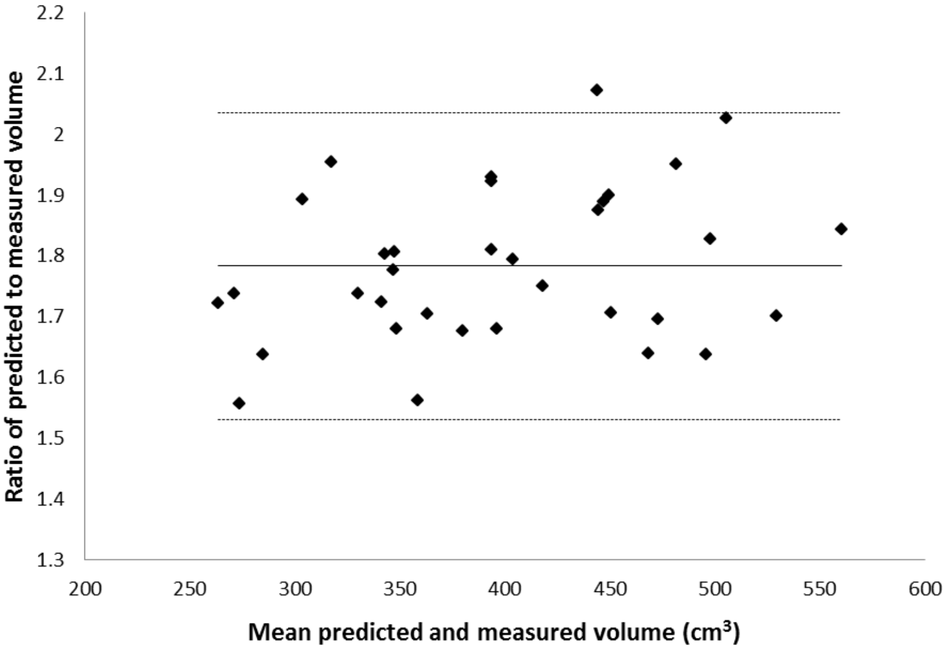

Figure 2.5: Limits-of-agreement plot showing the ratio between the volume predicted from Scott et al. (1999) (*Procedure A*) and measured volume (water displacement) against the mean of the two methods, for 34 whole feet (medial plus lateral claw), right hind and left front, from 17 dairy cows.

Interpretation: Solid line is the mean ratio: 1.78. Dashed lines are the 95% limits-of-agreement, range 1.53 to 2.04.

The minimum, maximum and mean values for the proximal, distal, abaxial groove length and dorsal border variables and claw volume are shown in Table 2.2 for both front and hind feet to allow comparison with the findings of Scott et al. (1999).

For both proximal and distal, front and hind limbs, the average total of the medial and lateral claws are similar in both studies. However for the length of the abaxial groove and the dorsal border the mean values were 20% larger than those reported by Scott et al. (1999), while mean measured volume was approximately 58% of that reported by Scott et al. (1999).

Table 2.2: Summary of the mean values and ranges for the variables reported by Scott et al. (1999) compared to the values obtained in the present study.

Variable		**Proximal (cm)**		**Distal (cm)**		**Length abaxial groove (cm)**		**Dorsal border(cm)**		**Volume (cm^3)**	
Foot		**Front**	**Hind**	**Front**	**Hind**	**Front**	**Hind**	**Front**	**Hind**	**Front**	**Hind**
Scott et al. (1999)	**Mean (range)**	19.3 (16.4-21.4)	18.2 (15.8-19.9)	22.3 (18.5-26.1)	20.9 (17.6-24.5)	9.9 (7.3-11.5)	8.9 (6.9-11.0)	12.9 (11.1-17.0)	13.4 (10.6-16.0)	530 (374-722)	459.7 (329-633)
	SD	1.20	0.90	2.20	2.40	1.00	0.90	1.20	1.10	96.90	78.90
Present study	**Mean (range)**	19.5 (16.4-2.4)	18.4 (16.3-0.5)	21.6 (18.1-24.8)	20.9 (17.2-24.8)	10.3 (12.1-14.7)	10.6 (9.3-13.3)	15.6 (13.6-6.65)	16.1 (14.4-17.5)	303.9 (214-394)	267.3 (193.5-355)
	SD	1.54	1.27	2.01	2.02	1.22	1.15	0.85	0.90	56.01	48.50
Present study as % of Scott et al. (1999)		101.3	100.8	96.9	100.0	122.0	119.6	121.0	120.0	57.3	58.2

Procedure B

This whole-foot model was generated using all parameters in a backwards linear regression

Five potential models were generated (Table 2.3). The model with the lowest AIC was chosen as Model B:

Predicted volume = -458.78 + (13.55*total proximal) + (15.5*total distal) + (1.69*total toe angle)

There was a significant association between predicted and measured volume (R^2=0.897, P<0.001) and CCC and mCCC were both high (0.95 and 0.90, respectively). Figure 2.6 illustrates the concordance of the model.

The mean difference between predicted and measured volume for Model B was 0.02 cm^3 and the 95% limits-of-agreement for this model were -34.97 to 35.34 cm^3. There was no trend for the mean to increase with the size of the difference (R^2=0.028, *P*=0.345) (Figure 2.7.)

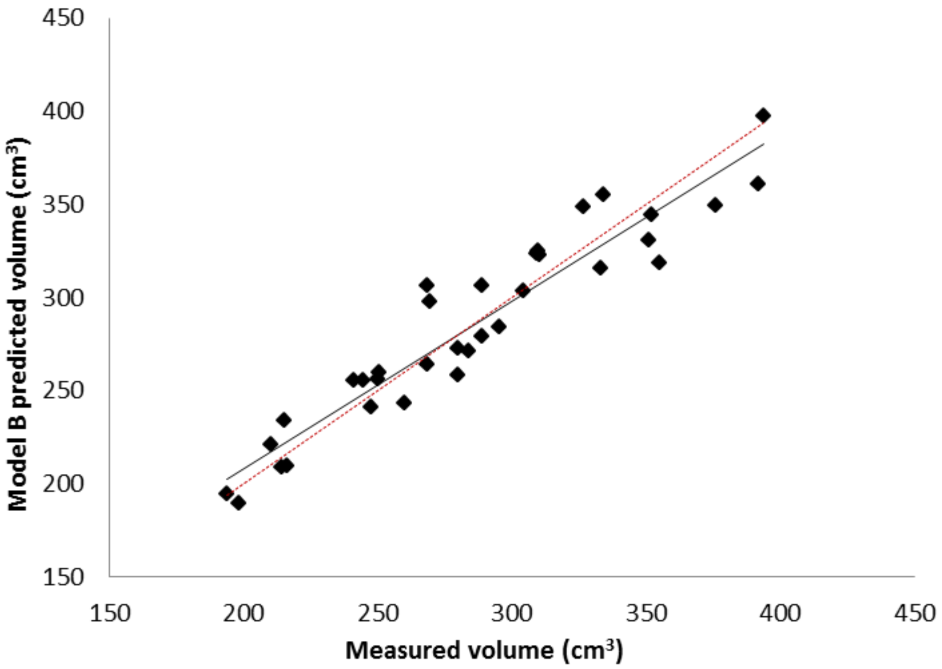

Figure 2.6: Scatter plot of whole foot predicted volume obtained using Model B against measured volume (water displacement)) from 34 feet (right hind and left front), from 17 dairy cows.
Interpretation: Solid line is line of best fit for the model (R^2=0.897, *P*<0.001), dashed line is the line of identity.

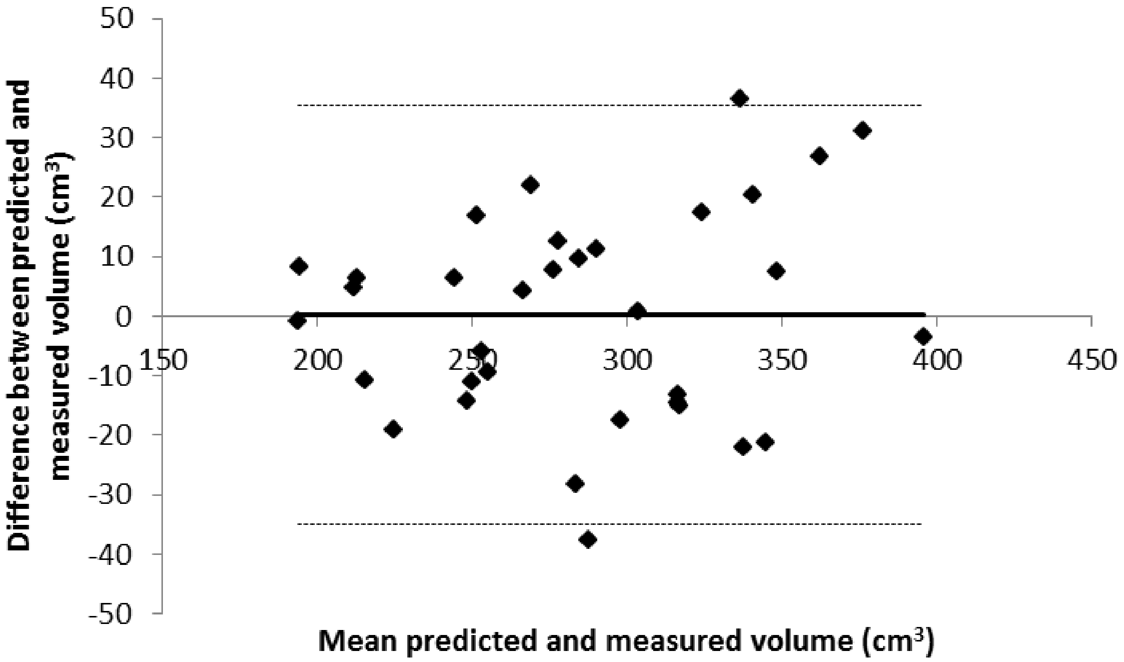

Figure 2.7: Limits-of-agreement plot showing the difference between predicted volume Model B and measured volume (water displacement) against the mean of the two methods (cm^3).

Interpretation: Solid line is the mean difference 0.02 cm^3. Dashed lines are the 95% limits-of-agreement range -34.97 to 35.34 cm^3. There was no significant trend in the data (R^2=0.028, *P*=0.345).

Table 2.3: Summary of models suggested for procedure B

Model	Model Summary	R^2	Adjusted R^2	AIC	C*p*
1	(Constant), age and foot categories, total[a] dorsal border (B), total toe angle (A), total proximal (D), total abaxial groove (F), total distal (E)	0.907	0.882	96.7	8
2	(Constant), foot category, total dorsal border (B), total toe angle (A), total proximal (D), total abaxial groove (F), total distal (E)	0.907	0.887	94.7	6.0
3	(Constant), total dorsal border (B), total toe angle (A), total proximal (D), total abaxial groove (F), total distal (E)	0.907	0.890	92.8	4.1
4	(Constant), total dorsal border (B), total toe angle (A), total proximal (D), total distal (E)	0.906	0.893	90.8	2.2
5	(Constant), total toe angle (A), total proximal (D), total distal (E)	0.897	0.887	90.2	2.8

[a] , total figures include sum of measurements from medial and lateral claws; AIC, Akaike Information Criterion; C*p*, Mallow's index

Procedure C

This procedure took the variables present in the equation reported by Scott et al. (1999) (total[a] proximal, total distal, and total length of the abaxial groove) and entered them into a whole foot model. The resulting (Model C) was:

Predicted volume = -351.41 + (20.17*total proximal + (10.15* total distal + (3.43* medial + lateral abaxial groove)

There was a significant association between predicted and measured volume (R^2=0.879, P<0.001) and CCC and mCCC were both high (0.94 and 0.88, respectively). Figure 2.8 illustrates the concordance of the model.

The mean difference between predicted and measured volume for Model C was 0.10 cm^3 and the 95% limits-of-agreement for this model were -38.10 to 38.31 cm^3. There was no trend for the mean to increase with the size of the difference (R^2=0.034, *P*=0.300).

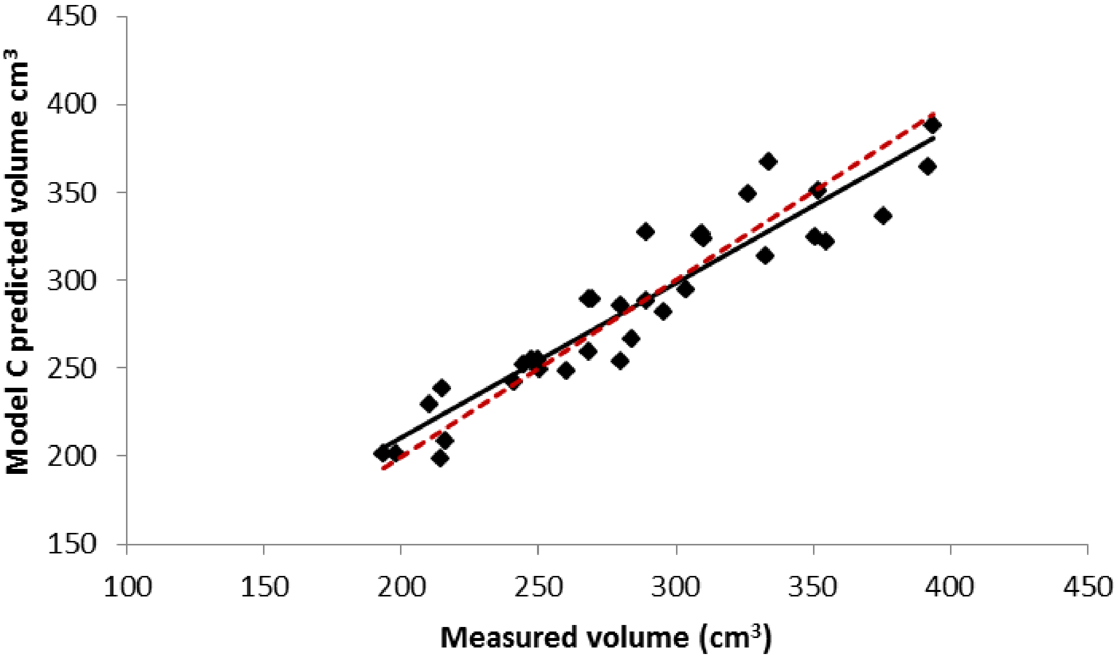

Figure 2.8: Scatter plot of predicted volume obtained using whole-foot Model C against measured volume (water displacement) obtained for 34 feet (right hind and left front), from 17 dairy cows.

Interpretation: Solid line is line of best fit for the model (R2=0.879, P<0.001), dashed line is the line of identity.

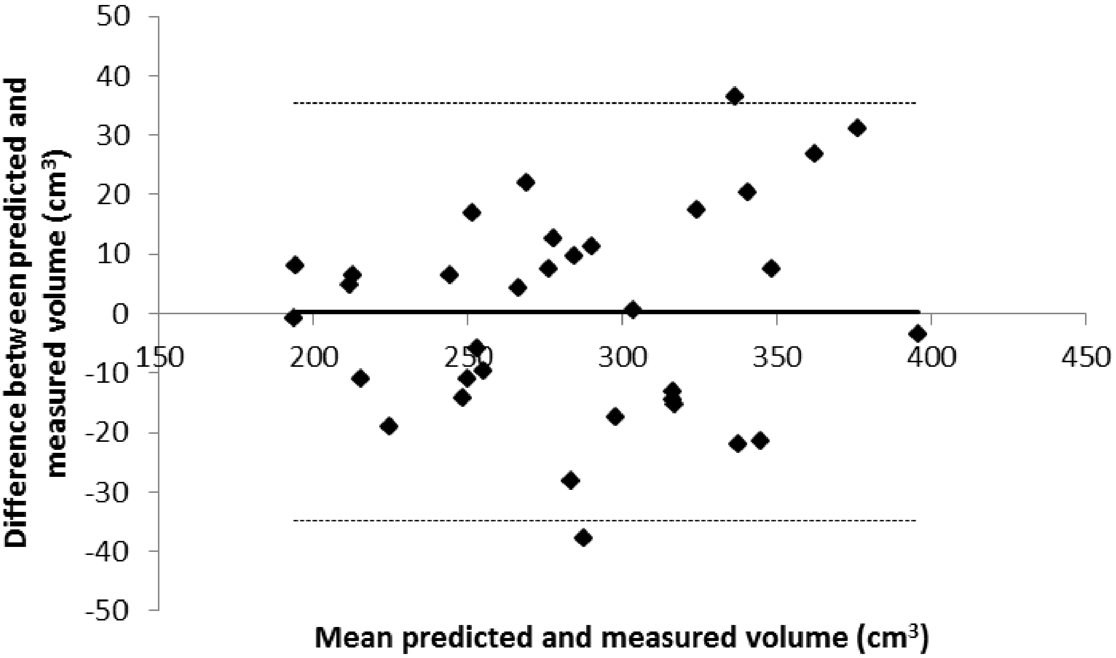

Figure 2.9: Limits-of-agreement plot showing the difference between predicted volume Model C and measured volume (water displacement) against the mean of the two methods (cm^3).

Interpretation: Solid line is the mean difference 0.10 cm^3. Dashed lines are the 95% limits-of-agreement range -38.10 to 38.31 cm^3. There was no significant trend in the data (R^2=0.034, *P*=0.300).

Procedure D

This procedure took the Model C variables and performed a backwards regression to see if further improvement of the model was possible. The best model which resulted (Model D) was:

Predicted volume = -346.11 + (23.79*total[a] proximal) + (8.52*total distal)

There was a significant association between predicted and measured volume (R^2=0.875, P<0.001 and CCC and mCCC were both high (0.94 and 0.88, respectively). Figure 2.10 illustrates the concordance of the model.

The mean difference between predicted and measured volume for Model D was -0.18 cm^3 and the 95% limits-of-agreement for this model were -39.0 to 38.6 cm^3. There was no trend for the mean to increase with the size of the difference (R^2=0.034, *P*=0.295).

The best whole-foot models for each procedure are summarized in Table 2.4.

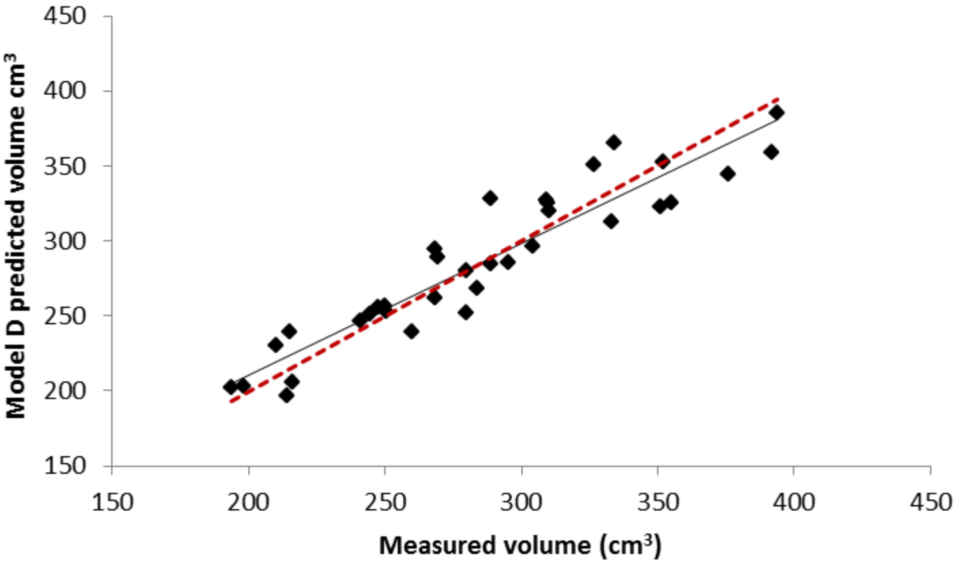

Figure 2.10: Scatter plot of predicted volume obtained using whole-foot Model D against measured volume obtained for 34 (right hind and left front), from 17 dairy cows.

Interpretation: Solid line is line of best fit for the model $R^2=0.875$, $P<0.001$), dashed line is the line of identity.

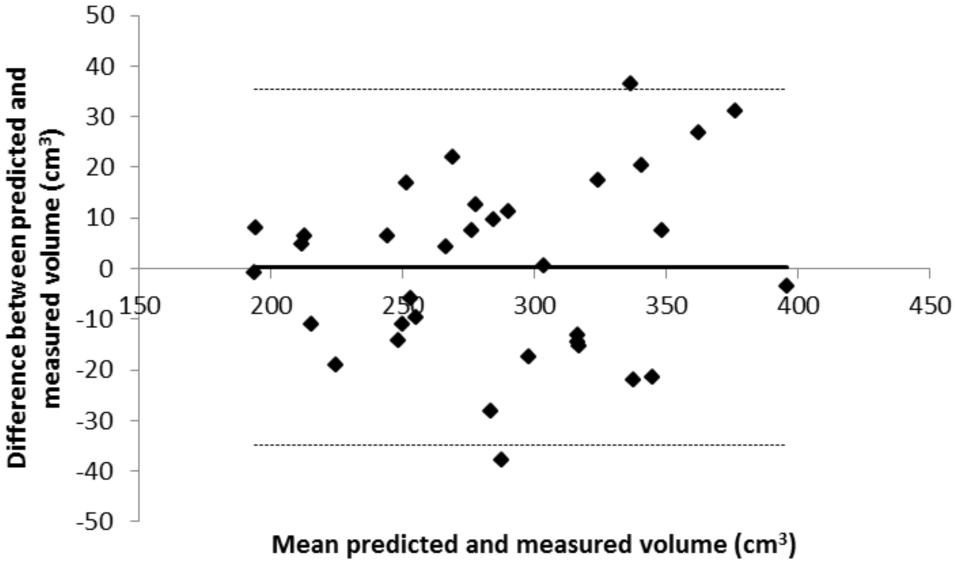

Figure 2.11: Limits-of-agreement plot showing the difference between predicted volume Model D and measured volume (water displacement) against the mean of the two methods (cm^3).

Interpretation: Solid line is the mean difference 0.18 cm^3. Dashed lines are the 95% limits-of-agreement range -39.0 to 38.6 cm^3. There was no significant trend in the data ($R^2=0.034$, $P=0.295$).

Table 2.4: Summary of the whole foot models investigated.

Best model for prediction of whole foot volume (cm^3) for each of the procedures A, B, C and D		R^2	R^2Adj	CCC	mCCC	Mean diff (cm^3)	SD
A	(17.19*total[a] distal) + (7.47*total abaxial groove) + (45.27*total proximal) - 798.5	0.877	0.873	0.17	0.13	223.97	58.86
B	-458.78 + (13.55*total proximal) + (15.5*total distal) + (1.69*total toe angle)	0.897	0.887	0.95	0.90	0.19	17.6
C	-351.41 + (20.17*total proximal) + (10.15* total distal) + (3.43* total abaxial groove)	0.879	0.866	0.94	0.88	0.10	19.1
D	-346.11 + (23.79*total proximal) + (8.52* total distal)	0.875	0.867	0.94	0.88	-0.19	19.4

[a], total figures include sum of measurements from medial and lateral claws; CCC, Lin's concordance coefficient; mCCC , Laio's improved concordance coefficient; Mean diff, mean difference between the prediction from the model and the water displacement measurement (cm^3); SD, Standard deviation of the difference between the two methods.

Experiment 1: Volumetric modelling - Individual claw modelling

Procedure E:

An individual-claw model was generated by Stepwise linear regression, performed on all variables. The model with the lowest AIC (see Table 2.5) (Model E) was:

Predicted volume = -113.55 + (26.58*Proximal) + (15.48*Claw category) + (-13.59*Age category)

There was a significant association between predicted and measured volume (R^2=0.804, P<0.001), but both CCC and mCCC were moderate (0.89 and 0.80, respectively). Figure 2.12 illustrates the concordance of the model.

There was a small but significant trend for the mean difference to increase with the size of the difference (R^2=0.057, *P*=0.049). However, because the size of the effect was small, the limits-of-agreement were calculated using the standard deviation of the difference between the two measures. The mean difference obtained between the predicted and measured volumes was - 0.040 cm^3, and the 95% limits-of-agreement were -25.01 to 24.92 cm^3.

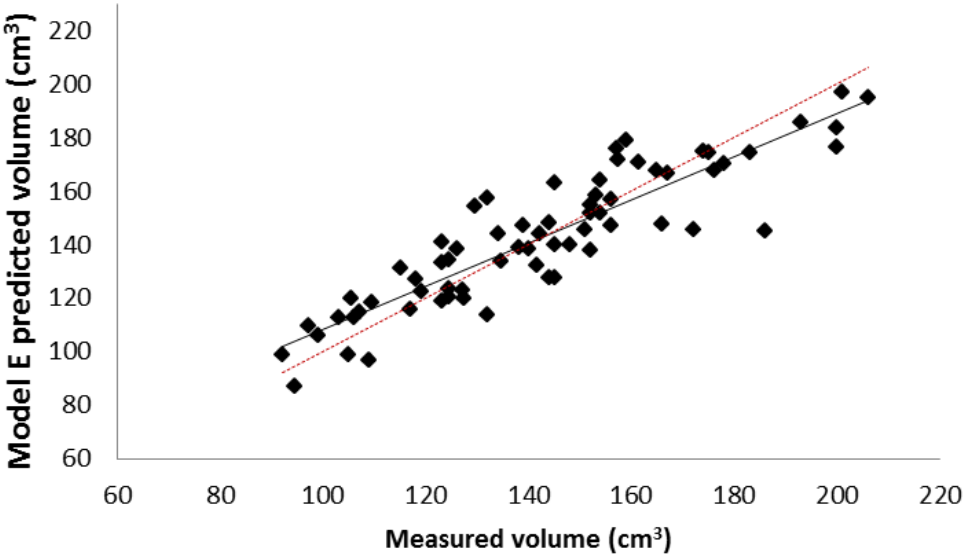

Figure 2.12: Scatter plot of predicted volume obtained using Model E against measured volume (water displacement) for the individual claws (medial and lateral) from 34 feet (right hind and left front), of 17 dairy cows.

Interpretation: Solid line is line of best fit for the model (R^2=0.804, *P*<0.001), dashed line is the line of identity).

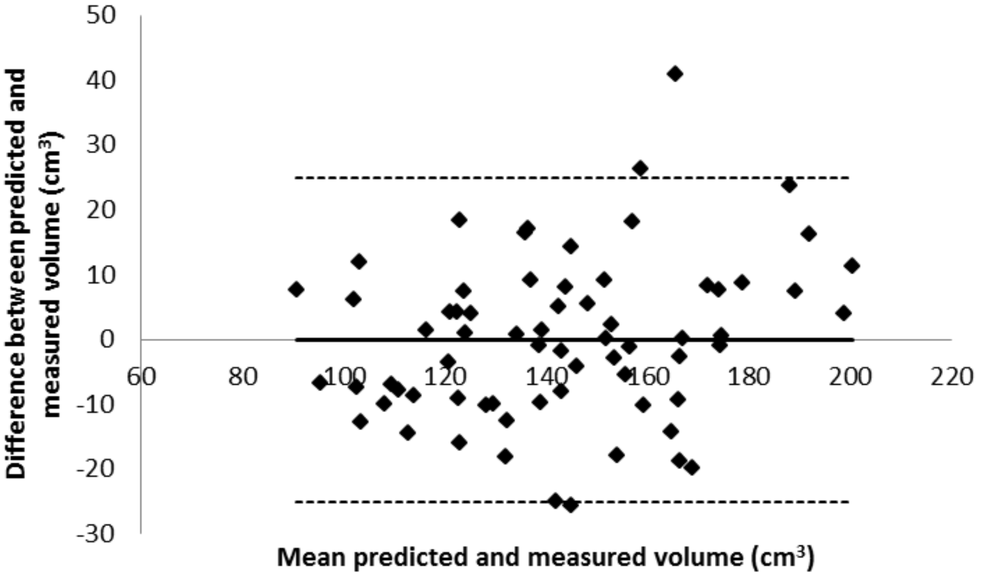

Figure 2.13: Limits-of-agreement plot showing the difference between the volumes (cm^3) predicted by Model B and measured volume (water displacement) against the mean of the two methods.

Interpretation: Solid line is the mean difference (-0.040), dashed lines are the 95% limits-of-agreement (-25.01 to 24.92 cm^3). Trend line for data points R^2=0.057, *P*=0.049.

Procedure F:

An individual claw model was developed by backwards linear regression, performed on all variables. Four models were generated. The model with the lowest value for AIC and C*p* closest to the number of variables included in the model was (Model F):

Predicted volume = -151.52 + (7.98*Foot category) + (8.32*Claw category) + (13.23*Claw length) + (21.52*Proximal) + (-6.62*Distal) + (5.32*Abaxial groove height) + (-14.16*Age category)

There was a significant association between predicted and measured volume (R^2=0.846, P<0.001) and CCC and mCCC were 0.92 and 0.85, respectively both higher than Model E. Figure 2.14 illustrates the concordance of the model.

Model F exhibited a mean difference between the methods of 0.048 cm^3 and 95% limits-of–agreement of -22.10 to 22.20 cm^3 (Figure 2.15). There was no significant trend in the data (R^2=0.044, *P*=0.087).

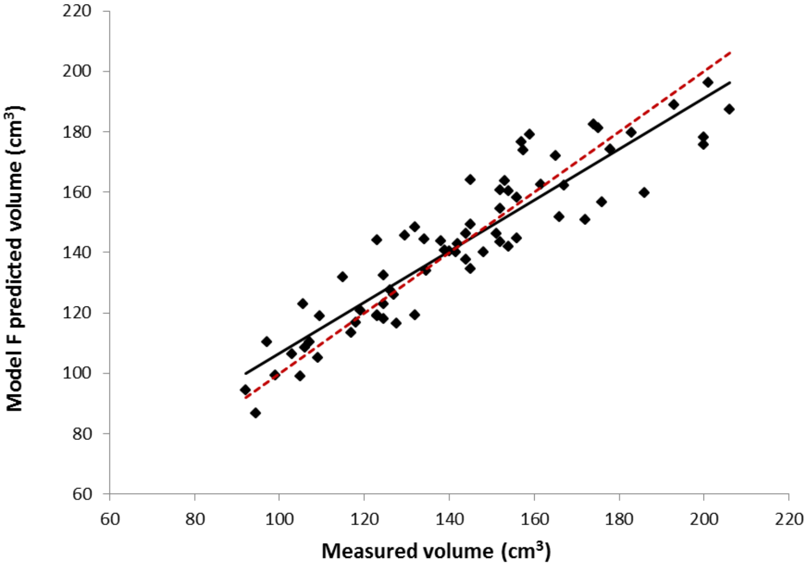

Figure 2.14: Scatter plot of predicted volume obtained using Model F against measured volume (water displacement) for the individual claws (medial and lateral) from 34 feet (right hind and left front), from 17 dairy cows.

Interpretation: Solid line is line of best fit for the model (R^2=0.846, *P*<0.001), dashed line is the line of identity.

Table 2.5: Summary of models generated for Procedure E

Model	Model Summary	R^2	Adjusted R^2	AIC	C*p*
1	(Constant), Proximal (D)	0.828	0.681	93.5	2
2	(Constant), Proximal (D), and claw category	0.878	0.765	90.8	-14
3	(Constant), Proximal (D), age and claw categories	0.897	0.795	90.6	-18

Table 2.6: Summary of models generated for Procedure F

Model	Model Summary	R^2	Adjusted R^2	AIC	C*p*
1	(Constant), age, claw and foot categories dorsal border (B), toe angle (A), claw width (G), abaxial groove(F), claw length (C), proximal (D), distal (E)	0.852	0.826	160.3	11
2	(Constant), age, claw and foot categories dorsal border (B), toe angle (A), abaxial groove(F), claw length (C), proximal (D), distal (E)	0.851	0.828	158.5	9.5
3	(Constant), age, claw and foot categories, toe angle (A), abaxial groove(F), claw length (C), proximal (D), distal (E)	0.849	0.829	156.9	8.3
4	(Constant), age, claw and foot categories , abaxial groove(F), claw length (C), proximal (D), distal (E)	0.846	0.828	155.6	7.6

AIC, Akaike Information Criterion; C*p*, Mallow's index

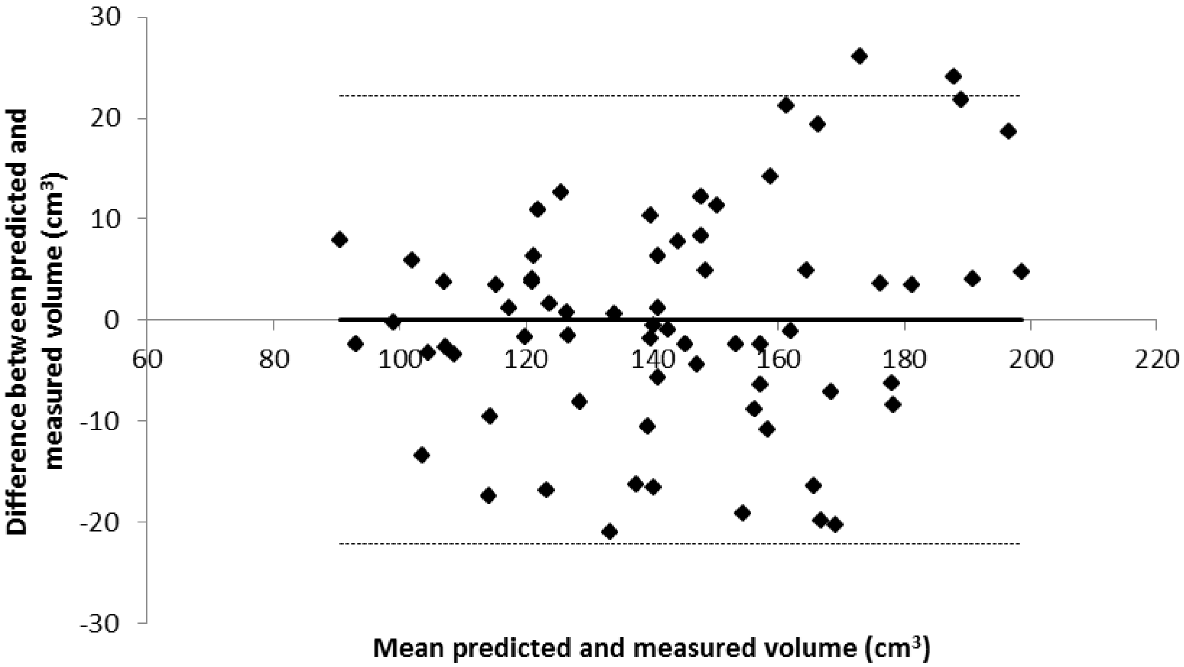

Figure 2.15: Limits-of-agreement plot showing the difference between the volumes (cm^3) predicted by Model F and the measured volume (water displacement).

Interpretation: Solid line is the line of fit (0.048), dashed lines are the 95% limits-of-agreement (-22.10 to 22.20 cm^3). There was no significant trend in the data (R^2=0.044, *P*=0.087).

Procedure G.

An individual claw model was investigated by backwards linear regression, performed on those variables that would translate easily to the live animal (i.e. claw width and claw length were not entered).

Five models were proposed (Table 2.7) AIC and Mallow's C*p* Index were calculated and the best model selected (Model G):

Predicted volume = -116.84 + (13.18*Claw category) + (24.38*proximal) + (4.49*abaxial groove) + (-14.62*Age category)

Table 2.7: Summary of models generated for procedure G

Model	Model Summary	R^2	Adjusted R^2	AIC	C*p*
1	(Constant), age, claw and foot categories, dorsal border (B), toe angle (A), abaxial groove(F), proximal (D), distal (E)	0.816	0.791	162.7	9
2	(Constant), age, claw and foot categories, dorsal border (B), abaxial groove(F), proximal (D), distal (E)	0.816	0.795	160.8	7.1
3	(Constant), age, claw and foot categories, dorsal border (B), abaxial groove(F), proximal (D)	0.816	0.798	158.8	5.1
4	(Constant), age, and claw categories, dorsal border (B), abaxial groove(F), proximal (D)	0.815	0.800	157.0	3.4
5	(Constant), age, and claw categories, abaxial groove (F), proximal (D)	0.814	0.802	155.2	1.9

There was a significant association between predicted and measured volume ($R^2=0.814$, $P<0.001$) and CCC and mCCC were 0.90 and 0.81, respectively. The values were both higher than Model E and not much lower than Model F. Figure 2.16 illustrates the concordance of the model.

For this model, the association between mean and difference approached significance ($R^2=0.054$, $P=0.056$). Mean difference was -0.024 cm^3, with the 95% limits-of-agreement ranging from -24.37 to 24.32 cm^3 (Figure 2.17).

The best individual-claw models for each procedure are summarized in Table 2.8.

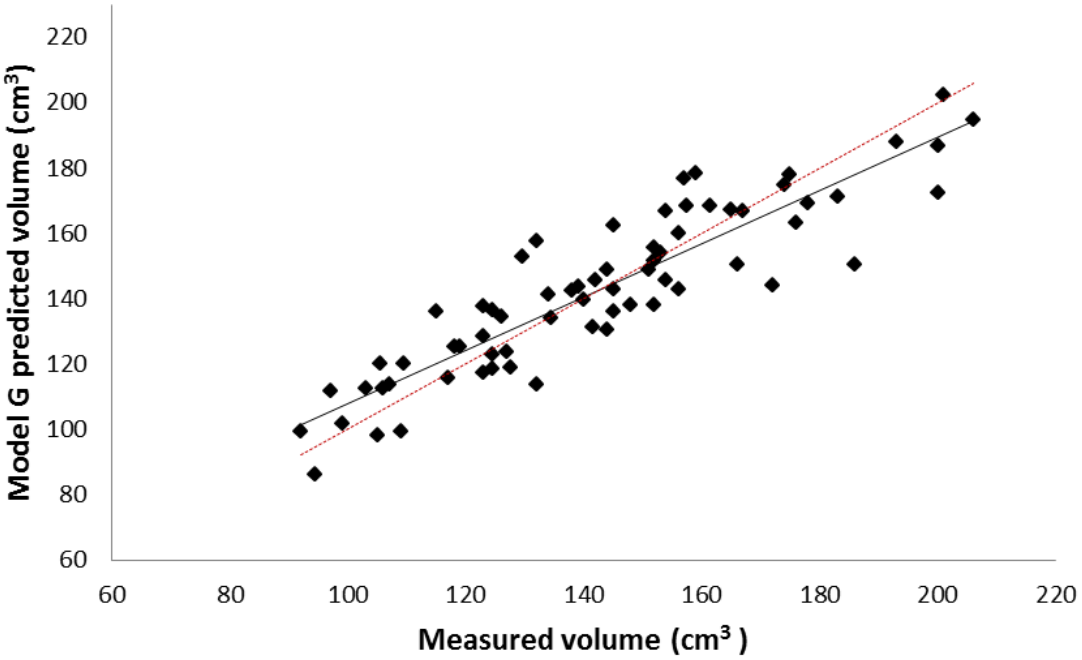

Figure 2.16: Scatter plot of predicted volume obtained using Model G against measured volume (water displacement) for the individual claws (medial and lateral) from 34 feet (right hind and left front), from 17 dairy cows.

Interpretation: Solid line is line of best fit for the model ($R^2=0.814$, $P<0.001$), dashed line is the line of identity.

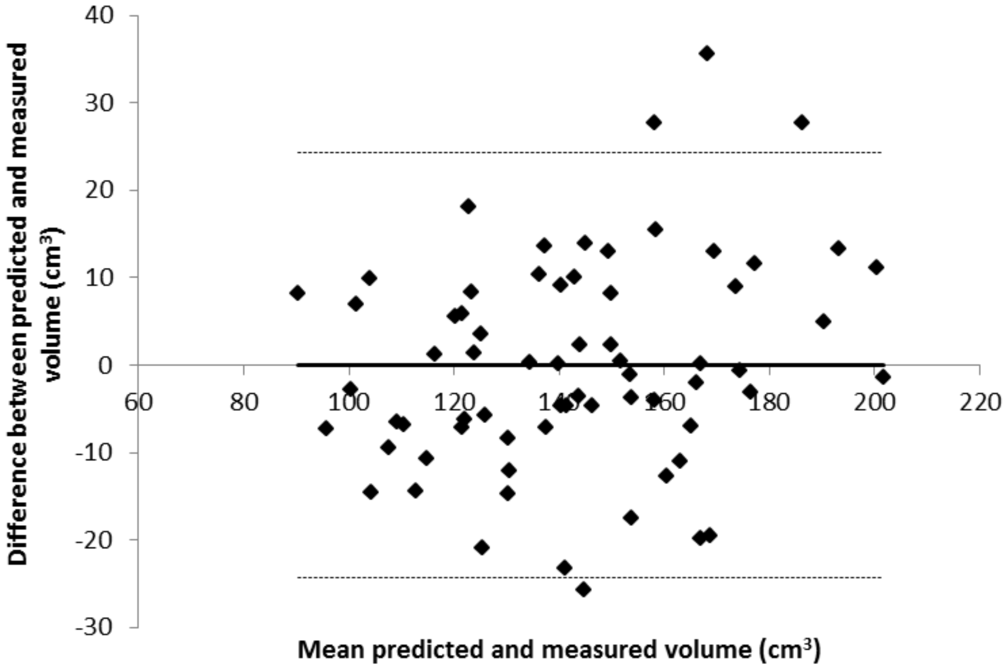

Figure 2.17: Limits-of-agreement plot showing the difference between the volumes (cm^3) predicted by Model G and the measured volume (water displacement).

Interpretation: Solid line is the mean difference (-0.024), dashed lines are the 95% limits-of-agreement (-24.37 to 24.32 cm^3). The trend in the data approached significance at (R^2=0.054, *P*=0.056).

Table 2.8: Summary of the individual-claw models investigated.

Best model for prediction of individual claw volume (cm^3) for each of the procedures E, F and G.		**R^2**	**R^2Adj**	**CCC**	**mCCC**	**Mean diff**	**SD**
E	-113.55 + (26.58*Proximal) + (15.48*Claw category) + (-13.59*Age category)	0.804	0.795	0.89	0.80	-0.04	12.48
F	-151.52 + (7.98*Foot category)+(8.32*Claw category) + (13.23*Claw length) + (21.52*Proximal) + (-6.62*Distal) + (5.32*Abaxial groove height)+(-14.16*Age category)	0.846	.828	0.92	0.85	0.048	11.07
G	-116.84+(13.18*Claw category)+(24.38*proximal)+(4.49*abaxial groove)+(-14.62*Age category)	0.814	.802	0.90	0.81	-0.024	12.17

CCC, Lin's concordance coefficient; mCCC, Laio's improved concordance coefficient; Mean diff, mean difference between the prediction from the model and the water displacement measurement (cm^3); SD, Standard deviation of the difference between the two methods. For summary of linear regression procedures used see Table 2.1.

Experiment 2: Investigation the relationship between predicted volume and calliper measurements of sole thickness and soft tissue depth.

In total 156 claws from 41 animals were included in the analysis. Data from eight claws was excluded because the distance between the calliper estimates was < 25 mm i.e. the distance between Site 1 and Site 2 was too short.

Sole thickness

There was a small but significant relationship between the predicted volume and measured sole thickness at Site 1 ($R^2=0.053$, $P<0.004$). At Site 2 this relationship was stronger ($R^2=0.087$, $P<0.001$) (see Figure 2.18 and 2.19), respectively.

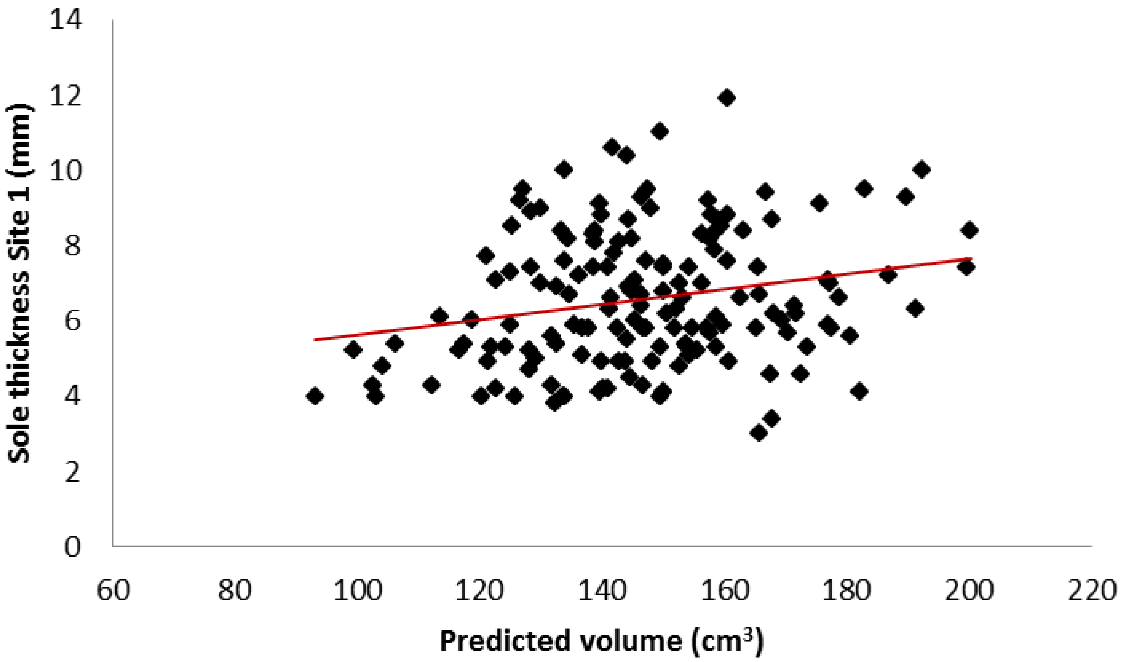

Figure 2.18: Predicted claw volume (cm^3) plotted against sole thickness (mm) at Site 1 (tip of the distal phalanx), as measured with electronic callipers, in the frozen claw, for 156 claws from the left front and right hind feet of 41 dairy animals. Missing data accounts for eight claws.
Interpretation: Solid line is the line of best fit ($R^2=0.053$, $P<0.004$).

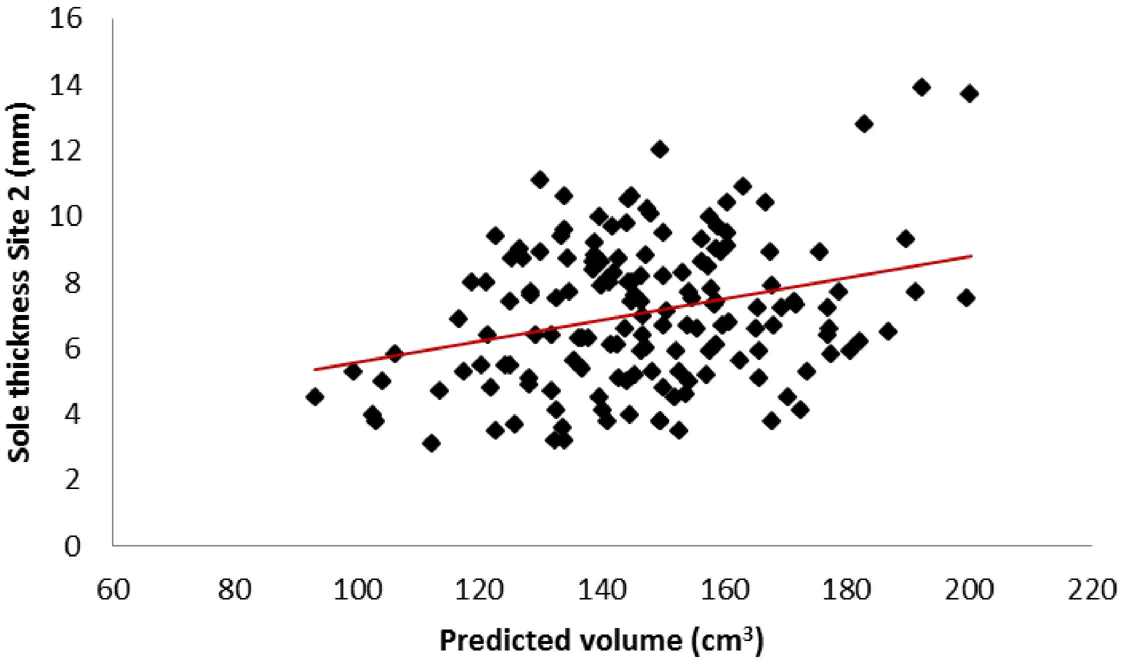

Figure 2.19: Predicted claw volume (cm^3) plotted against sole thickness (mm) at Site 2 (25mm towards the heel from the tip of the distal phalanx) measured with electronic callipers, in the frozen claw, for 156 claws from the left front and right hind feet of 41 dairy animals. Missing data accounts for 8 claws.

Interpretation: Solid line is the line of best fit (R^2=0.087, *P*<0.001).

Soft tissue depth

There was a small but significant relationship between the predicted volume and measured soft tissue thickness (R^2=0.110, P<0.001) at Site 1 (Figure 2.20). At Site 2 this relationship was stronger (R^2=0.192, P<0.001). Figure 2.21 illustrates the relationship at Site 2.

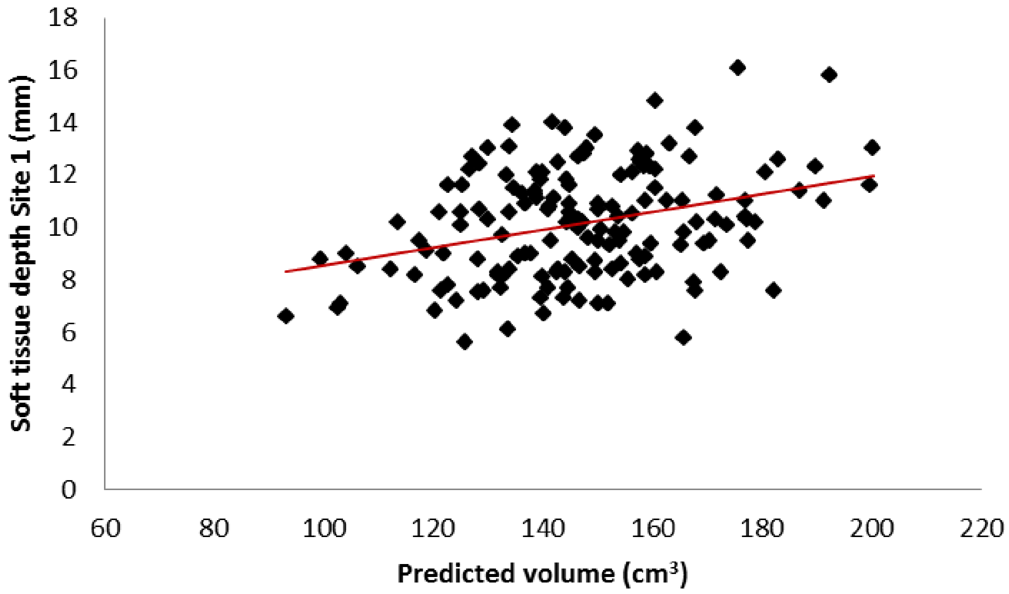

Figure 2.20: Predicted claw volume (cm^3) plotted against soft tissue depth (mm) at Site 1 (tip of the distal phalanx) measured with electronic callipers, in the frozen claw, for 156 claws from the left front and right hind feet of 41 dairy animals. Missing data accounts for eight claws.

Interpretation: Solid line is the line of best fit (R^2=0.110, *P*<0.001)

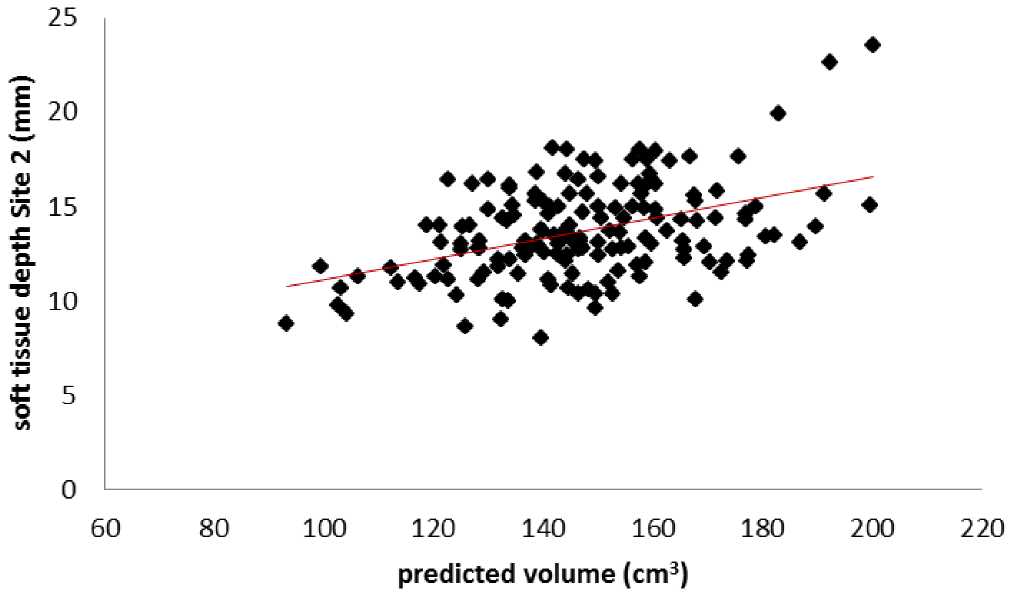

Figure 2.21: Predicted claw volume (cm^3) plotted against soft tissue depth (mm) at Site 2 (25mm towards the heel from the tip of the distal phalanx) measured with electronic callipers, in the frozen claw, for 156 claws from the left front and right hind feet of 41 dairy animals. Missing data accounts for eight claws.

Interpretation: Solid line is the line of best fit (R^2=0.192, *P*<0.001)

Discussion

The first aim of this study was to evaluate how well the model developed by Scott et al. (1999) created in trimmed even beef cows feet worked in feet collected from New Zealand dairy animals. Correlation between measured volumes and predicted volumes using that model suggested that it worked well (r=0.877, *P*<0.001). However, correlation is a measure of association rather than agreement (Altman and Bland, 1983), and this study has highlighted the importance of also assessing agreement. Two measures of agreement were used in this analysis: Concordance (via concordance correlation coefficients) and limits-of-agreement. The first evaluates how well the data clusters around the line of identity, the second analyses the distribution of the difference between the methods. Together they allow the search for biased or homogenous (clumpy) data (Atkinson and Nevill, 1997). Both of the concordance correlation coefficients used showed that volumes predicted using the Scott et al. (1999) model were markedly different from measured volumes obtained; and the limits-of-agreement demonstrated that the difference between the two methods increased with hoof (whole-foot) volume (Figure 3) even though the ratio between the two measures remained constant. The predicted volume using the Scott et al. (1999) model was on average, 1.78 times the measured volume, with a 95% limits-of-agreement of 1.53 to 2.04.

One potential reason for this difference may be inaccuracies in the measurement of volume using water displacement. The claws for this study were neither clipped nor fully disarticulated as described by Scott et al. (1999). Furthermore, the assayed claws were of all shapes and sizes, rather than trimmed and uniform as described by other studies (Phillips et al. 1996; Scott et al. 1999). However, it is extremely unlikely that these factors would have resulted in such a large discrepancy between this study and that of Scott et al. (1999). Moreover, measuring claws which have not been trimmed to a standardised shape could mean that the model more realistically reflects the range of claw conformation seen in the live animal prior to human intervention.

This study used a different method to measure water displacement to that of Scott et al. (1999), because it was not possible to determine easily when equilibrium had been achieved. A similar problem seems to have been encountered by Clark et al. (2004) who, in their study relating claw volume to the risk of sand cracks, collected displaced water over a fixed 5 minute period rather than simply recording the total amount of water displaced. It is unlikely that this difference in method would have had such a dramatic effect on measured volume as seen in Table 2.2, particularly as the measurements were made to the nearest 0.5 cm^3 and the correlation between the two measurements per claw was very high ($r=0.966$; $P<0.001$). Similarly measurement error in the conformation variables, while undoubtedly present, would not impact on the outcome to this extent. The mean value was used for modelling and the intra-assay coefficient for the two measurements taken ranged from 0.55% to 1.06%, although measures were not blinded or separated by time this does indicate that the landmarks could be employed with acceptable accuracy for field application.

One unavoidable limitation of this study was the restricted number of heifer limbs available for volume assessment. Only four of the seventeen animals were 30 months old or less. Nevertheless, age category was selected in the final model, supporting Scott's contention that a model developed from young beef animals might not be directly applicable to animals of other ages. None the less, the effect of age was relatively small. The best individual claw model predicted that cattle 30 months old and under would have claws that were each 14.62 cm^3 smaller than older cattle, a figure which is much smaller than the difference of 253 cm^3

Conformational differences between beef and dairy animals have been previously described (Browne et al., 2007). A small (n=8) post mortem study revealed statistically significant differences with respect to toe angle, claw and heel height, as well as sole thickness and soft tissue depth between the claws of beef and dairy heifers. In dairy heifers toe angle was shallower and sole width, heel and claw height smaller. Sectioned claws revealed this to be true for sole horn thickness and soft tissue depth too Browne et al. (2007).

The marked overall reduction in volume for this study compared to Scott et al. (1999) cannot simply be explained in terms of a reduction in the size of conformational parameters contributing to the volume calculation. If the dairy feet studied were just "scaled down" versions of the beef feet studied by Scott et al. (1999), a reduction of approximately 20% would be expected for each parameter. However, the values for dorsal border and abaxial groove length were notably larger (Table 2.2) in the dairy animals used for the present study (for the hind limbs 16.08 cm vs. 13.40 cm and 10.64 cm vs. 8.90 cm, respectively) whilst the proximal and distal values equated to those of Scott et al. (1999). These observations indicate that the relationship between foot (claw) conformation parameters is not the same in the feet of beef and dairy cattle and may well be the explanation of the large difference between measured and predicted volume. This supports the search for a more appropriate model, aligned to type, age and the production system represented in the current study. In other words because the dairy feet parameters differ in their relationship to each other, it is reasonable to expect that their relationship to claw volume would differ; a conclusion which is borne out by the differences seen in the final equations generated. Ideally the volume equations created in the present study should be tested on another set of dairy cattle feet to confirm and if necessary refine the model for the New Zealand dairy animal.

The best whole-foot model, developed using linear regression modelling of variables in the present study, was Model B. This model produced high concordance coefficients (CCC 0.95, mCCC 0.90) and acceptable 95% limits-of-agreement (-35.0 to 35.3 cm^3) for whole-foot volume, i.e. if the model predicted a whole-foot volume of 300 cm^3 measured volume would be between 265 and 335 cm^3, 95% of the time.

Interestingly, the first parameters that dropped out of the model during the regression process were the age and foot categories, which is in agreement with Scott et al. (1999). An attempt to improve on Model B by forcibly entering the parameters of Scott et al. (1999) resulted in Model C, whose concordance coefficients (CCC 0.94, mCCC 0.88) and 95% limits-of-agreement (-38.1 to 38.3 cm^3) for whole-foot volume, were not as good as for Model B. Using backwards rather than stepwise regression was also unsuccessful in improving concordance or agreement.

Modelling whole foot volume rather than individual medial and lateral claws means that examination of changes in, and comparisons between, the individual claws cannot be made. Clark et al. (2004) found that claw position was significantly linked to claw volume and also the presences of vertical fissures. Bryan et al. (2012) demonstrated a link between the magnitude of the difference in claw height between the two claws of a foot, and the incidence of lameness in dairy cows. This implicates claw volume (particularly differences in claw volume) as a factor which can alter the risk of lameness, and highlights the possibility of prophylaxis via selection on phenotype as proposed by Phillips et al. (1996). The ability to examine individual claw volume against changes in conformational parameters and lesion development was a driver for the development of an individual claw model as a more useful tool for the evaluation and investigation of cattle lameness.

For individual claw volume, Model F was the most successful, with concordance coefficients of (CCC 0.92, mCCC 0.85) and 95% limits-of-agreement range (-22.1 to 22.2cm^3). Thus predicted value of 150cm^3 would be between 128 and 172 cm^3, 95% of the time.

However, to aid the use of a volume estimation model in the live animal (Phillips et al., 1996; Scott et al., 1999) only parameters easy to obtain under field conditions were used to produce (Model G). Concordance coefficients for this model were CCC 0.90, mCCC 0.81, with a 95% limits-of-agreement range -24.4 to 24.3 cm^3. Therefore for Model G, a predicted value of 150cm^3 could be between 126 and 174cm^3, 95% of the time. This meant that the use of these more practical measurements resulted in a small loss in concordance of the model and an increase of 4 cm^3 in the range of the limits of agreement. The advantages associated with the increased feasibility of this model in the field more than outweigh the small decrease in agreement between predicted and measured volumes.

Model G (Predicted claw volume = -116.84 + [13.18*Claw category] + [24.38*proximal] + [4.49*abaxial groove] + [-14.62*Age category]) was used to predict the volume of 156 claws from 41 animals and, thereafter, to allow linear regression of this predicted volume against calliper measures of sole thickness and soft tissue depth. Small but significant relationships between claw volume and both sole thickness and soft tissue depth were found at Site 2 in particular. Such an outcome would be compatible with the functional anatomy of the two sites used. Site 2 incorporates elements of the digital cushion which plays a protective role within the claw, protecting internal structures by absorbing the force of impact applied during locomotion (Räber et al., 2004), therefore it would be reasonable to expect that the capacity of this cushion (reflected in the measure soft tissue depth) would have a relationship to the volume of the claw.

This result supported the use of individual claw Model G to assess the relationship between volume and other conformational parameters in the live animal over time (Chapter 6).

Conclusion

In conclusion, this study has supported the initial hypothesis and in turn demonstrated the importance of evaluating agreement between measurement methods, as well as demonstrating correlation between them. Development of a targeted model to predict claw volume in the New Zealand dairy animal has strongly suggested that beef and dairy animals have distinct differences in their claw conformation and supports the proposal that the age of animal is important in this model (Scott et al., 1999). The investigation of variables which can be readily assayed in the live animal, in addition to individual claw volume modelling, has the potential to allow the monitoring of conformational change alongside individual claw lesion extent and severity scoring.

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Chapter three: Validation of a portable ultrasound machine for the assessment of sole thickness in the New Zealand dairy cow.

Introduction

Sole horn offers protection to the underlying soft tissues contained within the claw capsule. In cattle, the wall and sole of the heel should form the main weight bearing surface of the claw for the transmission of forces during locomotion (Toussaint Raven, 2003). Without sufficient sole horn, trauma can lead to hoof horn haemorrhage and subsequent adverse effects on horn growth, wear, quality and claw conformation (van Amstel and Shearer, 2001).

In lactating cattle, exposure to abrasive surfaces and walking tracks result in the loss of the natural concavity of the sole surface and the inclusion of additional regions of the sole in to the weight bearing surface (Tranter and Morris, 1992; Livesey and Laven, 2007; Telezhenko et al., 2009). Sole horn is inherently softer than wall horn (Budras et al., 1996) and is thought to grow more slowly (Greenough et al., 1990; van Amstel and Shearer, 2001), all of which suggest that sole horn is more vulnerable to wear than wall horn. Sole horn wear is most prominent along the abaxial margin of the claw ground surface and wear rate varies along this margin such that it is greater in the middle section than at the extremities (Tranter and Morris, 1992). Consequently, the junction between wall and sole horn (the white line) is at risk when there is expansion of the weight bearing surface to include the sole resulting in increased mechanical loading across the white line.

The white line is composed of three portions (inner, outer and middle: Budras et al., 1996) and structural differences within the white line regions have been demonstrated (Kempson and Logue, 1993; Budras et al., 1996). The composite structural architecture of the white line is aligned to resist separation between the white line and adjoining wall and sole horn while acting to allow transfer of dynamic forces (Kempson and Logue, 1993). Increased exposure to shearing forces and load bearing extending onto the sole surface and the interruption of quality horn production can weaken the white line horn (van Amstel and Shearer, 2001) and risk white line separation and disease.

Further to this it has been proposed that the disparity in horn growth rates either side of the white line may act to induce tension across the white line and in turn lead to white line disease (Livesey and Laven, 2007). In fact, Budras et al. (1996) revealed that horn growth rate varies within the white line itself and lesions are particularly associated with areas of high cell turnover (rapid horn production) within the length of the white line - especially the abaxial and axial extremities. Such areas are vulnerable to alteration in circulation as well as mechanical pressures. Insults such as altered mechanical loading and poor quality horn production related to circulatory change will facilitate the development of white line lesions (Livesey and Laven, 2007), sole bruising, foreign body penetration and development of sole abscesses (Tranter and Morris, 1992).

Thickness of the sole horn in lactating cattle therefore has a significant impact on resistance to lameness. Toussaint Raven (2003) proposed that a sole thickness of approximately 7 mm at the toe and 5 mm in the centre of the sole would provide adequate protection against outside mechanical and chemical influences under most environmental conditions. This idea was supported by van Amstel et al. (2003), who stated that optimal sole thickness may also be dependent on environmental and management conditions. No data are available on optimal sole thickness for dairy cattle in the pasture-based systems which predominate in New Zealand.

Measurement of sole thickness would therefore be useful for predicting potential problems and for confirming the involvement of thin soles in lameness outbreaks. Shearer and van Amstel (2001) described how thin soles could be detected by digital pressure, but this is an insensitive and subjective technique (van Amstel et al., 2003), with limited utility for identifying changes in sole thickness. An alternative technique is the use of toe length as a proxy for sole thickness (Shearer and van Amstel, 2001; Toussaint Raven, 2003; Nuss and Paulus 2006). However, the agreement between dorsal wall length and sole thickness is probably not sufficient for it to be used as a surrogate measure of sole thickness: van Amstel et al. (2002) reported that a toe length of 7.5 cm was associated with a sole thickness of between 4 and 14 mm. Additionally, inadequate sole thickness ('thin soles') is often considered to be a significant problem in heifers (e.g. Dewes, 1978) and it is quite possible that the relationship between toe length and sole thickness will differ between heifers and cows, as heifers are still growing and tend to have shorter toes than adult cows (Hahn et al., 1984; Ahlström et al., 1986; Offer et al., 2000).

Monitoring sole horn growth and wear could potentially provide useful information on sole thickness (which ultimately depends on the balance between growth and wear of the sole horn). Tranter and Morris (1992) were able to measure sole wear rates by creating grooves in the sole horn but were not able to estimate sole growth. Without a definitive figure for sole growth, sole wear rates are of limited value. For example, increased wear could actually be accompanied by thickening of the sole due to an increase in the growth rate, as is seen in wall horn (Livesey and Laven, 2007).

Ultrasonography is probably the most practical method of measuring sole thickness *in vivo.* Kofler et al. (1999) showed that it was possible, using a high specification ultrasound machine (Sonoline Versa Pro, Siemens), to image the internal structures of the bovine claw. With the ultrasound transducer applied to the sole surface, Kofler et al. (1999) were able to estimate sole and soft tissue thickness in claws from bovine cadavers. They reported that the correlations between ultrasound estimates of sole thickness and anatomical measurements (made using mechanical callipers) of the same parameters in the claws after sectioning were highly significant (r was between 0.88 and 0.91). They did not assess agreement between values obtained by the two methods of estimation and it is clear from the data which they presented that there were differences between the two measures even though they were highly correlated. For example in claws with a sole thickness >5mm, mean sole thickness estimated using ultrasound was 6.4 mm, whereas that measured after sectioning was 7.6 mm.

Kofler et al. (1999) also attempted to image the internal structures of the bovine claw using a portable ultrasound machine (Logic 100 machine, Kranzbühler) but they reported that they were not able to reliably identify the structures. The images obtained with a portable machine were of lower quality than those produced by a higher specification machine perhaps due to the poorer resolution of the transducer. In contrast, van Amstel and co-workers (van Amstel et al., 2003, 2004a) reported that they were able to measure sole thickness using portable machines (an Aloka 500 and a Toshiba SSA320, respectively). However, although both papers showed images where the inner margin of the sole could be clearly seen, neither demonstrated how accurately the ultrasound measurements reflected actual sole thickness i.e. the agreement between the two methods.

Hypothesis: It was postulated that a portable ultrasound machine, could be used for the estimation and monitoring of mean sole thickness and/or distance to distal phalanx (external sole surface to palmar/plantar surface of the distal phalanx) in a representative group of animals. Or, as an alternative, a portable machine could be utilised to categorise animals as having thin or adequate sole thickness.

This chapter aims to compare measures obtained using a portable ultrasound machine (fresh claw material) to those estimated with electronic callipers (claws frozen and sectioned). Estimates will be obtained by both methods for sole thickness (external sole surface to internal sole surface) and the distance to distal phalanx (i.e. from the external sole surface to the distal phalanx). Values from the two methods of estimation will subsequently be compared to establish if the agreement is sufficient to allow ultrasound to be used as a non-invasive instrument to monitor changes in sole thickness over the course of lactation in first lactation dairy heifers.

Materials and methods

The distal limbs were collected from 24 dairy cows. The age, breed and cause of death of each animal were recorded. Eight had died within the previous 24 hours, three had been euthanased on-farm and the remainder were collected from a local abattoir. None of the cattle had died/been euthanased for conditions related to lameness.

Each limb was identified on removal using an elastic band of a specified colour (red: right fore, yellow: left fore, blue: left hind, green: right hind) which remained with the limb throughout processing. The limbs were washed after collection to remove any mud or manure, and a latex glove was placed over the sawn end of the limb to prevent moisture loss. All four limbs from each animal were placed in an individual plastic bag, sealed with a cable tie, and stored at 3 to

5°C, prior to ultrasound assessment. All ultrasound measurements were completed within 72 hours post mortem.

Ultrasound assessment of claws

Ultrasonography was undertaken using a Mindray DP 6600 (Mindray, Szechuan, China) portable ultrasound machine with a variable frequency, linear array probe. Prior to examination the sole surface of each claw was lightly pared and the transducer was placed along a line perpendicular to and bisecting the line from the abaxial groove to the axial border, which was also perpendicular to a line from the end of the axial white line to the abaxial border (modified from Lischer et al., 2002). The probe was protected by a vinyl glove containing acoustic coupling gel and additional gel was also applied to the claw surface to aid contact between the claw surface and transducer. The ultrasound measurements were taken at two sites; the tip of the distal phalanx (Site 1) and 25 mm towards the heel (Site 2). All measurements were completed with the probe set at a frequency of 5 MHz. This frequency was established as the best of the available settings on the ultrasound machine (5, 7.5 or 10 MHz.) for efficient imaging and recognition of the internal surface of the sole. To confirm the level of the internal sole surface ahead of the current study an excoriated hypodermic needle was inserted into a sectioned claw during ultrasonography. The internal sole surface was visualised as a thin hyperechoic line of a continuous or interrupted nature above an anechoic region as described by Kofler et al. (1999) and van Amstel et al. (2003) in their studies.

Once an image was obtained the ultrasound screen was frozen to capture a static image and two measurements immediately taken at each site; the distance to the distal phalanx from the external sole surface (DP) and the distance from the external sole surface to the internal sole surface (STh).

Calliper measurement of distance to distal phalanx and sole thickness

Following ultrasound assessment the limbs were frozen at –20°C for at least 24 hours before being sectioned. The frozen claws were sectioned using a band saw sagittally along the line where the transducer had been placed. DP and STh were measured using electronic callipers at Sites 1 and 2

Assessment of dorsal border and toe angle relationship to sole thickness.

Feet from a total of 41 individual animals were used (distal limbs sourced and processed as above) to investigate the relationship between dorsal border length (cm), toe angle (°) and calliper measurements of sole thickness and soft tissue thickness (mm). The majority (32/41) were made within 96 hours, but for five animals measurements were made within 5-7 days, and for four animals within 8 - 9 days of death.

Statistical analysis

The correlation between calliper and ultrasound measurements was evaluated for each site using Pearson's correlation coefficient (r).

Limits-of-agreement plots (Bland and Altman, 1999) were used to examine the agreement between the two methods, and the potential use of the variables investigated for prediction and monitoring of sole thickness and distance to distal phalanx in the live animal.

Univariate analysis of covariance was then used to evaluate the effect of limb and claw on the relationship between distance to distal phalanx measured using ultrasound and STh measured using callipers, for Site 1. The limits-of-agreement method was then used to estimate the agreement between these two measures for claws of the hind foot only.

Receiver operator characteristic (ROC) curves were employed to investigate the ability of ultrasound measurement of distance to distal phalanx at Site 1 to categorise hind claws as having thin or adequate sole thickness, using a threshold of <5 mm sole thickness (by calliper estimation) to define a thin sole (van Amstel et al., 2003). Two cut off points were chosen using this analysis. The first cut-off point, the value below which sole thickness was likely to be <5 mm, was identified by maximising the specificity but maintaining sensitivity at >0.9. The second cut-off point, the value above which sole thickness was likely to be ≥5 mm, was identified in the same way.

The relationship between dorsal border length, toe angle and calliper sole thickness for the 41 individuals assessed was investigated using Pearson's correlation coefficient (r) and backwards linear regression (R^2). All statistical analyses were undertaken using SPSS18 (SPSS Inc. Chicago, USA).

Results

Ultrasound assessment of claws

Images were obtained from 92 of the 96 claws assessed. No ultrasound images could be obtained from the claws of Cow No 32. In addition, sole thickness could not be measured at Site 11 in two claws and at Site 22 in five claws.

The correlations between the estimates for sole thickness and distance to the distal phalanx obtained using ultrasound and those derived from measurements taken on the sectioned hoof are presented in Table 3.1.

All four calliper measurements were highly correlated (r ranged from 0.71 to 0.92, $P<0.001$). The correlation between the four ultrasound measurements was lower (r ranged from 0.24 to 0.74), but remained significant ($P<0.001$ for all comparisons, except between STh at Site 2 and the two DP measures, which were $P=0.028$ and 0.025).

The comparison of the ultrasound and electronic calliper measurements showed that the correlation was higher for estimates of DP than STh and for measurements made at Site 1 than Site 2.

The correlations between the calliper and ultrasound measurements of distance to distal phalanx (DP1 and DP2) were both >0.84 ($P<0.001$), whereas for STh1 the correlation was 0.51 (P<0.001) and for STh2 there was no significant correlation (r=0.10, P=0.343). At both Sites 1 and 2, there was a higher correlation between the ultrasound estimate of DP and the calliper STh than there was between ultrasound DP and the ultrasound estimate of STh. At Site 1, the correlation between ultrasound DP1 and calliper STh1 was r=0.80 compared with r=0.51 for the two STh measurements. At Site 2 the correlations coefficients were r=0.71 and r=0.10, respectively.

Further evaluation of the association between measurements using ultrasound and anatomical measurements was restricted to DP1 (ultrasound and calliper) and calliper sole thickness at Site 1. Figure 3.1 illustrates the relationship between DP as measured using ultrasound and calliper measures of STh and DP at Site 1.

Table 3.1: Pearson correlation coefficients for estimates of sole thickness (STh) and distance to distal phalanx (DP) measured using ultrasound (US) in the intact claw and using electronic callipers after sectioning of the frozen claw on a band saw (A). Measurements were made at the tip of the distal phalanx (Site 1) and 25 mm along the solar surface of the claw towards the heel (Site 2).

Numbers emboldened: *P*<0.01; Numbers in italics: *P*<0.05
Number of claws recorded by ultrasound measure: STh1: 90; STh2: 87; DP1:92; DP2: 92. All calliper measurements were from a total of 96 claws

	US DP1	**US STh2**	**US DP2**	**A STh1**	**A DP1**	**A STh2**	**A DP2**
US STh1	**0.74**	**0.46**	**0.45**	**0.51**	**0.60**	**0.35**	**0.37**
US DP1		*0.24*	**0.62**	**0.80**	**0.89**	**0.06**	**0.57**
US STh2			*0.24*	0.17	*0.24*	0.10	0.21
US DP2				**0.60**	**0.64**	**0.71**	**0.84**
A STh1					**0.92**	**0.81**	**0.71**
A DP1						**0.77**	**0.75**
A STh2							**0.90**

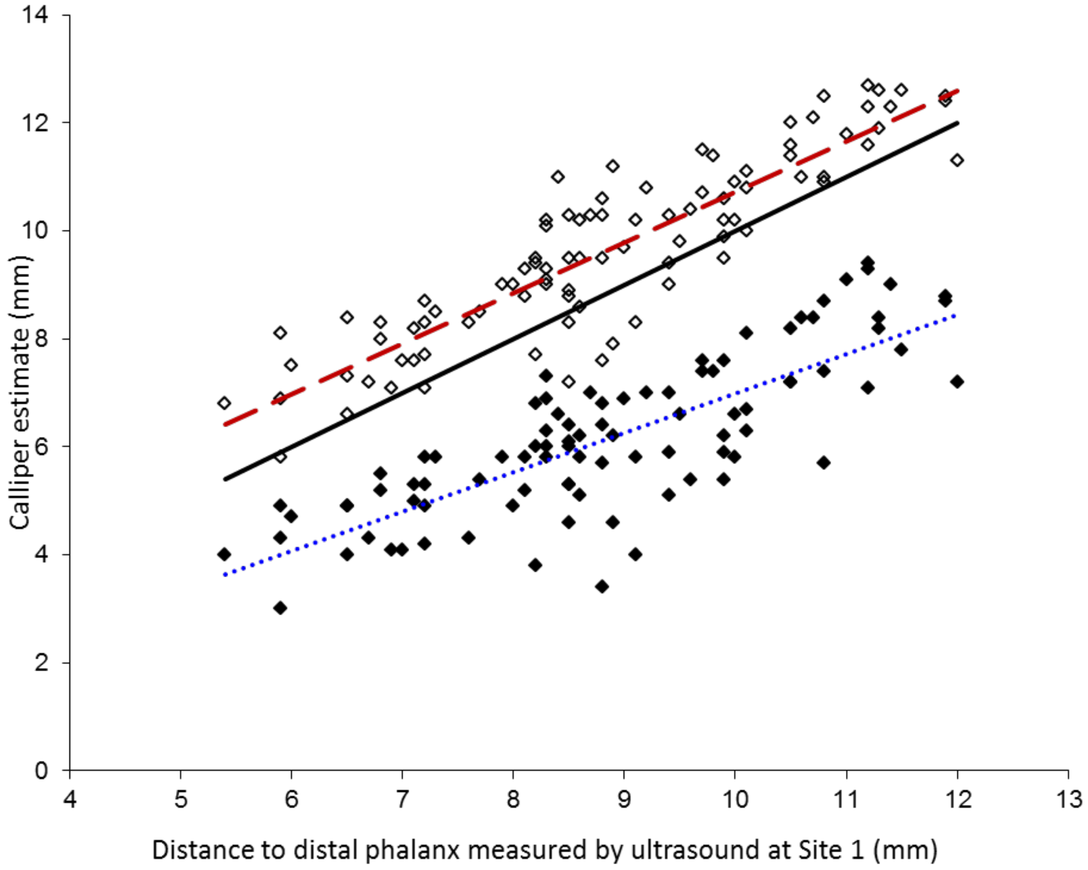

Figure 3.1: Comparison of distance from the outer surface of the sole to the distal phalanx (DP), measured at the tip of distal phalanx as determined by ultrasonography, compared with the distances measured at the same site using electronic callipers after sectioning of frozen claws on a band saw. (Data for 92 intact claws, from 23 cows).

Interpretation: Filled diamond - calliper measurement of sole thickness, closed diamond - calliper measurement of DP. Long dashed line is line of best fit for the two DP measures, dotted line is the line of best fit for ultrasound DP and calliper-measured sole thickness, solid line is line of equality.

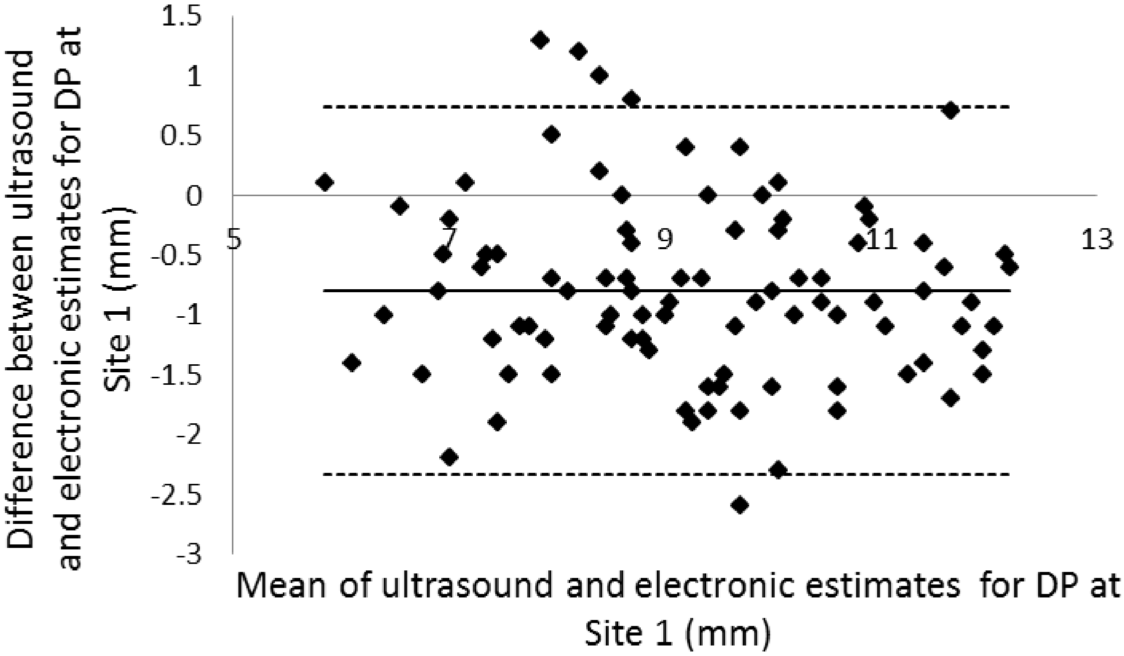

Figure 3.2: Limits-of-agreement plot showing the difference between ultrasound and electronic measures of distance to distal phalanx (mm) against the mean of the two methods (mm) at Site 1. Interpretation: Solid line is the mean difference (0.8 mm). Dashed lines are the 95% limits-of-agreement range, -2.33 to 0.74 mm. There was no significant trend in the (R^2 adj=0.001, P=0.299).

Figure 3.2 demonstrates that the average estimate of DP1 using ultrasound in the intact foot was lower than that obtained using callipers after freezing and sectioning. The mean difference between the two measures was 0.8 mm (SEM 0.08) and there was no significant association between the difference and the mean of the two values (Adjusted R^2=0.001, *P*=0.299). Thus, for an ultrasound estimate of DP1 of 10.0 mm, the mean calliper measure would have been 10.8 mm. The range of the 95% limits-of-agreement was 3.1 mm, indicating that for an individual ultrasound estimate of DP1 of 10 mm, 95% of calliper measurements would have been between 9.3 and 12.3mm.

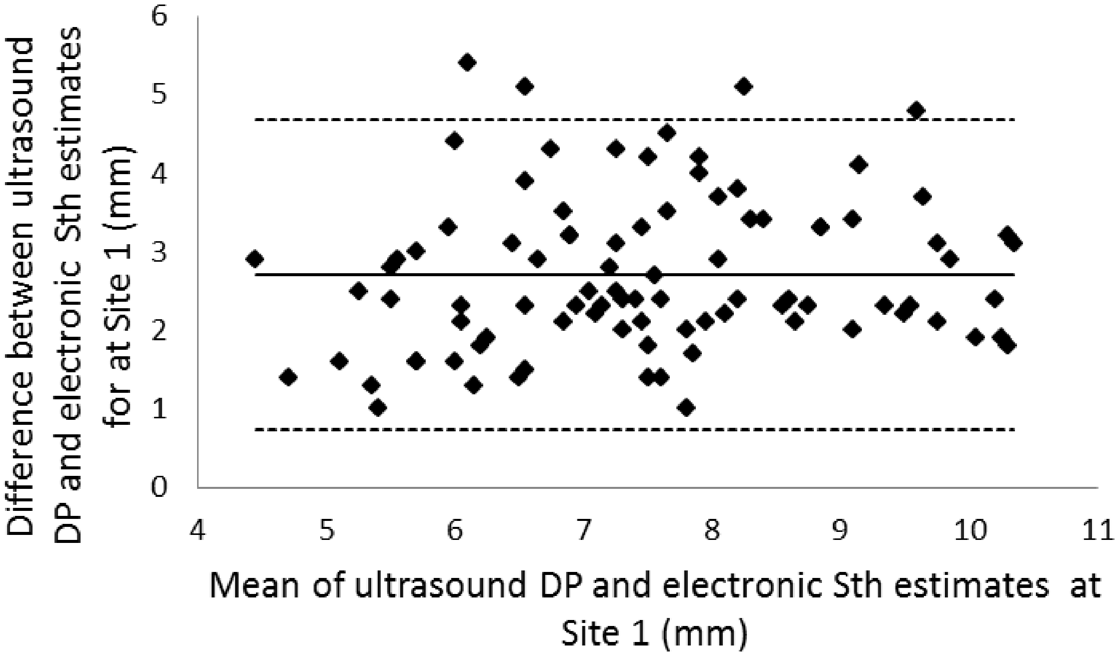

Figure 3.3: Limits-of-agreement plot showing the difference between ultrasound measurement of distance to distal phalanx and electronic measure of sole thickness at Site 1 against the mean of the two methods.

Interpretation: Solid line is the mean difference 2.7 mm. Dashed lines are the 95% limits-of-agreement range, 0.7 to 4.7 mm. There was no significant trend in the data (R^2 adj=0.009, *P*=0.182).

With respect to the agreement between calliper estimated sole thickness and ultrasound values distance to distal phalanx, the mean difference between the two measures was 2.7 mm (SEM 0.10) and there was no significant association between the difference and the mean of the two values (Adjusted R^2=0.009, *P*=0.182). Thus, for an ultrasound estimate of DP at Site 1 of 10.0 mm the mean calliper measure of sole thickness would have been 7.3 mm. The range of the 95% limits-of-agreement was 4 mm, indicating that for an individual ultrasound estimate of DP1 of 10mm, 95% of calliper measurements would have been between 5.3 and 9.3mm.

Prediction of sole thickness using the distance from the outer surface of the sole to the distal phalanx

Prior to using DP as a predictor of sole thickness, the effect of foot and claw (and their interaction) on the relationship between DP and sole thickness was evaluated. Table 3.2 shows the mean values for DP, sole thickness and thickness of soft tissue, calculated using electronic callipers, for each foot and claw at Site 1.

Table 3.2: Effect of foot and claw on mean distance from the outer surface of the sole to the distal phalanx (P3), sole thickness and soft tissue depth (distance to P3 – sole thickness) at the tip of the distal phalanx (Site 1) as measured using electronic callipers after sectioning of the frozen hoof. (RH=right hind; LF=left front)

Foot	Claw	Distance to P3 (mm) (SEM)	Sole thickness (mm) (SEM)	Soft tissue (mm) (SEM)
LF	**Medial**	10.1 (0.39)	6.4 (0.31)	3.8 (0.15)
	Lateral	10.7 (0.34)	6.7 (0.33)	4.0 (0.11)
RH	**Medial**	9.3 (0.39)	5.9 (0.35)	3.3 (0.15)
	Lateral	9.4 (0.35)	6.4 (0.33)	3.0 (0.13)

Analysis of covariance for the calliper measurements of DP and STh showed that there was a significant effect of foot (*P*<0.001) but no effect of claw (*P*=0.719) or interaction between claw and foot (P=0.124). Further analysis of between-subject effects showed that the effect of foot was only significant for DP (*P*=0.005) and not sole thickness (*P*=0.263).

Analysis of the effect of claw and foot on the relationship between the ultrasound estimation of DP and the calliper measurement of sole thickness at the same site found no significant effect of claw (*P*=0.36), foot (*P*=0.16) or any significant interaction (*P*=0.2). However, when claw was excluded, the estimated depth of soft tissue (i.e. DP minus sole thickness) at Site 1 was significantly greater for front than back feet (3.0 vs. 2.5 mm (SEM 0.13 and 0.16, respectively: *P*=0.015). Therefore, further analysis was undertaken using data from right hind feet only.

Figure 3.4 is a limits-of-agreement plot of the mean of the calliper sole thickness at Site 1 (STh1) and the ultrasound estimate of DP1 against the difference between those two measures. The mean difference between the two measures was 2.5 mm (SEM 0.15) and there was no significant association between the difference and the mean of the two values (Adjusted R^2=-0.001, *P*=0.33). Thus, for an ultrasound estimate of DP1 of 8.0 mm, on average the calliper measure of STh1 would have been 5.5 mm. The range of the 95% limits-of-agreement was 4.2 mm.

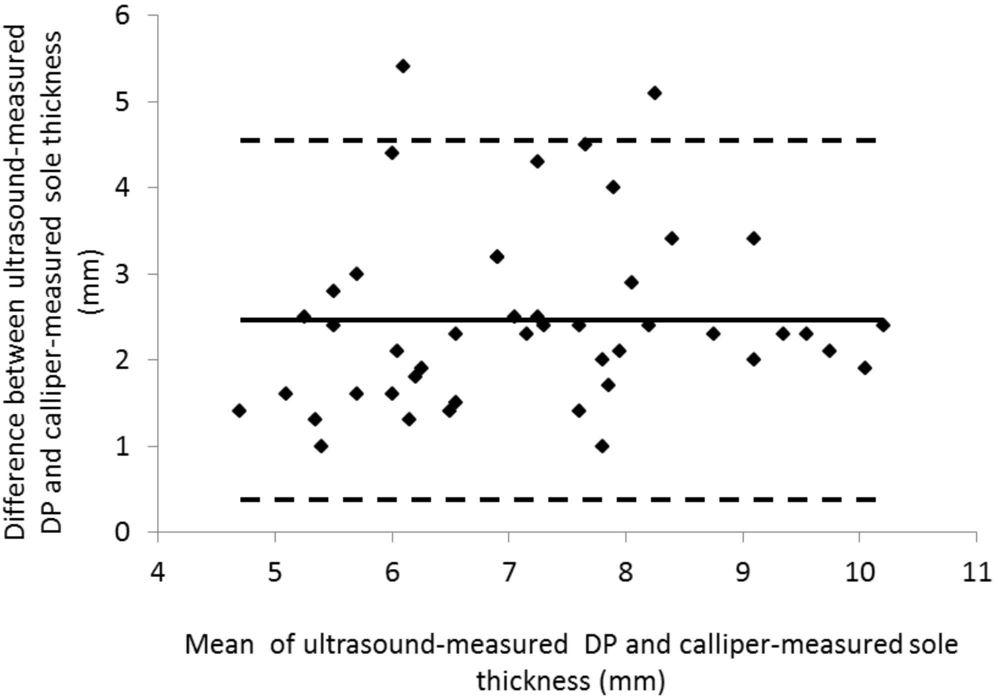

Figure 3.4: Limits-of-agreement plot of ultrasound measurement of the distance from the outer surface of the sole to the distal phalanx (DP) at the tip of the distal phalanx (Site 1) and calliper measurement of sole thickness at the same site after sectioning of the frozen claw, in 46 claws from the right hind foot of 23 cows.

Interpretation: Solid line is the mean difference between the two measures 2.5 mm. Dashed lines are the 95% limits-of-agreement range, 0.4 to 4.5.

Receiver operator characteristics curve for the categorisation of sole thickness (thin/marginal/adequate)

On the basis of the calliper estimate of sole thickness at Site 1, the claws were then categorised into either thin (<5 mm) or adequate (≥5 mm). Figure 3.5 shows the receiver operator characteristic (ROC) curve for use of ultrasound measurement of DP to categorise soles into thin or adequate in the hind feet. The area under the ROC curve was 0.92 (95% CI: 0.81 to 1.0), indicating that measurement of DP using ultrasound was highly discriminatory between adequate soles of ≥5 mm and <5 mm. The ROC curve analysis showed that an ultrasound DP of <7.0 mm had a specificity of 0.667 and a sensitivity of 0.941 in identifying a thin sole (i.e. one that was <5 mm thick); giving a positive likelihood ratio of 11.3 and a negative likelihood ratio of 0.399. In other words, a claw with an ultrasound DP of <7.0 mm was >11 times more likely to have a thin sole than one ≥5 mm, and a claw with an ultrasound DP of >7.0 mm was >2.5 times more likely to have a sole thickness ≥5 mm thick than one <5 mm. The sensitivity and specificity for an ultrasound DP of >8.25 mm in identifying a sole thickness ≥5 mm were 0.917 and 0.794, respectively, giving a positive likelihood ratio of 4.45 and a negative likelihood ratio of 0.099.

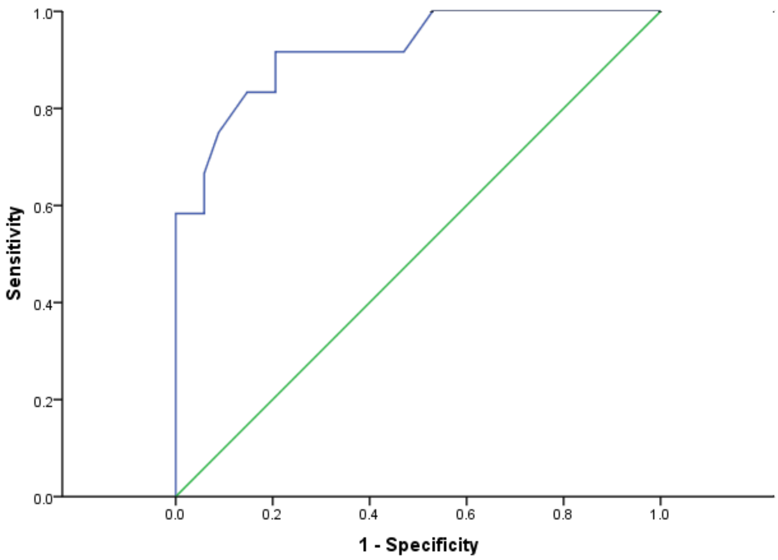

Figure 3.5: Receiver operator characteristic (ROC) curve for use of ultrasound measurement of distance from the outer surface of the sole to the distal phalanx to diagnose thin soles (<5 mm) in cadaver feet. Area under curve = 0.92, 95% CI: 0.81 to 1.0.

Relationship between sole thickness and dorsal border, toe angle at Sites 1and 2.

Data collected from 41 individuals for toe angle, dorsal border length, sole thickness at Sites 1 and 2 had normal distribution (Kolmogorov-Smirnov test). Means for each foot and claw are presented in Table 3.3.

Table 3.3: Mean toe angle, dorsal border length (from intact claw post mortem) and sole thickness at Site 1 and Site 2 (electronic callipers after sectioning of the frozen hoof) for each foot claw combination as measured in 164 claws from 41 cattle. (RH=right hind; LF=left front)

Foot	Claw	Toe angle (°) (SEM)	Dorsal border length (cm) (SEM)	Sole thickness Site 1 (mm) (SEM)	Sole thickness Site 2 (mm) (SEM)
LF	**Medial**	45.2 (0.75)	8.1 (0.08)	6.7 (0.26)	7.2 (0.34)
	Lateral	47.1 (0.57)	7.9 (0.06)	7.0 (0.28)	7.3 (0.34)
RH	**Medial**	43.6 (0.63)	8.0 (0.08)	6.4 (0.29)	7.0 (0.33)
	Lateral	43.0 (0.77)	8.0 (0.11)	6.5 (0.28)	7.0 (0.35)

For a dorsal border length of 7.5cm (combined hind and front foot data), mean sole thickness at Site 1 was 6.6 mm (range 4 to 8.8 mm), while at Site 2 mean sole thickness was 6.8 mm (range 3.8 to 8.7 mm).

Sole thickness at both Sites 1 and 2 was significantly correlated to the length of the dorsal border (r=0.16 and 0.2, respectively; *P*=0.037 and 0.011, respectively: Figure 3.5) and toe angle (r=0.17 and 0.2, respectively; *P*=0.03 and 0.01, respectively: Figure 3.6). There was no significant effect of claw or foot on the relationship between sole thickness (at either Site 1 or 2) and dorsal border length or toe angle (*P*>0.13).

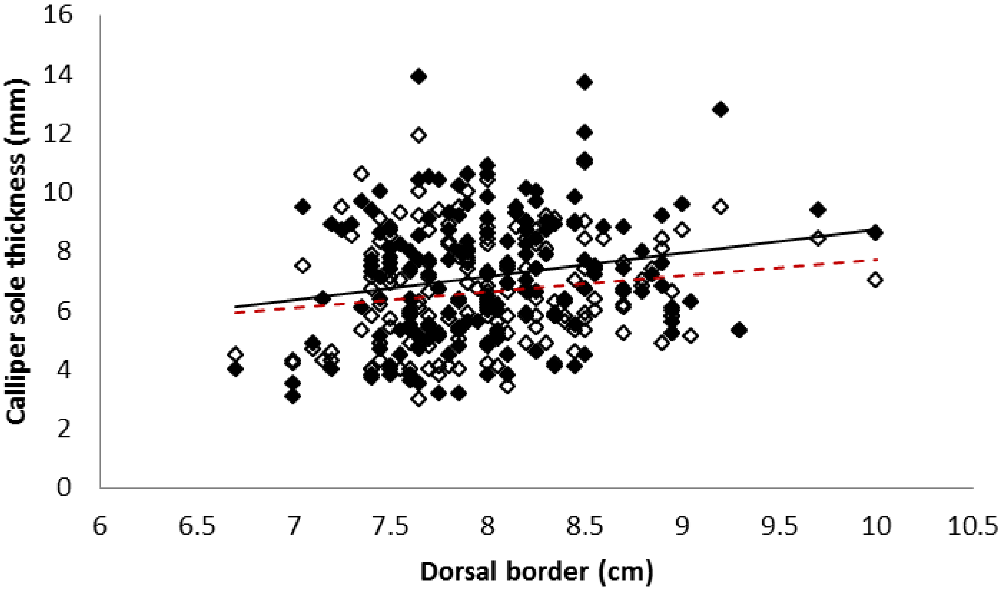

Figure 3.6: Scatterplot of dorsal border length (cm) against sole thickness, measured with electronic callipers at Site 1 (tip of the distal phalanx) and 2 (25mm along the sole surface towards the heel from Site 1). for both claws of 82 feet (left fore and right hind) from 41 dairy animals)

Interpretation: Site 1 open diamonds, line of best fit is dashed (red); Site 2 filled diamonds, line of best fit solid (black)

Backwards linear regression showed no significant effect of foot or claw category, with the best model for each site using the dorsal border and toe angle variables only:

Site 1 sole thickness (mm) = 0.134*Toe angle (degrees) + 1.145*Dorsal border (cm) - 8.53

(r=0.34, Adjusted R^2=0.104, P<0.001)

Site 2 sole thickness (mm) = 0.194*Toe angle (degrees) + 1.675*Dorsal border (cm) - 14.95

(r=0.41, Adjusted R^2=0.155, P<0.001).

The correlation between dorsal border length and toe angle was negative (r=-0.519, *P*<0.001).

Discussion

This validation study was undertaken to establish that there was sufficient agreement between ultrasound estimates of the distance to distal phalanx (DP) and sole thickness (STh) from a portable ultrasound machine and the actual anatomical values of the distances (measured in the frozen claw using electronic callipers). A comparison of methods using limits-of-agreement plots allowed the predictive value of the obtained ultrasound measurements for DP and STh to be evaluated along with the two specified collection sites before the application of the technique in the hind claws of live animals.

The images of the internal structures of the claw that were obtained in this study were largely similar to those reported in the studies by Kofler et al. (1999) and van Amstel et al. (2003, 2004a, 2004b). There was only one individual animal (No.32) for which no image of the internal structures could be obtained. When the claws were sectioned, this animal had sole thicknesses of between 8-11 mm at Site 1 and 12-13.9 mm at Site 2, coupled with soft tissue depth between 12.6-15.8 mm at Site 1 and 17.4-23.5 mm at Site 2. These values exceeded measures obtained from other animals in the study and support the findings of other studies (Kofler et al., 1999; van Amstel et al., 2003, 2004a) which reported failure to obtain ultrasound measurements from individual animals with thick (>10mm), hard or dry sole horn.

While Kofler et al. (1999) successfully imaged the internal sole surface as a thin hyper echoic line in 86/100 claws; they reported that the line was either interrupted or incomplete in 8/86 (9.3%) claws. This interruption was also a finding in the present study at a similar frequency 13/90 (14.4%). Confidence that the interrupted line seen with the portable machine in the present study was the internal sole surface as described by Kofler et al. (1999), having been established by positioning an excoriated hypodermic needle at the point of interest. The extra freeze-thaw cycle for claws in Kofler et al. (1999) does not therefore seem to influence imaging of the internal sole surface. This conclusion fits with the work of Boettcher et al. (2014) who reported that freezing did not alter the biomechanical properties of the suspensory apparatus in bovine claw horn. Minimal claw preparation was undertaken before obtaining ultrasound images in this study so as to not alter sole thickness or cause greater sole thinning. Other authors have reported the need to remove dry flaky horn in order to prevent trapped air interfering with ultrasound penetration (Kofler et al. 1999), or have performed the ultrasonography after claw trimming procedures (van Amstel and Shearer, 2002, 2003, 2004b). Despite these minor methodological differences, good surface contact was achieved, most likely due to the relatively smooth and non-flaky nature of the sole horn encountered. Ultrasound coupling was additionally facilitated by the placement of the transducer probe into a glove filled with acoustic ultrasound gel, which moulded to the contour of the sole.

The present study used two measurement sites along a repeatable line that ran parallel to the long axis of the claw. This line was employed to allow collection of histopathological specimens for light microscopy (Chapter 5) from standard sites (after Lischer et al., 2002; Räber et al., 2004) and proved to be a practical method for probe application and landmark recognition. Nonetheless, the site of probe application in the present study differed from those used previously. For example, van Amstel et al. (2003, 2004a, 2004b) used a site at the confluence of white line Zones 1 and 2, and Zone 5 of the sole (International hoof map; Greenough and Vermunt, 1994), a region observed to be clinically affected in cows with thin soles (van Amstel et al., 2004a). Alternatively, Kofler et al. (1999) took measurements at three sites along an oblique longitudinal line on the sole surface using bony features for landmarks (ultrasonography was also attempted on the dorsal wall). Differing probe application sites means that study results are not necessarily transferable as they are estimating sole dimensions in different parts of the claw. If the technique of ultrasonography becomes more widely applied it may be possible to discern the most advantageous placement position for a given management environment or clinical condition.

The results in this study agree with those reported by Kofler et al. (1999), inasmuch as correlations between ultrasound and calliper measurements of the distance to distal phalanx were high for all sites measured. On the other hand, there were only moderate correlations between the two methods of measuring sole thickness at Site 1 and no significant correlations at Site 2; whereas Kofler at al. (1999) reported high correlations for these measures.

For distance to distal phalanx, electronic calliper measurements taken while the claw was still frozen were larger than those made using ultrasound (mean 0.8 mm, SEM 0.08). This bias was consistent across all values of distance to distal phalanx; it is likely that at least some of this difference was a freezing artefact related to expansion of soft tissue elements (though not hoof horn) during freezing. Kofler et al. (1999) pointed out that in defrosted sectioned claws bulging of adipose tissue from the cut fat pads occurred; and that this was one of the potential reasons for the reduced correlation between soft tissue estimates measured by ultrasound compared to calliper estimates in their study.

Kofler et al. (1999) used computer tomography to image ten defrosted claws and obtained high correlation coefficients for both calliper and ultrasound estimates when compared to computer tomography (CT) estimates (r=0.96-0.91, and 0.83-0.89 respectively). Computer tomography has been considered to be a ‘gold standard’ for the study of foot conformation and hoof volume in the horse by Labens et al. (2013), who reported the technique to be more sensitive to quite small volume changes (for example pre and post corrective hoof trimming) than a photographic modelling technique. The use of CT would allow investigation of the effect of freezing on the dimensions of the distance to distal phalanx and soft tissue depth and confirm whether freezing had indeed contributed to the difference between methods.

For Site 1, in the present study, the 95% limits-of-agreement between the ultrasound and calliper estimates of the distance to distal phalanx was relatively small (3.1 mm), which indicates reasonable agreement between the two measures. In contrast, the 95% limits-of-agreement between ultrasound distance to distal phalanx and calliper estimates of sole thickness were 30% larger at 4.2 mm (hind feet only). Therefore, for an individual ultrasound estimate of distance to distal phalanx at Site 1 of 8.0 mm, 95% of calliper measurements of sole thickness at the same site would have been between 3.5 mm and 7.6 mm. So while estimation of the mean sole thickness for a group of animals would be associated with a small standard error of the mean, for prediction of sole thickness in an individual animal the 95% limits-of-agreement were unacceptably wide ranging across the values for sole thickness which are currently regarded as being either adequate (7 mm; Toussaint Raven, 2003) or too thin (<5 mm; van Amstel et al. 2004a).

However, an alternative to actually measuring sole thickness is simply to identify thin soles. van Amstel et al. (2004a) defined thin soles as those which had a sole thickness of <5 mm.

The ROC curve and associated analyses show that if this were to be used as a diagnostic criterion ultrasound measurement of distance to the distal phalanx at Site 1 in the hind claw would be an effective site and method of discriminating between cattle with thin soles <5 mm from those with adequate sole thickness (≥5 mm). Hind claws with a distance to distal phalanx of <7.0 mm (as assessed by ultrasound) were 11.3 times more likely to have thin rather than adequate soles, while claws with a distance to distal phalanx >8.25 mm were 10 times more likely to be adequate than thin. So, for this ultrasound machine and probe, hind claw soles with a distance to distal phalanx of <7.0 mm can be defined as thin, soles with a distance to distal phalanx of >8.25 mm can be defined as adequate and those with distance to distal phalanx between 7.0 and 8.25 mm as falling in a marginal zone where classification is not clear cut. The analysis was performed for hind claws because they are more frequently the site of lameness, and there is probably consequent merit in suggesting that future live animal studies should also focus on hind claws. Individual machines will vary in their cut off values and therefore it would be good practice to run a pilot study to assess the performance of a machine before using it in the live animal.

In this study, there was a small but significant relationship between dorsal border length and sole thickness, which supports the observations of Toussaint Raven (2003) that these features are related. For a dorsal border length of 7.5 cm (combined hind and front foot data) the present study demonstrated a mean sole thickness of 6.6 mm (range 4.0. to 8.8 mm) at Site 1 and 6.8 mm (range 3.8 to 8.7 mm) at Site 2. The mean and range of values obtained are smaller and narrower than the values reported by van Amstel et al. (2002), where the mean sole thickness at dorsal border length 7.5 cm was 8.2 mm (range 4 to 14 mm) but critically the values obtained in the present study still span the accepted thin: thick threshold of 5 mm, making dorsal border a less useful indicator of claw sole thickness than an ultrasound estimate of distance to distal phalanx. The range of values for sole thickness at 7.5 cm differs slightly to the findings of Nuss et al. (2011), who report a consistent relationship between dorsal border of 7.5 cm and a threshold sole thickness >5 mm. This finding, as with those of Chapter 2, indicates that “dairy” conformation is different to the beef breeds studied by other workers.

While the regression models show that the use of both toe angle and dorsal border to predict sole thickness is more accurate than either variable used alone see Table 3.4. Based on the correlations obtained this study suggests the use of toe angle and dorsal border together would not be as successful as the use of ultrasound estimation of distance to distal phalanx.

Table 3.4. Comparison of the correlations between actual sole thickness (as determined with electronic callipers) and different external estimates of sole thickness for 41 individuals.

Variable	Pearson's correlation coefficient (r) Site 1	Pearson's correlation coefficient (r) Site 2
Dorsal border	0.16	0.2
Toe angle	0.17	0.2
Dorsal border and toe angle	0.34	0.41
Ultrasound distance to distal phalanx	0.8	0.71

Conclusion

Supporting the initial hypothesis, it was concluded that, applying the methods used in this study, the Mindray DP 6600 portable ultrasound could be used to accurately estimate the mean distance to distal phalanx for a group of animals within a herd. However, it was not considered sufficiently accurate for the direct estimation of sole thickness in the individual animal. Nonetheless, data generated from the study would enable a sole thickness category to be allocated to a given hind claw. These conclusions apply solely to the ultrasound machine and probe used in this study, and similar validations should be undertaken prior to field use if other machines are to be used.

So, for research purposes, measurement of distance to distal phalanx can be used to follow the impact of factors such as environment, time after calving and nutrition on sole thickness. In the clinical situation, measurement of distance to distal phalanx can be used to identify whether groups of animals (such as freshly calved heifers) have thin soles, or to identify animals with thin soles prior to the development of lameness so that management changes such as milking once per day or reducing distance walked can be implemented for those animals.

Publication

Contents from this chapter have been published in:

Laven, L., Margerison, J., & Laven, R. (2010). Measurement of sole thickness and distance to distal phalanx using a portable ultrasound machine. In Roland Sumner (Ed.), Proceedings of the New Zealand Society of Animal Production Vol. 70 (pp. 240-242). Massey University, New Zealand: New Zealand Society of Animal Production (Inc.).

Laven, L. J., Margerison, J. K., & Laven, R. A. (2011, December 5). Validation of a portable ultrasound machine for estimating sole thickness in dairy cattle in New Zealand. New Zealand Veterinary Journal, 60(2), 123-128. doi:10.1080/00480169.2011.644215

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Chapter four: Histological preparation pilot study

Introduction

The collection of histological samples from claw material is technically demanding since it contains horn and bone as well as the soft tissue of the corium. Samples can be collected from exungulated material; however, this may disrupt areas of interest such as the dermoepidermal junction (Ossent and Lischer, 1997). Sectioning of intact claws is therefore preferable. If this is to be done, then slices of hoof must be obtained from the whole structure. This is usually achieved using a band saw (e.g. Maclean, 1971; Lischer et al., 2002), although the use of a hand saw (Singh et al., 1992) and a vibrating electrical saw (Kempson and Logue, 1993) are also reported. The band saw can be used with both frozen (Lischer et al., 2002; Tarlton et al., 2002; Räber et al., 2004) and fresh material (Nilsson, 1963; Maclean, 1971; Andersson and Bergman, 1980; Thoefner et al., 2005). For operator safety, cutting frozen hoof is preferred (Hogan M., personal communication). In contrast, fresh tissue is better for histology, although the artefacts associated with freezing do not completely preclude the use of frozen sections for histology (Baraibar and Schoning, 1985). Indeed, previous studies of hoof horn using frozen tissue have shown that the impact of freezing on the structures in the hoof does not exempt the use of frozen tissue for testing of structural integrity. For example, Tarlton et al. (2002) undertook a pilot study in which all four hind claws were collected from one animal so that comparison could be made between two fresh and two frozen claws. No influence of freezing was demonstrated when the material was compared under biomechanical testing and then histological examination to examine the site of failure within the tissue. Their conclusions that frozen tissue was suitable for such testing and examination are supported by Boettcher et al. (2014) who reported that freezing seemed not to affect the mechanical properties of the suspensory structures of the hoof, and by others (Mulling et al., 1999; Lischer et al., 2002; Räber et al., 2004) who used frozen material to examine the layers of the hoof epidermis, the suspensory apparatus, and digital cushion architecture, respectively. After collection, samples need to be embedded, cut and then mounted. Embedding of samples in methylmethacrylate has been described (Boosman et al., 1989; Budras et al., 1996; Mulling et al., 1999; Hendry et al., 2001) but, more commonly, tissue is embedded in paraffin blocks (Maclean 1971; Andersson and Bergman, 1980; Thoefner et al., 2005; Danscher et al., 2010). Paraffin blocks are usually cut on a microtome, but cryosectioning of non-embedded tissue has also been employed on material from both fresh and frozen hooves (Mulling et al., 1999; Hendry et al., 2001; Räber et al., 2004).

Once cut, sections need to be mounted on slides and stained. Across studies in the literature, the most widely used staining technique is haematoxylin and eosin (H&E). This common stain produces good nuclear and intra-nuclear detail in blues and black (due to haematoxylin) while connective tissue fibres and cell cytoplasm are stained shades of pink and orange (eosin) (Gamble and Wilson, 2002). A wide variety of other staining techniques have been reported, with the most common being the Periodic acid-Schiff (PAS) method which is particularly useful for highlighting basement membranes (Thoefner et al., 2005). Van Gieson's method (vGE), also known as an elastin stain, is a trichrome method, principally for the differentiation of muscle and connective tissue, which stains nuclei blue-black; collagen red and other tissues yellow (Lamar Jones, 2002). The benefit of this stain lies in its ability to highlight features of blood vessels, including the internal elastic membrane of arterioles, and the interface between smooth muscle and surrounding loose connective tissue. Decalcification of bone tissues, to aid sectioning and stain penetration (Callis, 2002) has been reported in some studies (Boosman et al., 1989; Singh et al, 1992; Lischer et al., 2002).

The variety of different technical approaches and staining methods employed throughout the literature suggest that there is not a single agreed 'gold standard'. Methodology would undoubtedly reflect the technical equipment and expertise available to the individual authors and, as a result, comparison between studies. The lack of impact of freezing on parameters such as biomechanical strength as well as the successful use of frozen sections for histology, combined with the advantages of storage and operator safety which using frozen sections provides, strongly supports the use of frozen sections. However, limited attention in the published literature has been paid to trying to improve the quality of sections created from frozen tissue.

Difficulties in processing frozen tissue were emphasised by the first sections created from frozen claws for this thesis. To create these sections, the entire distal limb was frozen and sectioned on a band saw; samples were placed in neutral buffered formalin for fixation, and then processed and embedded in paraffin wax before sectioning on a microtome. Initial evaluation of section quality indicated poor overall retention of tissue morphology, limiting the utility of the sections for histology-based investigations.

Hypothesis: Although microscope slides prepared from fresh claw samples are of higher quality than those obtained from frozen claw tissue, it is difficult to prepare such slides. It was postulated that alteration of processing procedures could be used to create microscope slides from frozen tissue samples which would result in prepared slides of improved quality and thereby allow a wider range of assessments to be performed on them.

The aim of this study was, therefore, to identify whether better quality sections could be made by altering the processing of sections made from frozen tissue and compare these results to similarly processed tissue from sections collected from fresh hooves.

Materials and methods

The distal limbs of five cows were collected post mortem. All five had died of conditions unrelated to lameness within the previous 24 hours and entered the Massey University Post Mortem facility. The age, breed and cause of death of each animal were recorded.

Each limb was identified on removal from the carcase using an elastic band of a specified colour (red, right front; yellow, left front [LF]; blue, left hind; green, right hind [RH]) which remained with the limb throughout. After collection, the limbs were washed to remove any mud or manure, and a latex glove placed over the proximal end of the limb to prevent moisture loss. All four distal limbs from each animal were placed in an individual plastic bag, sealed with a cable tie, and stored at 3 to 5°C. The LF and RH feet of three animals were frozen at -20°C for at least 24 h. The LF and RH feet of the other two animals were processed in the fresh state, within 48 hours of collection.

The frozen claws were sectioned on the band saw along the line AB shown in Figure 4.1 and a thin sagittal section of claw approximately 3mm thick produced. A section from the sole area below the distal phalanx was collected for this study. The section included sole horn, distal phalanx and the interposing soft tissue, including the digital cushion. The tissue samples were fixed in 10% buffered formalin for at least 6 weeks. The fresh claws were sectioned in the same position on the band saw to produce a sagittal slice of claw approximately 10mm thick. The whole slice was fixed by immersion in 10% buffered formalin for at least 6 weeks before processing.

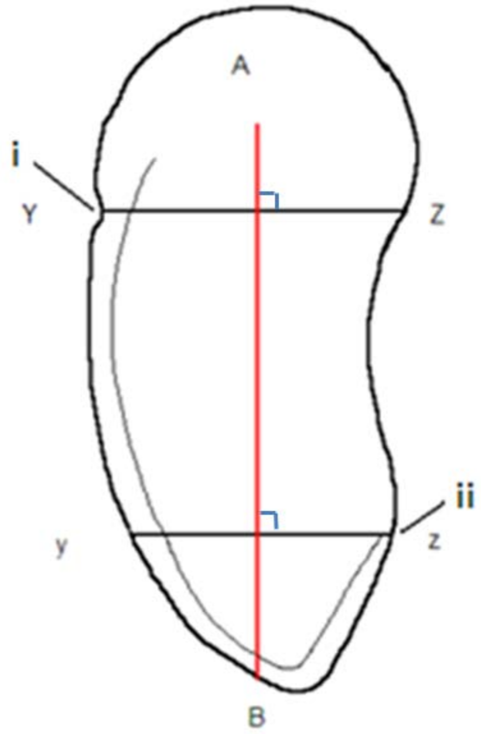

Figure 4.1. Plantar view of bovine claw; sagittal and perpendicular reference points for dissection. The frozen claws were sectioned along the line AB. Line AB bisected lines YZ, and yz (sited at the abaxial groove (i) and axial white line (ii) respectively, modified from Lischer et al. (2002).

Immediately prior to processing the fixed tissue (from either fresh or frozen claw) was rinsed with distilled water and trimmed. A sample of the fresh-cut fixed tissue was cut (with a scalpel blade) from the fixed claw slice, then all samples were trimmed in the same way; bone removed at the periosteum and horn reduced to a minimum. Trimmed samples (approximately 3 mm thick) were then placed into one of six different processing solutions for 24-48 hours prior to sectioning. Each processing solution was tested with one sample from a frozen claw and one sample from a fresh claw. The processing solutions contained phosphate buffer alone, sucrose solution alone or a mixture of the two, with varying concentration of sucrose (see Table 4.1).

Table 4.1: Processing methods used for fresh and frozen specimens of claw tissue.

Processing protocol	Solution	frozen cut (a)		fresh cut (b)	
1	Phosphate buffer	26 LF sole section		51 LF sole section	
		FAST	SLOW	FAST	SLOW
2	Phosphate buffer + 0.3M sucrose	26 RH sole section		51 LF sole section	
		FAST	SLOW	FAST	SLOW
3	Phosphate buffer + 20% sucrose	25 LF sole section		51 RH sole section	
		FAST	SLOW	FAST	SLOW
4	20% sucrose no buffer	25 LF sole section		51 RH sole section	
		FAST	SLOW	FAST	SLOW
5	10% sucrose no buffer	24 LF sole section		53 sole section	
		FAST	PARA	FAST	PARA
6	Phosphate buffer and 10% sucrose	24 RH sole section		53 sole section	
		FAST	PARA	FAST	PARA

FAST – mounted and snap frozen in liquid nitrogen and then sectioned on the cryotome at approx. -21°C
SLOW – mounted and allowed to cool in cabinet, then sectioned on the cryotome at approx. -21°C
PARA – mounted in wax block and cut on microtome at room temperature

Effect of altering cryosectioning method (***Protocols 1-4)***

For Protocols 1-4 (see table 4.1), trimmed samples were removed from their processing solutions and divided into two with a scalpel blade. Both of these tissue samples were then sectioned on a cryotome, with one sample frozen following a SLOW freezing process and the other a FAST freezing process. For the FAST freezing procedure, the tissue sample was attached to the cryotome chuck (metal) with optimal cutting temperature medium (OCT) (Tissue Tek, Sakura) and snap frozen. This was achieved by dipping the metal stalk of the chuck into a small quantity of liquid nitrogen and maintaining the tissue sample in the vapour for approximately 10 seconds. For the SLOW freezing procedure, the tissue was also attached to the cryotome chuck with OCT, and then was allowed to cool against the thermal mass (cooled bulk) of the cryotome cabinet which was already established at a temperature of -21 °C. This procedure resulted in a slower more uniform freezing of the tissue over about 20 seconds, and was aided by proprietary coolant spray applied to the top surface. Cryosectioning was then performed at a cabinet temperature of -21 to -22 °C; the tissue was aligned on an angle to the blade and faced up before sections were cut at a thickness of 5 to 6 µm.

Paraffin block mounting vs. fast cryosectioning (***Protocols 5 and 6)***

The remaining tissue samples (n=4) were used to compare the FAST freeze and cryosectioning procedure with embedding in a paraffin block (PARA) and sectioning on a microtome. Mounting in methylmethacrylate was rejected due to expense and availability of equipment. For samples designated PARA sections were cut at 3 to 4 µm thick. The resulting sections were mounted on positively charged slides and stained with H&E in an automatic processor. The slides obtained for each protocol were examined and compared by light microscopy.

Results

Cutting on the band saw

Frozen claws were of a uniform hardness throughout the tissue and allowed finer sections to be cut and harvested than from the fresh material. In fresh tissue, the blade had to move through multiple interfaces of hard (bone and horn) to soft (corium, tendon, skin) tissue. Each boundary impeded the smooth transition of the cutting blade, and meant that the minimum thickness of the resulting section was approximately 10 mm, as below this threshold thickness the teeth of the band saw ripped sections of soft tissue out of the sagittal slice, disrupting the architecture completely.

Cryosection mounting

Care was needed to build up the OCT medium in a good block around the tissue sample, and attention to the exclusion of bubbles from this medium was important for clean sections. A straight edge cut across the mounted block with a scalpel blade, behind the tissue section aided the acquisition of uniform sections from the block. The tissue sample was aligned such that a corner of horn was the first element to encounter the blade, thereby allowing resistance to sectioning and compression of trailing tissues to be minimized. Some difficulty was experienced in getting the horn to adhere to the slides on mounting, but this was partially mitigated by the use of positively charged slides.

Effect of freezing speed

For sections created on the cryotome, snap freezing with liquid nitrogen (FAST) resulted in better sections and cell morphology than did the spray-assisted cooling procedure in the cold cabinet environment (SLOW). The best cryosection results with respect to ease of cutting and section integrity were obtained when the processing solution included sucrose. Sections from solutions containing sucrose were notably easier to cut, particularly the 20% solution. However, inclusion of sucrose led to swelling of the tissues, particularly adipose tissue. This was evident to the naked eye on processing, as the adipose tissue stood proud of the rest of the tissue sample when taken out of the processing solution for trimming. The swelling was also reflected in the cellular morphology on histological examination, as all cells had a plump inflated appearance and the slide as a whole had a closed uniform texture.

Artefacts were seen microscopically in all sections, but particularly the SLOW frozen sections, which often showed knife marks and a shattering effect. The SLOW process did not preserve red blood cells intact as well as the FAST one. However, the difference in slide quality, cell and tissue morphology, seen between SLOW and FAST cooling procedures was not as distinct as that seen between samples that were collected initially from fresh *versus* frozen claws.

Fresh versus frozen claw tissue

Collection and processing of fresh tissue resulted in sections with intact red blood cells within the vasculature, as opposed to the amorphous pink material that was more common with the frozen cut samples. Despite this drawback, some areas of the internal structure of the claw capsule appeared to survive the freezing process with less damage: for example the dorsal lamellae were intact and their architecture was recognisable. On the other hand, frozen sections contained more halo artefacts around blood vessels than did fresh cut tissue.

Discussion

This study looked at alternative processing methods to identify whether the quality of sections obtained from tissues that had been previously frozen could be improved. In addition, it also compared results from fresh-collected tissue. Fresh-collected tissue yielded superior histological sections across all protocols. However, the collection of samples from claws on the band saw was safer and more targeted with less disruption of tissue when frozen feet were processed rather than fresh feet.

Across the protocols some refinements to section production and improvements to final section quality were made. Cryotomy was particularly successful where sucrose was used as a cryoprotectant / molecular lubricant in the fixation process. However, while sucrose had some beneficial ‘molecular lubricant’ properties this was negated by the osmotic artefact and distortion that it caused to tissue morphology. The exact mechanism of adipose swelling with sucrose was not determined, but even at a physiological osmolarity of approximately 10% there remains a sucrose concentration gradient in force. It was considered that a lack of tissue stabilisation during fixation contributed to the effect of sucrose upon the tissue. It was also noticeable the effect of sucrose was greater at the higher concentrations.

Artefacts such as shattering or knife marks in the sections created on the cryotome were a regular feature. ‘Shattering’, as opposed to knife marks, can result from selection of a sub-optimal cutting temperature. The presence of many different tissue elements (hoof wall, adipose and connective tissue) with different cutting temperature optima in the studied tissues means that the temperature used was almost certainly not the optimal temperature for all tissues. This dilemma makes shattering hard to eliminate. However, the FAST cooling procedure produced fewer artefacts then the SLOW cooling procedure.

Light microscopy showed that, as well as there being fewer artefacts for the FAST cooling procedure than the SLOW cooling procedure, overall section quality was better, particularly in terms of the preservation of cellular morphology. However, the FAST process did not result in a consistent improvement in section quality above the use of standard histological techniques to embed frozen collected tissue in paraffin blocks.

Comparison of the section quality obtained using the standard techniques from frozen cut samples in the present study to the images presented in Tarlton et al. (2002) showed that the sections created for the current study had better maintenance of tissue integrity and cellular morphology; so, although the sections created were not of sufficient quality for detailed histopathological examination, they were clearly sufficient for the type of examination undertaken by Tarlton et al. (2002).

The data in the literature generally reflect these conclusions. Studies describing detailed histopathological changes as a result of a laminitic episode all used fresh tissue (Nilsson, 1963; Maclean, 1971; Boosman et al., 1989, 1991; Thoefner et al., 2005). Using fresh tissue, changes were evident with blood vessel architecture (dilation, damage, and sclerosis), red and white blood cell presence within vessels and tissues or the detailed architecture of the dermoepidermal junction of the sole or dorsal lamellae. In contrast, studies evaluating the suspensory apparatus, lamellar morphology, or connective tissues and fat pads of the digit all successfully used frozen material (Lischer et al., 2002; Tarlton et al., 2002; Knott et al., 2007; Räber et al., 2004).

It appears then, that some structures within the claw capsule are more robust with respect to the freezing process, so they can be studied using frozen material. The present results suggest that the use of frozen material is more appropriate in the search for evidence of a pathological process (e.g., vascular remodelling or lamellar morphology) rather than for detailed histopathology (e.g. examination of basement membrane stretch or diapedesis of red blood cells). In particular, sections from frozen tissue therefore lend themselves more to the use of techniques such as morphometry.

Conclusion

The initial hypothesis was not supported. Although none of the protocols improved the histological appearance of sections from frozen hoof tissue, standard procedures yielded histological sections which compared favourably with images in published studies (e.g. Tarlton et al., 2002). Nevertheless, the use of frozen material limits the type of assessments that can be performed on the histological sections obtained compared to fresh material. Conversely, for techniques such as morphometry, the benefits of frozen tissue such as the collection of material over an extended time period and increased operator safety on the band saw during processing make it a suitable choice.

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Chapter five: Morphometric evaluation of the vasculature in light microscopy sections of the bovine claw

Introduction

In cattle, both acute and chronic laminitis (also termed “coriosis”: Blowey, 2005) cause lameness. However, it is the so-called ‘subclinical’ form of laminitis (SLS), which is characterised, at least initially, by hoof horn haemorrhages and soft yellow horn, that has been considered to be the most important laminitis-related cause of lameness (Vermunt, 1992). Despite its importance, the pathophysiology of SLS and its role in the aetiology of lameness have not been effectively explained. Indeed, over recent years, the perception of the role of SLS in claw horn lesions has changed from a point where all claw horn lesions were thought to be related to some form of SLS (Bradley et al., 1989; Smilie et al. 1996) to one where SLS is just one of a number of factors which can result in claw horn haemorrhage and disruption (Westwood et al., 2003; Danscher et al., 2010). This change in perception of the role of SLS has been accompanied by a change in opinion regarding its aetiopathogenesis. Nocek (1997) described SLS as a condition which had multiple risk factors (including bedding, exercise, behaviour, age, reproductive status and nutrition), while Cook et al. (2004) used the term laminitis only in relation to lameness resulting from subacute ruminal acidosis. Even with this limited use of the term, there is still much uncertainty; in particular regarding the means by which subacute ruminal acidosis produces laminitis. Westwood et al. (2003) reviewed the relationship between nutrition and lameness in pasture-fed dairy cattle and proposed several mechanisms by which acidosis and subsequent laminitis could occur. However, the current hypothesis (Webster et al., 2005; Greenough, 2007) is that subacute ruminal acidosis leads to vascular and metabolic disturbances within the claw, resulting in claw horn haemorrhages and the production of poor quality horn. Nonetheless, the details of this process need to be properly elucidated.

As visible hoof horn haemorrhage is the main feature linked with SLS, histopathological examination of tissue sections from affected claws may be able to cast some light on the aetiopathogenesis of the lesions. Several authors have set out to define the histopathological features of bovine laminitis (Nilsson, 1963; Maclean, 1971; Thoefner et al., 2005; Mendes et al., 2013) and both the acute and chronic forms of disease have been compared in affected and normal control animals. Although similar lesions have been reported for both forms of laminitis, the intensity and maturity of lesions differs between the acute and chronic forms due to the time-scale involved. For example, vascular changes in acute laminitis include dilatation, hyperaemia and oedema of the blood vessels (Nilsson, 1963; Maclean, 1971; Andersson and Bergman, 1980; Thoefner et al., 2005), whereas oedema is not common in chronic laminitis although there is sometimes distension or dilation of capillaries and blood vessels (Maclean, 1971; Andersson and Bergman, 1980).

Changes to the lamellae and epidermal basal cells are seen in acute laminitis (Maclean, 1971; Thoefner et al., 2005). In contrast, the basal cell morphology of chronically affected animals has been reported as being normal (Maclean, 1971; Boosman et al., 1989; Thoefner et al., 2005). Haemorrhage or diapedesis of red blood cells (Nilsson, 1963; Maclean, 1971; Boosman et al., 1989) feature regularly in descriptions of acute laminitis, but are not usually described for the chronic form. Various white blood cell types have been reported as located within the tissue in both acute and chronic laminitis (Nilsson, 1963; Maclean, 1971; Andersson and Bergman, 1980; Boosman et al., 1989), although the significance of the presence of these cells is unclear since this has also been reported in normal animals (Maclean, 1971; Andersson and Bergman, 1980).

Acute laminitis cases often feature extensive thrombosis of blood vessels and capillaries (Nilsson, 1963; Maclean, 1971; Andersson and Bergman, 1980; Boosman et al., 1991). In chronic cases, thrombi are more prominent and have often been subject to remodelling (Maclean, 1971; Andersson and Bergman, 1980). Shunt formation may also be present (Maclean, 1971; Boosman et al., 1989).

Changes to the vasculature such as arteriosclerosis, fibrosis of the tunica intima and adventitia (Singh et al., 1994) and enlargement of the tunica media (Andersson and Bergman, 1980) have been reported for acute laminitis but are more extensive in the chronic condition. Indeed several studies have shown that histologically moderate to advanced vascular sclerosis is the most consistent finding in chronic laminitis (Maclean, 1971; Andersson and Bergman, 1980; Boosman et al., 1989).

These vascular changes could allow haemorrhage due to laminitis subsequent to subacute ruminal acidosis to be distinguished from hoof horn haemorrhage resulting from external trauma, as although the trauma could result in vascular repair the ongoing chronic changes in histology associated with laminitis would be absent.

The suggestion that histological evaluation of claw lesions may help to elucidate the aetiology of the haemorrhage was made by Norton and Stalker (2005) in their report on the claw horn lesions present in New Zealand dairy at the time of slaughter. A histological assessment could be used to better understand the prevalence of lesions attributable to SLS in cull cows as an indicator of the importance of the disease within the national dairy herd.

However, undertaking a survey of the presence of SLS in the feet of cull cows is fraught with difficulty, since such cows are sent for slaughter in small numbers without a predetermined schedule. Hence, logistically, it would be necessary to collect feet as they become available and store them for later processing. Again, logistical considerations dictate that storage of tissue in a frozen form the most feasible means of managing such specimens. Freezing mitigates time constraints for collection and processing of material, avoiding the need for mass collection. Collection of samples for histology from frozen material has been described by several authors (Lischer et al., 2002a; Tarlton et al., 2002; Räber et al., 2004). However, some deleterious effects on section quality have been described in the form of freezing artefacts (Baraibar and Schoning, 1985) which might limit the usefulness of sections for histopathological examination.

In the presence of such artefacts, morphometry has advantages over histopathological examination. Morphometry allows the quantitative assessment of structures that are unaffected by the freezing process (such as the blood vessels), even though histological details of vulnerable structures such as individual red and white blood cells are lost.

Vascular remodelling can be detected by measuring blood vessel lumen diameter and wall thickness and calculating the ratio between lumen area and lumen plus tunica intima and media area (Mulvany, 1999). Such measurements are robust and repeatable (Fernie and Lamb, 1985) and allow for a wide range in vessel size and the response to fixation (Palvesky et al., 1989). As it is postulated that vascular remodelling is characteristic of SLS, such morphometrical examination could be used to assess whether SLS lesions are present within histological sections of bovine of claw tissue.

As yet, morphometry has not been used to study the blood vessels of the bovine hoof. There are therefore no data on how factors such as site within claw, and hoof conformation parameters (e.g. toe length, toe angle, sole thickness) affect morphometry. Such data are needed before the changes in hoof vasculature associated with laminitis or with external trauma can be studied.

Hypothesis: Morphometrical techniques could be used to assess vascular damage in the claw of first lactation heifers, on the basis that the ratio of vessel lumen area to lumen plus tunica intima and media area would be reduced in a region of the claw subjected to increased mechanical trauma during locomotion when compared to one with less insult and protection in the form of the digital cushion. Because vascular remodelling results in alteration of vessel walls, it was postulated the ratio L/V would be altered in animals that had experienced trauma. Furthermore, it was postulated that the ratio would be positively correlated to conformational parameters such as soft tissue depth and sole thickness. Such findings would infer greater protection of the underlying tissue and show a relationship to the extent and severity of claw horn haemorrhage present within the claws.

The aims of this study were to:

1. Use morphometry to examine the claws of non-lame New Zealand dairy animals to quantify the ratio of vessel lumen area to lumen plus tunica intima and media area of claw blood vessels
2. Relate that ratio to location within the claw and conformational parameters of the claw
3. Relate that ratio to the presence or absence of hoof horn haemorrhage in the claws.

Materials and methods

Sample preparation

The distal limbs were collected from 28 dairy cows, 7 of which had died within the previous 24 hours of conditions unrelated to lameness, 3 had been euthanased on-farm and the remainder (18) were collected from a local abattoir. The age, breed and cause of death of each animal were recorded (as for Chapter 3).

Each limb was identified on removal from the carcase using an elastic band of a specified colour (red: right front, yellow: left front, blue: left hind and green: right hind) which remained with the limb throughout processing. The limbs were then washed to remove any mud or manure, and a latex glove was placed over the sawn end of the limb to prevent moisture loss. All four limbs from each animal were placed in individual plastic bags, sealed with a cable tie, and stored at 3 to 5°C until anatomical measurements were completed. The limbs were then frozen at -20 °C until used for histology. All histological sections were harvested from claws processed and frozen within 48 hours of death.

Seven anatomical measurements were made on both claws of all feet (Figure 5.1). All measurements were taken twice. Apart from toe angle, all measurements were made using a flexible tape measure, to the nearest mm. Toe angle was measured using an engineer's angle finder, to the nearest degree. For linear measures, the difference between the two estimates was never more than two millimetres, whilst toe angle replicates were within one degree of each other. The mean values were used for analyses.

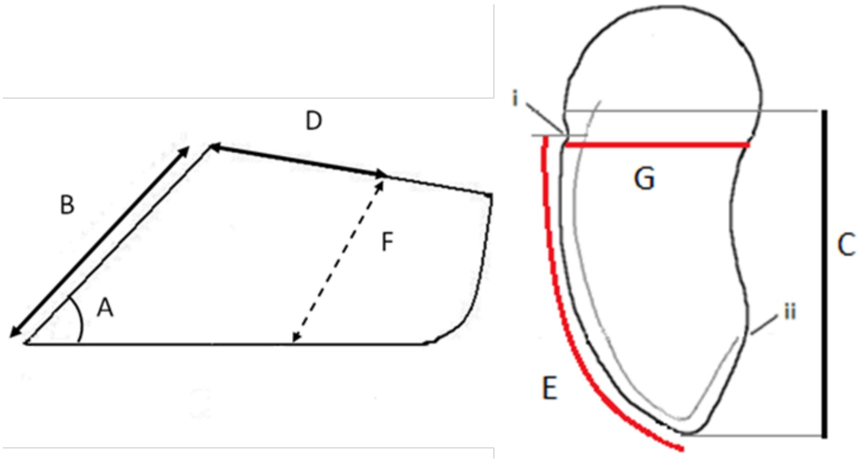

Figure 5.1: Variables measured on both claws of the hind feet of dairy cows. Measures adapted from Vermunt and Greenough (1995) and Scott et al. (1999).

i	Abaxial groove;
ii	End of the axial white line;
A.	**Toe angle:** Angle of dorsal border to weight-bearing surface;
B.	Length of **dorsal border** from skin: horn junction at coronary band to apex of toe;
C.	**Claw length** on the palmar/plantar surface from apex of toe to point where ground contact is lost (cm);
D.	Distance from the point where the coronary band meets the flexure of the dorsal border to the abaxial groove along the coronary band (**proximal**);
E.	Distance from the apex of the toe to the abaxial groove along the wall horn **(distal)**;
F.	Length of the **abaxial groove** from the coronary band to base of claw;
G.	**Claw width** on the palmar/plantar surface, at widest point of claw in area of sole/bulb junction.

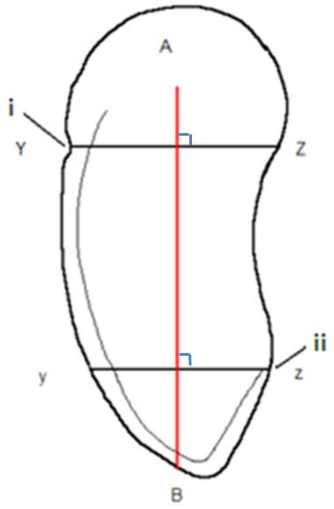

Figure 5.2. Plantar view of bovine claw; sagittal and perpendicular reference points for dissection. The frozen claws were sectioned along the line AB. Line AB bisected lines YZ (sited at the abaxial groove: i), and yz (sited at the axial white line: ii). Modified from Lischer et al. (2002a).

External sole and white line lesions

The line YZ line was drawn on the sole and lesions present on the white line or sole surface were outlined (Figures 5.2 and 5.3).

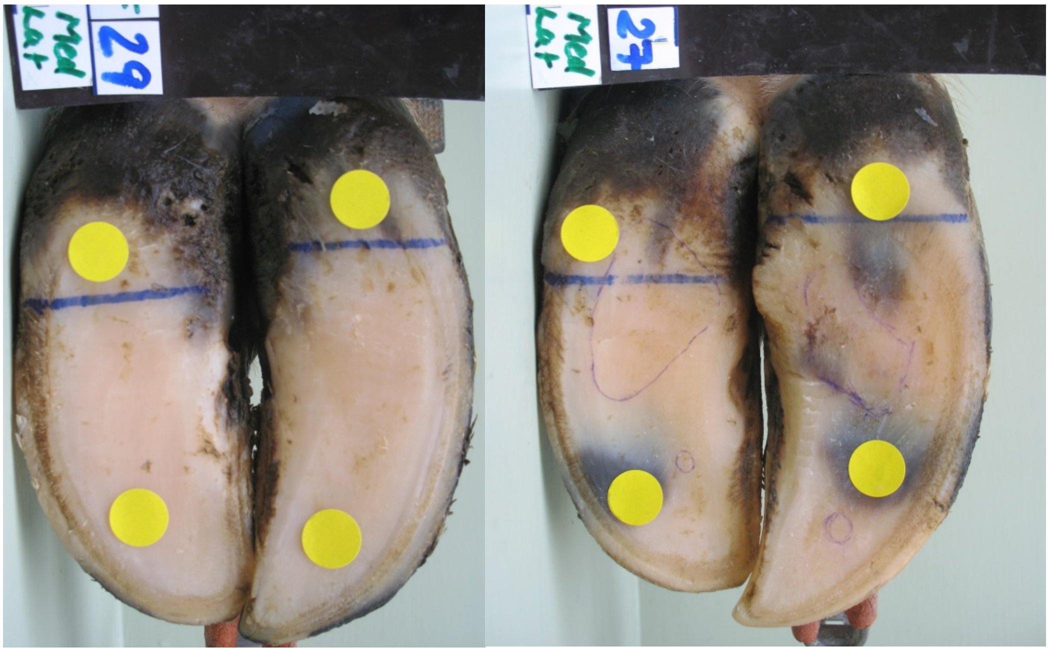

Figure 5.3: Palmar view of the cleaned surface of left front feet from Cows 29 and 27 showing line Y-Z and calibration dots applied (1.4 cm) as for the limb identification dots were: Red right front, yellow left front, blue left hind, green right hind. Grade one (mild, speckled diffuse ‘paintbrush’) lesions are outlined in cow 27 (right).

Each limb was mounted in a specially made photographic box which allowed the foot to be clamped at a set distance from the mounted camera and aligned parallel to it. Adhesive paper dots of 1.4 cm diameter were placed on each claw, one in the region of the toe and one in the region of the heel, for calibration purposes, then a digital image was recorded (Power shot A640, Canon) taken on the macro setting, so that their images filled the display. Each image included foot and animal identification (Figure 5.3).

The presence of haemorrhage was scored from the images obtained. A claw was positive for a white line lesion if haemorrhage was evident in the white line contained within zones 1, 2 or 3 (Figure 5.4: International hoof map; Greenough and Vermunt, 1994). A sole horn lesion was present if haemorrhage was seen in zones 4 or 5 (distal to the line YZ).

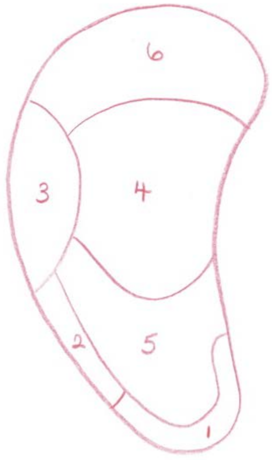

Figure 5.4: Diagram of the international hoof map, developed to allow recording of the location of lesions within the claw. Separate zones are defined for the white line and sole regions and named by number (1-6) (Greenough and Vermunt, 1994).

Anatomical studies

After measurement and photography was complete, the left front and right hind limbs were processed for ultrasound validation (Chapter 3) and then frozen at -20°C for at least 24 hours before being sectioned on the band saw along the line AB (Figure 5.2).

Electronic calliper measures of sole and soft tissue thickness were made (as described in Chapter 3 under: calliper measurement of distance to distal phalanx and sole thickness).

Samples were collected for histology from the lateral claw of the right hind and the medial claw of the left front feet. Two standard locations (based upon Räber et al., 2004) were used for the collection of samples (see Figure 5.5):

Site 1: a thin ≈3 mm thick sagittal slice of the axial surface of the abaxial portion of the sectioned claw was made and tissue was collected from the area directly below the concavity of the distal phalanx containing the digital cushion.

Site 2: a transverse sliver (≈ 3mm thick) was cut through the flexor tuberosity of the distal phalanx across the remaining abaxial portion of the claw.

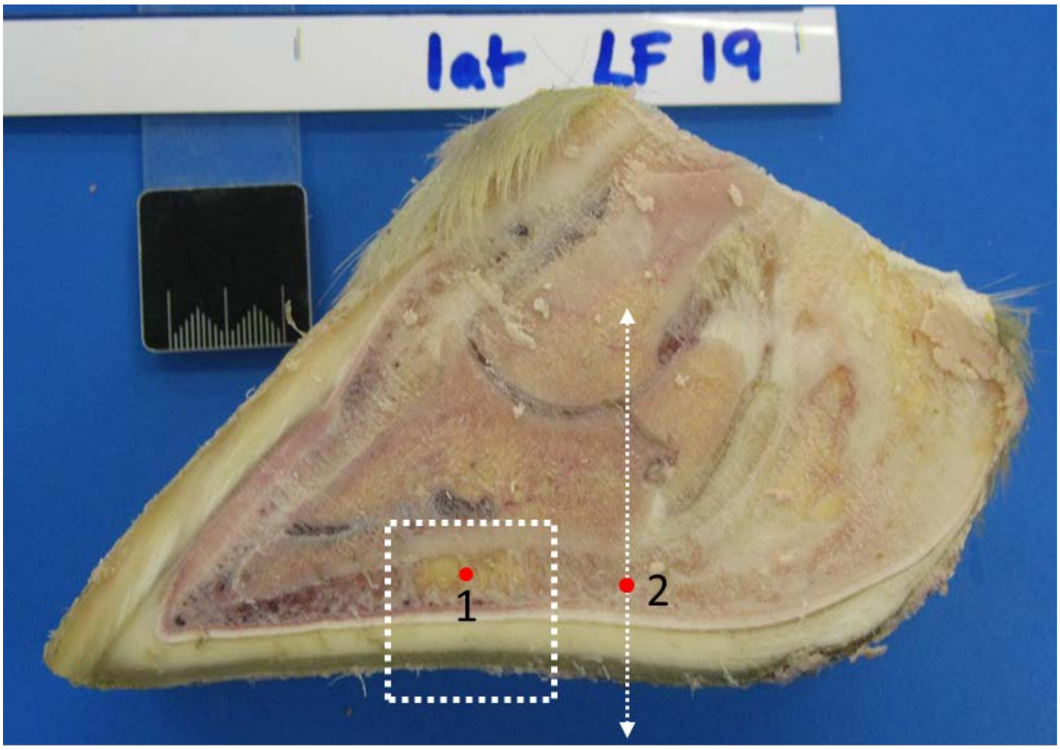

Figure 5.5: A frozen claw sectioned on the band saw along line AB (as shown in Figure 5.2), demonstrating the two collection sites for histological specimens.

Site 1: tissue from the area below the concavity of the distal phalanx contained the digital cushion.
Site 2: tissue collected from a transverse cut through the flexor tuberosity of the distal phalanx.

The samples were placed immediately after collection into 10% buffered formalin for fixation. They remained in the fixative for at least 30 days before further processing. A scalpel blade was used to dissect the bone out of each sample at the level of the periosteum, and any horn (wall or sole) in the sample was trimmed as much as possible to leave only a small portion to be cut on the microtome. The trimmed samples were mounted in wax and 3 to 4 µm sections cut on the microtome and mounted for staining with haematoxylin and eosin (H&E).

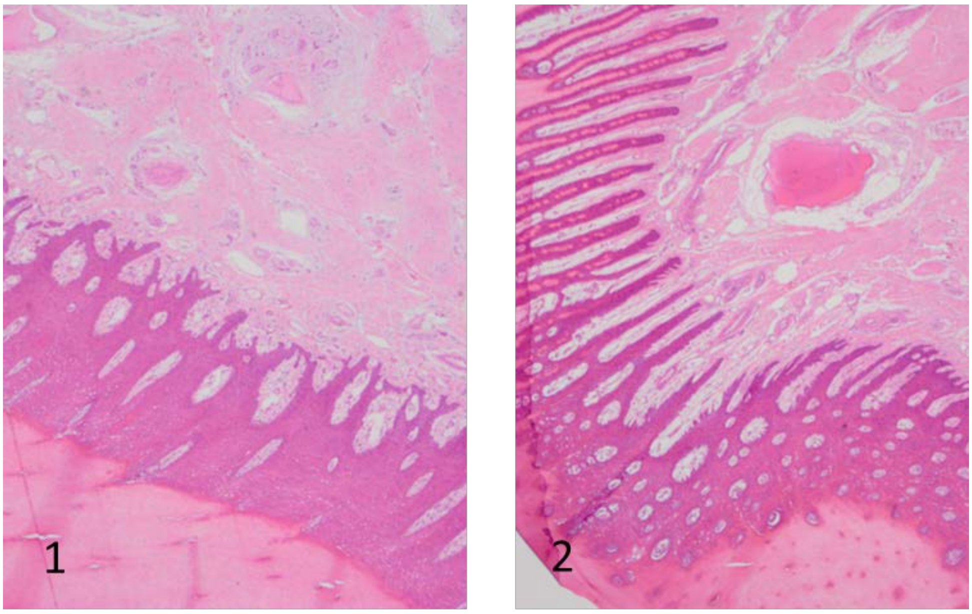

Figure 5.6: Photographic images of haematoxylin and eosin stained sections (low power x2.5) from two regions within the claw: Site 1; tissue from directly below the concavity of the distal phalanx; Site 2; tissue from a transverse cut through the flexor tuberosity of the distal phalanx.

Morphometry

The area between the papillae forming the sole horn and the digital cushion were examined by light microscopy for blood vessels in the H&E stained sections collected from Site 1. These were evaluated in a randomised order. To determine the point at which examination of the fields started, a dice was thrown and that number of x10 magnification fields were omitted before examination started. Thereafter, a castellated search pattern was used.

Blood vessels needed to satisfy the following criteria for inclusion in the study:

1) From the arterial side of the circulation. This was defined by (1) prominent nuclei in tunica intima; and (2) visible tunica media with at least one layer of smooth muscle cells i.e. spindle-shaped cells with elongated, round-ended nuclei in longitudinal section and acidophilic cytoplasm (Eurell, 2006).
2) Cut in good circular cross section

3) In order to avoid size related inconsistencies in vessel morphology, all selected vessels were small enough in cross section to be completely included within one x40 field of magnification on the microscope.

The first ten blood vessels on each slide, which satisfied the selection criteria, were photographed at x40 magnification, upon which a 100 µm calibration bar was superimposed. Files were then exported as TIFF files for image analysis.

This entire process was repeated for Site 2, imaging the first 10 blood vessels found in the region below the pedal bone (i.e. excluding the white line area).

Image analysis: Blood vessel morphometry

Images were opened in ImageJ software (ImageJ.nih.gov), and calibration set using the 100 µm calibration bar. The diameter of each blood vessel was recorded along the vertical axis. The area of the lumen was measured by outlining the cells of the tunica intima; and the area of the tunica media plus the lumen and intima was measured by outlining the tunica media.

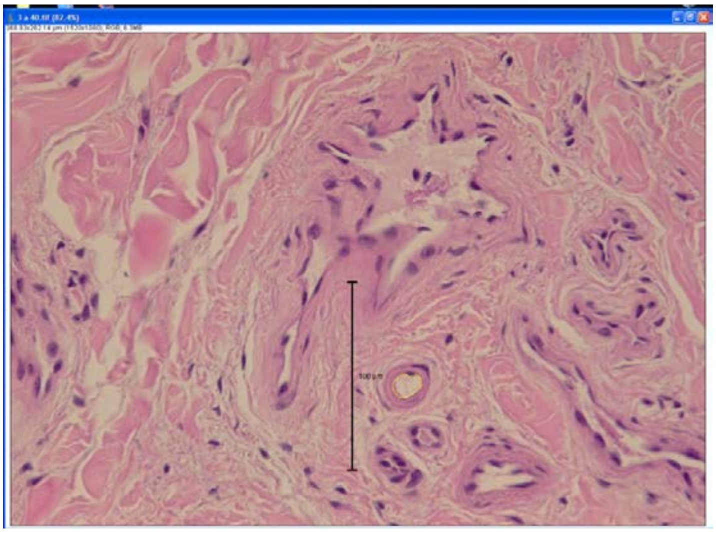

Figure 5.7A: Screen shot of haematoxylin and eosin stained tissue (x40) collected from Site 1 (area below the flexor tuberosity of the distal phalanx). The 100 µm bar was superimposed on the image at capture.

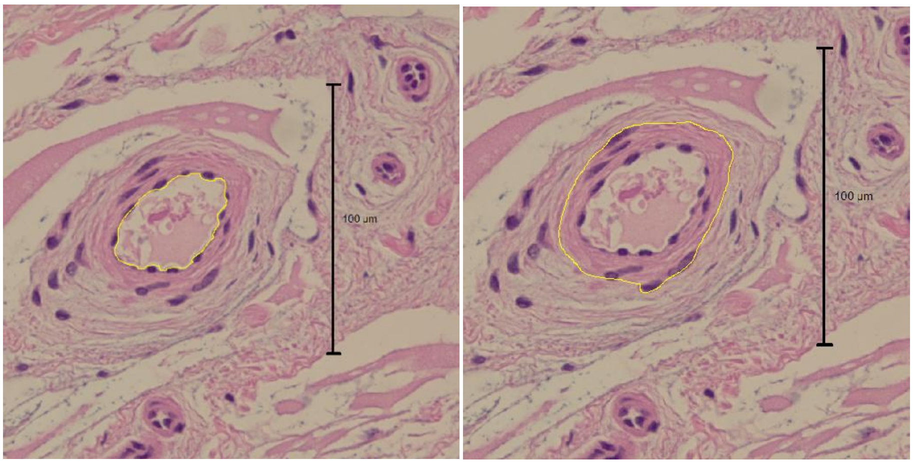

Figure 5.7B: Photographic images of haematoxylin and eosin stained tissue (x40) collected from Site 2 (area below the flexor tuberosity of the distal phalanx). Illustrating the ImageJ outline (yellow) of the lumen area (left) and the area within the boundary of the tunica media (right). Bar is 100 µm.

Statistical analysis

Only data from individuals where information from the left front medial claw and right hind lateral claw were both available were used in the analysis. All outcome variables were tested for normality and, where appropriate, transformed as required before data analysis. Data were analysed using correlations, linear regression and univariate linear mixed models.

All mixed models were built with foot, site and vessel as the variables used to identify repeated observations within the same cow. In addition, foot (left front medial claw or right hind lateral claw), site (1 or 2) and their interactions were entered into the model as fixed effects and cow was included as a random effect. In all models the right hind lateral claw and Site 2 were designated as the reference foot and site for the model.

For the mixed models the covariance structure chosen was that which had the lowest Akaike information criterion (AIC). Diagonal covariance structure was selected for all models.

After analysis, the residuals from each model were explored to ensure that the residuals were normally distributed and check for any relationship between the values predicted by the model and the residuals generated. Individual models are described in more detail below. All statistical analyses were undertaken using with SPSS 20 (IBM, SPSS, New York, USA).

Model 1: The effect of foot and site on the ratio of lumen area to the total area (L/V).

The ratio of lumen area to the total area was not normally distributed; so it was square root transformed (Figure 5.8) and this value (SQRT L/V) was used as the outcome variable in a repeat measures linear mixed model. Where foot site and vessel were the variables used to identify repeated observations within the same cow and foot, site, and interaction between foot and site were entered into the model as fixed effects and cow was included as a random effect.

Figure 5.8 illustrates the distribution of the raw (i.e. untransformed) L/V ratios. The distribution was right skewed, with a mean ratio of 0.25. Transformation of the data was explored and a square root transformation used to normalise the data (mean square root ratio: 0.48). The square root transformed data satisfied the Kolmogorov-Smirnov test for normality (P=0.155).

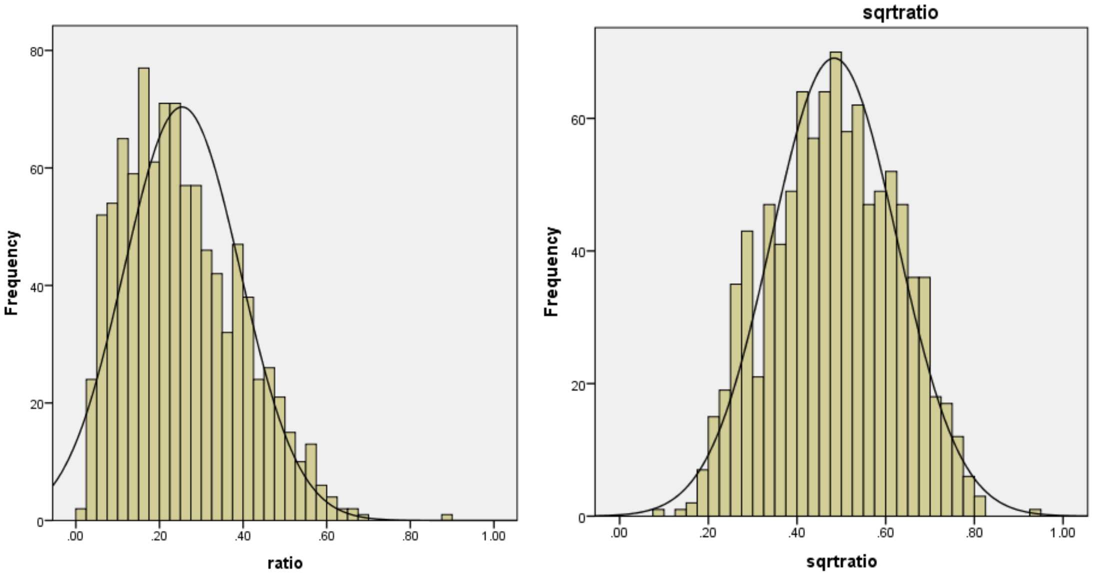

Figure 5.8: left panel: Frequency histogram for raw data, ratio (vessel lumen area: tunica media + tunica intima + lumen areas), with superimposed normal curve. Right panel: Frequency histogram for square root transformed data, Square root ratio (vessel lumen area: tunica media + tunica intima + lumen areas), with superimposed normal curve.

Model 2: The effect of hoof conformation on L/V.

SQRT L/V was used as the outcome variable in a repeat measures linear mixed model. Where foot site and vessel were the variables used to identify repeated observations within the same cow and foot, site, and interaction between foot and site were entered into the model as fixed effects and cow was included as a random effect. However, in addition to foot, site and interaction between foot and site, hoof conformation measurements were added as covariates to the model. The anatomical variables available for analysis were: toe angle, dorsal border length, claw width, claw length, proximal length, distal length, abaxial groove length, sole thickness, distance to distal phalanx, soft tissue thickness at Site 1 and soft tissue thickness at Site 2.

In order to reduce the risk of multicollinearity, the data for these variables were all checked for normally and then entered into a Pearson's correlation matrix (Table 5.1). The variables were then chosen for the model ensuring that none of the variables used were highly correlated ($r>0.5$) with the other variables included in the model. The choice of variables was based on minimising the number of variables in the model and, if possible, utilising variables which were easy to measure in the live claw.

This resulted in toe angle, dorsal border length, claw width, abaxial groove length, sole and soft tissue thickness at Site 1 and soft tissue thickness at Site 2 being entered into the model as covariates. A backwards stepwise elimination procedure was then used to create the final model to describe the relationship between hoof conformation and SQRT L/V. Factors were eliminated based upon P value until all factors were significant in the model ($P<0.05$).

Investigation of the effect of vessel size on L/V.

The ratio of lumen area to the total area was used in order to limit the effect of vessel size on the wall area in analysis. To confirm that this was the case, the effect of vessel size on SQRT L/V was investigated using linear regression and graphing.

Lumen area was not normally distributed Figure 5.9 illustrates the distribution of the raw (i.e. untransformed) lumen area data. The distribution was right skewed, with a mean lumen area of 559 μm^2 ± 29, transformation of the data was explored and $\log_{10}$ transformation used to normalise the data (mean $\log_{10}$ lumen area: 2.45 ± 0.016). After $\log_{10}$ transformation the data satisfied the Kolmogorov-Smirnov test for normality ($P=912$). Figure 5.10 shows the strong positive relationship between SQRT L/V. and $\log_{10}$ transformed lumen area data.

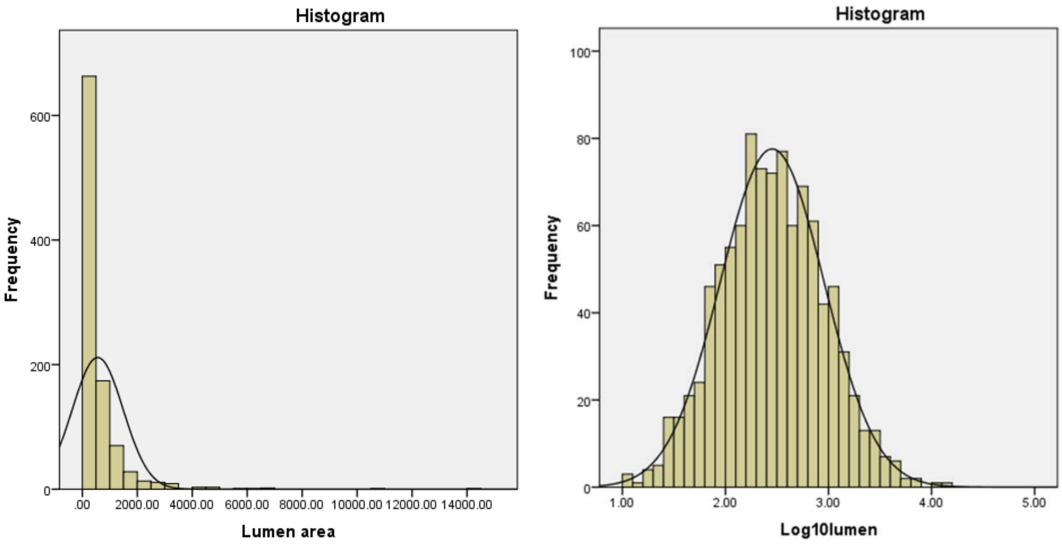

Figure 5.9: left panel: Frequency histogram for raw data, vessel lumen area, with superimposed normal curve. Right panel: Frequency histogram for log$_{10}$ transformed lumen area data with superimposed normal curve.

Model 3 and 4: The effect of section and foot and conformational measures on SQRT L/V with log$_{10}$ lumen as a covariate.

For Models 3 and 4, the analyses used for Models 1 and 2 (respectively) were repeated with the addition of (i) log$_{10}$ lumen area as a covariate and (ii) the interaction between log$_{10}$ lumen area and site in the models.

Model 5: The effect of lesion score for the white line and sole haemorrhages on SQRT L/V.

To assess whether there was any association between white line or sole horn haemorrhage and SQRT L/V Model 4 was repeated with lesion score category for sole and for white line horn (normal: 0, abnormal [i.e. any lesion score]: 1) included as fixed effects.

Table 5.1: Correlation matrix: Pearson's correlation coefficient matrix for anatomical variables in the foot. Emboldened figures: correlation is significant at the 0.01 level (2-tailed). Italic figures: correlation is significant at the 0.05 level (2-tailed). N is given in the second row of each line.

Pearson's Correlation coefficient (n)	Dorsal border length (cm)	Claw length (cm)	Claw width (cm)	Proximal (cm)	Distal (cm)	Abaxial groove length (cm)	Sole thickness Site 1 (mm)	Depth to distal phalanx Site 1 (mm)	Soft tissue depth Site 1 (mm)	Sole thickness Site 2 (mm)	Depth to distal phalanx Site 2 (mm)	Soft tissue depth Site 2 (mm)
Toe angle (degrees)	**-0.442** 54	**-0.375** 54	0.187 54	**0.349** 54	-0.198 54	**0.373** 54	0.150 48	*0.347* 48	**0.468** 48	*0.334* 48	**0.438** 48	0.268 48
Dorsal border length (cm)		**0.590** 54	0.252 54	0.232 54	**0.535** 54	*0.328* 54	0.239 48	0.218 48	0.036 48	0.102 48	0.150 48	0.116 48
Claw length (cm)			**0.363** 54	**0.436** 54	**0.705** 54	0.053 54	0.076 48	0.109 48	0.096 48	0.022 48	0.054 48	0.072 48
Claw width (cm)				**0.538** 54	**0.563** 54	*0.320* 54	-0.002 48	0.201 48	**0.430** 48	0.042 48	0.200 48	*0.345* 48
Proximal (cm)					**0.693** 54	**0.532** 54	0.216 48	**0.426** 48	**0.517** 48	0.281 48	**0.483** 48	**0.471** 48
Distal (cm)						0.208 54	0.056 48	0.236 48	0.399 48	-0.029 48	0.186 48	**0.457** 48
Abaxial groove length (cm)							0.289 48	**0.443** 48	**0.424** 48	**0.390** 48	**0.520** 48	*0.333* 48
Sole thickness Site 1 (mm)								**0.883** 48	0.084 48	**0.838** 48	**0.752** 48	-0.068 48
Depth to distal phalanx Site 1 (mm)									**0.542** 48	**0.778** 48	**0.793** 48	0.140 48
Soft tissue depth Site 1 (mm)										0.151 48	*0.337* 48	**0.418** 48
Sole thickness Site 2 (mm)											**0.886** 48	-0.106 48
Depth to distal phalanx Site 2 (mm)												*0.368* 48

Results

From 28 animals (56 processed claws: left front, medial and right hind, lateral claws) microscopy sections were obtained for 27 individual cows (54 claws) for Site 1 and 22 individuals (44 claws) for Site 2. Some claws were lost to analysis either because the paraffin-blocked tissue disintegrated on the microtome, or due to poor quality of the final stained section.

Model 1: The effect of foot and site on the ratio of lumen area to total vessel area (L/V).

Estimated marginal means of the SQRT L/V by foot were:

Left front medial claw: 0.489 (95 % CI: 0.476 to 0.501)
Right hind lateral claw: 0.475 (95 % CI: 0.463 to 0.487)

This difference was not significant (*P*=0.120).

Estimated marginal means of the SQRT L/V by site were:

Site 1: 0.495 (95 % CI: 0.483 to 0.506)
Site 2: 0.469 (95 % CI: 0.456 to 0.482)

Site within claw (1 or 2) was significant (P = 0.003). There was no interaction between site and foot (P=0.528).

Model 2: The effect of hoof conformation on SQRT L/V.

Following the backwards stepwise elimination of variables a final equation was generated:

SQRT L/V= 0.421 + 0.03*foot + 0.036*site + 0.03*dorsal border length (cm) - 0.068*claw width (cm) + 0.018*length of abaxial groove (cm)

For each unit increase in dorsal border length, the SQRT L/V ratio increased by 0.03 (95% CI: 0.015 to 0.047). For each unit increase in claw width, the ratio decreased by 0.068 (95% CI: 0.089 to 0.046). For each unit increase in the length of the abaxial groove, SQRT L/V increased 0.018 (95% CI: 0.002 to 0.033).

There was a significant effect of foot (*P*=0.014) and Site (P<0.001) on SQRT L/V

Estimated marginal means of the SQRT L/V by foot and site were:

Left front medial claw, Site 1: 0.494 (95 % CI: 0.480 to 0.508)

Left front medial claw, Site 2: 0.494 (95 % CI: 0.480 to 0.508)

Right hind lateral claw, Site 1: 0.475 (95 % CI: 0.461 to 0.489)

Right hind lateral claw, Site 2: 0.475 (95 % CI: 0.461 to 0.489)

There was no significant interaction between site and foot (P=0.496).

The effect of vessel size on SQRT L/V.

The relationship between the SQRT L/V and vessel size as represented by $\log_{10}$ lumen area is illustrated in the scatterplot (Figure 5.10).

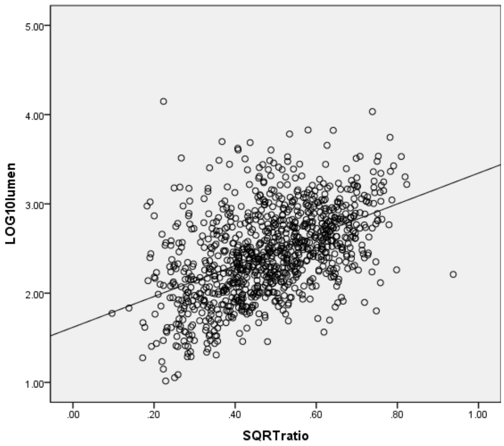

Figure 5.10: Scatterplot $\log_{10}$ lumen area versus SQRT L/V for all sections $R^2=0.24$ (P<0.001)

Linear regression revealed that the relationship between vessel size and SQRT L/V, while positive for both Sites 1 and 2, differed in slope between sections (Figure 5.11). The strength of the relationship varied between Site 1 and Site 2 ($R^2=0.11$ and 0.45, respectively; both P<0.001).

It was concluded that the use of the SQRT L/V did not account adequately for vessel size and subsequent models should include $\log_{10}$ lumen and interaction between $\log_{10}$ lumen and site in order to reflect effect of vessel size on SQRT L/V.

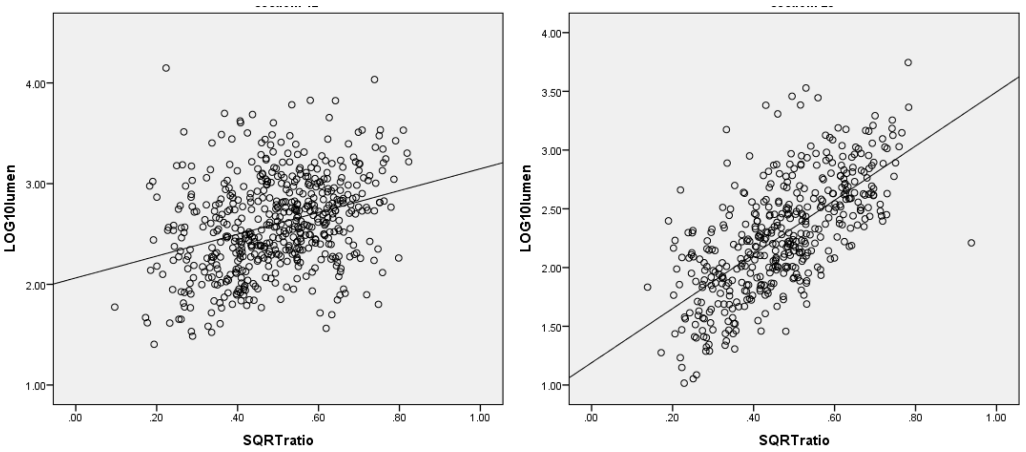

Figure 5.11: Scatterplots $\log_{10}$ lumen area versus SQRT L/ V. Left panel: Site 1 $R^2=0.11$. Right panel: Site 2 $R^2=0.45$.

Model 3: The effect of foot and site on SQRT L/V with log $_{10}$ lumen as a covariate.

There was no significant effect of foot at the $P<0.05$ level ($P=0.087$) or interaction between foot and site ($P= 0.654$) on SQRT L/V. However, site within claw was significantly related to SQRT L/V ($P<0.001$). Estimated marginal means of the SQRT L/V generated for each site were:

Site 1: 0.482 (95 % CI: 0.470 to 0.493)
Site 2: 0.509 (95 % CI: 0.499 to 0.519)

The model demonstrated a significant relationship between the SQRT L/V and vessel size as represented by $\log_{10}$ lumen ($P<0.001$). For each unit increase in $\log_{10}$ lumen, SQRT L/V increased by 0.202 (95% CI: 0.183 to 0.221). There was a significant interaction between $\log_{10}$ lumen and site ($P < 0.001$); compared to Site 2, in Site 1 each unit increase in $\log_{10}$ lumen decreased SQRT L/V by 0.101 (95% CI: 0.132 to 0.071) i.e. the increase in SQRT L/V resulting from an increase in $\log_{10}$ lumen, was less in Site 1 than in Site 2.

Model 4: The effect of hoof conformation on SQRT L/V with log $_{10}$ lumen as a covariate.

Following the backwards stepwise elimination of variables a final equation was generated:

SQRT L/V= 0.141 + 0.203*(site) - 0.002*toe angle - 0.047*claw width + 0.04*length of abaxial groove + 0.196*$\log_{10}$ lumen - 0.093*($\log_{10}$ lumen area*site)

(Where angles are in degrees and other measurements are in cm or cm^2)

Thus, for each unit increase in toe angle, SQRT L/V decreased 0.002 (95% CI: 0.004 to 0.0009), for each unit increase in claw width, SQRT L/V decreased 0.047 (95% CI: 0.065 to 0.03) and for each unit increase in the length of the abaxial groove, SQRT L/V increased 0.038 (95% CI: 0.025 to 0.051).

There was no significant effect of foot (P=0.116) or interaction between foot and site (P= 0.699) on SQRT L/V. However, site within claw was significantly related to SQRT L/V (P<0.001). Estimated marginal means of the SQRT L/V generated for each site were:

Site 1: 0.483 (95 % CI: 0.472 to 0.494)
Site 2: 0.506 (95 % CI; 0.496 to 0.515)

The SQRT L/V was smaller at Site 1 than Site 2 (as also in Model 3).

The model demonstrated a significant relationship between the SQRT L/V and vessel size as represented by $\log_{10}$ lumen ($P<0.001$). For each unit increase in $\log_{10}$ lumen, SQRT L/V increased by 0.196 (95% CI: 0.178 to 0.214).

There was a significant interaction between $\log_{10}$ lumen and site ($P<0.001$). Each unit increase in $\log_{10}$ lumen decreased SQRT L/V in Site 1 by 0.093 (95% CI: 0.123 to 0.064) when compared to Site 2 i.e. for vessels of equal size, the increase in SQRT L/V resulting from an increase in $\log_{10}$ lumen, was less in Site 1 than in Site 2.

Model 5: The effect of lesion score category for the white line and sole haemorrhages on SQRT L/V.

Neither white line lesion score category nor sole lesion score category had a significant relationship to SQRT L/V (P=0.578 and 0.973, respectively).

Discussion

This study was undertaken in an effort to better understand the prevalence of lesions attributable to SLS in cows managed under the pastoral dairying systems of New Zealand. The plan was to use morphometry to quantify the ratio of vessel lumen area to lumen plus tunica intima and media area of claw blood vessels and thereafter relate that ratio to external and internal conformational parameters of the claw, and the presence of claw horn haemorrhage in the form of lesion scores.

Histological samples were collected from frozen claws and processed into paraffin blocks. More technical difficulty was experienced in creating sections for light microscopy for samples from Site 2 (which had a greater amount of horn in the sample) than Site 1, but sufficient slides were obtained for the study. Application of the methodology described was successful; ten vessels matching the criteria were obtained for each site and the L/V ratio was calculated following image analysis.

Models 1 and 2 demonstrated a significant relationship between L/V ratio and the site of collection within the foot (SQRT L/V being smaller in Site 2 than Site 1) which initially appeared to align well with the working hypothesis that L/V ratio reflects vascular remodelling, as well as sitting well with published observations regarding the increased vascular change under light microscopy in the region of the claw from which Site 2 was collected (Boosman et al., 1989; Lischer et al., 2002a; Singh et al., 1992; van Amstel and Shearer, 2006).

The regions sampled for this study were chosen to mirror sites previously reported in the literature (Räber et al., 2004). They reflect areas of the claw exposed to different levels of mechanical insult. Site 1 was a region protected by the sole which incorporated a well-developed portion of the digital cushion; it is not a site of initial impact during locomotion nor is it a noted area for frequent claw horn haemorrhage. In comparison, Site 2 was collected from an area of the claw with a high potential for ‘internal’ trauma from the flexor tuberosity of the distal phalanx upon the underlying soft tissues (Lischer et al., 2002a, b; Tarlton et al., 2002) and was in the location of the ‘typical ulcer site’ described by several authors (e.g. Boosman et al., 1989; Ossent and Lischer, 1997; Vermunt, 1992; van Amstel and Shearer, 2006), where bruising or breach of the sole horn is common. Site 2 was also close to the region of impact and energy transfer during locomotion (Carvalho et al., 2005).

It was initially thought that the differences seen in the SQRT L/V between the two regions of the claw were due to increased vascular remodelling and repair in Site 2 compared with Site 1, because of differences between sites in the mechanical protection of the vessels and differences in the potential for traumatic insult (internal or external).

Such a disparity would imply that it is not only systemic diseases such as laminitis which affect the SQRT L/V, as a systemically mediated insult would be expected to produce similar levels of vascular damage throughout the claw and so should not result in the SQRT L/V differing significantly between the two sites. This is particularly so for laminitis as the histological changes it produces are widespread through the claw (Nilsson et al., 1963; Maclean, 1971; Andersson and Bergman, 1980; Thoefner et al., 2005), so vascular damage would also be widespread.

However, a strong relationship between SQRT L/V and Log_{10} lumen area revealed (Figure 5.9). Therefore, the smaller SQRT L/V seen at Site 2 in Models 1 and 2 could have been due to the vessels being smaller in this region. Model 3, which included $\log_{10}$ lumen area as a covariate showed that this was indeed the case and that for the same size vessel SQRT L/V was less in Site 1 than Site 2, in opposition to the suggestion that differences in SQRT L/V were due to increased vascular remodelling and repair in Site 2 compared with Site 1.

The finding of an association between the SQRT L/V and vessel size was not anticipated from the literature on morphometry, which suggested that use of the calculated ratio obviated the need to account for vessel size. For example, Palvesky et al. (1989) initially grouped vessels by size, but failed to find any difference in L/V ratio between vessels of different sizes. Perhaps the discrepancy between the present and previous studies arises because the current study does not have an applied treatment, exposure or intervention to allow before and after evaluation of the tissues and is thus observational and descriptive in nature. This is in contrast, for example, to Hart (1980) who examined the morphometry of brain parenchymal vessels after subarachnoid haemorrhage in cats, Amann et al. (2002) who examined the effect of DL-α-tocopherol treatment on vessel structure in the cardiovascular system in renal failure in rats, and Akinaga et al. (2009) who examined the response of vessels to chronic exposure to air pollutants in mice.

Nevertheless, the present results show that there is different vessel morphology in different regions and the entire claw cannot be assumed to start with the same baseline L/V ratio value. This study suggests that, when using morphometry, a measure of vessel size such as lumen area (Akinaga et al., 2009) should accompany the calculated ratio to allow the effect of size on the ratio to be incorporated in the model. Other size markers used in the literature include vessel diameter (Kashani et al., 1986; Pavelsky, 1989; Stopa, 2008), the size of intimal nuclei and wall thickness (at the short axis), though wall thickness is considered less reliable than the length of the internal elastic limiting membrane between tunica intima and tunica media, (Fernie and Lamb, 1985).

The present study shows that morphometry alone cannot be used to substantiate any conclusions about aetiology or pathogenesis of vascular changes. The use of vascular morphometry to assess slides from animals in affected groups compared to a normal control group would help to clarify how site within the claw affects SQRT L/V. This would help elucidate whether the smaller ratios seen in different sites are purely related to physiological and/or functional effects or whether pathophysiological changes, related to a defined diseased state, are also involved.

Model 4 examined the relationship between SQRT L/V and the conformational data obtained from the studied claws, while accounting for vessel size. A significant relationship was obtained between conformational variables and the SQRT L/ V. Given that the recent literature points to the importance of protection from internal structures such as the digital cushion (Räber et al., 2004; Bicalho et al., 2009) it was surprising that the anatomical variables entered into Models 2 and 5 that could be considered 'protection' variables did not feature in the modelling at all. It was the 'conformation' related variables which featured in the final models (dorsal border length, claw width and abaxial groove length for Model 2 and toe angle, claw width and abaxial groove length for Model 4). Räber et al. (2004) demonstrated that the digital cushion matures as cows reach their third parity, after which fat content declines and connective tissue increases. The animals in the present study were non-lame and younger parous cattle, so, from this perspective, the digital cushion should be mature and performing a good protective function.

Under the working hypothesis that the SQRT L/V reflects vascular remodelling in the pododermal structures, it might have been expected that a positive relationship between soft tissue dimensions or sole thickness of the individual claws and the SQRT L/V of the vessels would be present but this was not the case. Possible explanations include that the sites of sample collection (used to create the sections) and for measurements of sole and tissue depth are not identical which may have reduced the strength of any relationship present. Further to this, the body condition score of the animals used in the current study was unknown and recent ultrasound studies have shown that the effectiveness of the digital cushion could be reduced if they were in poor condition (Bicalho et al., 2009). It may simply be that the external measures of conformation used do represent changes in protection within the claw and the internal measures from the dead claw are not an improvement on these live measures.

No relationship was elucidated between SQRT L/V and lesion score status (Model 5, for the current study claws were categorised by lesion score normal vs. abnormal). This may well be because these non-lame animals had relatively few low-grade lesions and were not selected to be normal or exhibit gross pathological change but rather represent a convenience sample of cull dairy claws. The lesions recorded on these claws were very mild when compared to the feet examined in the live cow studies detailed in this thesis (Chapters 6-8)

Despite quite widespread use to assess vascular change in the field of human medical and physiological research, morphometry has not been previously applied in an attempt to quantify change related to claw conditions, lesions or conformation. Indeed, the only reported use of morphometry with regard to the bovine claw (Singh et al., 1992) has been to compare overgrown and normal claws of beef and dairy claws. They assessed the length of the dermo-epidermal junction, mean height and area of the stratum spinosum and stratum germinativum, mean number, height, thickness, and area of dermal papillae in the sole and coronary regions of the claw. From that study, Singh et al. (1992) identified that overgrown claws of dairy cattle had a smaller number of higher papillae in the sole region, with a shorter dermo-epidermal junction, than did normal claws; a finding which integrates well with the work of Hirschberg et al. (2001) and Hirschberg and Plendl (2005).

Further work to evaluate the morphometric changes seen in discrete, defined conditions may see it play a useful role in evaluating vascular change alongside histopathology. Morphometry has the advantage of being inexpensive and simple in comparison to techniques such as micro perfusion corrosion casting and subsequent light or scanning electron microscopy described by Hirschberg et al. (1999).

Conclusion

In conclusion, this study supported the initial hypothesis by the successful use of morphometry to obtain a ratio of vessel lumen area to lumen plus tunica intima and media area for hoof blood vessels in non-lame New Zealand dairy cows. Marked differences in the ease of creating histological sections between the Sites 1 and 2 were experienced; however the L/V ratio was simple to obtain for both sites. However, the initial hypothesis proved to be incorrect with respect to the relationship between vessel site within the claw and vascular remodelling. Using the transformed data, it was found that site within the claw was important because there was a variation in size of blood vessels between locations, and that this variation itself influenced the magnitude of the L/V ratio. The hypothesised relationship to conformational variables was demonstrated with SQRT L/V related not just to vessel size and Site, but also to the conformational variables claw width and abaxial groove length. In contrast the hypothesised association between the SQRT L/V and claw horn lesions was not proven.

Further research

Assessment of sections from animals thought to have coriosis would establish whether changes in vascular morphometry are present in those cases and what form they take. Multiple sections throughout the claw would be examined, preferably using both morphometry and histopathology to allow maximum information to be collected and integrated. To allow this, fresh collection of samples immediately following slaughter would be advisable. Special staining such as Perl's Iron hemosiderin may help distinguish between recent haemorrhage in tissues and more mature haemorrhage that has undergone change and macrophage ingestion. Location of such stained remnants may help to differentiate chronic trauma from more acute vascular incidents where remodelling and repair has not had time to occur.

Publication

Preliminary results were accepted for **ORAL PRESENTATION** at the International Ruminant Lameness Conference in Bristol August 2013. Morphometric analysis of blood vessel architecture in histological sections of the bovine claw - method and preliminary findings. Authors: LJ Laven, RA Laven, M Perrott, N Lopez-Villalobos, and TJ Parkinson.

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Chapter six: Estimations of claw conformation and volume change over lactation for two sequential cohorts of first lactation heifers

Introduction

The changes to claw conformation that occur over time have been addressed in many live animal studies (see Vermunt and Greenough, 1995). Of the characteristics listed by Vermunt and Greenough (1995), toe angle (Offer et al., 2000; Loberg et al., 2004; Leach et al., 2005; Telezhenko et al., 2009) and dorsal border length (Vokey et al., 2001; Manske et al., 2002; Telezhenko et al., 2009; Fjeldaas et al., 2011) feature prominently in the literature. Other characteristics such as heel height (Fregonesi and Leaver, 2001; Somers et al., 2005; Kremer et al., 2007; Ouweltjes et al., 2009), heel angle (Kremer et al., 2007), claw diagonal (Nüske et al., 2003; Somers et al., 2005; Kremer et al., 2007; Ouweltjes et al., 2009), claw length (Vermunt and Greenough, 1996; Kremer et al., 2007; Baird et al., 2009) and claw height (Somers et al., 2005) have also been described. Scott et al. (1999) described a method to estimate claw volume, which was initially greeted by a degree of acclaim (Vermunt, 1999), although no subsequent application to the live animal has been reported. In consequence, the value of using claw volume in the investigation of the development of lesions and lameness has not yet been assessed.

The literature review has shown that results for many conformational parameters are inconsistent even within the same system. Seasonal patterns are seen but significant differences can arise between years (Boelling and Pollott, 1998; Offer et al., 2000). Inconsistency may relate to the timescale of studies (i.e. being too short to allow for conformational change to be seen; Boelling and Pollott, 1998; Kremer et al., 2007), or perhaps the composition of the study population introducing variability into conformational estimates e.g. by incorporating mixed breeds or a range of ages (Ahlström et al., 1986). In particular, descriptions of conformational changes over lactation in pasture-based systems are limited (Tranter and Morris, 1992).

Offer et al. (2000) demonstrated the vulnerability of heifers entering the milking herd to the development of claw lesions during their first lactation and the increased risk of foot disease (claw lesions and increased locomotion score) in subsequent lactations. They concluded that it is important to maximise claw health in young stock and first lactation heifers to improve their future lameness status.

Hypothesis: First lactation heifers (approx. 24 months at calving) managed in a New Zealand pastured based dairy system will experience a modification of their claw conformation throughout lactation during their - maturation and growth-and due to increased exposure to concrete, track surfaces and long distance walking. The collection of data from both claws of both hind feet will give more information than assessment of one foot or nominated claw only.

From the literature it is expected that changes of conformation in pasture-based animals will be of an intermediate between the extremes of straw or rubber flooring and concrete or asphalt surfaces. For example, dorsal border length and toe angle are expected to increase and reduce respectively, but not to the extremes described for animals managed exclusively on straw or with rubber surfaces.

It is postulated that claw volume will increase over the course of lactation to reflect animal maturation and growth, perhaps with a size bias towards the lateral claw or a dominant hind limb. In addition to allowing the prediction of claw volume, the collection of proximal coronary band, distal wall and abaxial groove lengths are expected to add to the available parameters for monitoring claw shape. Collection over two consecutive years will allow the stability of the parameters to be ascertained.

The aim of the present study was to measure and evaluate the changes in claw conformation seen in two consecutive cohorts of first-calving heifers during their first lactation. Animals entered the milking herd after calving and were managed in a pasture-based system. To achieve the aim conformational data were collected to allow the application of the *in vitro* model, developed in Chapter 2, to: (1) estimate and monitor claw volume; and (2) to investigate the relationship between claw volume and variables of claw conformation. To ensure that there would be a broad range of descriptors of the claw; measurements were taken of toe angle, length of dorsal border, abaxial groove length and the proximal and distal distances from the abaxial groove to the dorsal flexure of the claw.

Materials and Methods

Animals

Year 1: Twenty five heifers of mixed breed (Friesian and Friesian/Jersey crossbreed) from a single dairy herd (Massey University No.4 herd) were recruited into the study as they calved during the 2008/2009 season.

Year 2: Twenty nine Friesian heifers were recruited to the study from the same dairy herd in the 2009/2010 season.

Animals were managed, on the same farm unit, in a typical New Zealand pasture based system, in which milking animals were kept solely at pasture and were milked in a rotary parlour, to which they walked twice a day. All animals were approximately 24 months of age at calving (Year 1 mean; 23.7 ± 0.3 months, range 22.3 to 29.3, Year 2 mean; 23.7 ± 0.08 months, range 23.0 to 24.5).

All live animal procedures performed were approved by the Massey University Animal Ethics Committee. Protocol numbers Year 1: MUAEC 08/25 and Year 2: MUAEC 09/59.

Conformational measurements

In Year 1, the heifers were examined on five occasions during their first lactation, approximately 10, 60, 110, 160 and 220 days post calving (DPC). In Year 2, to allow for an increased number of animals, examinations were made on only four occasions during the first lactation, at approximately 10, 60, 120, and 230 DPC.

The heifers were restrained in purpose designed foot crush (WOPA, Netherlands) for efficient lifting of hind limbs. Each foot was lifted, cleaned and if necessary, washed and dried. Surface preparation was kept to a minimum. Conformational measurements were made on both claws of the hind feet (Figure 6.1). All measurements were made using a flexible tape measure, except for toe angle (measured using an engineer’s angle finder) and claw height (for which electronic callipers were used).

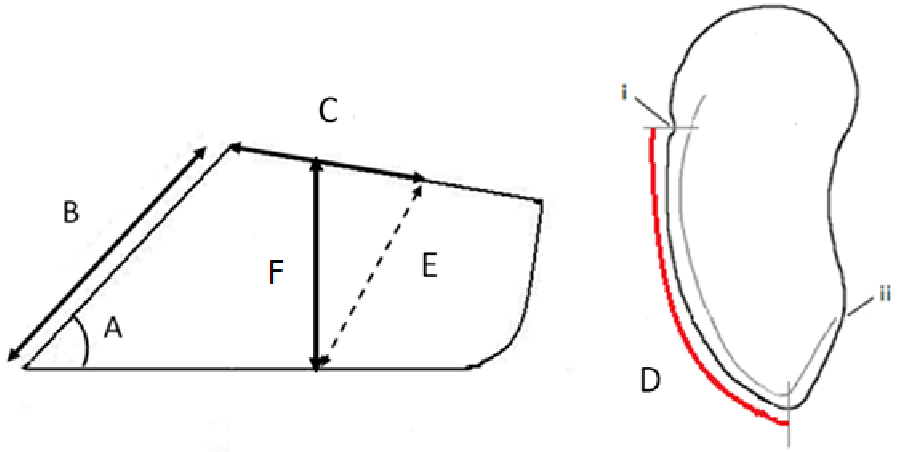

Figure 6.1: Left and right panels show the six variables measured on both claws of the hind feet of 54 first lactation dairy heifers. Left panel: Abaxial view of claw. Right panel: The plantar surface of the sole: (i) the abaxial groove, (ii) end of the axial white line. Measures adapted from Vermunt and Greenough (1995) and Scott et al. (1999).

A. **Toe angle:** Angle of dorsal border to weight-bearing surface;
B. Length of **dorsal border** from skin: horn junction at coronary band to apex of toe;
C. Distance from the point where the coronary band meets the flexure of the dorsal border to the abaxial groove along the coronary band (**proximal**);
D. Distance from the apex of the toe to the abaxial groove along the wall horn (**distal**);
E. Length of the **abaxial groove** from the coronary band to base of claw;
F. **Height of the claw** at the distal end of the abaxial groove.

Volume estimation

Individual claw volume was predicted for the medial and lateral claws of both hind feet using the model created in Chapter 2.

Predicted claw volume = -116.84 + (13.18*Claw category) + (24.38*C) + (4.49*E) - 14.62

(Claw category: lateral claw=1, medial claw=0, C: proximal, E: abaxial groove length).

Statistical analysis

All outcome variables were assessed for normality and graphical exploration of the raw data performed to illustrate of interaction between time, foot and claw over the course of lactation.

A univariate linear mixed model was applied to each outcome variable in turn, to investigate the effect of foot, claw within foot and time on each variable in Year 1 and Year 2. A correlation matrix (Spearman’s ranked correlation, ρ) was used to examine the relationship between the change in each variable between Days 10 and 220 (Year1) and Days 10 and 230 (Year 2).

All mixed models were built with claw, foot and time as the variables used to identify repeated observations within the same cow. In addition, foot (left or right hind), claw within foot (medial or lateral in left or right hind), time (categorical variable based on the examination number post calving) and the interactions between claw or foot and time were entered into the model as fixed effects. Cow was included as a random effect. For all models a random intercept was included to account for individual variation between cows; testing confirmed that in all cases this inclusion was appropriate as the variance of the intercept was >0 (Wald Z test). The designated reference categories for claw within foot and time in the models were medial claw and the final examination on Day 220 or 230, unless otherwise indicated.

The covariance structure of the repeated measures was decided with lowest Akaike information criterion (AIC). The residuals for each model were assessed for normality and the relationship between the values predicted by the model, for the variable under investigation, and the residuals generated by the model were assessed as a measure of fit.

All statistical analyses were undertaken using SPSS version 20 (IBM, SPSS, New York, USA).

Results

The mean values for toe angle, dorsal border, length of the abaxial groove, proximal, distal and predicted volume for each foot and claw combination were tabulated and the data presented in graph form. Graphical illustrations use raw data and average group timings throughout lactation rather than actual DPC.

In Year 1, animals were examined on five occasions throughout their first lactation, approximately Days 10 (mean 13 DPC; range 3-32), 60 (62; 55-74), 110 (110; 104- 117), 160 (160; 153-176) and 220 (221; 205-254) post calving. The number of animals examined at each time point was twenty five with the exception of Day 10 (24 animals).

In Year 2, they were examined on four occasions throughout their first lactation, approximately Days 10 (mean 14 DPC; range 7-25), 60 (62; 57-81), 120 (119; 110-137) and 230 (228; 220-238) days post calving. Twenty-nine animals were examined at each time point except Day 230 (28 animals).

Toe angle Year1

Mean toe angles for both hind feet (Year 1) are tabulated for each claw and time combination in Table 6.1.

Table 6.1: Mean (SEM) toe angle (degrees) from raw data for each foot, claw, and time combination. Angles were recorded with an electronic angle finder in both hind limbs of 25 first lactation heifers on five occasions on Days 10, 60,110, 160 and 220 post calving.

		Year 1: Toe angle (degrees) (SEM)				
Foot	**Claw**	**Day 10***	**Day 60**	**Day 110**	**Day 160**	**Day 220**
LH	**Medial**	43.4 (0.54)	43.8 (0.52)	44.4 (0.73)	44.5 (0.51)	44.6 (0.77)
	Lateral	44.4 (0.66)	44.1 (0.63)	43.7 (0.75)	45.1 (0.67)	44.1 (0.70)
RH	**Medial**	42.8 (0.55)	43.7 (0.54)	45.1 (0.66)	45.2 (0.63)	44.2 (0.48)
	Lateral	44.6 (0.77)	44.6 (0.77)	43.7 (0.77)	45.1 (0.67)	44.1 (0.61)

*n=24

The distribution of toe angle data for all claws is shown in Figure 6.2. This demonstrates an outlier (220 days post calving). However, on investigation this animal (575) was found to be among the suspected outliers at each time point; this cow had a consistently shallow toe angle at each recording point. Removal of this cow did not alter the model's outcome markedly, so it was kept in all analyses.

The best model for toe angle in Year 1 had a first-order autoregressive covariance structure of the repeated measures. Neither foot, nor claw position within foot, had a significant effect on toe angle (*P*=0.368 and 0.101, respectively). However, comparing claws within the same foot, lateral claw toe angle tended to be smaller in the left hind foot but, was greater in the right hind foot, mean difference:

Left hind foot: -0.52° (95 % CI: -1.67 to +0.62)
Right hind foot: 1.35° (95 % CI: 0.20 to 2.49)

There was a significant effect of time on toe angle (*P*<0.001), such that toe angle was maximal on Day 160. Toe angle on Day 220 was significantly greater than on Day 10 (*P*=0.024), not significantly different from Days 60 or 110 (*P*=0.402 and 0.108 respectively) and tended to be smaller than on Day 160 (*P*=0.059). There was no interaction between foot and time (*P*=0.684) but a significant interaction (*P*=0.008) between claw within foot and time (Figure 6.3).

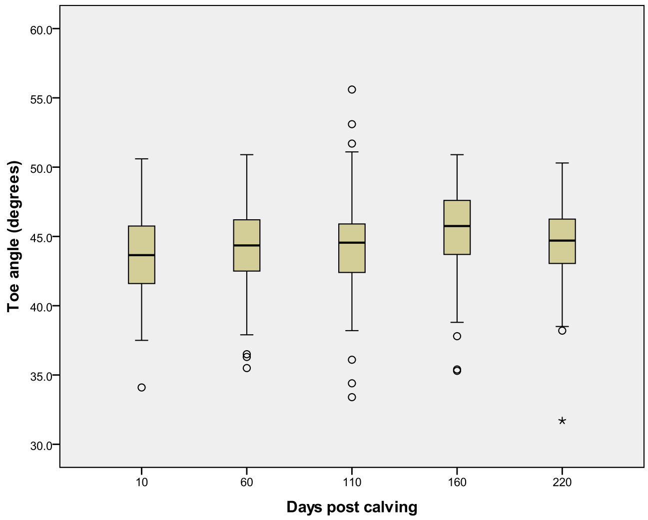

Figure 6.2: Relationship between time since calving and toe angle (Year 1) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Interpretation: The line in the box marks the median and the central box spans the quartiles. Lines extend from the box to the smallest and largest observations that are not suspected outliers. Observations more than 1.5 times the interquartile range (1 step) from the box are plotted individually as suspected outliers (open circles). Observations more than 2 steps from the box are plotted individually (stars) and are probable outliers.

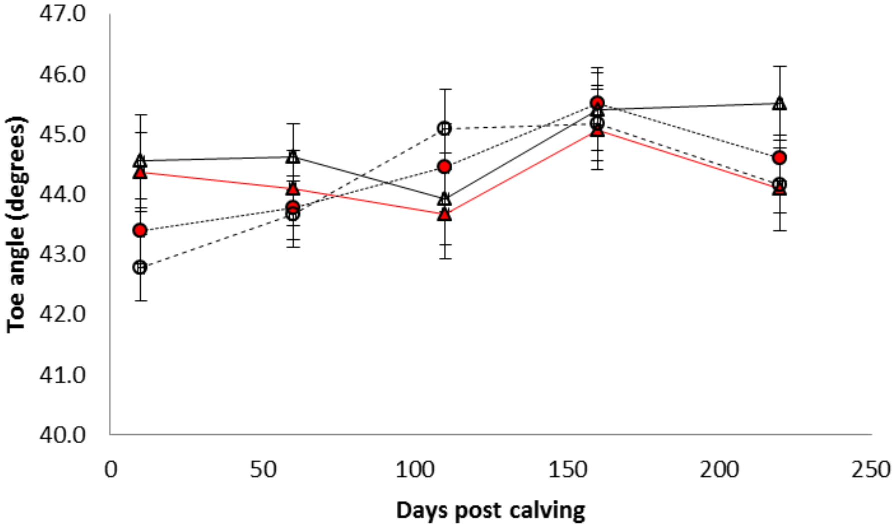

Figure 6.3: Mean (SEM) toe angle (degrees) for the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Toe angle Year 2

Mean toe angles for both hind feet (Year 2) are tabulated for each claw and time combination in Table 6.2 and shown as a boxplot in Figure 6.4.

Table 6.2: Mean (SEM) toe angle (degrees) from raw data for each foot, claw, and time combination. Angles were recorded with an electronic angle finder in both hind limbs of 29 first lactation heifers on Days 10, 60, 120, 160 and 230 post calving.

		Year 2: Toe angle (degrees) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230 ***
LH	**Medial**	44.5 (0.42)	45.0 (0.53)	44.1 (0.42)	43.0 (0.49)
	Lateral	45.3 (0.53)	44.4 (0.55)	45.0 (0.62)	44.3 (0.60)
RH	**Medial**	44.6 (0.51)	43.3 (0.54)	44.1 (0.62)	40.9 (0.53)
	Lateral	45.7 (0.66)	45.1 (0.66)	46.0 (0.59)	45.0 (0.61)

*n=28

The best model for toe angle in Year 2 had heterogeneous first-order autoregressive covariance structure. There was no significant effect of foot (*P*=0.971) on toe angle. However, the effect of claw position within foot was significant (*P*<0.001). Comparing claws within the same foot, toe angle was relatively even in both claws of the left hind foot, whilst in the right hind, lateral claw toe angle was greater than medial claw (Figure 6.5), mean difference:

Left hind foot: 0.25° (95 % CI: -0.75 to +1.24)
Right hind foot: 4.13° (95 % CI: 2.88 to 5.38)

There was a significant effect of time on toe angle (*P*<0.001), with a decrease in angle over the entire period to Day 230 (*P*<0.001). There was no interaction between foot and time (*P*=0.140). However, the interaction between claw within foot and time was significant (*P*=0.004): Figure 6.5.

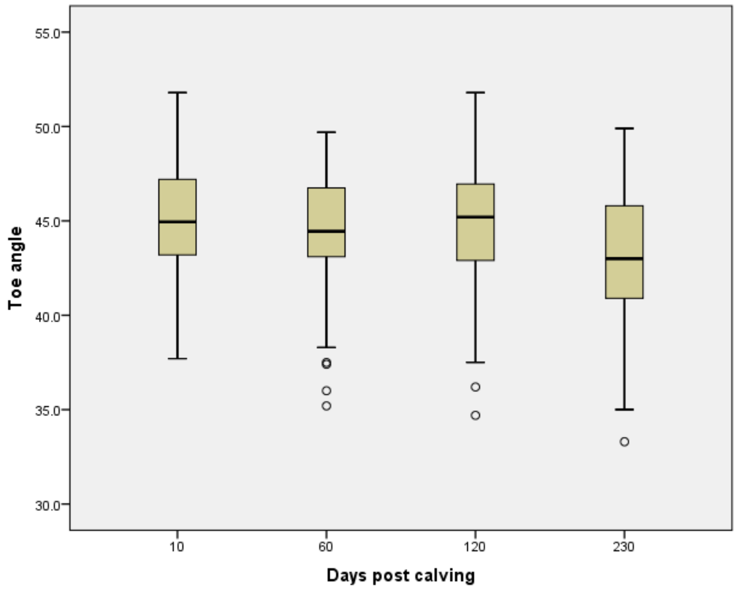

Figure 6.4: Relationship between time since calving and toe angle (Year 2) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 120 and 230 post calving. For interpretation of the box and whisker plots see Figure 6.2.

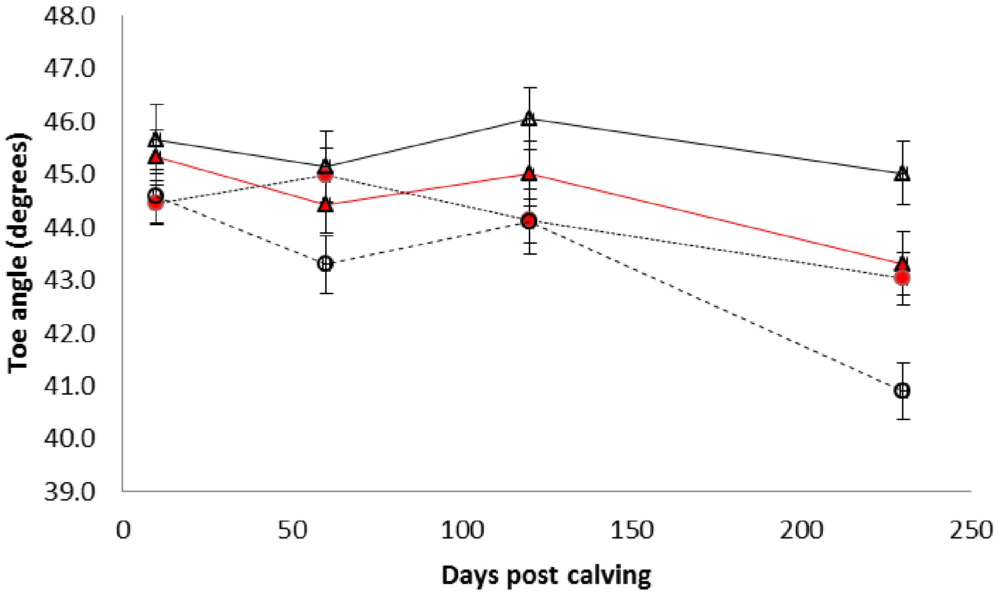

Figure 6.5: Mean (SEM) toe angle (degrees) for the medial and lateral claws of both hind limbs of 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Dorsal border Year 1

Mean dorsal border lengths for both hind feet (Year 1) are tabulated for each claw and time combination in Table 6.3 and shown as a boxplot in Figure 6.6.

Table 6.3: Mean (SEM) dorsal border length (cm) from raw data for each foot, claw, and time combination. Borders were measured with a flexible tape measure in both hind limbs of 25 first lactation heifers on Days 10, 60, 110, 160 and 220 post calving.

		Year 1: Dorsal border (cm) (SEM)				
Foot	**Claw**	**Day 10***	**Day 60**	**Day 110**	**Day 160**	**Day 220**
LH	**Medial**	7.3 (0.09)	7.1 (0.08)	7.2 (0.08)	7.2 (0.06)	7.4* (0.09)
	Lateral	6.8 (0.10)	7.0 (0.10)	7.2 (0.10)	7.3 (0.09)	7.4 (0.10)
RH	**Medial**	7.1 (0.07)	7.1 (0.08)	7.2 (0.08)	7.3 (0.09)	7.4 (0.08)
	Lateral	7.1 (0.07)	7.1 (0.08)	7.2 (0.08)	7.3 (0.09)	7.4 (0.08)

*n=24, 258 removed from data set recording error.

The best model for dorsal border length in Year 1 had a heterogeneous first-order autoregressive covariance structure. There was a significant (*P*=0.043) effect of foot on dorsal border length.

The effect of claw position within foot was significant (*P*=0.001) (Figure 6.7). However, comparing dorsal border length in claws of the same foot, mean difference in was negligible.

There was a significant effect of time on dorsal border length (*P*<0.001), with maximum values being seen on Day 220. Values on Day 160 were not significantly lower than those on Day 220 (*P*=0.128), but differences on all other days were significant (Day 10: *P*=0.003, Day 60: *P*=0.013, Day 110: *P*=0.016). There was no significant interaction between foot and time (*P*=0.748) but significant interaction was present between claw within foot and time (*P*<0.001): Figure 6.7.

The model residuals did not satisfy the Kolmogorov-Smirnov test. Graphical examination of residuals, identified five data points, of which, after cross-reference to the initial box plot

(Figure 6.6) four were included as possible outliers. When these five data points were removed from the data set the residuals were normally distributed. However, this removal did not alter the outcome of the mixed model markedly so all data points were retained in the analysis.

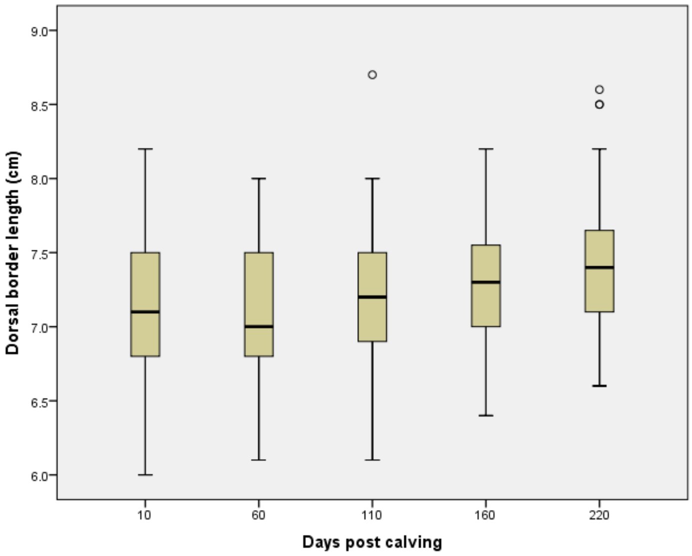

Figure 6.6: Relationship between time since calving and dorsal border length (Year 1) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving. For interpretation of the box and whisker plots see Figure 6.2.

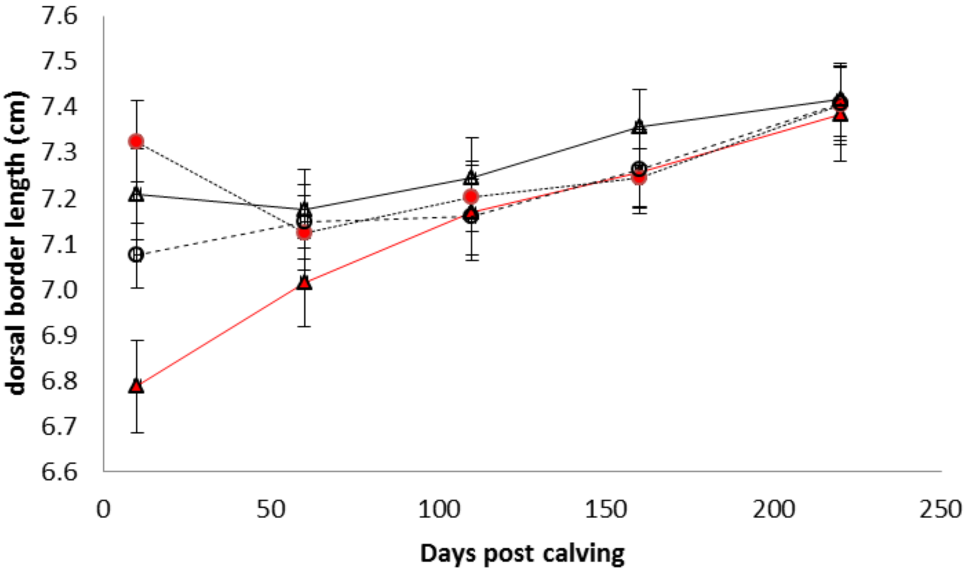

Figure 6.7: Mean (SEM) dorsal border length (cm) for the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Dorsal border Year 2

Mean dorsal border lengths for both hind feet (Year 2) are tabulated for each claw and time combination in Table 6.4 and shown as a boxplot in Figure 6.8.

Table 6.4: Mean (SEM) dorsal border length (cm) from raw data for each foot, claw, and time combination. Borders were measured with a flexible tape measure in both hind limbs of 29 first lactation heifers on Days 10, 60, 120 and 230 post calving.

		Year 2: Dorsal border (cm) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230***
LH	**Medial**	7.6 (0.05)	7.3 (0.06)	7.4 (0.07)	7.8 (0.06)
	Lateral	7.4 (0.06)	7.2 (0.05)	7.2 (0.06)	7.7 (0.07)
RH	**Medial**	7.6 (0.07)	7.3 (0.07)	7.4 (0.07)	7.8 (0.08)
	Lateral	7.4 (0.04)	7.2 (0.05)	7.3 (0.04)	7.7 (0.06)

*n=28

The best model for dorsal border length in Year 2 had a first-order autoregressive covariance structure. There was no effect of foot (*P*=0.497) on dorsal border length, but the effect of claw position within foot was significant (*P*<0.001). Comparing claws in the same foot, dorsal border tended to be shorter in lateral claws than medial claws but by only a very small amount, mean difference:

Left hind foot: -0.11 cm (95 % CI: -0.23 to +0.01)
Right hind foot: -0.10 cm (95 % CI: -0.22 to +0.02).

There was a significant (*P*<0.001) effect of time on dorsal border length, such that the length was maximal on Day 230. Minimum values occurred on Day 60, representing a significant (*P*<0.001) decrease from values on Day 10. There was no interaction between either, foot and time (*P*=0.500) or claw within foot and time (*P*=0.748): Figure 6. 9.

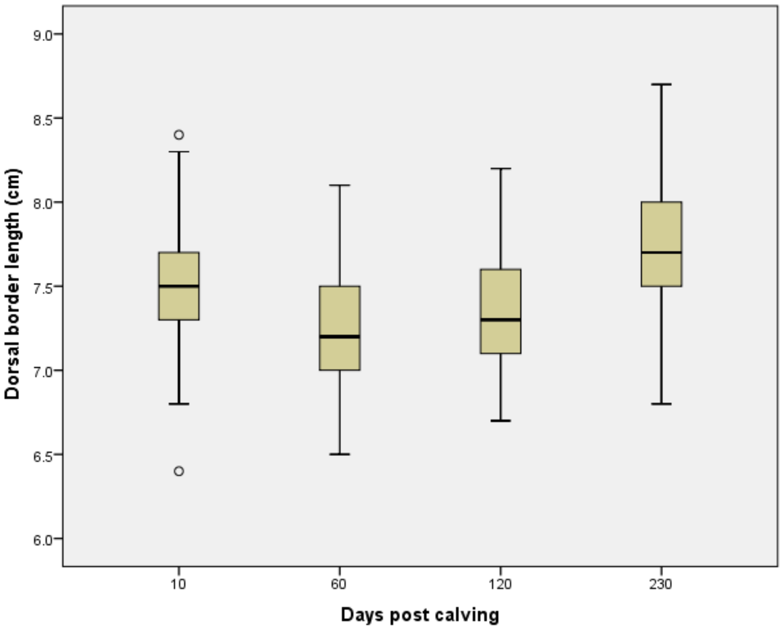

Figure 6.8: Relationship between time since calving and dorsal border length (Year 2) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 120 and 230 post calving. For interpretation of the box and whisker plots see Figure 6.2.

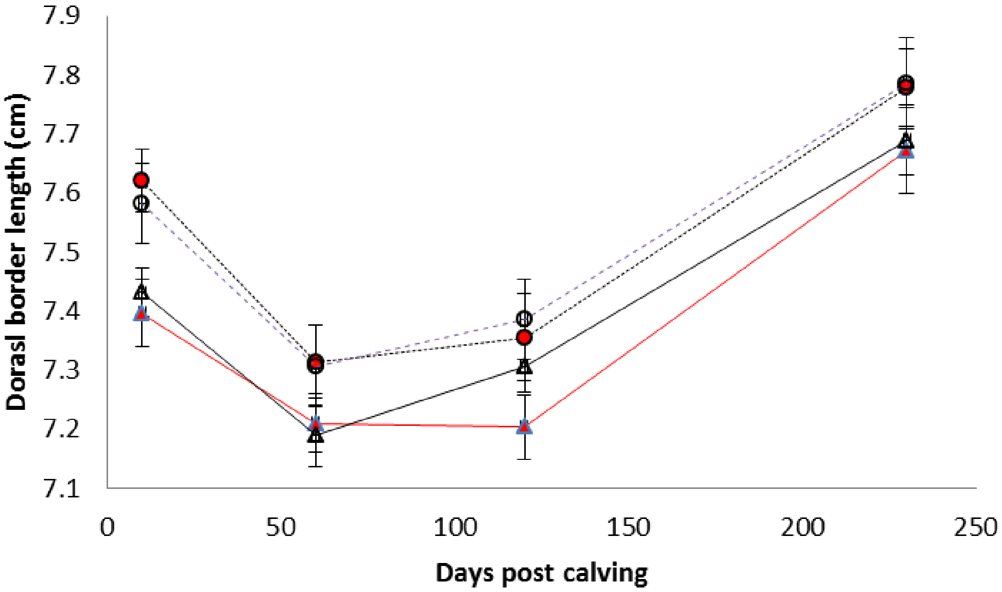

Figure 6.9: Mean (SEM) dorsal border length (cm) for the medial and lateral claws of both hind limbs of 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Abaxial groove Year1

Mean abaxial groove lengths for both hind feet (Year 1) are tabulated for each claw and time combination in Table 6.5 and shown as a boxplot in Figure 6.10.

Table 6.5: Mean (SEM) abaxial groove length (cm) from raw data for each foot, claw, and time combination. Lengths were measured with a flexible tape measure in both hind limbs of 25 first lactation heifers on five occasions on Days 10, 60,110, 160 and 220 post calving.

		Year 1: Abaxial groove length (cm) (SEM)				
Foot	**Claw**	**Day 10***	**Day 60**	**Day 110**	**Day 160**	**Day 220**
LH	**Medial**	4.3 (0.08)	4.3 (0.07)	4.4 (0.07)	4.6 (0.07)	4.7 (0.07)
	Lateral	4.2 (0.06)	4.3 (0.06)	4.3 (0.05)	4.6 (0.07)	4.7 (0.08)
RH	**Medial**	4.3 (0.06)	4.3 (0.07)	4.3 (0.07)	4.5 (0.06)	4.6 (0.07)
	Lateral	4.4 (0.07)	4.4 (0.04)	4.4 (0.07)	4.7 (0.07)	4.7 (0.07)

*n=24

The best model for abaxial groove length in Year 1 had first-order autoregressive covariance structure. There was no significant effect of foot on abaxial groove length (*P*=0.679). The effect of claw position within foot was significant (*P*=0.042): Figure 6.11. However, the difference between claws of the same foot in abaxial groove length was very small and likely to be biologically unimportant.

There was a significant (*P*<0.001) effect of time on abaxial groove length. Abaxial groove length was maximal on Day 220, although the difference from values on Day 160 was not significant (*P*=0.168). There was no interaction between either foot and time (*P*=0.577) or claw within foot and time (*P*=0.906): Figure 6.11.

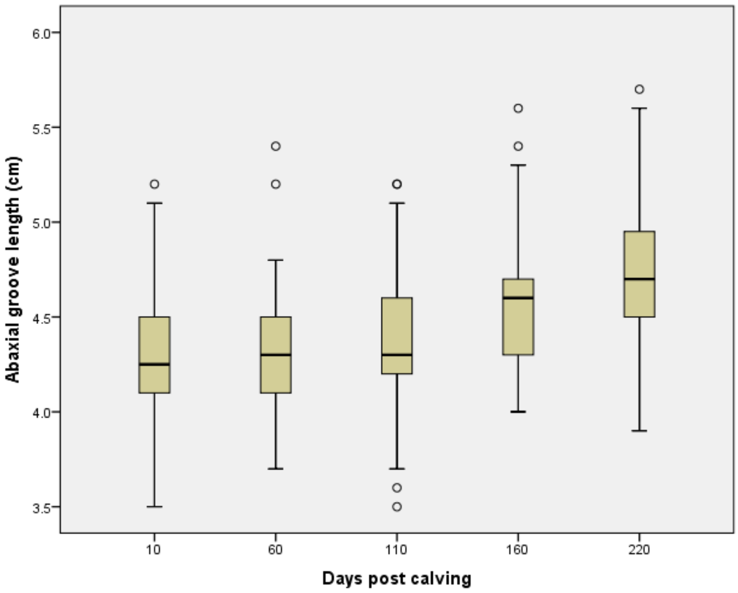

Figure 6.10: Relationship between time since calving and abaxial groove length (Year 1) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving. For interpretation of the box and whisker plots see Figure 6.2.

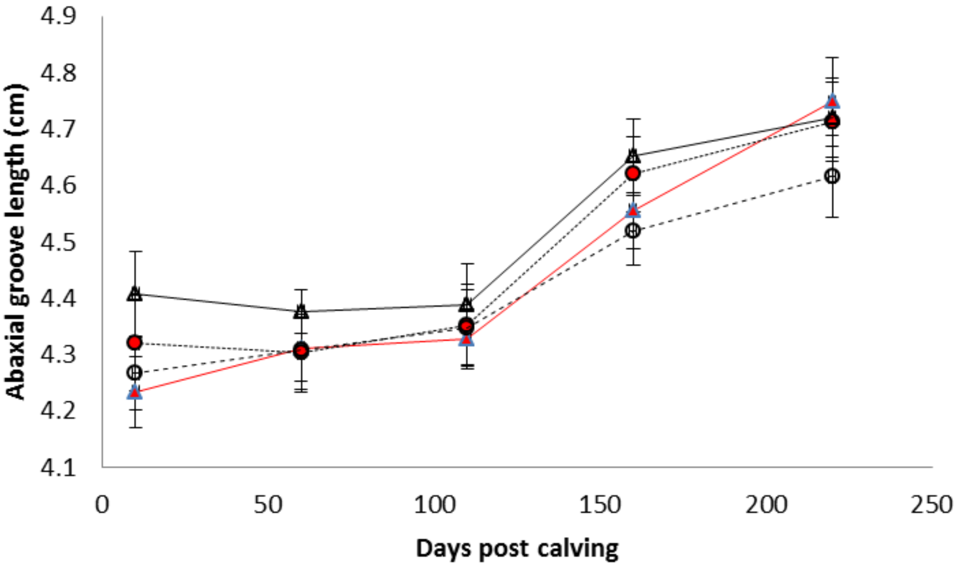

Figure 6.11: Mean (SEM) abaxial groove length (cm) for the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Abaxial groove Year 2

Mean abaxial groove lengths for both hind feet (Year 2) are tabulated for each claw and time combination in Table 6.6 and shown as a boxplot in Figure 6.12.

Table 6.6: Mean (SEM) abaxial groove length (cm) from raw data for each foot, claw, and time combination. Lengths were measured with a flexible tape measure in both hind limbs of 29 first lactation heifers on Days 10, 60, 120 and 230 post calving.

		Year 2: Abaxial groove length (cm) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230***
LH	**Medial**	5.0 (0.05)	4.3 (0.06)	4.3 (0.04)	4.5 (0.06)
	Lateral	5.0 (0.07)	4.4 (0.05)	4.4 (0.05)	4.8 (0.05)
RH	**Medial**	5.0 (0.06)	4.4 (0.06)	4.3 (0.06)	4.5 (0.06)
	Lateral	5.1 (0.07)	4.4 (0.05)	4.5 (0.06)	4.8 (0.07)

*n=28

The best model for abaxial groove length in Year 2 had first-order autoregressive covariance structure. There was no significant (*P*=0.427) effect of foot on abaxial groove length. The effect of claw position within foot was significant (*P*<0.001). Comparing claws of the same foot, abaxial groove length was greater in lateral than medial claws, a difference that was particularly evident at the end of lactation (Figure 6.13). Mean difference was of similar magnitude in both feet:

Left hind foot: 0.29 cm (95 % CI: 0.14 to 0.44)
Right hind foot: 0.36 cm (95 % CI: 0.21 to 0.51)

There was a significant (*P*<0.001) effect of time on abaxial groove length, such that length decreased between Days 10 and 60, thereafter rising gradually until Day 230 (although without regaining the initial value on Day 10). Compared to Day 230, abaxial groove length was not significantly different on Day 60 (*P*=0.160) and was lower on Day 120 (*P*=0.007). There was no interaction between foot and time (*P*=0.890) but interaction between claw within foot and time was significant (*P*=0.031): Figure 6.13.

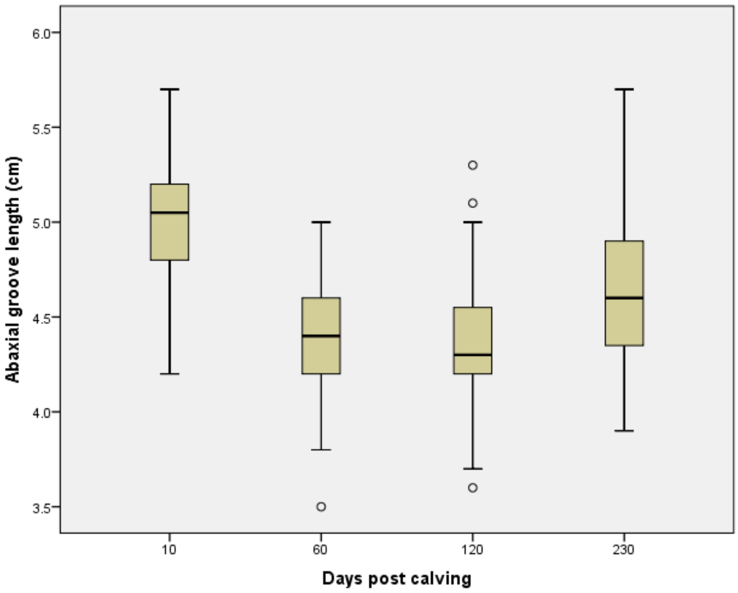

Figure 6.12: Relationship between time since calving and abaxial groove length (Year 2) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 120 and 230 post calving. For interpretation of the box and whisker plots see Figure 6.2.

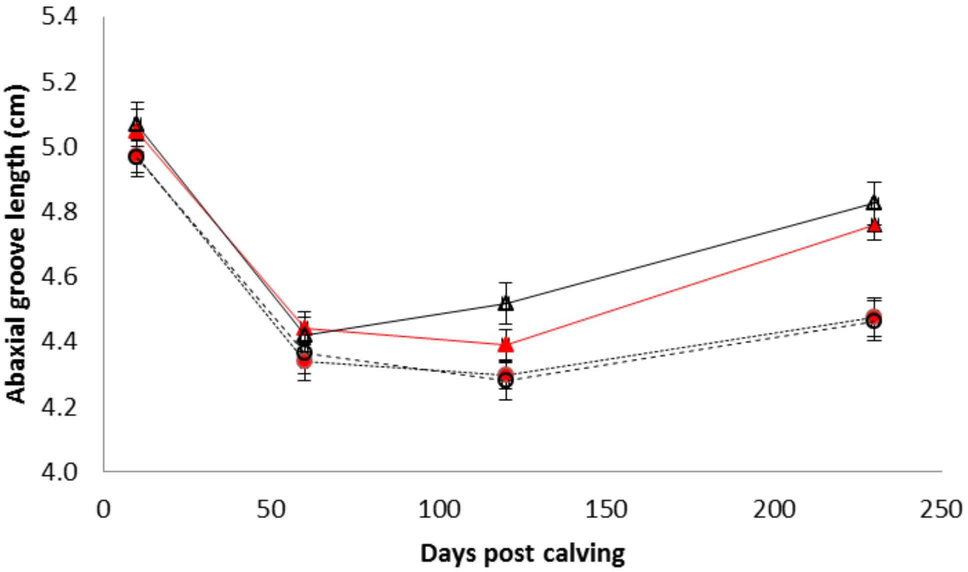

Figure 6.13: Mean (SEM) abaxial groove length (cm) for the medial and lateral claws of both hind limbs of 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Proximal: Length of coronary band from flexure of the dorsal border to the abaxial groove Year 1

Mean proximal coronary band lengths (proximal) for both hind feet (Year 1) are tabulated for each claw and time combination in Table 6.7 and shown as a boxplot in Figure 6.14.

Table 6.7: Mean (SEM) proximal coronary band length (cm) from raw data for each foot, claw, and time combination. Lengths were measured with a flexible tape measure in both hind limbs of 25 first lactation heifers on five occasions on Days 10, 60,110, 160 and 220 post calving.

		Year 1 Proximal coronary band (cm) (SEM)				
Foot	**Claw**	**Day 10***	**Day 60**	**Day 110**	**Day 160**	**Day 220**
LH	**Medial**	8.0 (0.07)	7.9 (0.11)	8.2 (0.09)	8.2 (0.08)	8.3 (0.08)
	Lateral	7.9 (0.09)	8.0 (0.06)	8.1 (0.08)	8.1 (0.09)	8.2 (0.09)
RH	**Medial**	8.0** (0.09)	8.1 (0.08)	8.1 (0.09)	8.2 (0.09)	8.2 (0.08)
	Lateral	7.9 (0.09)	7.8*** (0.07)	8.0 (0.09)	8.2 (0.07)	8.4 (0.08)

*n=24 (no 593 10 days), **n= 23 additional missing data (420) ***n= 24 recording error missing (25)

The best model for the length of the proximal coronary band in Year 1 had a first-order autoregressive covariance structure. There was no significant (*P*=0.128) effect of foot on length, but the effect of claw position within foot was significant (*P*=0.002). Comparing claws in the same foot proximal coronary band length tended to be shorter in the lateral claws of the left hind but was greater in the lateral claws of the right hind, mean difference:

Left hind foot: -0.13 cm (95 % CI: -0.27 to +0.003)
Right hind foot: 0.17 cm (95 % CI: 0.04 to 0.31)

There was a significant effect of time on length (*P*<0.001), such that values were highest on Day 220. Values were significantly lower than on Day 220 on Days 10, 60 and 110 (*P*<0.001, *P*<0.001 and *P*=0.003, respectively), but the difference on Day 160 was not significant (*P*=0.972). There was no interaction between either foot and time (*P*=0.194) or claw within foot and time (*P*=0.140): Figure 6.15.

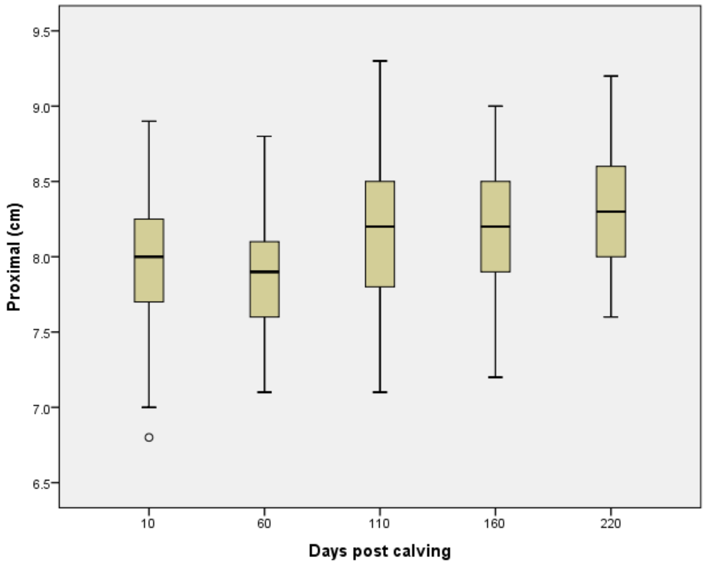

Figure 6.14: Relationship between time since calving and proximal coronary band length (Year 1) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving. For interpretation of the box and whisker plots see Figure 6.2.

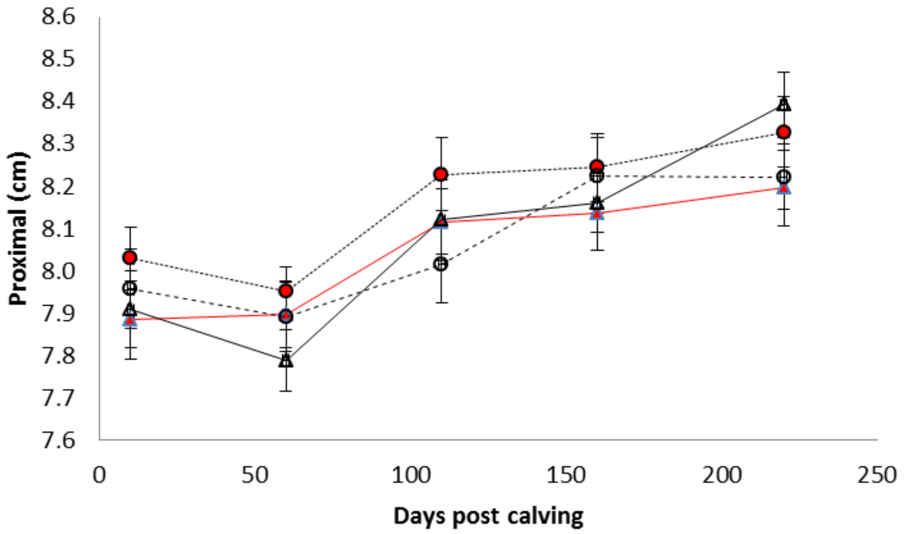

Figure 6.15: Mean (SEM) distance from the point where the coronary band meets the flexure of the dorsal border to the abaxial groove along the coronary band (Proximal, cm) for the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Proximal: Length of coronary band from flexure of the dorsal border to the abaxial groove Year 2

Mean proximal coronary band lengths (proximal) for both hind feet (Year 2) are tabulated for each claw and time combination in Table 6.8 and shown as a boxplot in Figure 6.16.

Table 6.8: Mean (SEM) proximal coronary band length (cm) from raw data for each foot, claw, and time combination. Lengths were measured with a flexible tape measure in both hind limbs of 29 first lactation heifers on Days 10, 60, 120 and 230 post calving.

		Year 2 Proximal coronary band (cm) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230***
LH	**Medial**	8.2 (0.07)	8.1 (0.08)	8.3** (0.06)	8.6 (0.07)
	Lateral	8.2 (0.06)	8.1 (0.07)	8.2 (0.07)	8.4 (0.08)
RH	**Medial**	8.2 (0.08)	8.2 (0.07)	8.2 (0.08)	8.6 (0.08)
	Lateral	8.1 (0.06)	8.1 (0.07)	8.2 (0.06)	8.4 (0.09)

* n=28 (cow 345 missing data), ** n=28 (cow 123 missing data)

The best model for the length of the proximal coronary band in Year 2 had first-order autoregressive covariance structure. There was no significant effect (*P*=0.516) of foot on length. However, there was a significant (*P*=0.005) effect of claw position within foot. Proximal coronary band length tended to be shorter in lateral than medial claws of both feet, mean difference:

Left hind foot: -0.13 cm (95 % CI: -0.27 to 0.00)
Right hind foot: -0.11 cm (95 % CI: -0.25 to +0.03)

There was a significant (*P*<0.001) effect of time on proximal coronary band length. Compared to length on Day 220, values on all other days were lower (all *P*<0001). There was no interaction between either foot and time (*P*=0.719) or claw within foot and time (*P*=0.936): Figure 6.17.

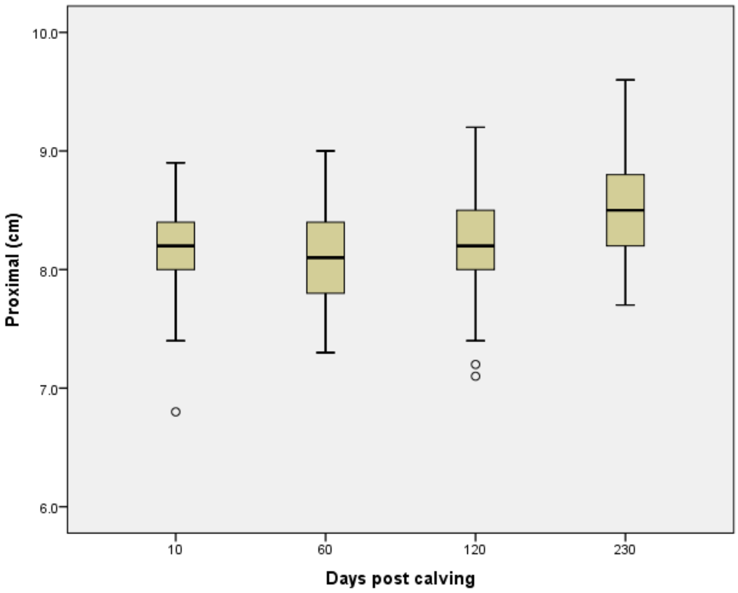

Figure 6.16: Relationship between time since calving and proximal coronary band length (Year 2) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 120 and 230 post calving. For interpretation of the box and whisker plots see Figure 6.2.

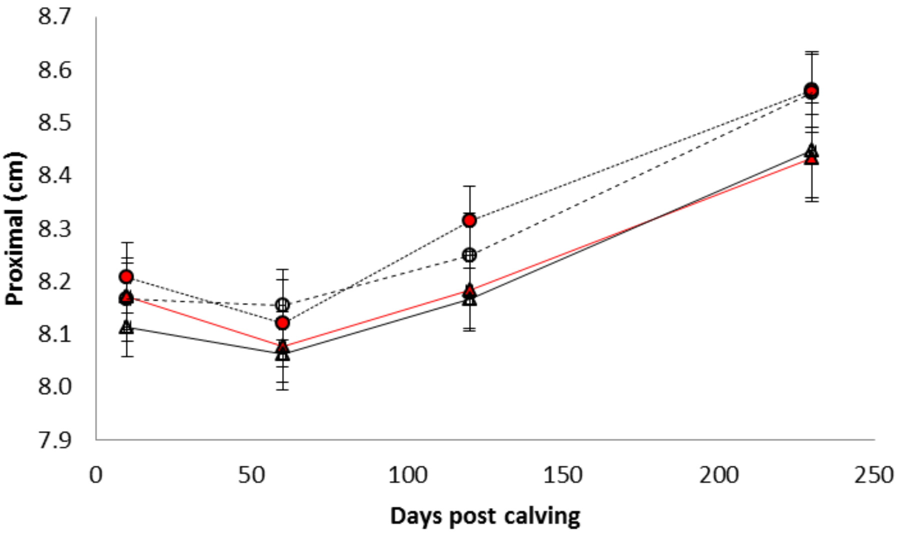

Figure 6.17: Mean (SEM) distance from the point where the coronary band meets the flexure of the dorsal border to the abaxial groove along the coronary band (Proximal, cm) for the medial and lateral claws of both hind limbs of 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Distal: Length of abaxial wall from the apex of the toe to the abaxial groove at ground surface Year 1

Mean distal wall lengths (distal) for both hind feet (Year 1) are tabulated for each claw and time combination in Table 6.9 and shown as a boxplot in Figure 6.18.

Table 6.9: Mean (SEM) distal wall length (cm) from raw data for each foot, claw, and time combination. Lengths were measured with a flexible tape measure in both hind limbs of 25 first lactation heifers on five occasions on Days 10, 60,110, 160 and 220 post calving.

		Year 1: Distal wall length (cm) (SEM)				
Foot	**Claw**	**Day 10***	**Day 60**	**Day 110**	**Day 160**	**Day 220**
LH	**Medial**	9.5 (0.07)	9.2 (0.07)	9.1 (0.13)	9.3 (0.09)	9.3 (0.09)
	Lateral	9.9 (0.19)	9.6 (0.16)	9.8 (0.14)	9.9 (0.19)	10.1 (0.17)
RH	**Medial**	9.5 (0.11)	9.5 (0.11)	9.4 (0.12)	9.5 (0.12)	9.6 (0.16)
	Lateral	9.7 (0.17)	9.6 (0.12)	9.5** (0.15)	9.8 (0.14)	10.0 (0.15)

*n=24 (no 593 Day 10), **n= 24 additional missing data cow 21 recording error

The best model for distal wall length in Year 1 had heterogeneous first-order autoregressive covariance structure. There was no effect (*P*=0.564) of foot on distal wall length. There was a significant (*P*=0.008) effect of claw position within foot. Comparing claws of the same foot distal length was greater in lateral than medial claws but the difference between claws was smaller in the right hind foot. Mean difference:

Left hind foot: 0.80 cm (95 % CI: 0.49 to 1.11)
Right hind foot: 0.41 cm (95 % CI: 0.06 to 0.77)

Although there was a significant effect of time on distal wall length (*P*<0.001), compared to Day 220, mean distal wall length was not significantly different on Days 10, 60, 120 or 160 (*P*=0.698, *P*=0.881, *P*=0.533 and *P*=0.845, respectively). There was no interaction between either foot and time (*P*=0.476) or claw within foot and time in the model (*P*=0.289): Figure 6.19.

The residuals of model were not normally distributed and did not satisfy the Kolmogorov-Smirnov test (P<0.05). Graphical examination of the residuals identified 8 data points, of which, after cross-reference to the initial box plot (Figure 6.18) all were included as possible outliers. When these data points were removed from the data set the residuals were normally distributed. However, this removal did not alter the outcome of the mixed model markedly so all data points were retained in the analysis.

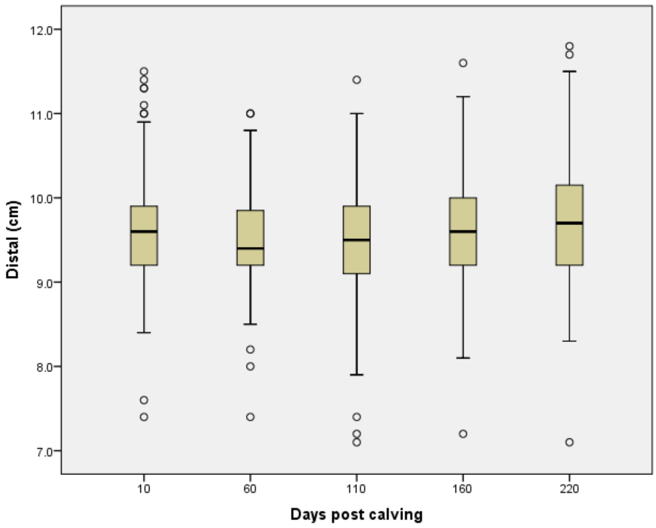

Figure 6.18: Relationship between time since calving and distal wall length (Year 1) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving. For interpretation of the box and whisker plots see Figure 6.2.

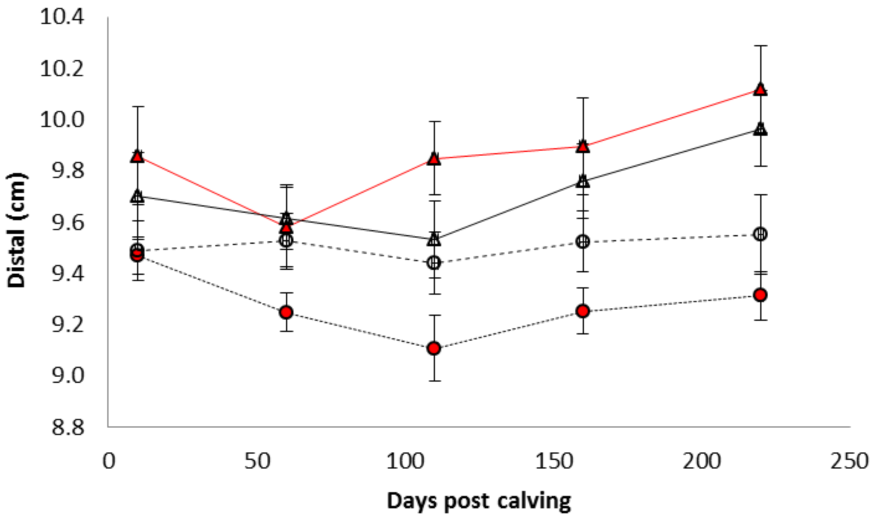

Figure 6.19: Mean (SEM) distance from the apex of the toe to the abaxial groove along the wall horn (Distal, cm) for the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Distal: Length of abaxial wall from the apex of the toe to the abaxial groove at ground surface Year 2

Mean distal wall lengths (distal) for both hind feet (Year 2) are tabulated for each claw and time combination in Table 6.10 and shown as a boxplot in Figure 6.20.

Table 6.10: Mean (SEM) distal wall length (cm) from raw data for each foot, claw, and time combination. Lengths were measured with a flexible tape measure in both hind limbs of 29 first lactation heifers on Days 10, 60, 120 and 230 post calving.

		Year 2: Distal wall length (cm) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230***
LH	**Medial**	9.2 (0.08)	9.7 (0.08)	9.7 (0.08)	10.2 (0.10)
	Lateral	9.2 (0.08)	9.6 (0.09)	9.8 (0.10)	10.1 (0.09)
RH	**Medial**	9.2 (0.10)	9.7 (0.10)	9.7 (0.08)	10.3 (0.12)
	Lateral	9.6 (0.08)	9.5 (0.09)	9.5 (0.09)	9.8 (0.13)

* n=28 (cow 345 missing data)

The best model for distal wall length in Year 2 had heterogeneous first-order autoregressive covariance structure. The effect of foot approached, but did not reach statistical significance (*P*=0.091) of foot on distal but the effect of claw position within foot was significant (*P*=0.001). Comparing claws in the same foot, distal wall length tended to be shorter in lateral claws of the left hind foot. While, mean difference between claws was greater in the right hind foot and lateral claws where shorter. Mean difference:

Left hind foot: -0.03 cm (95 % CI: -0.28 to 0.21)
Right hind foot: -0.53 cm (95 % CI: -0.86 to -0.19)

There was also a significant effect of time on distal wall length (P<0.001), such that length was maximal on Day 230 and significantly greater than on any other day (all *P*<0.001). There was no between either foot and time (*P*=0.688) or claw within foot and time (*P*=0.463): Figure 6.21.

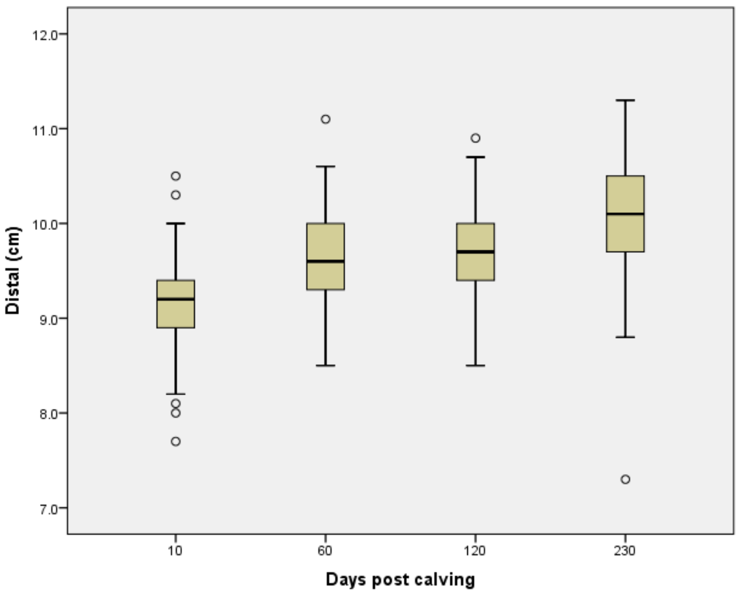

Figure 6.20: Relationship between time since calving and distal wall length (Year 2) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 120 and 230 post calving. For interpretation of the box and whisker plots see Figure 6.2.

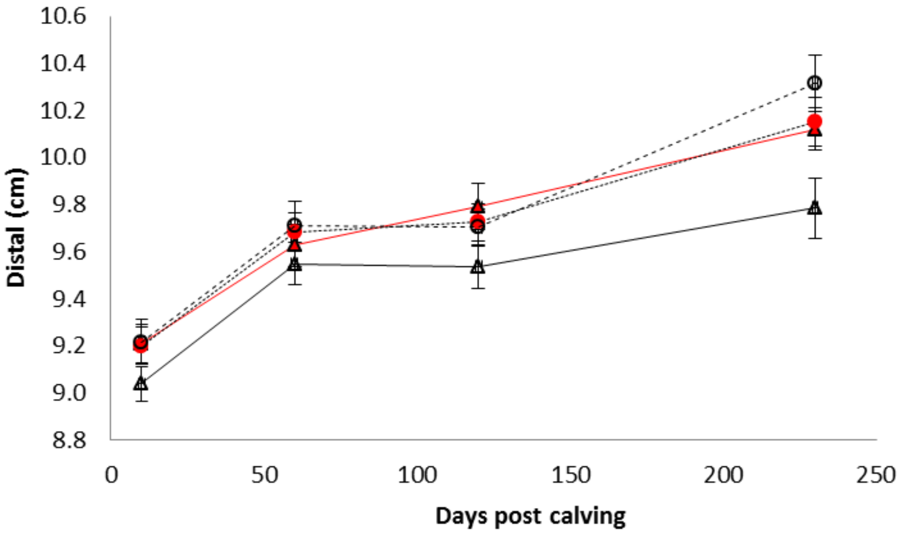

Figure 6.21: Mean (SEM) distance from the apex of the toe to the abaxial groove along the wall horn (Distal, cm) for the medial and lateral claws of both hind limbs of 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Change in predicted claw volume over lactation for two sequential cohorts of first lactation heifers Year 1

Mean predicted claw volumes for Year 1 are tabulated for each claw and time combination in Table 6.11 and are illustrated as boxplot in Figure 6.22.

Table 6.11: Mean (SEM) predicted claw volume (cm^3) calculated from raw data for each foot, claw, and time combination. For both hind limbs of 25 first lactation heifers on five occasions on Days 10, 60,110, 160 and 220 post calving.

		Year 1: Predicted claw volume (cm^3) (SEM)				
Foot	**Claw**	**Day 10***	**Day 60**	**Day 110**	**Day 160**	**Day 220**
LH	**Medial**	83.7 (2.0)	81.7 (1.5)	88.7 (2.2)	90.3 (2.0)	92.7 (2.2)
	Lateral	92.9 (2.3)	93.6 (2.0)	99.0 (2.0)	100.5 (2.1)	102.9 (2.2)
RH	**Medial**	81.7** (2.4)	80.3 (2.1)	83.5 (2.3)	89.3 (2.3)	89.7 (1.9)
	Lateral	94.3 (2.3)	91.3*** (1.8)	99.4 (2.4)	101.6 (1.8)	107.5 (2.0)

*Day 10 n=24 cows, **n=23 note: recording error. *** n=24 cows, note: one outlier, recording error.

The best model for predicted claw volume in Year 1 had first-order autoregressive covariance structure. There was no significant effect of foot (*P*=0.147) on calculated claw volume. The effect of claw position within foot was significant (*P*<0.001). Comparing claws of the same foot, lateral claws had greater volume than their medial partners. Mean difference was greater in the right hind foot:

Left hind foot: 10.1 cm^3 (95 % CI: 6.9 to 13.4)
Right hind foot: 17.8 cm^3 (95 % CI: 14.6 to 21.1)

There was a significant effect of time on predicted claw volume (*P*<0.001), but no interaction between either foot and time (*P*=0.292) or claw within foot and time (*P*=0.138). Thus, there was a gradual increase in claw volume between Days 60 and 220; values were significantly lower on Days 10, 60 and 110 than on Day 220 (all *P*<0.001). Values on Day 160 were not significantly different (*P*=0.840) from those on Day 220 (Figure 6.23).

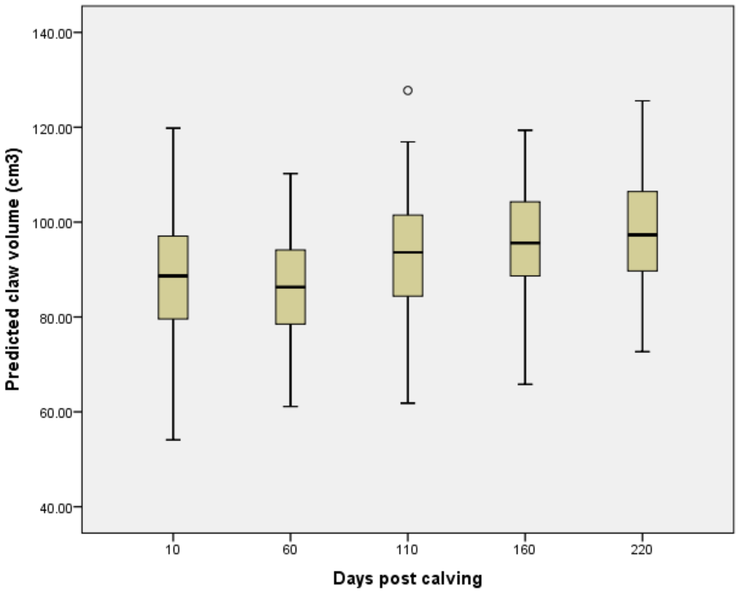

Figure 6.22: Relationship between time since calving and predicted claw volume (Year 1) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving. For interpretation of the box and whisker plots see Figure 6.2.

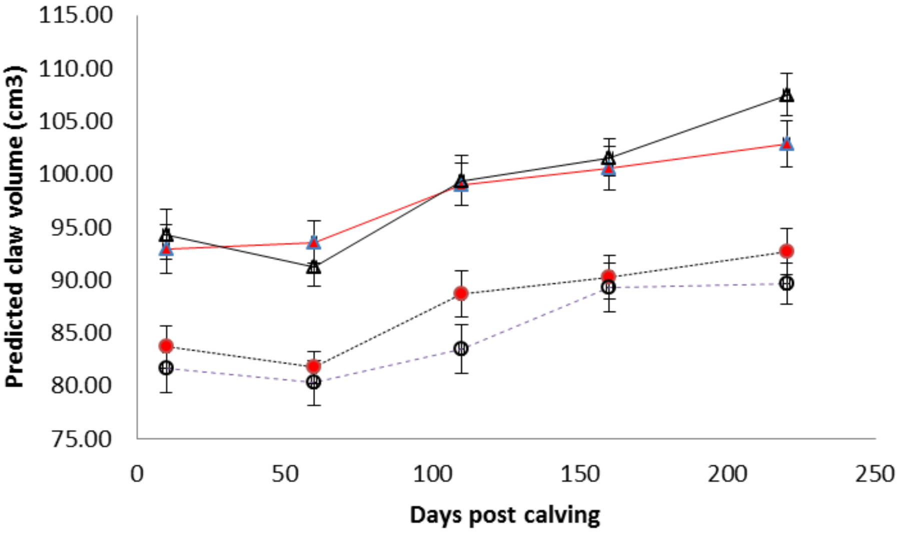

Figure 6.23: Mean (SEM) Predicted claw volume (cm^3) calculated for the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)

Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Change in predicted claw volume over lactation for two sequential cohorts of first lactation heifers Year 2

Mean predicted claw volumes for Year 2 are tabulated for each claw and time combination in Table 6.12 and are illustrated as a boxplot in Figure 6.24.

Table 6.12: Mean (SEM) predicted claw volume (cm^3) calculated from raw data for each foot, claw, and time combination. For both hind limbs of 29 first lactation heifers on Days 10, 60, 120 and 230 post calving.

		Year 2: Predicted claw volume (cm^3) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230***
LH	**Medial**	90.93 (1.69)	86.00 (1.97)	90.50 (1.58)	97.34 (1.80)
	Lateral	103.63 (1.56)	98.55 (1.60)	100.93 (1.70)	108.67 (2.03)
RH	**Medial**	89.91 (1.90)	86.96 (1.76)	88.85 (2.05)	97.21 (1.88)
	Lateral	102.29 (1.41)	98.12 (1.67)	101.08 (1.58)	109.31 (2.25)

*n=28

The best model for predicted claw volume in Year 2 had first-order autoregressive covariance structure. There was no significant effect of foot (*P*=0.640) on predicted claw volume, but there was a significant (*P*<0.001) effect of claw position within foot. Comparing claws of the same foot, lateral claws had greater predicted volume than their medial partners but the difference between claws was similar in both feet, mean difference:

Left hind foot: 11.3 cm^3 (95 % CI: 7.9 to 14.7)
Right hind foot: 12.1 cm^3 (95 % CI: 8.7 to 15.5)

There was a significant (*P*<0.001) effect of time on predicted claw volume, with a gradual increase in claw volume over lactation. There was no interaction between either, foot an time (*P*=0.759) or claw within foot and time (*P*=0.974): Figure 6.25.

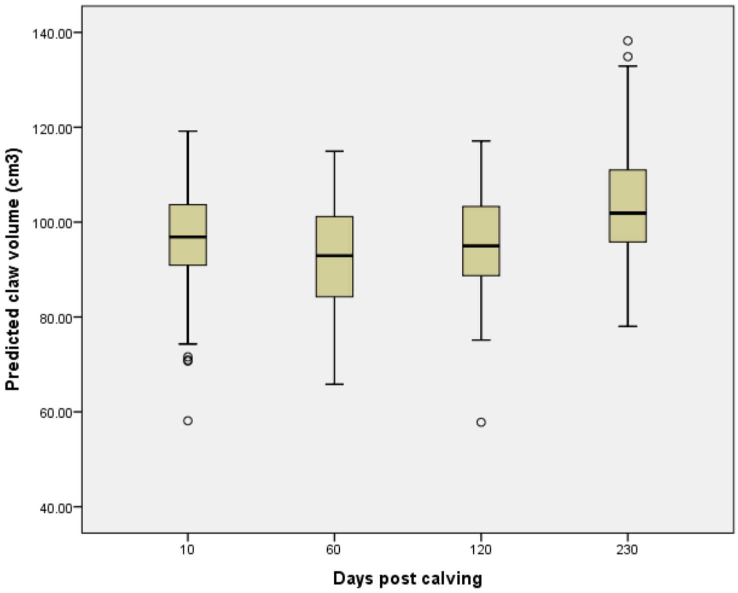

Figure 6.24: Relationship between time since calving and predicted claw volume (Year 2) in the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 120 and 230 post calving. For interpretation of the box and whisker plots see Figure 6.2.

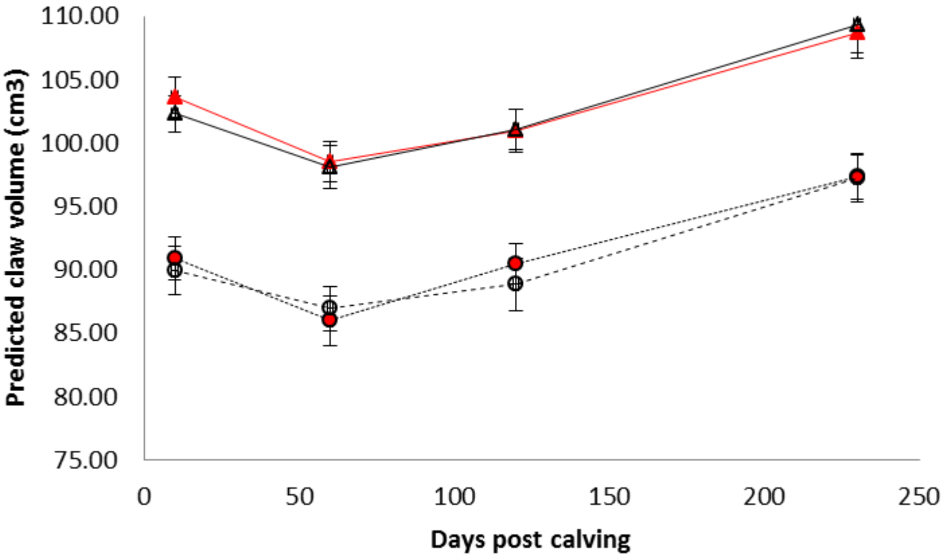

Figure 6.25: Mean (SEM) predicted claw volume (cm^3) calculated for the medial and lateral claws of both hind limbs of 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Change in variables over time: last estimate minus first estimate for each variable

The mean change values between first and last examination in each year are tabulated in Table 6.13. For both Years 1 and 2 the distribution of data for change in dorsal border length and change in abaxial groove length data Year 1 was not normal (Kolmogorov-Smirnov test) and so Spearman's ranked correlation (rho) was used to assess correlation between the changes in the individual variables.

Year 1

The change in toe angle and change in dorsal border length show a small, negative correlation (ρ=-0.225, *P*<0.05). A similar relationship was seen between the change in proximal coronary band length and change in dorsal border length. A moderate, positive correlation (ρ=0.311, *P*<0.01) was present between the changes in abaxial groove length and change in dorsal border length and, as expected being part of the equation to calculate volume, change in proximal coronary band length was strongly correlated to change in predicted claw volume (ρ=0.978, *P*<0.01). However, change in abaxial groove length was not (ρ=0.016, *P*>0.05): Table 6.14.

Year 2

The changes in toe angle and dorsal border length showed a small, negative correlation (ρ=-0.196, *P*<0.05). Changes in proximal coronary band length were not significantly correlated to change in dorsal border in Year 2 but changes in distal wall length were correlated (ρ=0.329, *P*<0.01). Changes in abaxial groove length and dorsal border length were not significantly correlated in Year 2 (ρ=0.140, *P*>0.05), but changes in abaxial groove length and change in distal wall length had a moderate, negative correlation (ρ=-0.424, *P*<0.01). In Year 2, as expected being part of the equation to calculate volume, both changes in abaxial groove length and proximal coronary band length were significantly correlated to change in predicted claw volume (ρ=0.328 and 0.978, respectively; both *P*<0.01) Table 6.15.

Table 6.13: Mean change for each variable between first and last measurements (Day 220 minus Day 10) in Year 1 and Year 2.

Change between first and last estimation	n Year 1	Mean change (SEM)	n Year 2	Mean change (SEM)
Toe Angle (degrees)	96	0.83 ± 0.30	112	-1.95 ± 0.30
Dorsal Border Length (cm)	96	0.32 ± 0.06	111	0.22 ± 0.03
Proximal (cm)	95	0.35 ± 0.04	112	0.33 ± 0.03
Distal (cm)	96	0.10 ± 0.07	112	0.92 ± 0.05
Abaxial Groove Length (cm)	96	0.38 ± 0.04	112	-0.37 ± 0.04
Predicted Volume (cm^3)	95	10.25 ± 0.86	112	6.35 ± 0.84

Table 6.14: Correlation matrix: Spearman's rho correlation coefficient and significance for the change in each variable between first and last measurements (Day 220 minus Day 10) in Year 1. Emboldened figures: correlation is significant at the 0.01 level (2-tailed). Italic figures: correlation is significant at the 0.05 level (2-tailed).

Change between first and last estimation	Dorsal Border Length (cm)	Proximal (cm)	Distal (cm)	Abaxial Groove Length (cm)	Predicted Volume (cm^3)
Toe Angle (degrees)	*-0.225*	0.037	-0.184	-0.184	-0.001
Dorsal Border Length (cm)		*-0.241*	0.188	**0.311**	-0.181
Proximal (cm)			0.142	-0.163	**0.978**
Distal (cm)				-0.081	0.116
Abaxial Groove Length (cm)					0.016

Table 6.15: Correlation matrix: Spearman's rho correlation coefficient and significance for the change in each variable between first and last examination (Day 230 - Day 10) in Year 2. Emboldened figures: correlation is significant at the 0.01 level (2-tailed). Italic figures: correlation is significant at the 0.05 level (2-tailed).

Change between first and last estimation	Dorsal Border Length (cm)	Proximal (cm)	Distal (cm)	Abaxial Groove Length (cm)	Predicted Volume (cm^3)
Toe Angle (degrees)	*-0.196*	0.052	-0.134	-0.066	0.024
Dorsal Border Length (cm)		0.011	**0.329**	0.140	0.047
Proximal (cm)			-0.038	0.166	**0.978**
Distal (cm)				**-0.424**	-0.120
Abaxial Groove Length (cm)					**0.328**

Discussion

The present study was undertaken to describe the conformational changes seen over the production year and used variables commonly reported in the conformation literature (Vermunt and Greenough, 1995; Scott et al., 1999) to monitor conformation throughout lactation. Assessment spanned two years to provide an opportunity to look at which factors are stable and which are more volatile on a year-by-year basis. Farm replacement policy meant that the breed make-up of the heifer group was not identical between years. An increased number of animals were monitored in Year 2, but time constraints for the study personnel required a reduction in the number of examinations performed. After preliminary assessment of Year 1 data, Day 160 was dropped to ensure changes in both early (10-120 DPC) and late (220 DPC) lactation were captured. Although these factors were borne in mind, the study is of a descriptive nature and it is the knowledge of the underlying pattern of change within the pastoral system that is primarily of interest. The study was intended to highlight the utility of the different conformational variables assessed, to inform their use as instruments to monitor the impact of management change on conformation and their potential, in conjunction with mobility scoring and lameness recording, to aid investigation of outbreaks and lameness control planning.

The engineer's angle finder proved an efficient method for the estimation of toe angle and the expected difficulty of applying them to claws with excessively concave or convex dorsal borders was not encountered. This may be can probably be explained by the young age of these animals; i.e. that they had not had sufficient length of time in the milking herd to develop extremes of conformation.

Although no effect of claw was seen in Year 1, an effect was evident in Year 2, which supports the claim by Andersson and Lundström (1981) that both medial and lateral claws need to be assessed. No effect of foot on toe angle was seen in either year, supporting the studies which chose to assess only one hind foot per animal as having made a representative assessment of the claw status (Webster, 2001; Livesey and Laven, 2007; Telezhenko et al., 2009). However, in both years lateral claws of the right hind foot had greater toe angle than their medial counterparts with the difference between claws much smaller in the left hind foot. This would suggest that despite no effect of foot in the model, potentially more information is to be gained regarding change in toe angle if both hind feet and all four claws are assessed. Further research is required to elucidate whether this effect is consistent, relates to the pastoral system studied or perhaps the individual farm assessed.

Whilst there was a significant effect of time on toe angle in both years of the study, the magnitude of the change between assessments was only significant in the later stages of lactation. Toe angle increased in Year 1, but decreased in Year 2 (particularly in the medial claws). Medial claws in Year 2 had shallower toe angles which reduced in size over time to a greater extent than the lateral claws over time, but significant change was not seen until the second half of lactation in either claw category.

A difference in the magnitude or significance of toe angle change between years might be expected from the work of Boelling and Pollott (1998) who found that there was both a seasonal and year effect on toe angle. However, in that study, the pattern of toe angle change was consistent with the seasonal changes in environmental management described across both years. So the fact that the current study demonstrated an increase in toe angle for Year 1 and a decrease for Year 2 within the same system and over the same stages of the production year (seasonal calving) was unexpected. Furthermore, in Year 1 no claw effect was demonstrated whilst in Year 2 claw position was important. This inconsistent finding for the effect of time on toe angle within the same pastoral system is interesting and suggests that without an extreme environmental challenge such as housing on straw yards (soft) or concrete flooring (abrasive) the modification of toe angle during lactation is unpredictable and probably influenced by the balance of multiple factors rather than just one factor (as occurs in more extreme environments).

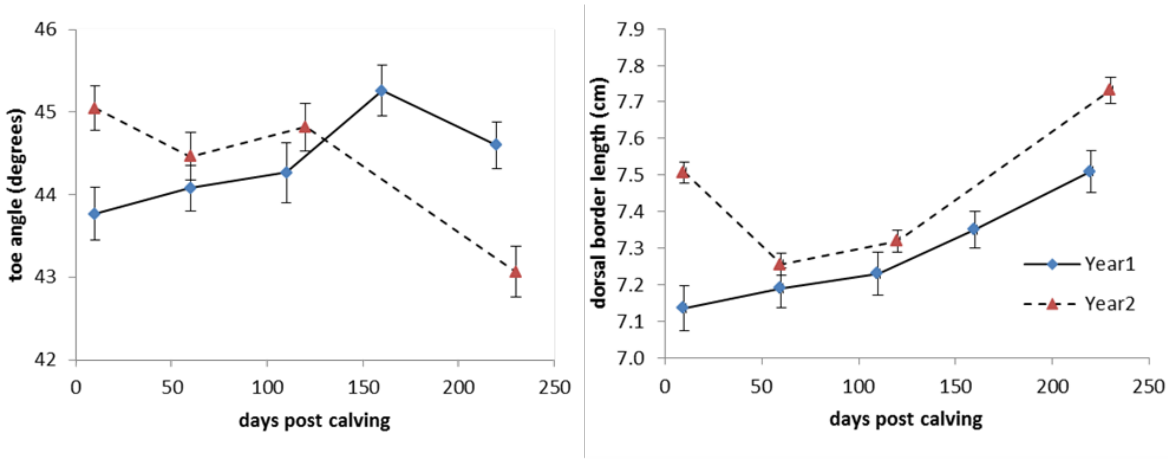

Figure 6.26: Left panel; mean (SEM) toe angle Year 1 and 2 (°). Right panel; mean (SEM) dorsal border length Year 1 and 2 (cm). For the medial and lateral claws of both hind limbs of dairy heifers followed over lactation. Year 1; n=25 examined on Days 10, 60, 110, 160 and 220 post calving. Year 2; n=29 examined on Days 10, 60, 120 and 230 post calving.

Some of the variability in toe angle between years in the present study may relate to breed difference between the two cohorts of animals (i.e. with a Friesian bias and less Jersey influence on conformation in Year 2 compared to the mixed breeding of Year 1); although the effect is not obvious and the two graphs cross mid lactation (see Figure 6.26). For animals of known breed (Livestock Improvement Company data) on Day 10: Year 1 mean (SD) toe angle was 42.88° (2.37) Friesians (10/23) and 44.23° (3.57) for cross breeds (13/23). Year 2 mean (SD) toe angle was 45.06° (2.84) Friesians (25/27) and 45.45° (3.18) for cross breeds (2/27).

To extend the findings of this study it would be interesting to collect further data on whether changes related to decreasing toe angle are consistent across medial and lateral claws, or whether increasing toe angle triggers a more exaggerated response in the medial claw than the lateral claw.

With regard to dorsal border length, the methodology chosen made it possible to slide the tape measure below the overlying hair to align it with the skin: horn junction at the coronet. The method was simple and allowed rapid assessment of both claws. As was the case for toe angle, measurement of both claws within a foot revealed a claw effect only in Year 2. However, for dorsal border an interaction between time and claw was present (P<0.05) in both years. Again no effect of foot on dorsal border was seen in either year supporting as representative the studies which chose to assess only one hind foot per animal (e.g. Offer et al., 2001; Telezhenko et al., 2009).

There was a significant effect of time on dorsal border length in both years, but the pattern of change differed between years; and it may be that the smaller lengths in Year 1 than Year 2 represented the higher proportion of Jersey and Jersey cross animals in Year 1. Nonetheless, in Year 1 the length of the dorsal border increased steadily as lactation progressed, even though the initial changes between Days 10 and 60 were not significant. After Day 60, the length of the dorsal border gradually increased although the overall change in length was quite small (mean of all claws across all time points ranged from 7.1 cm (0.05) to 7.4 cm (0.04), which is well below the magnitude of the changes that Gitau et al. (1997) associated with lameness and underrun sole. Thus, the lack of any increase in the length of the dorsal border between Days 10 and 60 probably suggests that the rate of claw horn wear was matched by the claw horn growth, whilst the subsequent small increase of 0.3 cm may reflect the growth of these animals over their first lactation. Ahlström (1986) and Offer et al. (2000) both showed that dorsal border length increased with lactation number, so further increase might be expected if the animals were to be followed for a longer period of time.

The pattern of dorsal border length change was not completely consistent between years (Figure 6.26). In Year 2, length decreased significantly between Days 10 and 60 before stabilising and gradually increasing over the remaining stages of lactation. This was initially explained by the proposal that claw horn growth was exceeded by horn wear in the early stages of lactation (Year 2), but that horn production then increased to match and later exceed the wear. However, analysis of claw horn growth and wear data for the period between Days 10 and 60 for both Year 1 and Year 2 cohorts (Data not presented) demonstrated that there was in fact a change in the growth rate of claw horn between the years rather than a change in the rate of wear, which was relatively consistent between the two years:

Daily growth rate: 0.25 mm/day ± 0.02 Year 1 and 0.15 mm/day ± 0.01 for Year 2.
Daily wear rate: 0.20 mm/day ± 0.03 Year 1 and 0.19 mm/day ± 0.02 for Year 2.

In other words, it appears that it was a higher growth rate in Year 1 as opposed to greater rate of wear in Year 2 which was the key driver of the effect of time the length of the dorsal border between Days 10 and 60.

As previously mentioned for toe angle, the discrepancy in dorsal border length behaviour between Years 1 and 2 may in some part be a result of the breed makeup of the cohorts, inasmuch as Friesian animals had larger feet with longer dorsal border length compared to the mixed bred animals. For animals of known breed on Day 10 mean (SD) dorsal border length was:

Year 1: 7.27 cm (0.49) Friesians (10/23), 6.98 cm (0.46) cross breeds (13/23).
Year 2: 7.49 cm (0.30) Friesians (25/27), 7.35 cm (0.21) cross breeds (2/27).

Despite low numbers crossbred animals in this study do appear to have shorter dorsal border length which may go some way to explaining why Year 2 mean values always exceed Year 1 values for dorsal border length. However, animals in Year 2 started the study with larger feet irrespective of breed perhaps an indication that animals were better grown at calving in Year 2. If true, this may have contributed to the difference in wall horn growth rate between years.

For dorsal border as for toe angle, there was not a consistent pattern over time in the pastoral system studied, reflecting the presence of multi-factorial influences on claw conformation which vary from year to year. This study supports the measurement of both medial and lateral claws with respect to change in dorsal border length over time and in addition the recommendation that both hind feet be assessed.

Taken together, the estimation of both toe angle and dorsal border length from individuals gives more information than the assessment of just one or the other of these variables. However, their assessment would perhaps be of more benefit in intervention studies where an imposed change would be expected to result in modification of claw conformation. In that circumstance, the noise and ambiguity related to general management, breed and maturation of the animal over time would be less influential on the final values for toe angle and dorsal border length.

Significant changes in the length of the abaxial groove occurred with time over both years of the study. The pattern of change was relatively similar to that of dorsal border length in the corresponding year. That is, an initial period when values were relatively static, followed by a significant increase over the over the second half of lactation for Year 1, and a fall between Days 10 and 60 followed by a gradual rise in Year 2, however, the initial value was not regained and mean change in abaxial groove length was negative in Year 2 (-0.37 ± 0.04). Change in abaxial groove length was positively correlated to change in dorsal border length in Year 1 ($\rho=0.311$, $P<0.01$) although the relationship was not significant in Year 2 (Tables 6.14 and 6.15). Perhaps more change was seen in Year 2 because the abaxial groove was longer than optimal when animals entered the milking herd after calving herd. This study suggests that abaxial groove length may be a useful parameter where only one hind foot is to be assessed although further evaluation would be needed before it was used in preference for dorsal border length.

The similarity of the patterns of change in the dorsal border and abaxial groove could be explained by both these variables being under the influence of dynamic wear at the distal ground surface. Net wear would underwrite the 'dip' that occurred between Days 10 and 60 in Year 2; i.e. before there is net growth and an increase in length later in lactation. However, the final value at Day 220 plus was similar for both cohorts, which lends weight to the notion of there being an optimal shape and size of claw for a given system.

Assessment of the length of the abaxial groove has only been described once before in the published literature by Scott et al. (1999) who assessed it alongside other linear measurements as a means of calculating foot volume. This parameter was also included in the final model for predicting claw volume developed in this thesis. After initial adjustments on entering the herd (Year 2) there was a small increase as time progressed and similar final values between years would suggest it is a useful contributor to the volume equation because it is influenced to a lesser extent by year on year changes than other conformation variables, instead being related more to the growth and maturation of the animal.

The length of the proximal coronary band (proximal) was readily obtained under field conditions. Smaller values were obtained for the Year 1 than Year 2 cohorts, following the trend in most of the data in the study. There was no effect of foot on proximal coronary band length in either year, but claw position and time were both significant in the model, and the pattern of change over time was the same between the two cohorts (i.e. little change over the first 120 days but then a gradual increase to the final assessment at 220 or 230 days). This suggests that proximal coronary band length is relatively independent of environmental factors such as wear which influence conformation, but is influenced more by the maturation of the animals over their first lactation and the associated increase in size (body, skeletal and claw).

The pattern of change in the length of the distal abaxial wall variable (distal) differed between years. The mean change over lactation in the Year 1 cohort was tiny (0.10 cm ± 0.07) and did not exhibit a clear temporal pattern, whereas, in Year 2, the magnitude of the changes was greater and values clearly increased in a linear manner with time (mean change; 0.92 cm ± 0.05). The effect of time on distal was of increased statistical significance in Year 2 compared to Year 1 (P<0.001 vs. P=0.05, respectively).

Moreover, distal was an interesting variable as in both years there was a significant difference between claws in the same foot for both feet. The difference was greater in one foot than the other, and both the foot exhibiting the greater difference and the claw with the shorter distal length changed between years studied:

Year 1: left hind foot exhibited the greatest difference and lateral claws > medial claws.

Left hind foot: 0.80 cm (95 % CI: 0.49 to 1.11)
Right hind foot: 0.41 cm (95 % CI: 0.06 to 0.77)

Year 2: right hind foot exhibited the greatest difference and medial claws > lateral claws.

Left hind foot: -0.03 cm (95 % CI: -0.28 to 0.21)
Right hind foot: -0.53 cm (95 % CI: -0.86 to -0.19)

Further research would be required to expand on this finding. The effect may stem from the existence of a natural “sidedness” among the animals (Phillips et al., 1996) with a position-related size-order being seen. Phillips et al. (1996) stated that diagonal pairing of feet with respect to claw size was seen in 26/30 animals i.e. left front and right hind or right front and left hind were similarly sized in their study.

Whilst it might initially have been expected that distal would be a variable to express obvious change over time (as a reflection of growth, overgrowth or medial deviation of the claws), this study has shown that it does not change very much over the course of lactation. The results may be a feature of first lactation heifers who have not been in the milking herd long enough to exhibit more extreme changes in claw conformation, or might simply be because the majority of change in distal claw length actually occurs between the heel and the abaxial groove and is therefore not captured by the distal measure used in this study. Moreover, even though the distal surface measured is in ground contact, the magnitude of distal was not influenced by increased net wear in the same way as dorsal border length or abaxial groove length, as in Year 2 distal rose between Days 10 and 60 when the other two variables were falling in value, probably because net wear was occurring. The impact of net wear on distal may be minimal because the measure is parallel rather than perpendicular in orientation to the ground surface compared to dorsal border or abaxial groove.

The predicted claw volume (which was itself calculated from abaxial groove length and proximal) was relatively independent of factors other than time. Thus, it demonstrated the same significant effect of time over lactation in both years and, although there was no effect of foot, medial claws were consistently lower in predicted volume than lateral claws in both years. Nevertheless, the difference between claws of the same foot was larger for the right hind in Year 1 but similar for both feet in Year 2. Claw volume therefore seems to be less influenced by environmental factors than some of the other variables assessed and therefore may provide a stable estimate for use in the investigation of conformation with respect to claw lesions and their severity in dairy cows. For example, to answer the question of whether large claws on small cows have a smaller lesion score compared to small clawed cows of the same body weight.

The strong correlation between change in proximal coronary band length and change in claw volume in both Year 1 and Year 2 however, suggests that there is not necessarily much advantage in estimating both proximal coronary band length and claw volume in the same animals. Although in Year 2 abaxial groove length also contributed to the change in claw volume (moderately correlated to change in volume ($\rho=0.328$, $P<0.01$), in contrast to Year 1, so further research regarding the utility of volume versus proximal coronary band length would be advisable before it was dropped in preference for proximal coronary band length alone.

Conclusion

The study described in this chapter set out to evaluate the changes in claw conformation, which develop in first lactation heifers over time in a pasture-based system. In support of the initial hypothesis change was documented over time. The traditional descriptors of conformation toe angle and dorsal border length were used (Vermunt and Greenough, 1995) in addition to variables discussed by Scott et al. (1999) in the calculation of foot volume in post mortem material. Abaxial groove length and the proximal and distal distances from the abaxial groove to the dorsal flexure of the claw were collected and investigated individually for their potential to monitor claw conformation and the two former measures used to estimate claw volume in the live animal. The influence of foot and claw position on the variables was examined in order to inform the production of efficient monitoring methods which capture maximum information on claw conformation.

No effect of foot (left hind vs. right hind) and a significant effect of claw position (medial vs. lateral) was a consistent finding across most variables and both years of the study. The study would appear therefore to support the assessment of both claws in one hind foot when recording claw conformation in a pastoral situation.

However, investigation of the relationship between claws of the same foot revealed that the mean difference between claws was not consistent in size and significance between foot (left vs. right) and year (1 vs. 2). Supporting the hypothesis, evidence strongly suggests that evaluation of both hind feet and all claws is advisable to capture maximum information. If a study is constrained to examine only one hind foot then this study would conclude that the foot should be the same foot at each examination.

The initial expectation that dorsal border length and toe angle would behave in a consistent way related to the impact of pasture-based animal management was not confirmed, the situation proved more complex. Toe angle, dorsal border length, and to a lesser extent distal and abaxial groove length were all influenced by factors that differed between the years assessed in this study. The balance between claw horn growth and wear is likely to be a major influence, modified by claw horn quality and environmental conditions such as rainfall, track conditions, concrete exposure and potentially breed. This volatility makes toe angle and dorsal border length in particular, useful for the evaluation and monitoring of conformational change within a given management system. For example, documenting a response to system change such as between pasture and a housed environment or the introduction of rubber mats to a concrete environment. However, the intervention being studied needs to have a greater impact on the claw than the background environment because there is general fluctuation and “noise” in these variables even when animals are continually at pasture.

As postulated, additional information was gained by monitoring proximal coronary band, distal wall and abaxial groove lengths. With further work Proximal coronary band length may be a simple measure to capture the information relating to claw size. Abaxial groove length was similar to dorsal border length with potentially some advantages if measuring only one foot. Distal wall length was not as useful as initially anticipated demonstrating year on year differences. Proximal coronary band length and predicted claw volumes appear to be more resistant to modification by such environmental and management factors over time, with consistent findings in Years 1 and 2. This in turn makes them less useful for assessing short term conformational change in response to an intervention but perhaps of more use for the investigation of the role of claw size in relation to lesion extent and severity and lameness. Predicted claw volume did indeed reveal a position related size-order which may relate to animal sidedness (Phillips et al., 1996). However, lateral and medial claws of a foot were found to display the same changes overtime.

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Chapter seven: Live animal estimations of distance to distal phalanx and sole thickness in a cohort of twenty five first lactation heifers

Introduction

Many studies involving the use of ultrasonography in the estimation of sole thickness or soft tissue dimensions of the bovine claw have focused on cadaver material (Kofler et al., 1999) or clinical assessment of live animals at one point in time to create an overall picture of lactation or a snapshot of the herd situation (van Amstel et al., 2003, 2004a; Bicalho et al., 2009; Machado et al., 2011). Recent improvements in the performance of portable ultrasound machines (Harris and Marks, 2009) have seen increased reports of the use of ultrasonography to assess the bovine foot in recent years. In particular, there are a number of reports which detail the use of ultrasonography in the investigation of the protective role played by the digital cushion in the development of claw lesions and lameness. Studies have assessed the link between body condition score and digital cushion dimensions, and the association between digital cushion dimensions and lesions such as sole ulcer in both the live animal (Bicalho et al., 2009; Machado et al., 2011) and cadaver material (Munk, 2013). However, although such reports indicate that ultrasonography may be a useful method for imaging the internal structures of the bovine claw in the live animal, there are no studies that have sequentially monitored a cohort of individuals throughout lactation to describe the normal changes that occur after calving within a given system.

Thin soles have been identified as a significant cause of lameness in housed cattle in the Northern Hemisphere (Van Amstel et al., 2004a; Sanders et al., 2009) where, in addition to excess wear caused by exposure to concrete and sand (Vokey et al., 2001; Sanders et al., 2009), inappropriate hoof trimming has also been implicated as a cause of thin soles (van Amstel et al., 2003). In Australian and New Zealand pasture based systems, excessive sole wear that occurs as a result of long walking distances could be involved the development of thin soles which are at risk of trauma (Dewes, 1978; Jubb and Malmo, 1991; Lawrence et al., 2011). However, although Tranter and Morris (1992) were able to demonstrate the extent to which sole horn wear occurs in NZ pasture based systems, no data exists regarding the actual sole thickness of animals in a pasture based system.

In addition, first lactation heifers are at particular risk from physiological changes occurring prior to and in the first few weeks after calving (Tarlton et al., 2002; Knott et al., 2007). Claw horn haemorrhage in the first lactation can have impact on claw horn haemorrhage and lameness subsequent lactations (Offer et al., 2000), making a method to monitor the impact of management and any environmental interventions on claw health a valuable tool. Compared to the subjective use of digital pressure or hoof testers to gauge the pliability and thickness of sole horn (Shearer and van Amstel, 2001; van Amstel et al., 2003) or imaging techniques such as radiography (Dobbs et al., 2004) and computerised tomography (Kofler et al., 1999) where safety issues, cost and time preclude routine field use, ultrasonographic assessment of the distance to distal phalanx (external sole surface to the surface of the distal phalanx) with a portable ultrasound machine is potentially an economic, non-invasive method with the potential to monitor the impact of the environment on sole thickness.

Chapter 3 established that a portable ultrasound machine Mindray DP 6600 (Mindray, Szechuan, China) could be used to estimate the distance to distal phalanx in cadaver material and that this estimate was well correlated to calliper measures of the same dimensions. The limits-of-agreement obtained were too wide to assess the sole thickness of animals on an individual basis (individual animals could only be placed into categories of thin, adequate and marginal with respect to sole thickness). However, agreement was sufficient to allow the assessment mean distance to distal phalanx in a group of animals. The present chapter aims to evaluate the use of a portable ultrasound machine to monitor changes of distance to distal phalanx in dairy heifers throughout their first lactation, building upon the pilot study performed in cadaver material and reported in the Chapter 3.

Hypothesis: Building on the findings of Chapter 3, it was postulated that the use of a portable ultrasound machine would allow non-invasive estimation of a mean distance to distal phalanx for a group of live animals throughout lactation. Direct estimation of sole thickness was not expected to be as successful and it was anticipated that the collection of repeat ultrasound images from the same individuals would encounter some difficulty in relation to the presence of claw horn lesions and expected loss of sole concavity (Tranter and Morris, 1992). It was envisaged that Site 1 would be more useful than Site 2 in the live animal when assessing distance to distal phalanx.

To confirm the anecdotal opinion that New Zealand dairy animals develop thin soles as a result of excessive sole horn wear due to the long distances walked the hypothesis was that the obtained group mean for distance to distal phalanx would fall consistently throughout lactation.

Materials and Methods

Animals

Year 1: Twenty five first lactation heifers of mixed breed (Friesian and Friesian/Jersey crossbreed) from a single dairy herd (Massey University No.4 herd) were recruited into the study as they calved in the 2008/2009 season. Animals were managed, on the same farm unit; in a typical New Zealand pasture based system, with heifers kept solely at pasture and being walked to milking twice a day in a rotary parlour. All animals were approximately 24 months of age at calving (mean: 23.7 ± 0.3 months, range 22.3 to 29.3 months).

Heifers were examined on five occasions throughout their first lactation, approximately 10, 60, 110, 160 and 220 days post calving. The range and mean values for days post calving are presented in Table 7.1. Twenty five animals were examined at each time (with the exception of Day 10 when twenty four animals were examined). Only one heifer became lame during the study (between the Day 60 and Day 110 examinations).

Table 7.1: Summary of the range and mean values obtained for each of five examinations, approximately 10, 60, 110, 160 and 220 days post calving (DPC) on 25 first lactation dairy heifers.

Examination	n	Mean (DPC)	Minimum (DPC)	Maximum (DPC)
Day 10	24	13	3	32
Day 60	25	62	55	74
Day 110	25	110	104	117
Day 160	25	160	153	176
Day 220	25	221	205	254

All procedures were approved by Massey University Animal Ethics Committee protocol number MUAEC 08/25

Ultrasound measurements

For each examination the heifers were restrained in purpose designed foot crush (WOPA, Netherlands) for efficient lifting of each hind limb. Each foot was lifted and cleaned (washed and dried), but surface preparation was kept to a minimum.

Ultrasound assessment of claws

Ultrasound measurements were recorded for all four claws of the hind feet with a portable ultrasound machine Mindray DP 6600 (Mindray, Szechuan, China) and variable frequency, linear array probe set at a frequency of 5 MHz. The transducer was placed on the claw perpendicular to and bisecting the line from the abaxial groove to the axial border, which was also perpendicular to a line from the end of the axial white line to the abaxial border (modified from Lischer et al., 2002). The probe was protected by a vinyl glove containing acoustic coupling gel, and additional gel was also applied to the claw surface to aid contact between the claw surface and transducer.

The internal sole surface was visualised as a thin continuous or interrupted, hyperechoic line and the distal phalanx was identified as a deeper more substantial hyperechoic line which attenuated the ultrasound beam. Once a clear image was obtained the ultrasound screen was frozen to capture a static image and ultrasound measurements immediately taken at two sites: Site 1; the tip of the distal phalanx and Site 2; 25 mm along the sole towards the heel. The two measures collected were, the distance to the distal phalanx from the external sole surface and the distance from the external sole surface to the internal sole surface, the sole thickness.

Sole thickness estimates at Site 2 are not reported due to the poor Pearson’s correlation coefficient (r) and associated *P* values obtained for this ultrasound estimate in the pilot study in Chapter 3.

To recapitulate those data: the correlations for ultrasound estimation of sole thickness at Site 2 were: calliper estimate sole thickness Site 1 (r=0.17, *P*=0.119); calliper estimate sole thickness Site2 (r=0.10, *P*=0.343); calliper estimate DP Site 1 (r=0.24, *P*=0.024); calliper estimate DP Site 2 (r=0.21, *P*=0.054).

Statistical analysis

A linear mixed model was produced for each outcome variable (distance to distal phalanx and sole thickness at Site 1 and Distance to distal phalanx at Site 2) in turn. All mixed models were built with claw, foot and time as the variables used to identify repeated observations within the same cow. In addition, foot (left or right hind), claw within foot (medial or lateral in left or right hind), time (categorical variable based on the examination number post calving) and the interactions between claw or foot and time were entered into the model as fixed effects. Cow was included as a random effect. For all models a random intercept was included to account for individual variation between cows; testing confirmed that in all cases this inclusion was appropriate as the variance of the intercept was >0 (Wald Z test).

The covariance structure of the repeated measures was decided with lowest Akaike information criterion (AIC), heterogeneous first-order autoregressive was selected as the best covariance structure for all outcome variables in the study. For each model the residuals were assessed for normality and the relationship between the model predicted values for the variable under investigation and the residuals generated by the model were assessed as a measure of fit.

In all models the claw within foot reference claw is medial and comparisons are made to Day 220 unless otherwise indicated. Statistical analyses were performed using SPSS 20 (IBM, SPSS, New York, USA) unless otherwise stated.

The relationship between Day 10 and the nadir for each variable was investigated using linear regression to give a value for R^2 (SPSS 18; SPSS Inc. Chicago, USA).

For distance to distal phalanx at Site 1, each claw was categorised into groups of thin (distance to distal phalanx of <7mm), marginal (distance to distal phalanx of 7 mm to 8.25mm), or adequate (distance to distal phalanx of >8.25 mm) sole thickness using the cut off points developed in Chapter 3. To investigate the utility of Day 10 estimates as a predictor of thin soles individual animals were categorised by their thinnest claw, at each time point. Contingency tables and a Relative Risk calculator (http://www.hutchon.net/ConfidRR.htm) were used to look at the progression of cows between categories between Days 10 and 110.

Results

Ultrasound measurement of distance to distal phalanx

Mean values were obtained for distance to distal phalanx at both sites, for both claws of both hind feet at each examination (Table 7.2).

Distance to distal phalanx at site one:

No effect of foot was demonstrated (*P*=0.599), but the effect of claw position within foot was significant (*P*<0.001). Comparing claws in the same foot, the distance to distal phalanx at Site 1 was greater in medial than lateral claws, with the size of the difference similar in both feet (Figure 7.2b). Mean differences were:

Left hind foot: -0.648 mm (95 % CI: -1.16 to -0.14)
Right hind foot: -0.652 mm (95 % CI: -1.00 to -0.31)

Time after calving had an effect on the mean value of distance to distal phalanx (*P*<0.001), however while there was no interaction between foot and time (*P*=0.240), the interaction between time and claw within foot approached significance (*P*=0.053): Figure 7.2a.

Mean distance to distal phalanx was at a minimum on Day 110. The difference between this nadir was significant on Days 10 (*P*=0.009) and Day 220 (*P*=0.050) and was not significant on Days 60 (*P*=0.256) or 160 (*P*=0.385).

The model residuals did not satisfy the Kolmogorov-Smirnov test. Graphical examination of residuals, identified three data points which, when cross-referenced to the initial box plot (Figure 7.1), corresponded to the possible outliers on that graph on Day 10 and all related to claws from the same cow (579). When these three data points were removed from the data set the residuals were normally distributed. However, this removal did not alter the outcome of the mixed model markedly so all data points were retained in the analysis.

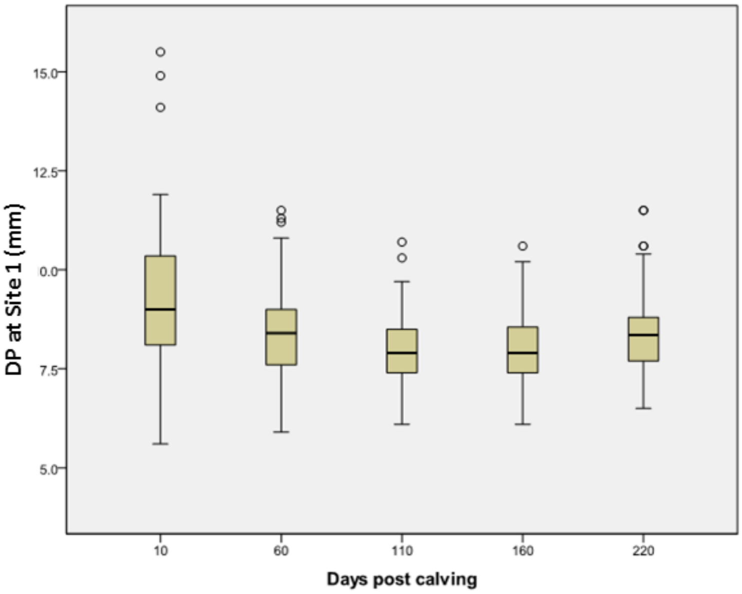

Figure 7.1: Relationship between time since calving and the distance from the external sole surface to the distal phalanx (DP) measured by ultrasound at Site 1, in the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Interpretation: The line in the box marks the median and the central box spans the quartiles. Lines extend from the box to the smallest and largest observations that are not suspected outliers. Observations more than 1.5 times the interquartile range (1 step) from the box are plotted individually as suspected outliers (open circles).

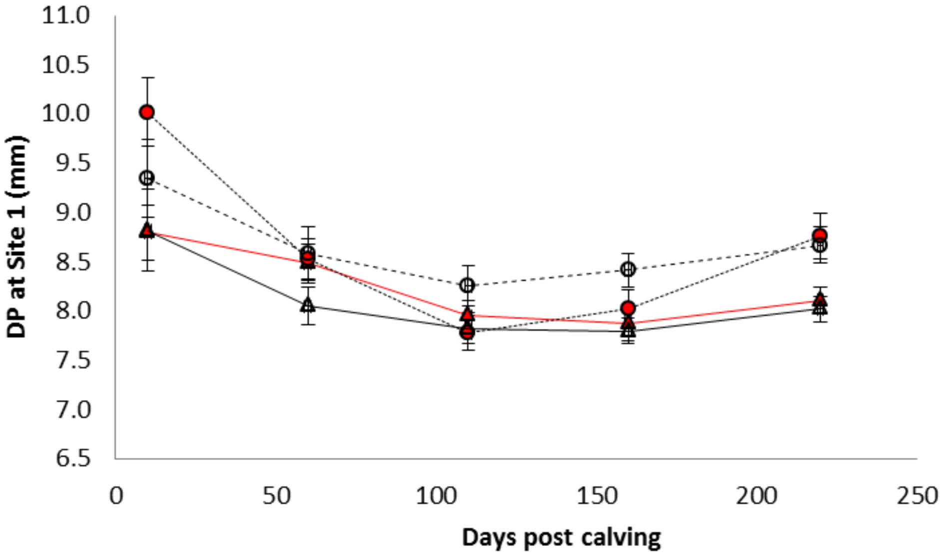

Figure 7.2a: Mean (SEM) depth to distal phalanx (DP) mm at Site 1 for the for the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

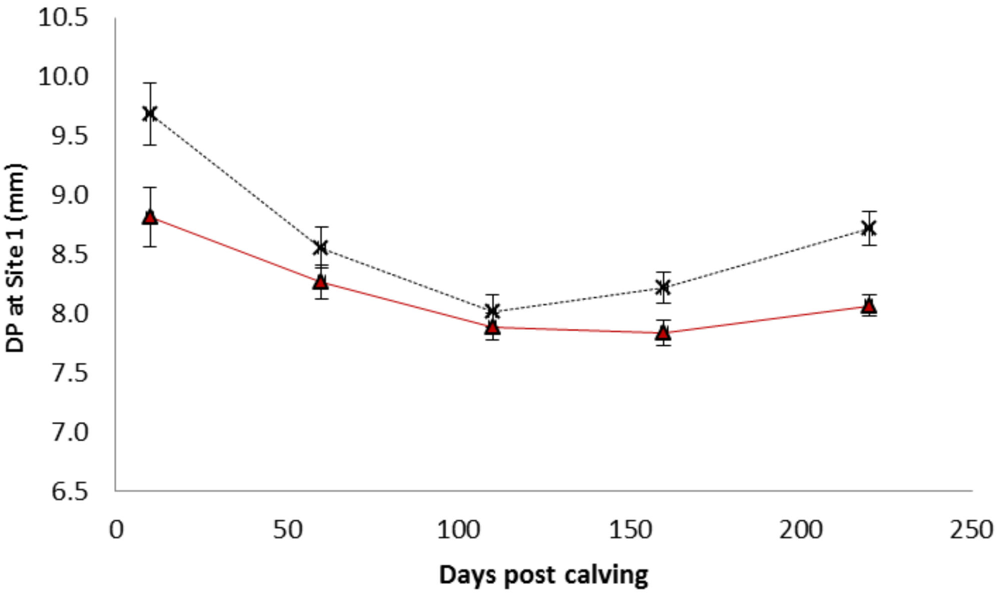

Figure 7.2b: Mean (SEM) depth to distal phalanx (DP) mm at Site 1 for the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Medial claws: **x**, dotted line; lateral claws: ▲, solid line (red)

Table 7.2: Effect of time after calving, foot and claw on mean distance to distal phalanx, at Site 1 (tip of the distal phalanx) and Site 2 (25mm towards the heel along the sole surface) in 25 first lactation dairy heifers. (RH=right hind; LH=left hind).

		Distance to distal phalanx (mm) (SEM)									
		Day 10		**Day 60**		**Day 110**		**Day 160**		**Day 220**	
N (animals)		24	24	25	25	25	25	25	25	25	25
Foot	**Claw**	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
LH	**Medial**	10.0 (0.35)	11.4 (0.31)	8.5 (0.21)	10.5 (0.21)	7.8 (0.17)	9.9 (0.23)	8.0 (0.20)	9.8 (0.22)	8.8 (0.23)	10.5 (0.22)
	Lateral	8.8 (0.28)	11.8 (0.29)	8.5 (0.19)	11.5 (0.29)	8.0 (0.15)	11.2 (0.20)	7.9 (0.18)	11.2 (0.20)	8.1 (0.13)	11.4 (0.26)
RH	**Medial**	9.4 (0.39)	10.8 (0.34)	8.6 (0.28)	10.6 (0.22)*	8.3 (0.20)	10.3 (0.18)	8.4 (0.17)	10.2 (0.22)	8.7 (0.18)	10.4 (0.21)
	Lateral	8.8 (0.42)	11.9 (0.29)	8.0 (0.19)	11.1 (0.29)	7.8 (0.16)	11.0 (0.22)	7.8 (0.13)	11.0 (0.18)	8.0 (0.12)	11.0 (0.27)

*n=24 (cow 578 missing data)

Distance to distal phalanx at site two:

The effect of foot was not significant (P=0.471), however there was an effect of claw position within foot (P<0.001). In contrast to the findings at Site 1, the distance to distal phalanx at Site 2 was greater in the lateral claw (Figure 7.4b compared to Figure 7.2b), Mean differences were:

Left hind foot: 0.90 mm (95 % CI: 0.35 to 1.46)
Right hind foot: 0.62 mm (95 % CI: 0.09 to 1.14)

Time after calving had an effect on the mean value of distance to distal phalanx (*P*<0.001), such that the distance was at its minimum on Day 160 (Figure 7.5). Despite this, there was no significant difference (P<0.05) between the lowest point and the other examinations, although values on Days 10 and 60 approached significance: day 10 (*P*=0.053), day 60 (*P*=0.059), day 110 (*P*=0.527) and day 220 (*P*=0.219). There was no interaction between either foot and time (*P*=0.481) or claw within foot and time (*P*=0.163).

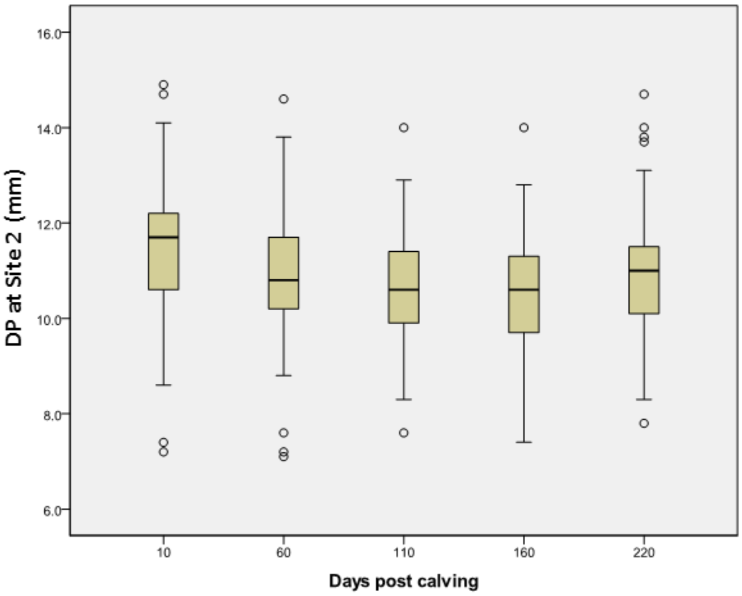

Figure 7.3: Relationship between time since calving and the distance from the external sole surface to the distal phalanx (DP) mm measured by ultrasound at Site 2, in the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving. For interpretation of the box and whisker plots see Figure 1.

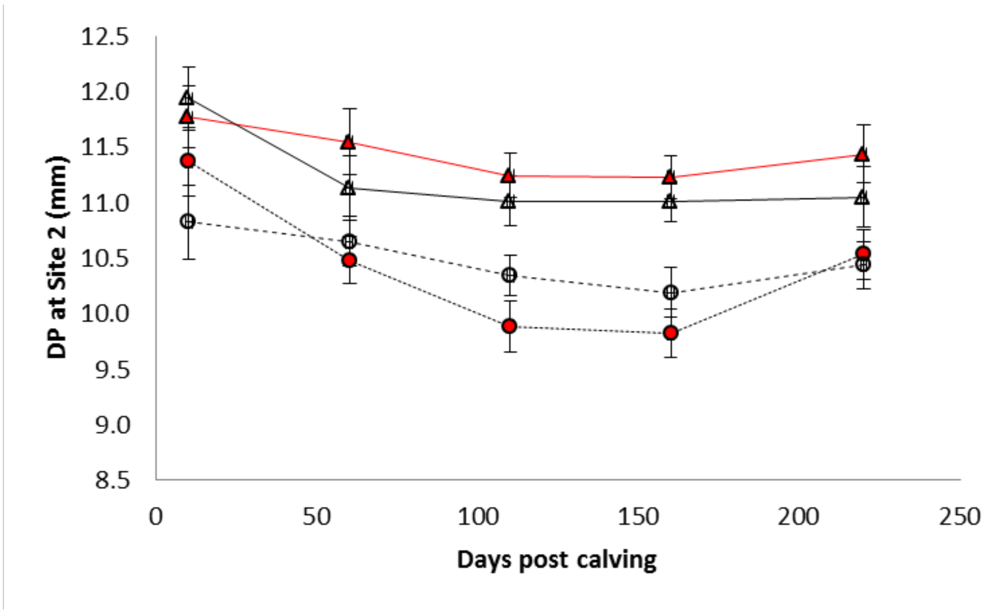

Figure 7.4a: Mean (SEM) depth to distal phalanx (DP) mm at Site 2 for the for the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

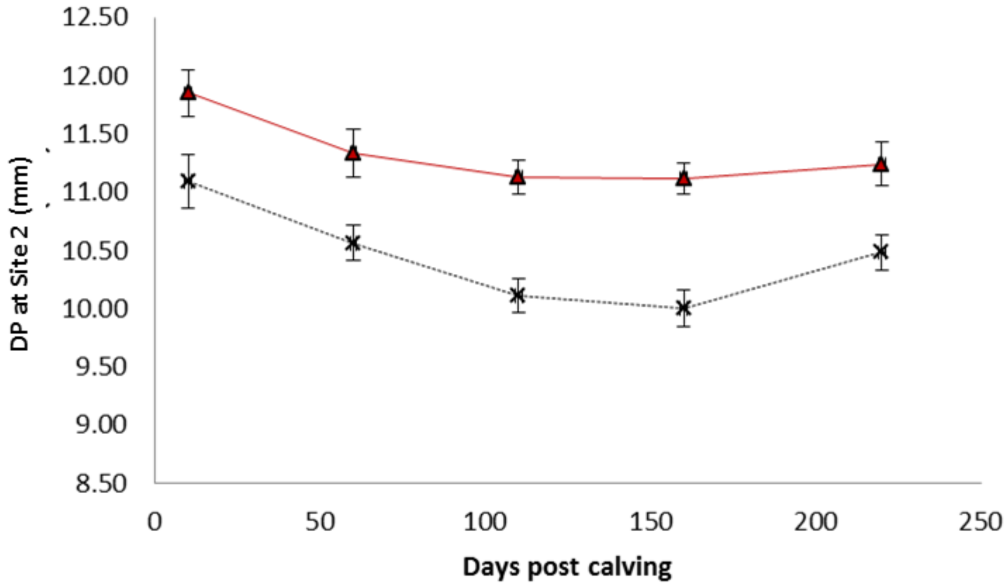

Figure 7.4b: Mean (SEM) depth to distal phalanx (DP) mm at Site 2 for the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Medial claws: **x**, dotted line; lateral claws: ▲, solid line (red)

Ultrasound measurement of sole thickness

Some difficulties were experienced in obtaining ultrasound measurements of sole thicknesses, and it was not possible to do so for every claw at each time point. Table 7.3 reports the mean values obtained for sole thickness at Site 1, for both claws of both hind feet at each examination, and an indication of the number of claws for which an estimate of sole thickness was successfully obtained.

Table 7.3: Effect of time after calving, foot and claw on mean sole thickness, at the tip of the distal phalanx (Site 1) as measured in 25 first lactation dairy heifers. (RH=right hind; LH=left hind).

		Sole thickness (mm) at Site 1 (SEM) (n)				
Foot	**Claw**	**Day 10**	**Day 60**	**Day 110**	**Day 160**	**Day 220**
LH	**Medial**	5.4 (0.22) 24	4.8 (0.13) 18	5.1 (0.16) 24	4.9 (0.12) 24	5.6 (0.12) 24
	Lateral	5.0 (0.16) 24	5.0 (0.14) 19	5.2 (0.15) 19	5.0 (0.11) 21	5.5 (0.11) 22
RH	**Medial**	5.6 (0.23) 24	5.0 (0.15) 18	4.8 (0.14) 20	4.9 (0.10) 24	5.4 (0.09) 23
	Lateral	5.5 (0.27) 24	5.1 (0.15) 22	4.9 (0.16) 17	4.9 (0.10) 18	5.5 (0.09) 23

Sole thickness at Site 1

There was no effect of foot (*P*=0.762) or claw position within foot (*P*=803) on sole thickness at Site 1. Within the same foot, sole thickness was similar in medial and lateral claws. Mean differences were:

Left hind foot: -0.05 mm (95 % CI: -0.36 to 0.26)
Right hind foot: 0.17 mm (95 % CI: -0.06 to 0.40)

Time after calving had an effect on the mean value of sole thickness (*P*<0.001), and in contrast to distance to distal phalanx measures, there was significant interaction between foot and time (*P*=0.038) (Figure 7.6) but not claw within foot by time (*P*=0.797). Thus, sole thickness was similar on Days 60, 110 and 160. Values on Days 10 (*P*=0.006) and 220 (*P*=0.003) were significantly higher than on other days: Figures 7.5 and 7.6.

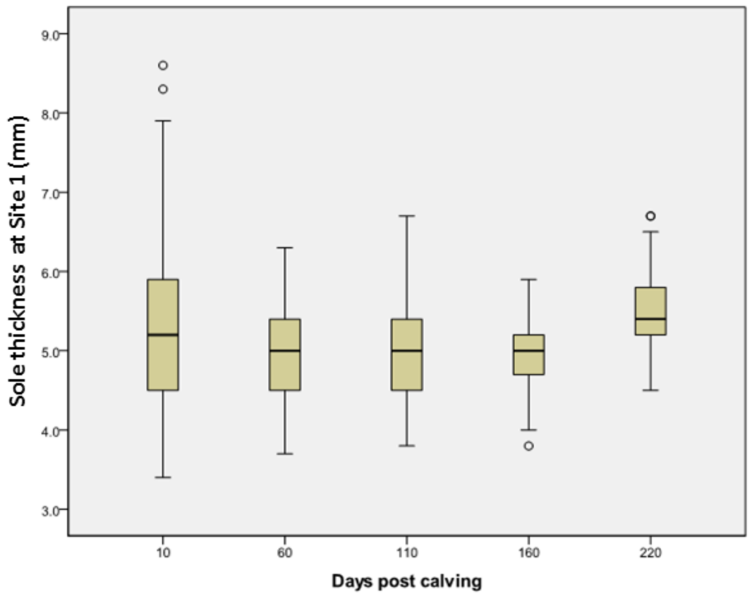

Figure 7.5: Relationship between time since calving and sole thickness mm measured by ultrasound at Site 1, in the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving. For interpretation of the box and whisker plots see Figure 1.

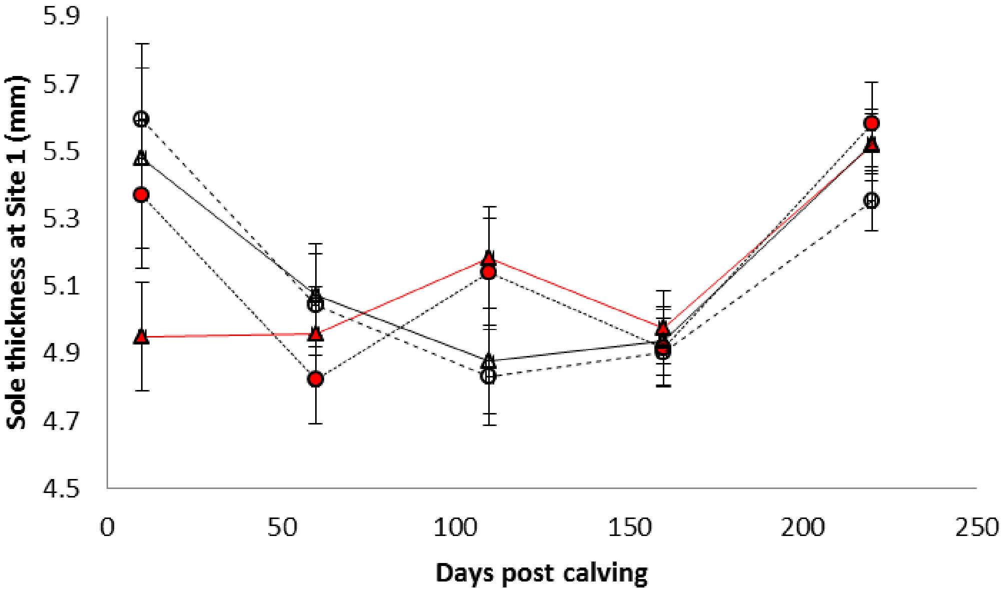

Figure 7.6: Mean (SEM) sole thickness at Site 1 (mm) for the for the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Relationship between initial depth on Day 10 and the nadir for each site

The variance obtained at each of the five examinations was not consistent, as illustrated in Figure 7.7. The effect was particularly pronounced for Site 1 measurements.

This phenomenon led to an investigation of the relationship between the initial value obtained on Day 10 and the change in the variable between that initial value and the point in lactation when the lowest group-mean value was obtained. That is, Day 110 for Site 1 variables and Day 160 for Site 2 variables.

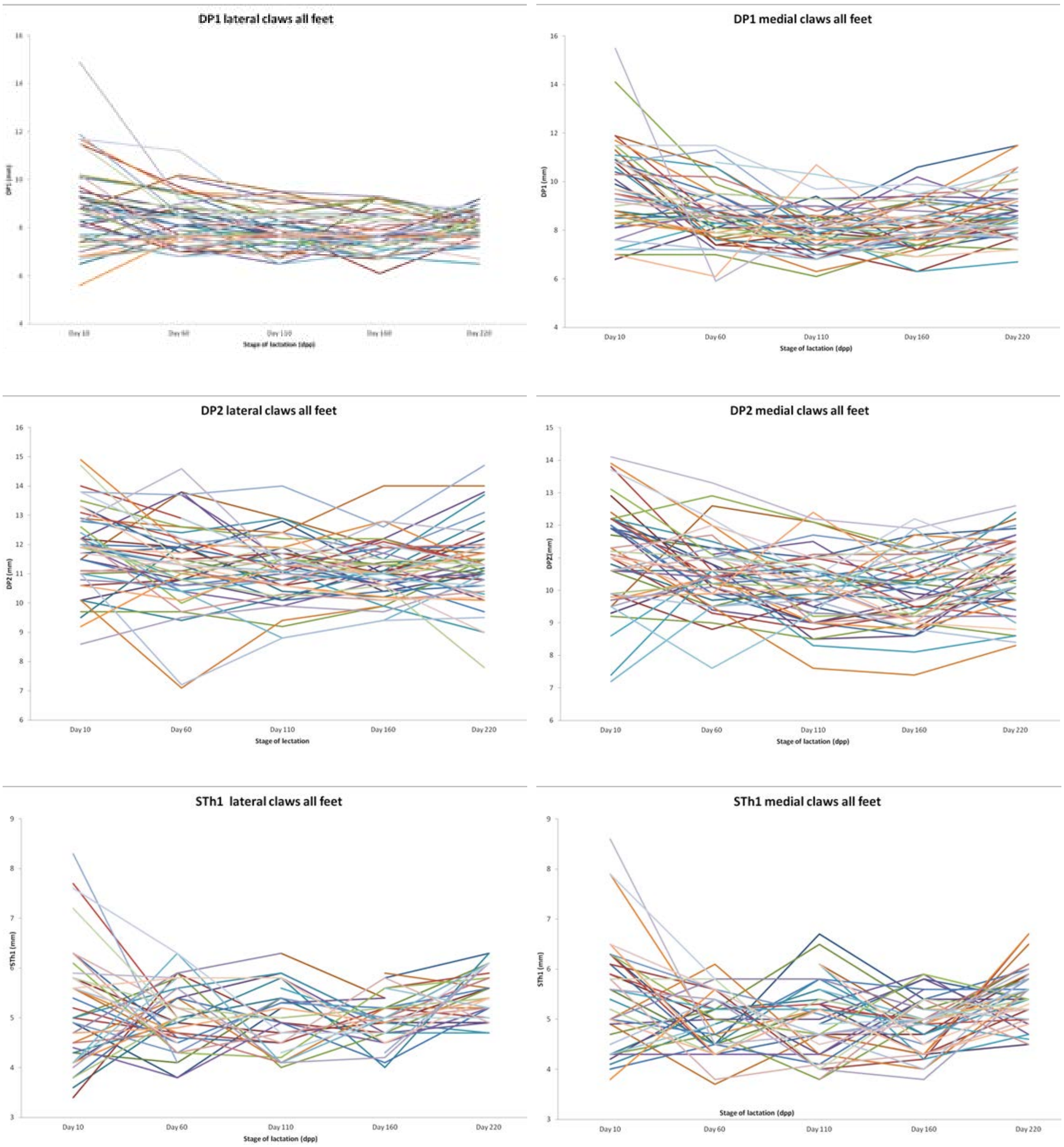

Figure 7.7: Multigraphs to illustrate distance to distal phalanx (DP) and sole thickness (STh) plots for individual animals for medial and lateral claws at Site 1 and Site 2.
Variance at Site 1 reduces over time, particularly in lateral claws. At Site 2 the distribution between claws is more even and the patterns of change less distinct. Sole thickness distribution at Site 1 follows a similar pattern to that of the DP at Site 1.

Distance to distal phalanx at Site 1:

The change in distance to distal phalanx at Site 1 between Days 10 (initial depth) and 110 (when the lowest group-mean value was obtained) was correlated to the depth on Day 10 for both lateral (R^2 Adjusted=0.816: $P<0.001$) and medial (R^2 Adjusted=0.788: $P<0.001$) claws. These data are illustrated in Figure 7.8.

Of the 96 claws measured on Day 10, 18 claws (from 10 heifers) had an increase in distance to distal phalanx between Days 10 and 110. The mean distance to distal phalanx of these claws on Day 10 was 7.3 mm ± 0.4, significantly smaller than the mean distance to distal phalanx of the 74 claws in which a decrease in distance to distal phalanx occurred (9.8 mm ± 0.4).

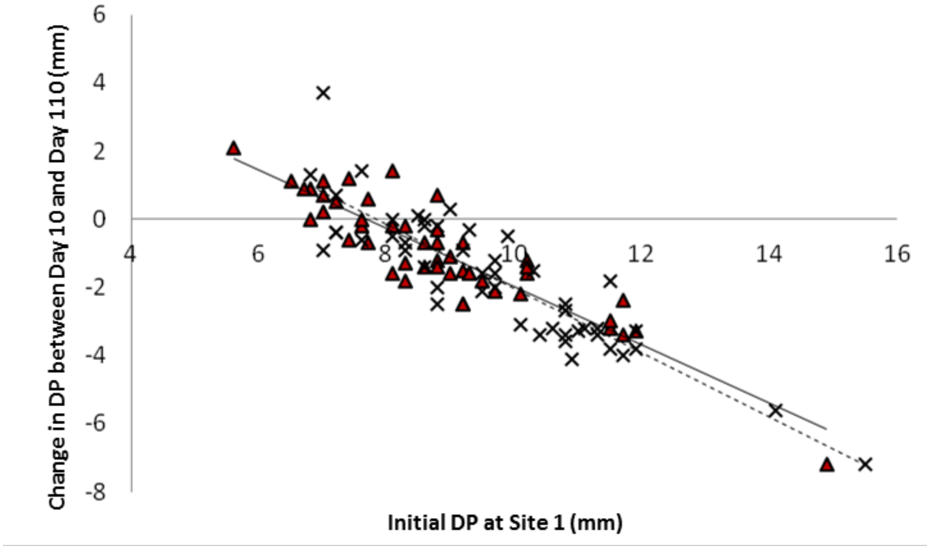

Figure 7.8: Scatterplot of the change in distance to distal phalanx (DP) between Days 10 and 110 versus the initial DP on Day 10, at Site 1 for the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Medial claws: x, dotted line; lateral claws: ▲ , solid line (red)

Distance to distal phalanx at Site 2:

The change in distance to distal phalanx at Site 2 between Days 10 and 160 was correlated to the initial depth on Day 10 for both lateral (R^2 Adjusted=0.519: $P<0.001$) and medial (R^2 Adjusted=0.570: $P<0.001$) claws, as illustrated in Figure 7.9.

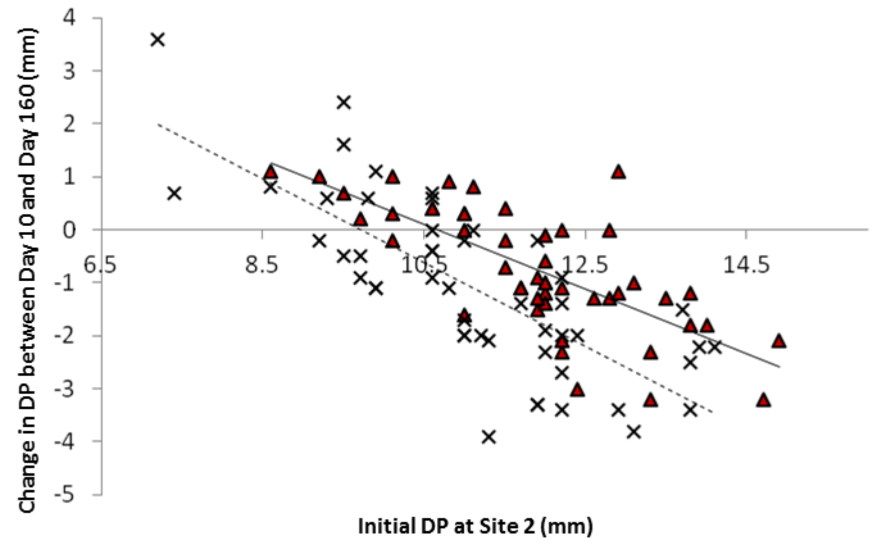

Figure 7.9: Scatterplot of the change in distance to distal phalanx (DP) between Days 10 and 160 versus the initial DP on Day 10, at Site 2 for the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving.

Medial claws: x, dotted line; lateral claws: ▲ , solid line (red)

Sole thickness at Site 1:

The change in sole thickness at Site 1 between Days 10 and 110 was correlated to the initial thickness on Day 10 for both lateral (R^2 Adjusted=0.688: P<0.001) and medial (R^2 Adjusted=0.612: P<0.001) claws. This illustrated in Figure 7.10.

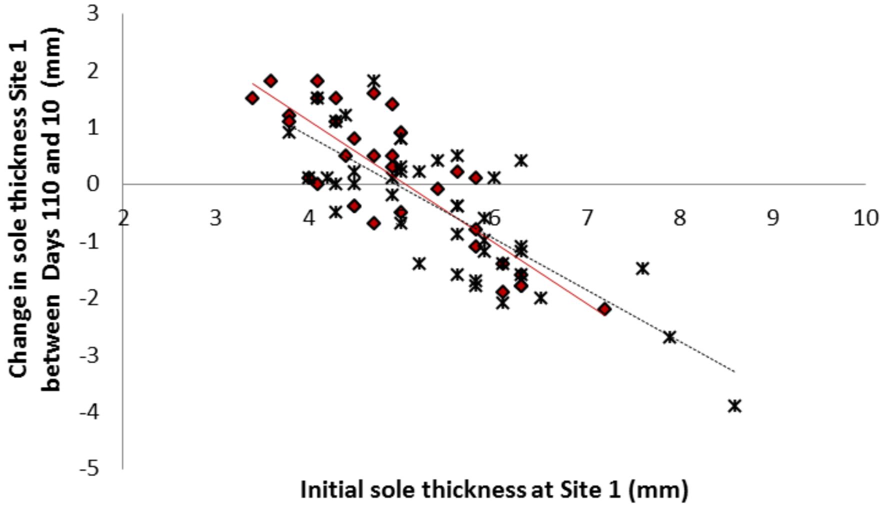

Figure 7.10: Scatterplot of the change in sole thickness between Days 10 and 110 versus the initial sole thickness on Day 10, at Site 1 for the medial and lateral claws of both hind limbs of 25 first lactation dairy heifers on Days 10, 60, 110, 160 and 220 post calving.
Lateral claws; ◆, solid line; medial claws; ✳, dotted line.

Sole thickness categories for studied claws Days 10-220

All claws

The cut off points for distance to distal phalanx at Site 1 developed in Chapter 3 were applied to Site 1 values in claws examined in the present study. A summary of the distribution of thin, marginal and adequate sole depths across lactation is given in Figure 7.11 and Table 7.4.

Overall, changes of sole depth category showed that, of the twelve claws that were categorised as thin on Day 110, six were adequate on Day 10 and six were thin or marginal. Claws which were marginal or thin on Day 10 were 2.4 (95 % CI: 0.9 to 6.9) times more likely to be thin on Day 110 than claws which were categorised as adequate on Day 10. In addition claws which were marginal or thin on Day 10 were 1.33 (95% CI 1.0–1.7) times more likely to be marginal or thin on Day 110.

Table 7.4: Contingency Tables for all claws of 24 first lactation dairy heifers showing the progression from one claw category to another between Days 10 and 110. Distances to distal phalanx (DP) at Site 1 were defined as Thin (<7 mm), Marginal (7-8.25 mm) or Adequate (>8.25mm).

		Day 110		
		Adequate / marginal N	**Thin Y**	
Day 10	**Thin / Marginal Y**	22	6	28
	Adequate N	62	6	68
		84	12	**96**

Day 10 yes (Y) is thin or marginal, no (N) is adequate
Day 110 Y is thin, N is marginal or adequate

All Claws (n=96)	Frequency	Day 10	Day 110
Adequate Day 10 and Day 110	62	no	no
Adequate Day 10 and Thin Day 110	6	no	yes
Thin /Marginal Day 10 but Adequate Day 110	22	yes	no
Thin /Marginal Day 10 and Thin Day 110	6	yes	yes

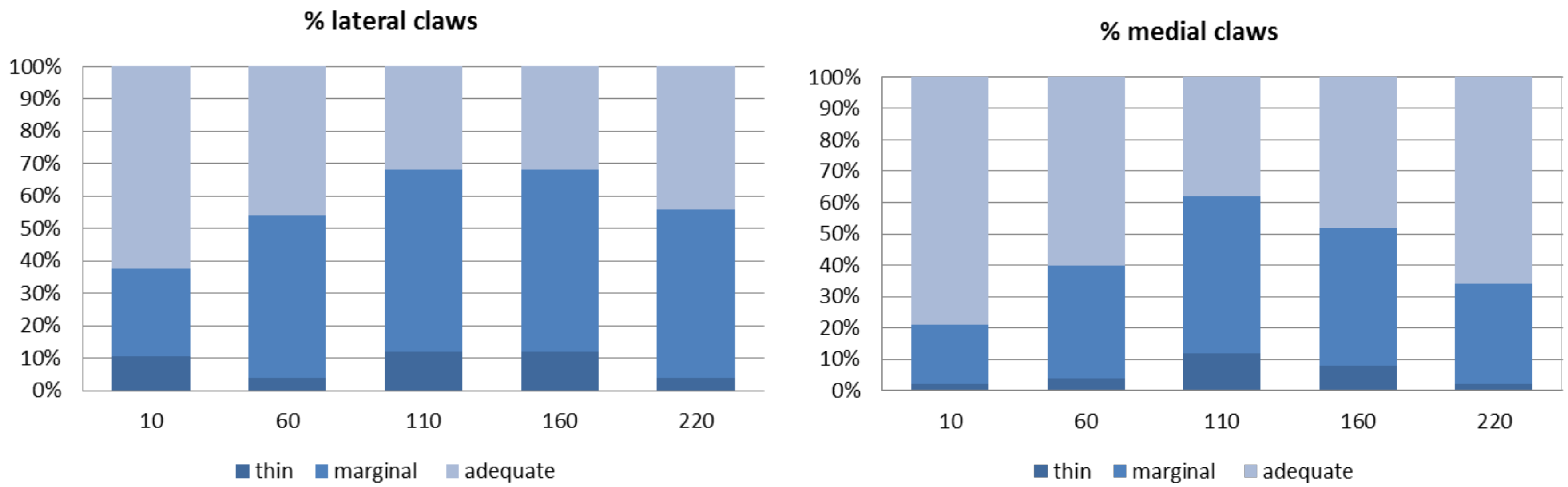

Figure 7.11: Sole thickness categories for the lateral and medial claws of both hind feet of 24 first lactation dairy cows on Days 10, 60, 110, 160 and 220. Distances to distal phalanx (DP) at Site 1 were defined as Thin (<7 mm), Marginal (7-8.25 mm) or Adequate (>8.25mm).

Table 7.5: Summary of the sole thickness categories for the lateral and medial claws of hind feet of 25 first lactation dairy cows on Days 10*, 60, 110, 160 and 220. Distances to distal phalanx (DP) at Site 1 were defined as Thin (<7 mm), Marginal (7-8.25 mm) or Adequate (>8.25mm).

DP Site 1 category	Days post calving Lateral claws					Days post calving Medial claws				
	10	60	110	160	220	10	60	110	160	220
Thin	5	2	6	6	2	1	2	6	4	1
Marginal	13	25	28	28	26	9	18	25	22	16
Adequate	30	23	16	16	22	38	30	19	24	33
Total number of claws	48	50	50	50	50	48	50	50	50	50

At the cow level

Of the eight heifers categorised as having thin soles (by thinnest claw) on Day 110, two were adequate on Day 10 and six were thin or marginal. Heifers which were categorised as having marginal or thin soles on Day 10 were 3.0 (95% CI: 0.8 to 12.0) times more likely to have thin soles on Day 110 than if they had had soles which were categorised as adequate on Day 10.

During the study, 31 claws (from 17 heifers) had at least one distance to distal phalanx (Site 1) measurement of <7.0 mm. Four claws from three animals had distances to distal phalanx of <7.0 mm on two occasions. No claw had a measurement of <7.0 mm on three or more occasions.

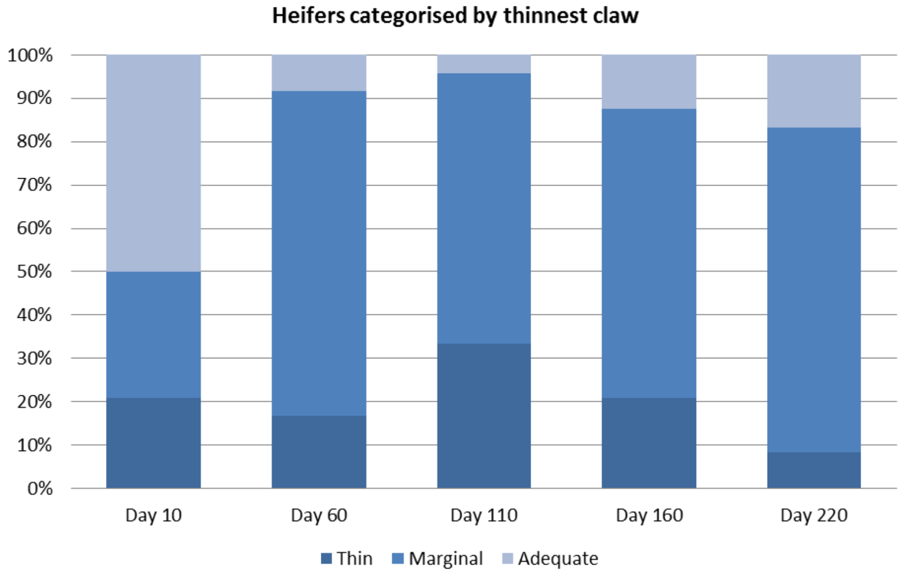

Figure 7.12: Sole thickness categories for 24 first lactation heifers categorised by their thinnest claw, on Days 10, 60, 110, 160 and 220. Distances to distal phalanx (DP) at Site 1 were defined as Thin (<7 mm), Marginal (7-8.25 mm) or Adequate (>8.25mm).

Table 7.6: Summary of the sole thickness categories for 24 first lactation dairy cows categorised by their thinnest claw, on Days 10, 60, 110, 160 and 220. Distances to distal phalanx (DP) at Site 1 were defined as Thin (<7 mm), Marginal (7-8.25 mm) or Adequate (>8.25mm).

DP Site 1 category	**Numbers of soles**				
	Days post calving				
	10	**60**	**110**	**160**	**220**
Thin	5	4	8	5	2
Marginal	7	18	15	16	18
Adequate	12	2	1	3	4
Total number of heifers	24	24	24	24	24

Table 7.7: Contingency Tables for medial claws of 24 first lactation dairy heifers to look at progression between Days 10 and 110 from one claw category to another. Thin distance to distal phalanx (DP) Site 1 <7 mm, Marginal DP Site 1 7-8.25 mm, Adequate DP Site1 >8.25mm.

		Day 110		
		Adequate / marginal N	**Thin Y**	
Day 10	**Thin / Marginal Y**	6	6	12
	Adequate N	10	2	12
		16	8	**24**

Day 10 yes (Y) is thin or marginal no (N) is adequate
Day 110 Y is thin, N is marginal or adequate

Heifers (n=24)	**Frequency**	**Day 10**	**Day 110**
Adequate Day 10 and Day 110	10	no	no
Adequate Day 10 and Thin Day 110	2	no	yes
Thin /Marginal Day 10 but Adequate Day 110	6	yes	no
Thin /Marginal Day 10 and Thin Day 110	6	yes	yes

Discussion

Inadequate protection of the sensitive tissues of the claw leads to tissue damage and increases the risk of lameness (Toussaint Raven, 2003). Thin soles provide insufficient protection (Toussaint Raven, 2003; Sanders, 2009; Mason et al., 2011) so an accurate measure of sole thickness, which can be used in the field, would be useful for predicting potential clinical problems and for confirming the involvement of thin soles in outbreaks of lameness. Previous studies (e.g. Sanders et al., 2009; Mason et al., 2011) have shown that ultrasound measurement of the internal structure of the claw is a useful technique in such cases.

However, in order to develop intervention studies specifically aimed at studying risk factors which impact on sole thickness, a technique is needed which can be used for multiple measurements on the same cows. Tranter and Morris (1992) were able to measure sole wear rates over the course of lactation by repeatedly creating grooves in the sole horn, but this technique did not estimate sole growth. Sole wear rates without sole growth rates do not provide information on how much protection is provided by the sole, as an increase in the wear rate of the sole could occur alongside an increase in sole thickness if there is an increase in the growth rate. Such a phenomenon has been reported for wall horn (Livesey and Laven, 2007), so is likely to also occur in sole horn. Ultrasound provides a non-invasive direct measure of sole thickness and is thus a better measure of sole protection than is sole wear. However, its use in the same animals over a long period of time has not been previously reported.

This is the first study which has shown that such multiple ultrasound measurements of hoof structure in the same animal are feasible and that it is thereby possible to follow changes in internal claw structure in a group of dairy heifers throughout their first lactation. It was not always possible to obtain an estimate for sole thickness and the mean changes in sole thickness at Site 1 described in this study are largely occurring within a range of approximately one millimetre (between 4.7-5.7 mm Figure 7.6). This coupled with the similar mean sole thickness values obtained on Days 60, 110 and 160 of lactation reduce the utility of this variable in monitoring change over time with the portable ultrasound in use.

The best measure used, i.e. the distance from the external surface to the distal phalanx, was quick and simple to measure taking less time to obtain than an estimate of sole thickness. Measurements were apparently unhindered by the presence of hoof horn haemorrhages. This utility, combined with the greater accuracy of the distance to distal phalanx measurement compared to sole thickness estimates with the portable machine (Chapter 3), means that measurement of distance to distal phalanx using a portable ultrasound machine has significant advantages over other methods of measurement of sole thickness for both research and clinical investigation (Mason et al., 2011).

Changes in distance to distal phalanx may not be solely due to changes in sole thickness, as distance to distal phalanx is a composite measure which includes both the sole and the underlying soft tissue between the sole and the distal phalanx. Thus, it is not possible to demonstrate conclusively that the changes seen in this study were due to changes in sole horn thickness alone, particularly as changes in soft tissue may not match changes in horn thickness. For example, van Amstel et al. (2004b) reported that in some animals with thin soles there may be hypertrophy of the soft tissues below the sole.

This has not been reported in New Zealand and, in the cadaver study reported in Chapter 3, it was found that distance to distal phalanx, measured using electronic callipers, was strongly correlated to actual sole thickness at both Site 1 and Site 2 (r=0.9 and 0.8, respectively). Furthermore, at the tip of the distal phalanx there is only a minimal amount of soft tissue (≈3 mm; Chapter 3) and no digital cushion (Räber et al., 2004), so at this site (Site 1) the majority of the change is likely to be hoof horn rather than soft tissue. At Site 2 there is more soft tissue, principally digital cushion (Räber et al., 2004), so changes at this site may reflect both soft tissue and hoof horn changes; indeed this may explain the greater variance in distance to distal phalanx at Site 1 than Site 2.

Although it cannot demonstrate conclusively that changes in distance to distal phalanx are due solely to changes in sole horn thickness as there are soft tissues elements, including the digital cushion, between the inner sole surface and the distal phalanx. As, both soft tissue thickness and sole thickness contribute to protection of the corium, so distance to distal phalanx may have actually some advantages over sole thickness as a measure of corium protection. Clinically it is the level of claw horn disease and lameness in relation to welfare and the economic consequences resulting from reduced productivity (with respect to milk yield, reproductive performance, and the expenses of treatment and culling from the herd) which are of interest. Whether the change is due to reduction in sole thickness or soft tissue is immaterial once there is inadequate protection of the vital underlying structures of the dermis and epidermis responsible for quality horn production. Thus it is considered that the advantages of measurement of distance to distal phalanx outweigh the disadvantages of not measuring sole thickness directly.

At the start of the study (Day 10) the heifers exhibited a wide spread of distance to distal phalanx values (e.g. from 5.6 to 14.9 mm for lateral claws at Site 1, includes cow 579), even though they had been reared together at pasture as a group from weaning. Factors such as breed, nutritional management prior to weaning, and disease may all have played a role in producing this wide spread. Further research in growing heifers is required to establish which of such effects, is the most important determinant of sole thickness.

At Site 1, mean distance to distal phalanx decreased between Days 10 and 110, indicating that sole thickness was also decreasing over this period. The most likely reason for this is that the rate of sole wear was greater than the rate of sole growth during this period of time. Tranter and Morris (1992) reported that the rate of sole wear in cattle at pasture tended to be higher in the first 4 months of lactation than later, so some of the decrease in sole thickness was probably due to this greater wear rate, although it is also feasible that some of the decrease could have been due to a slower rate of sole horn growth during this period. The only estimates of net sole horn growth rate reported in the literature relate to beef feedlot animals (Greenough et al., 1990). Greenough et al., (1990) sectioned claws from two cohorts of animals that were slaughtered sequentially to arrive at their estimates of; 0.16 cm / month ± 0.01 for calves and 0.05 cm / month ± 0.01, for yearlings in the study.

Interestingly, in the heifers in this study, this net wear of sole horn was not matched by net wear of wall horn. In these heifers during the period between Days 10 and 110, wall horn growth tended to be greater than wall horn wear (though net growth was not significantly greater than zero: *P*>0.05; Data not presented). This comparison highlights the value of measuring distance to distal phalanx or sole thickness as it shows that net wall horn growth can be positive when sole horn is getting thinner. For Site 2, later and less obvious changes were obtained with the lowest values at Day 160. Although the relationship with time was significant mean values fell within the confidence intervals of the next time period as the differences were small.

At site 1 medial claws had greater depth to distal phalanx than lateral claws throughout lactation while at Site 2 the opposite relationship was seen. For the sole thickness Site 1, the difference between claws was greatest at Day 10, medial claws started with a higher value and reduced between Days 10 and 60 to become smaller, but close in value to that of the lateral claw. Minimal difference between medial and lateral claws at Site 1 was seen at Day 110, when then lowest group mean was observed and supports this time point as a good stage in lactation to categorise claws by the established cut off points.

Although mean distance to distal phalanx decreased between Days 10 and 110, this was not the case for all claws. Overall, there was a significant correlation between distance to distal phalanx on Day 10 and the change in distance to distal phalanx between Days 10 and 110. As Figure 7.8 shows, claws with a distance to distal phalanx at Site 1 of <7.7 mm on Day 10 tended to increase in thickness, with thinner soles tending to increase more. A similar pattern was seen for distance to distal phalanx at Site 2 and sole thickness at site 1 Figures 7.9 and 7.10. This association between change with time and initial distance to distal phalanx was the key factor producing the reduction in variance of distance to distal phalanx between Day 10 and the other examinations. It also means that measuring distance to distal phalanx on Day 10 was only a moderate predictor of distance to distal phalanx on Day 110. Although a claw that was marginal or thin on Day 10 was 2.4 times more likely to be thin on Day 110 than a claw which was adequate on Day 10, only 21% (6/28) of such claws became thin, and 50% of claws which were thin on Day 110 had been adequate on Day 10. This confirms the importance of multiple measurements of dynamic structures, as single time point measures can be misleading. The data from the present study therefore suggest that adequate soles, which initially provided good protection to the underlying dermis, wore and became thinner, whilst mechanical loading of thin and marginal soles, which at least initially had had poorer protection, resulted in increased horn production probably reflecting stimulation of the epidermal keratinocytes (Greenough, 2007).

This study has shown that sole thickness changes significantly during lactation and that this can be detected using ultrasound. The results suggest that ultrasound measurement of sole thickness (either directly or via the measurement of distance to distal phalanx, depending on the ultrasound machine used) could be used as a standard technique for the investigation of lameness problems where sole thickness is believed to be an issue (Sanders et al., 2009; Mason et al., 2011). The technique is a useful addition to standard techniques such as growth and wear measurement and lesion recording which are currently used in intervention studies.

For such studies, these data provide a baseline for further comparison. This study was undertaken in pasture-based cattle where their only contact with concrete was around milking. Similar studies are required in housed cattle to show whether changes in sole thickness are more or less pronounced in such cattle and whether the timing of the nadir is similar. In pasture-based cattle, further studies are required to establish the impact of walking distance (to the parlour for milking) and standing times (in collecting yards and on feed pads) on sole thickness.

Conclusion

In support of the initial hypothesis, this study has shown that a portable ultrasound machine can be used to image internal claw structures in dairy heifers over lactation. However, the anticipated problems related to the impact of sole horn haemorrhage and loss of sole concavity, were not experienced. Although direct estimation of sole thickness using the portable machine Mindray DP 6600 (Mindray, Szechuan, China) was demonstrated to be less reliable than the use of distance to distal phalanx, position of the measurement was established to be important with Site 1 confirmed as preferable.

This study confirms the value of ultrasound as a research and clinical tool. In this study, mean distance to distal phalanx in the claws of 25 heifers decreased significantly after calving reaching a nadir at approximately 110 days post calving. However, the change in distance to distal phalanx was dependent on initial distance to distal phalanx, and the distance to distal phalanx of thinner claws tended to increase over this period. In contrast to the initial hypothesis the mean value of distance to distal phalanx was seen to stabilise as the end of lactation as approached, suggesting a dynamic response of the sole horn to a wear challenged rather than confirming a consistent loss of sole thickness throughout lactation.

Publication

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Chapter eight: Study of heel conformation changes in first lactation heifers

Introduction

Modification of heel conformation over time has been commented upon as a feature of dynamic conformational change by a number of authors (Hahn et al., 1984a; Vermunt and Greenough, 1996; Webster 2001; Livesey and Laven, 2007). The response to environmental modification has been investigated (Phillips and Schofield, 1994; Boelling and Pollott, 1998; Kremer et al., 2007; Ouweltjes et al., 2009) with inconsistent results and the impact of nutrition has also been assessed (Ahlström et al., 1986; Baird et al., 2009). The potential usefulness of monitoring conformational change at the heel is hampered by the challenge of obtaining a reliable estimate for features of heel conformation such as heel height and angle (Hahn et al., 1984b). Inconsistent findings across the literature may relate to this difficulty.

Bryan et al. (2012) discuss the existence of a difference in height between the claw ground surface of the lateral and medial claws within a foot in the New Zealand dairy cow. This difference in height was linked to a reduced time to lameness incidence (crude median days to lameness: disparity trimmed level, 38 days; control, 29 days). These reports tally with the observation of a developing asymmetry between the medial and lateral claws for heifers examined in Year 1 (Chapters 6 and 7) for conformational assessment and ultrasound evaluation of sole thickness and distance to distal phalanx. The discrepancy in heel height was particularly evident when the heel was viewed from above and behind in the lifted claw.

Nonetheless, it is clear from the literature review that heel depth can be difficult to assess accurately in the live animal.

Hypothesis: In consequence, a new method for estimation was developed, with the aim of monitoring the change in heel conformation over first lactation utilising set landmarks and linear measures rather than the collection of heel angles or heel heights. The working hypothesis was that as the length (i.e. linear distance) of the non-weight bearing region of the heel decreases with time while the weight bearing portion increases, and that this increase will be related to the area of the heel bulb within the claw ground surface which weight is bearing.

If this is confirmed, linear measures could be used future to examine the relationship between this weight bearing heel area and lesion development and severity in the sole areas and zone six of the international hoof map (Greenough and Vermunt, 1994).

This chapter aims to evaluate use of a novel method to monitor heel conformation throughout the first lactation of primiparous diary heifers and investigate how the obtained measures change with time, foot and claw position.

Materials and Methods

*A*nimals

Twenty nine first lactation Friesian heifers were recruited to the study from a single dairy herd (Massey University No. 4 herd) in the 2009/2010 season. They were managed in a typical New Zealand pasture based system, with heifers animals kept solely at pasture and being walked to milking twice a day in a rotary parlour. All animals were approximately 24 months of age at calving (mean; 23.7 ± 0.08 months, range 23.0 to 24.5).

Heifers Animals were examined on four occasions throughout their first lactation, approximately Days 10 (mean 14 dpc; range 7-25 dpc), 60 (62; 57-81), 120 (119; 110-137) and 230 (228; 220-238) days post calving. Data for all 29 animals are reported for each time point, with the exception of Day 230 (28 animals).

All procedures were approved by Massey University Animal Ethics Committee protocol number MUAEC 09/59.

Conformational measurements

At each examination heifers were restrained in purpose-designed foot crush (WOPA, Netherlands) for efficient lifting of both hind limbs. Each foot was lifted, cleaned (washed and dried). Surface preparation was kept to a minimum. For this study, three measurements were made on both claws of all feet (Figure 8.1). All measurements were made using a flexible tape measure, except for claw depth which was measured using electronic callipers.

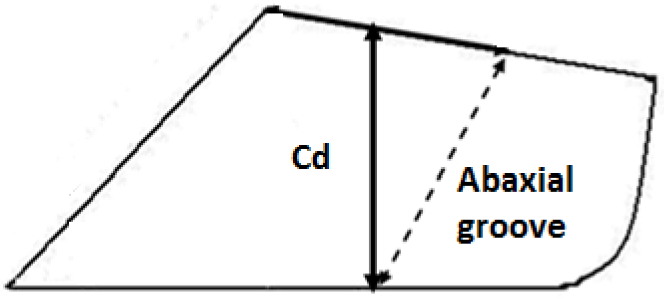

Figure 8.1: Abaxial view of claw showing the landmarks for claw depth measured on both claws of the hind feet in 29 dairy cows using electronic callipers). Cd: Height of the claw at the distal end of the abaxial groove.

Additional heel conformation measurements

The line YZ was drawn on the claw with a marker pen and the half-way point marked (measured with ruler). The extent of the heel surface above the line YZ that was weight bearing was also outlined (Figure 8.2) (H). The distance from the skin to heel-bulb-horn junction to meet this mark at ninety degrees (F) and the distance along the line F to the point (I) at which the tape measure crossed the line demarcating the weight bearing area of the heel (H) were recorded. The weight bearing length of the heel (G) was calculated by subtraction.

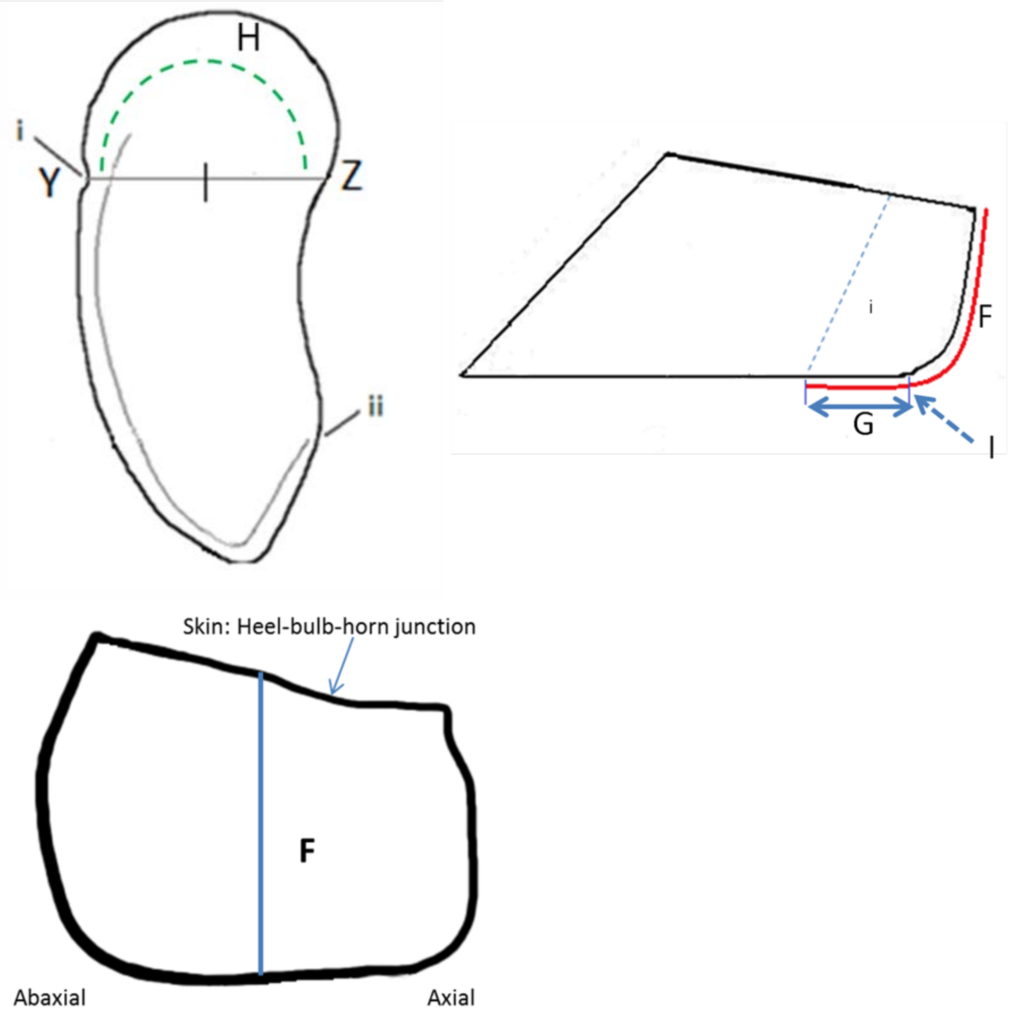

Figure 8.2: Top left panel shows the plantar surface of the sole, marked up to outline the weight bearing region of the heel horn (H) above the line YZ (drawn perpendicular to the tangent of the abaxial groove) and the demarcation of the half-way point measured by ruler.

Right panel: shows the abaxial view of a claw illustrating the variables (F) distance from the skin to heel-bulb-horn junction to meet the half way mark on line YZ at ninety degrees and (I) the point along the distance (F) at which the tape measure crossed the line demarcating the weight bearing area of the heel (H) which were measured on both claws of the hind feet. (G) The weight bearing length of the heel was calculated by subtraction. i the abaxial groove.

Bottom left panel: a rear view of the heel bulb showing the position of line F so as to meet the line YZ at ninety degrees.

Figure 8.3: Image of Cow 20 back right foot to show mark-up of the sole surface for data collection. Red dots, which are of known dimensions (diameter=1.2cm) are for calibration during image analysis.

Estimation of heel and sole weight bearing surface area.

The ends of the white line were marked on each claw. Lesions present on the white line or sole surface both above and below the line YZ were outlined with a marker pen. As lesion severity increased, severity was indicated by the use of dots within the area outlined. The line on the axial sole where weight bearing was lost was drawn on the sole to aid later image analysis.

An adhesive paper dot, 1.2 cm diameter, was placed on the sole of each claw for calibration purposes, before a digital image was recorded (Power shot A640, Canon) taken on the macro setting with the camera parallel to the sole surface of the claws so their image filled the display. Each image included foot and animal identification.

The process of image analysis was modified from Leach et al. (1998). Image J software (ImageJ.nih.gov), was used to measure the area of the heel bulb which was weight bearing above the line YZ and the claw surface area below the line YZ. Image analysis was completed following data collection and all images were analysed by one operator.

Statistical analysis

Data from each variable collected for the study were assessed for normality before analysis. Each conformational variable in turn was used as the outcome variable in a linear mixed model. Every model was built with claw, foot and time as the variables used to identify repeated observations within the same cow. In addition, foot (left or right hind), claw within foot (medial or lateral in left or right hind), time (discrete variable based on days post calving category 10, 60, 120 or 230 days post calving) and the interactions between foot and time and time by claw within foot were entered into the model as fixed effects and cow was included as a random effect. For all models a random intercept was included to account for individual variation between cows; testing confirmed that in all cases this inclusion was appropriate as the variance of the intercept was >0 (Wald Z test).

In all models the claw within foot reference claw is medial and comparisons are made to Day 230 unless otherwise indicated.

The covariance structure of the repeated measures was decided with lowest Akaike information criterion (AIC). Covariance structure was heterogeneous first-order autoregressive for claw depth and first-order autoregressive for weight bearing heel length, surface area of the weight-bearing heel and sole surface area. For the non-weight bearing heel length AIC was very close in value for both AR1 and ARH1 models; for raw data ARH1 was marginally the best, while for raw data AR1 was selected, model outcome was not impacted by covariance structure so the model with the lowest AIC was still used in preference.

For each model the residuals were assessed for normality and the relationship between the model predicted values for the variable under investigation and the residuals generated by the model were assessed as a measure of fit.

The relationships between the individual conformation variables assessed and the change in value for each variable between Days 10 and 230 were examined using Pearson's correlation coefficient. (Transformed data was used for the non-weight bearing heel length to ensure all variables were normally distributed).

All statistical analyses were undertaken using with SPSS 20 (IBM, SPSS, New York).

Results

Raw means for each foot and claw combination are reported. The raw data are displayed in boxplot form and graphed to illustrate the change in group mean against days post calving time point, with the SEM included for reference.

Claw depth at the distal end of abaxial groove

The mean values and SEM for raw claw depth data (sited at the distal end of the abaxial groove) are presented for each foot claw and time combination in Table 8.1 and Figure 8.4.

Table 8.1: Mean (SEM) (cm) claw depth (as measured with electronic callipers) for each foot, claw, and time combination in 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

		Claw depth (cm) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230***
LH	**Medial**	5.3** (0.07)	4.9 (0.07)	5.0 (0.06)	5.0 (0.06)
	Lateral	5.4** (0.07)	4.9 (0.06)	5.1 (0.05)	5.3 (0.05)
RH	**Medial**	5.3 (0.09)	4.9 (0.08)	4.9 (0.07)	5.2 (0.06)
	Lateral	5.2 (0.06)	4.9 (0. 05)	5.1 (0.06)	5.3 (0.05)

* n=28, ** n=28 cow 20 missing data

There was no effect of foot on claw depth (*P*=0.991). The effect of claw position within foot was significant (*P*=0.006). In the left foot there was a small but significant difference in claw depth between claws while in the right hind claws were of similar size, mean difference:

Left hind foot: 0.24 cm (95 % CI: 0.09 to 0.38)
Right hind foot: 0.04 cm (95 % CI: -0.11 to 0.19)

There was a significant effect of time on claw depth (*P*<0.001), with a decrease in claw depth between Days 10 and 60. Claw depth on Day 230 was significantly greater than on Days 60 and 120 (both *P*<0.001). There was no interaction between either foot and time (*P*=0.092) or claw within foot and time (*P*=0.183) at the *P*<0.05 level (Figure 8.5).

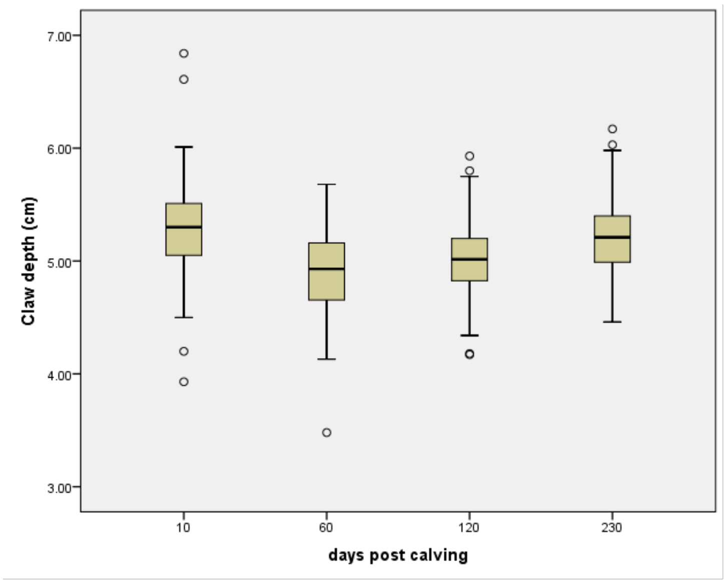

Figure 8.4: Relationship between time since calving and claw depth in the medial and lateral claws of both hind limbs of 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

Interpretation: The line in the box marks the median and the central box spans the quartiles. Lines extend from the box to the smallest and largest observations that are not suspected outliers. Observations more than 1.5 times the interquartile range (1 step) from the box are plotted individually as suspected outliers (open circles). Observations more than 2 steps from the box are plotted individually (stars) and are probable outliers.

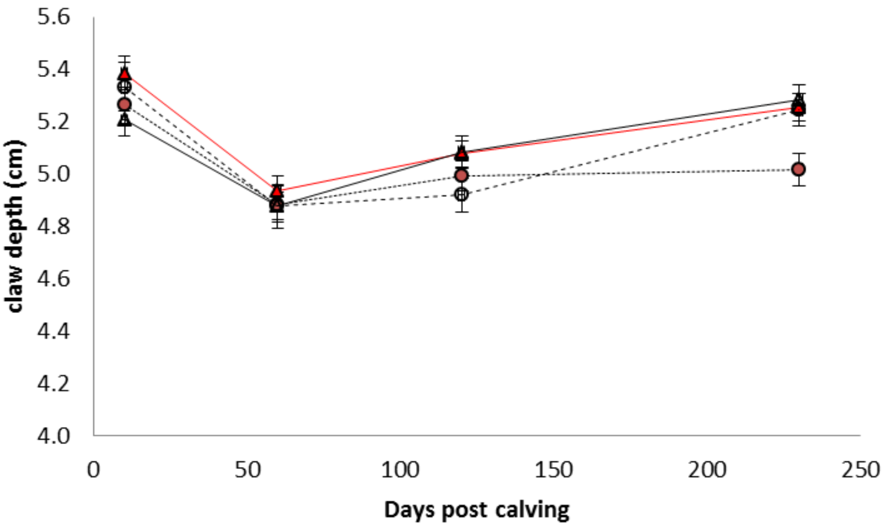

Figure 8.5: Mean (SEM) claw depth (cm) for the medial and lateral claws of both hind limbs of 29 dairy heifers, measured on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Non-weight bearing heel length

Data for non-weight bearing heel length are displayed for each foot, claw and time combination are presented in Table 8.2. The probable outlier 365 (see Figure 8.6) was examined as a potential recording error but the pattern change was the same for the other heel variables and the data point was retained.

Non-weight bearing heel length was the only variable that failed the Kolmogorov-Smirnov test for normality. However, graphical examination of the data did not reveal major deviation in the distribution and although transformation was explored residuals obtained from modelling the raw data were also normally distributed. There was no change in the outcome of the model and interpretation was simplified.

Table 8.2: Mean (SEM) (cm) non-weight bearing heel length (as measured with a flexible tape measure) for each foot, claw, and time combination in 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

		Non-weight bearing heel length (cm) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230***
LH	**Medial**	3.9** (0.11)	3.1 (0.09)	3.1 (0.07)	3.1 (0.07)
	Lateral	4.0 (0.08)	3.4 (0.08)	3.4 (0.08)	3.3 (0. 07)
RH	**Medial**	4.0 (0.10)	3.3 (0.10)	3.3 (0.07)	3.2 (0.08)
	Lateral	4.0 (0.09)	3.4 (0. 08)	3.3 (0.11)	3.3 (0.08)

* n=28, ** n=28 cow 249 missing data

There was no effect (*P*=0.108) of foot on non-weight bearing heel length, but the effect of claw position within foot was significant (*P*<0.001). The mean difference between claws of the same foot was similar in left and right hind feet; mean difference:

Left hind foot: 0.126 cm (95 % CI: -0.01 to 0.26)
Right hind foot: 0.128 cm (95 % CI: -0.02 to 0.27)

There was an effect of time on non-weight bearing heel length (*P*<0.001), with a fall in non-weight bearing heel length between Days 10 and 60. Non-weight bearing heel length was greater on Day 10 than Day 230 (*P*<0.001), but lengths on Days 60 and 120 did not differ significantly from Day 230 (*P*=0.403 and *P*=0.122, respectively). There was no interaction between either foot and time (*P*=0.973) or claw within foot and time in the model (*P*=0.672): Figure 8.7.

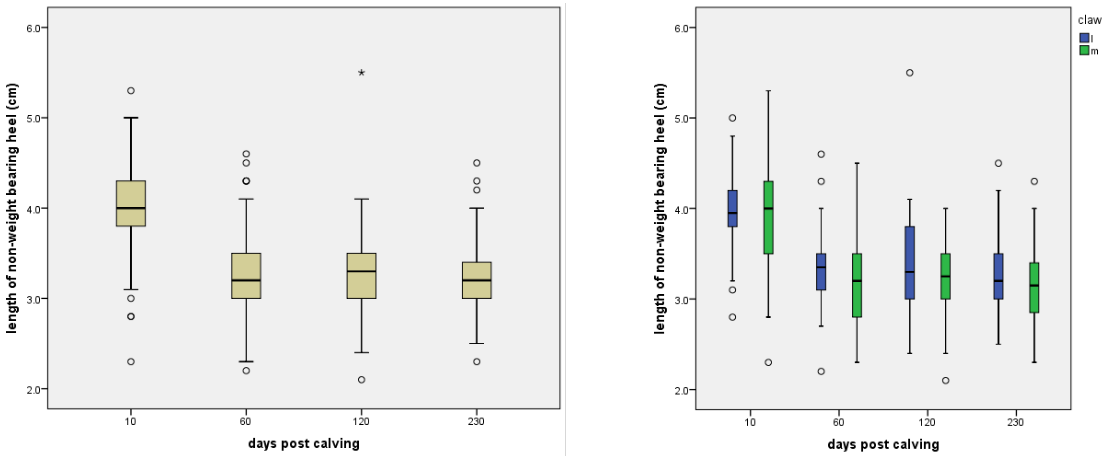

Figure 8.6: (Left panel) Effect of time since calving on non-weight bearing heel length in the medial and lateral claws of both hind limbs of heifers on Days 10, 60, 120 and 230 post calving. Right panel: Box plot split by claw (medial or lateral) at each time point. For interpretation of the box and whisker plots see Figure 8.4.

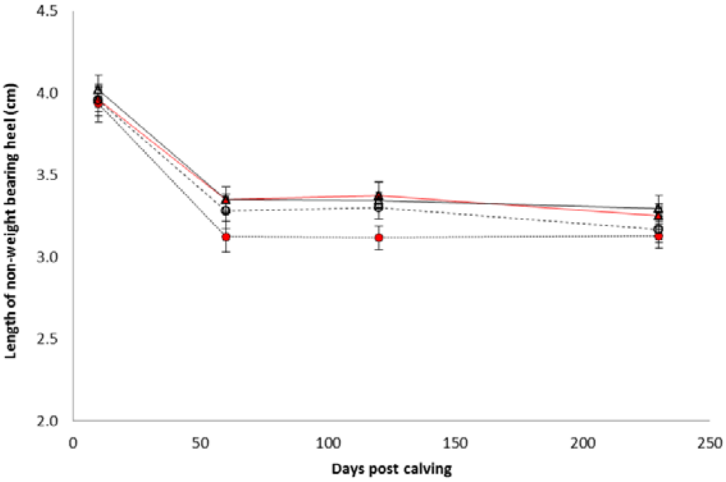

Figure 8.7: Mean (SEM) mean length of non-weight bearing heel (cm) for the medial and lateral claws of both hind limbs of 29 dairy heifers, measured on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Weight bearing heel length

The mean values and SEM for raw weight bearing heel length data are displayed for each foot, claw and time combination are presented in Table 3 and Figure 8.8.

Table 8.3: Mean (SEM) (cm) weight bearing heel length (as measured with a flexible tape measure) for each foot, claw, and time combination in 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

		Weight bearing heel length (cm) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230***
LH	**Medial**	2.5 (0.10)	2.7 (0.08)	2.7 (0.07)	2.8 (0.07)
	Lateral	2.6 (0.09)	2.4 (0.12)	2.4 (0.08)	2.9 (0.09)
RH	**Medial**	2.6 (0.10)	2.5 (0.09)	2.5 (0.08)	2.8 (0.09)
	Lateral	2.6 (0.08)	2.7 (0.06)	2.8 (0.09)	3.2 (0.10)

* n=28

There was no effect of foot (*P*=0.117) on weight bearing heel length, but the effect of claw within foot was significant (*P*<0.001). Lateral claws had longer weight bearing heel length than medial claws the difference between claws was greater in the right hind, mean difference:

Left hind foot: 0.13 cm (95 % CI: -0.09 to 0.35)
Right hind foot: 0.37 cm (95 % CI: 0.15 to 0.60)

There was a significant effect of time on weight bearing heel length (*P*<0.001), with gradual rise over lactation. Compared to weight bearing heel length on day 230, lengths on Days 10, 60 and 120 were all lower (*P*=0.051, *P*=0.009 and *P*=0.004, respectively). There was no interaction between foot and time (*P*=0.907) but significant interaction between claw within foot and time (*P*=0.004): Figure 8.9.

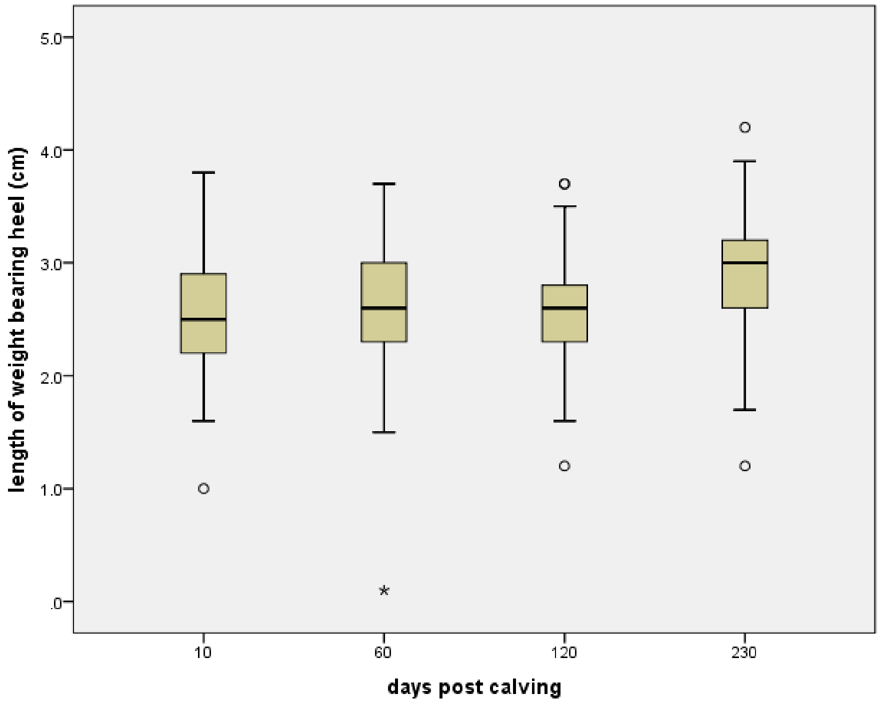

Figure 8.8: Relationship between time since calving and on weight bearing heel length in the medial and lateral claws of both hind limbs of 29 dairy heifers on Days 10, 60, 120 and 230 post calving. For interpretation of the box and whisker plots see Figure 8.4.

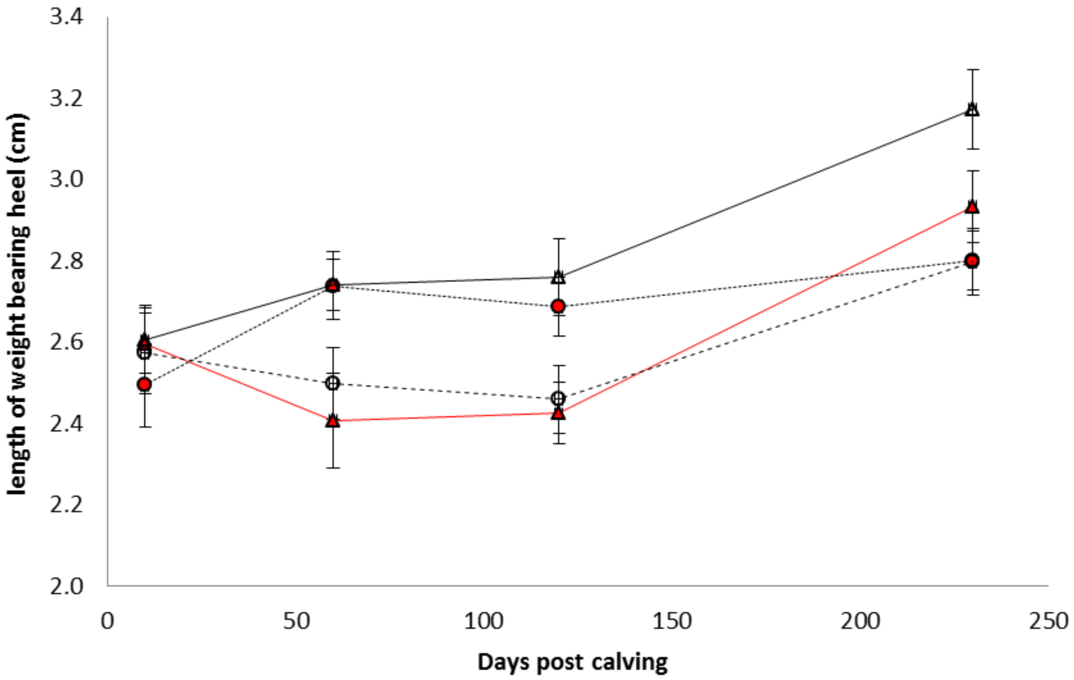

Figure 8.9: Mean (SEM) mean length of weight bearing heel (cm) for the medial and lateral claws of both hind limbs of 29 dairy heifers, measured on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Surface area of the weight-bearing heel

Data for surface area of the weight bearing heel are displayed for each foot, claw and time combination are shown in Table 8.4 and Figure 8.10.

Table 8.4: Mean (SEM) (cm^2) surface area of the weight bearing heel above the line YZ (estimated by image analysis) for each foot, claw, and time combination in 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

		Heel surface area - above the line YZ (cm^2) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230***
LH	**Medial**	6.86 (0.326)	6.93 (0.269)	7.90 (0.369)	7.97 (0.285)
	Lateral	8.19 (0.334)	7.41 (0.341)	7.87 (0.323)	9.18 (0.409)
RH	**Medial**	7.06 (0.358)	6.86 (0.327)	7.31 (0.359)	8.37 (0.338)
	Lateral	8.88 (0.390)	8.82 (0. 374)	9.14 (0.381)	10.31 (0.433)

* n=28

The effect of foot on surface area of the weight bearing heel was significant (*P*=0.001), as was the effect of claw within foot (*P*<0.001). The weight-bearing heel surface area was greater in lateral than medial claws but similar in both feet, mean difference:

Left hind foot: 1.23 cm^2 (95 % CI: 0.42 to 2.03)
Right hind foot: 1.94 cm^2 (95% CI: 1.13 to 2.74)

There was an effect of time on surface area of the weight bearing heel (*P*<0.001), with a rise in the surface area of the weight bearing heel between Days 60 and 230. Surface areas were lower on Days 10, 60 and 120 than on Day 230 (*P*=0.002, *P*<0.001, and *P*=0.012, respectively). There was no interaction between either foot and time (*P*=0.776) or claw within foot and time (*P*=0.262): Figure 8.11.

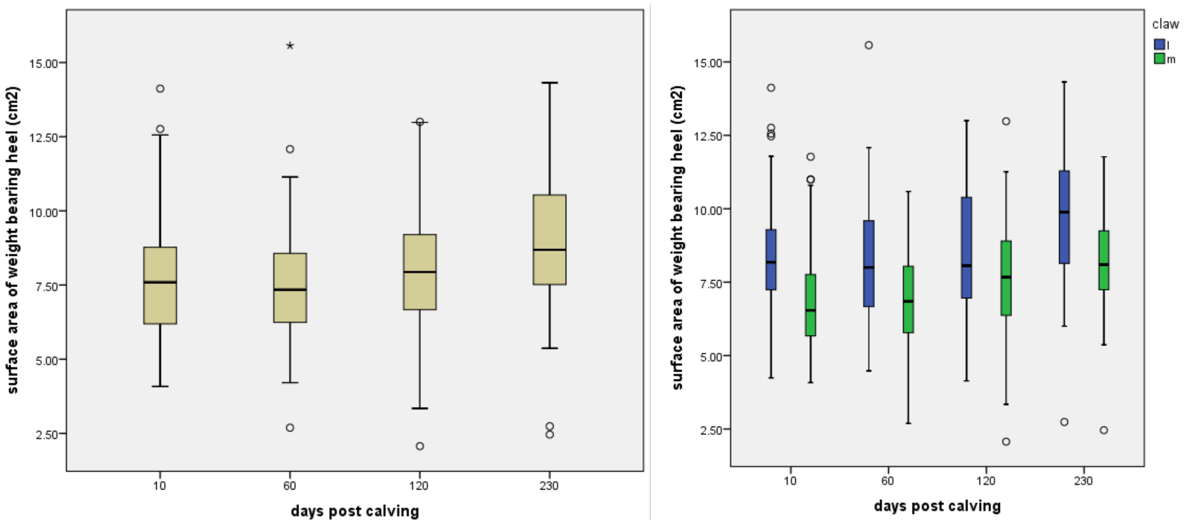

Figure 8.10: Left panel: Effect of time since calving on mean surface area of the weight bearing heel (cm^2) in the medial and lateral claws of both hind limbs of 29 dairy heifers on Days 10, 60, 120 and 230 post calving. Right panel: Box plot split by claw (medial or lateral) at each time point. For interpretation of the box and whisker plots see Figure 8.4.

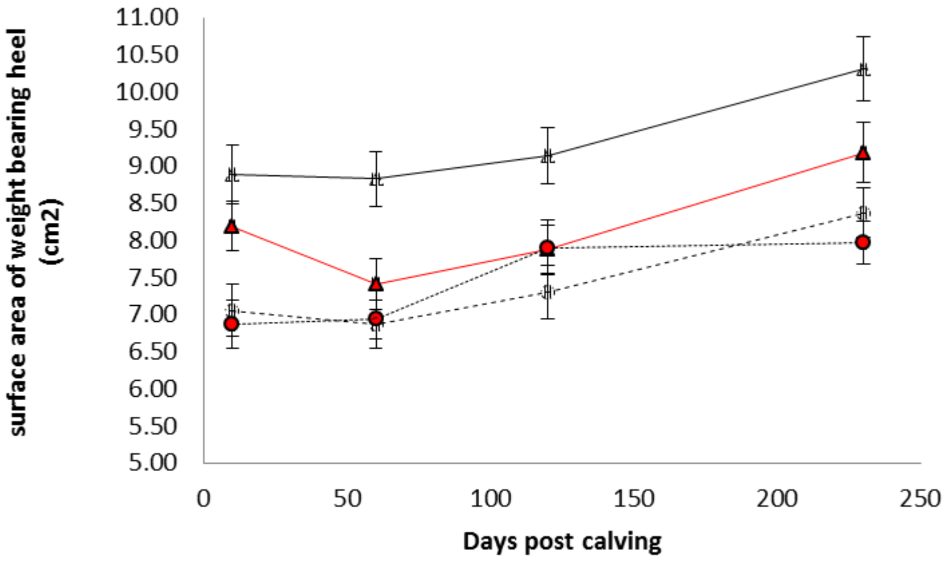

Figure 8.11: Mean (SEM) mean surface area of the weight bearing heel (cm^2) for the medial and lateral claws of both hind limbs of 29 dairy heifers, measured on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Sole surface area

Data for raw sole surface area are displayed for each foot, claw and time combination in Table 8.5 and Figure 8.12.

Table 8.5: Mean (SEM) (cm^2) sole surface area below the line YZ (estimated by image analysis) for each foot, claw, and time combination in 29 dairy heifers on Days 10, 60, 120 and 230 post calving.

		Sole surface area (cm^2) (SEM)			
Foot	**Claw**	**Day 10**	**Day 60**	**Day 120**	**Day 230***
LH	**Medial**	18.98** (0.414)	21.57 (0.461)	23.33 (0.387)	26.48 (0.480)
	Lateral	22.08** (0.420)	25.42 (0.487)	29.96 (0.606)	27.74 (0.417)
RH	**Medial**	19.11 (0.458)	23.17 (0.424)	23.84 (0.333)	26.13 (0.521)
	Lateral	21.29 (0.458)	24.27 (0. 510)	24.88 (0.418)	27.62 (0.394)

*n=28 , * n=28 missing data cow 234 no image for analysis

There was a significant (P=0.006) effect of foot on sole surface area; and the effect of claw within foot was also significant (P<0.001). Lateral claws had greater sole area than their medial partners, with a larger mean difference in the left than the right hind foot:

Left hind foot: 3.26 cm^2 (95 % CI: 2.26 to 4.27)
Right hind foot: 1.49 cm^2 (95 % CI: 0.49 to 2.50)

There was a significant (P<0.001) effect of time on the sole surface area, with a rise in the sole surface area between Days 10 and 230, such that values on Day 230 were significantly greater than on all other days (all P<0.001). There was and interaction between foot and time (P=0.054) but no interaction between claw within foot and time in the model time (P=0.594): Figure 8.13.

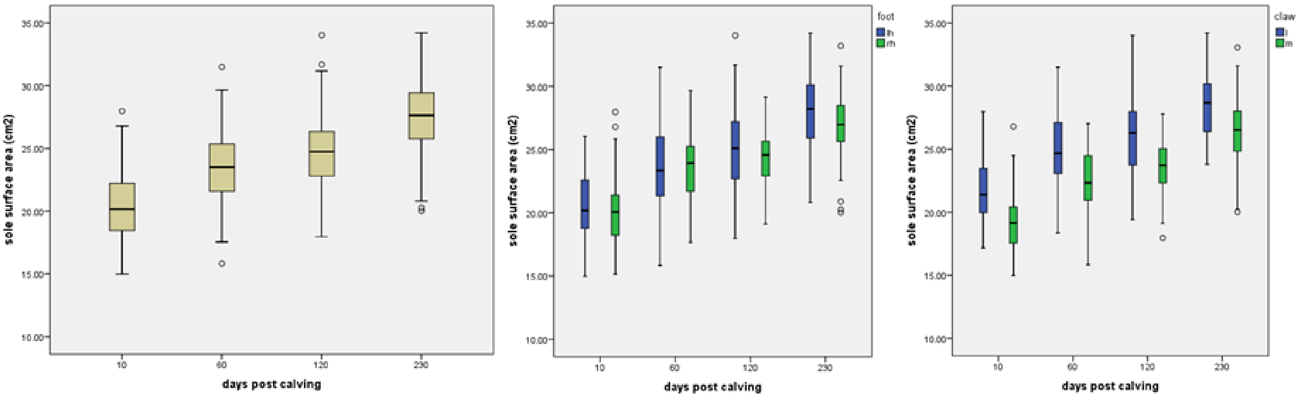

Figure 8.12 Left panel: Effect of time since calving on mean sole surface area (cm^2) in the medial and lateral claws of both hind limbs of 29 dairy heifers on Days 10, 60, 120 and 230 post calving. Centre panel: Box plot split by claw (medial or lateral) at each time point. Right panel: Box plot split by foot (left or right) at each time point. For interpretation of the box and whisker plots see Figure 8.4.

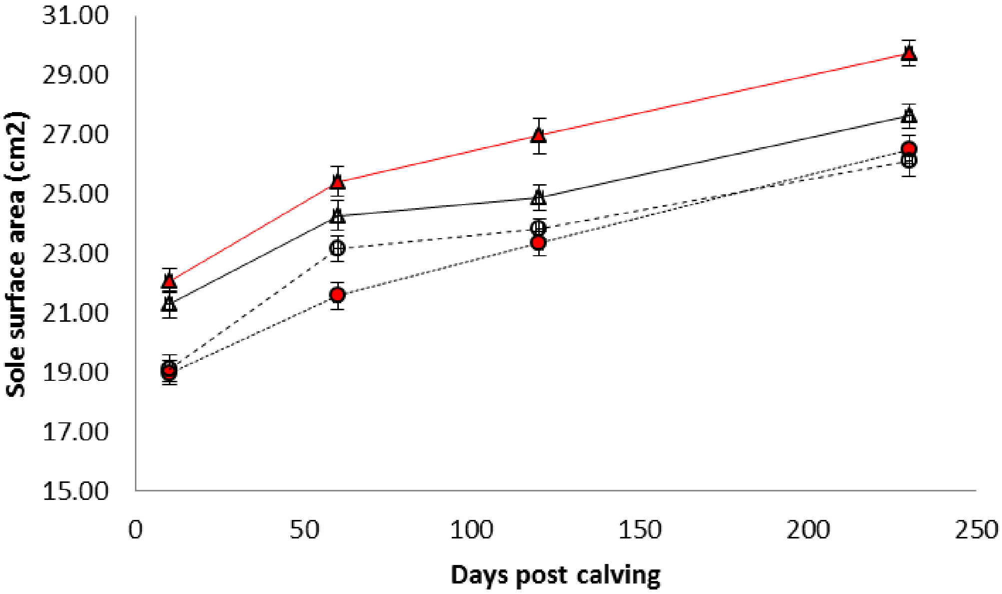

Figure 8.13: Mean (SEM) sole surface area (cm^2) for the medial and lateral claws of both hind limbs of 29 dairy heifers, measured on Days 10, 60, 120 and 230 post calving.

Left hind: medial claw ● dotted line; lateral claw ▲ solid line (red)
Right hind: medial claw ○ dashed line; lateral claw △ solid line (black)

Inter-variable relationships

The values for Pearson’s correlation coefficient between the individual variables are shown in Table 8.6.

Table 8.6: Pearson’s correlation coefficient (n) for claw depth, log 10 non-weight bearing heel length, weight bearing heel length, sole surface area , and area of the weight bearing heel of the medial and lateral claws of both hind limbs of heifers on Days 10, 60, 120 and 230 post calving. Numbers emboldened: $P<0.01$; Numbers in italics: $P<0.05$

Variable	**Sole surface area (cm^2)**	**Surface area of weight bearing heel (cm^2)**	**$\log_{10}$ Length of non-weight bearing heel (cm)**	**Length of weight bearing heel (cm)**
Claw depth (cm)	*-0.103* (456)	**0.193** (456)	**0.274** (457)	**0.179** (457)
Sole surface area (cm^2)		**0.217** (458)	**-0.346** (457)	*0.097* (457)
Surface area of weight bearing heel (cm^2)			**-0.264** (457)	**0.683** (457)
$\log_{10}$ Length of non-weight bearing heel (cm)				**-0.460** (459)

Differences in variables between first and last examination (i.e. Day 230 minus Day 10) were all normally distributed (one-sample Kolmogorov-Smirnov test). These data are shown in Table 8.7.

A high correlation (r=0.720; $P<0.01$) was present for the relationship between the change in change in weight bearing heel length and the change in surface area of weight bearing heel Figure 8.14. There were moderate negative associations between the change in non-weight bearing heel length and the change in length of weight bearing heel (r=-0.561; $P<0.01$) Figure 8.15, and the change in non-weight bearing heel length (cm) and change in surface area of weight bearing heel (r=-0.500; $P<0.01$): Figure 8.16. Small but significant relationships existed for change in weight bearing heel length and change in sole surface area (r=-0.397; $P<0.01$) and also change in sole surface area and change in surface area of weight bearing heel (r=-0.256; $P<0.01$): Figure 8.17. Less association was demonstrated for change in claw depth and change in length of the non-weight bearing heel (r=0.205; $P<0.05$) and change in claw depth and change in sole surface area (r=-0.194; $P<0.05$).

Table 8.7: Pearson's correlation coefficient (n) for changes in: claw depth, non-weight bearing heel length, weight bearing heel length, sole surface area , and area of the weight bearing heel for the medial and lateral claws of both hind limbs of heifers between Days 10 and 230 post calving.

Numbers emboldened: $P<0.01$; Numbers in italics: $P<0.05$

Variable	**Change in non-weight bearing heel length (cm)**	**Change in weight bearing heel length (cm)**	**Change in sole surface area (cm^2)**	**Change in weight bearing heel surface area (cm^2)**
Change in claw depth (cm)	*0.205* (109)	0.146 (109)	*-0.194* (108)	0.057 (108)
Change in non-weight bearing heel length (cm)		**-0.561** (111)	0.148 (109)	**-0.500** (109)
Change in weight bearing heel length (cm)			**-0.397** (109)	**0.720** (109)
Change in sole surface area (cm^2)				**-0.256** (110)

Table 8.8: Mean (SD) change between Days 10 and 230 for claw depth, non-weight bearing heel length, weight bearing heel length, sole surface area, and area of the weight bearing heel, of the medial and lateral claws of both hind limbs of heifers on Days 10, 60, 120 and 230 post calving.

Variable	**Mean change (SD)**	**n**
Change in claw depth (cm)	-0.10 (0.52)	110
Change in non-weight bearing heel length (cm)	-0.75 (0.57)	111
Change in weight bearing heel length (cm)	0.37 (0.65)	111
Change in sole surface area (cm^2)	7.09 (2.82)	110
Change in weight bearing heel surface area (cm^2)	1.27 (2.33)	110

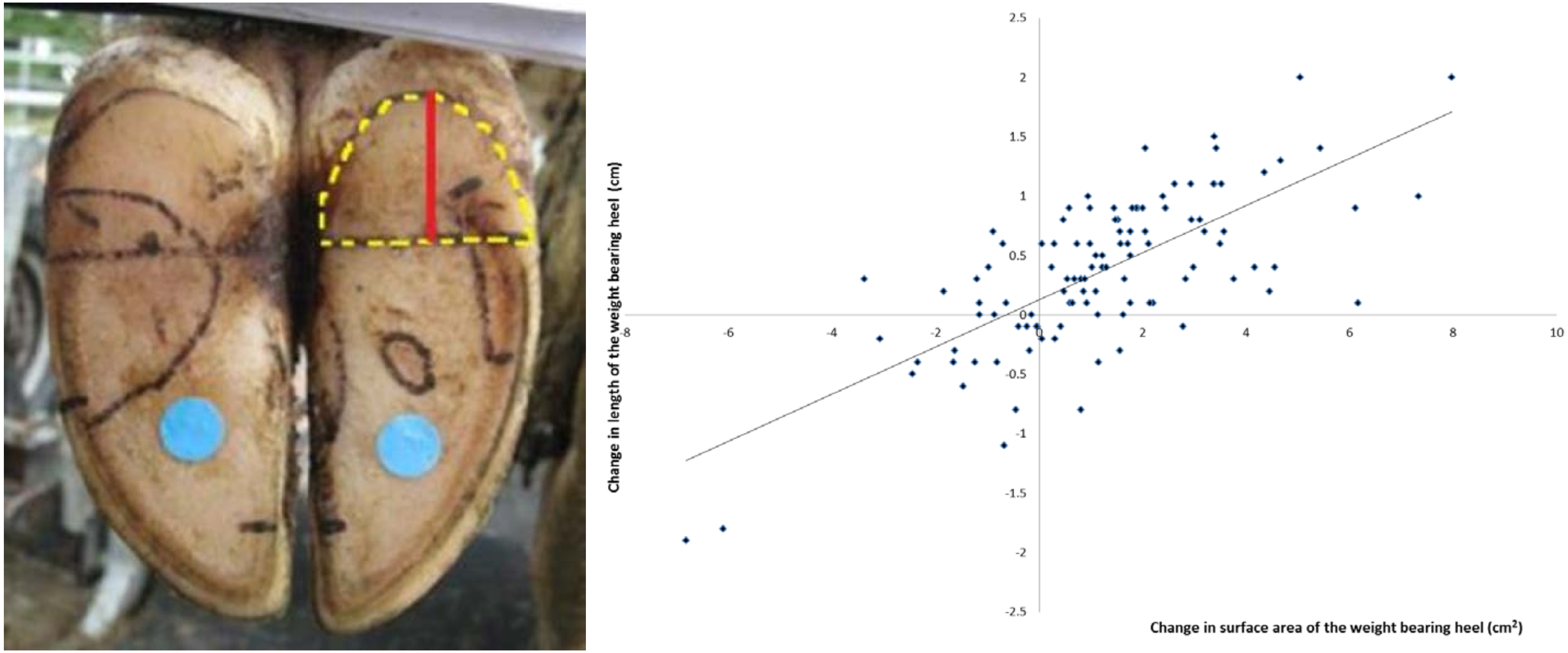

Figure 8.14: Left panel: photograph left hind claw with weight bearing surface area of heel, above line YZ outlined, and weight bearing heel length indicated. Right panel: Scatterplot; change in the surface area of the weight bearing heel (cm^2) against change in the length of the weight bearing heel (cm) for the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 120 and 230 post calving. y= 0.199x + 0.127, R^2=0.518.

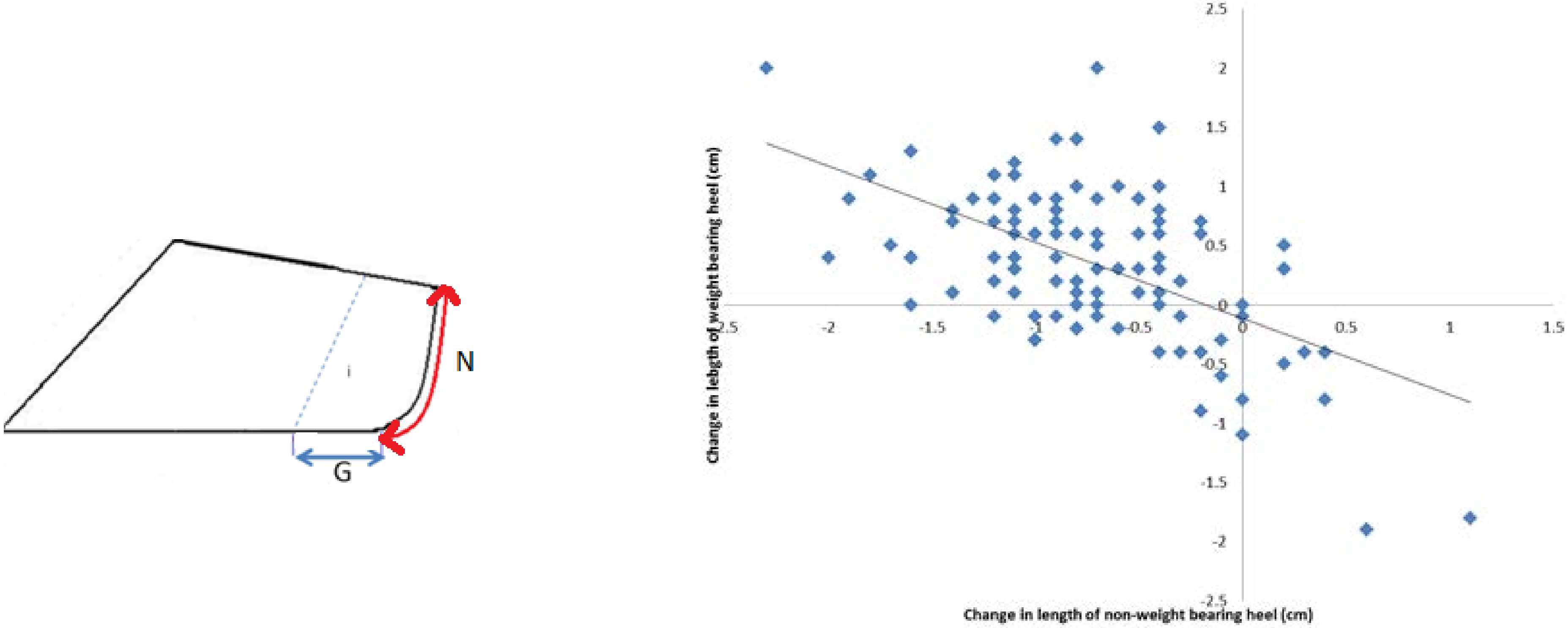

Figure 8.15: Left panel: shows the abaxial view of a claw illustrating (N) non-weight bearing heel length (G) the weight bearing length of the heel and (i) the abaxial groove. Right panel: Scatterplot; change in the length of the non-weight bearing heel (cm) against change in the length of the weight bearing heel (cm) for the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 120 and 230 post calving. y= -0.642x - 0.113, R^2=0.315.

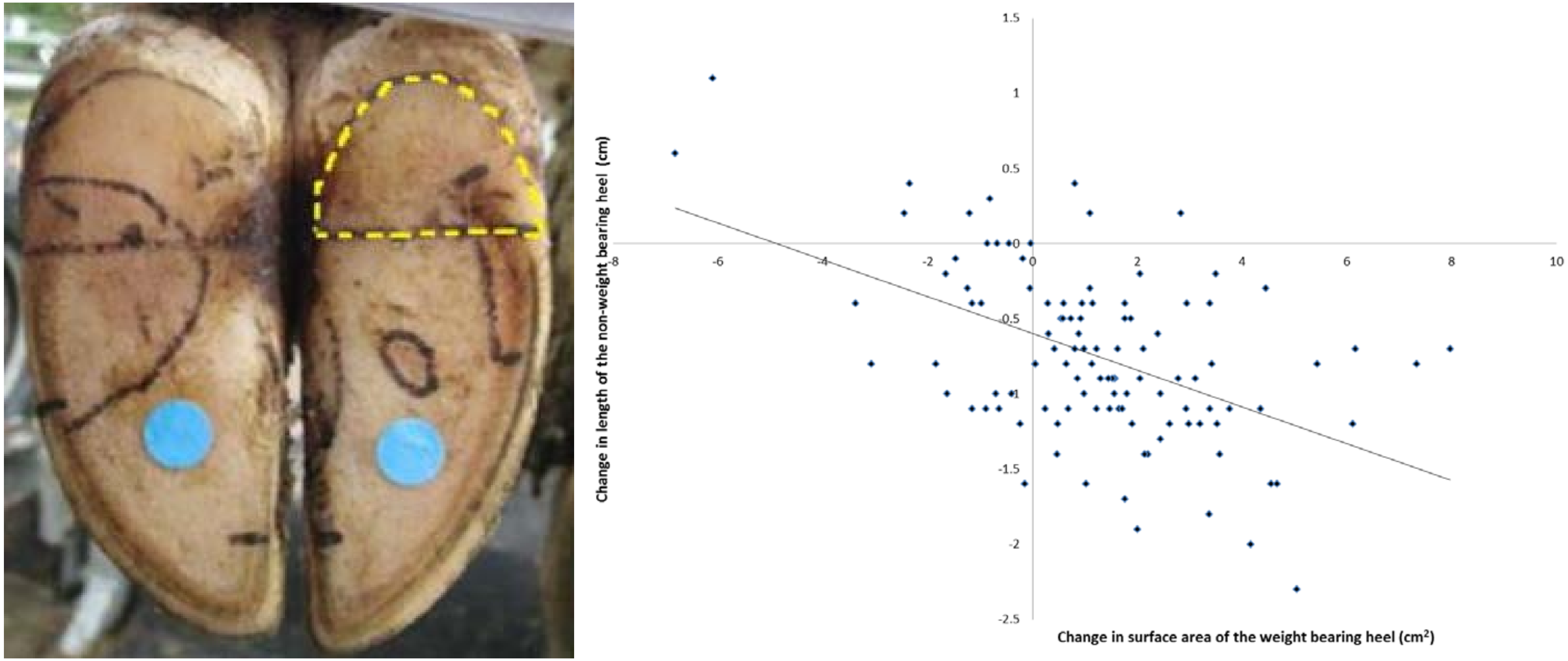

Figure 8.16: Left panel: photograph left hind claw with weight bearing surface area of heel, above line YZ outlined. Right panel: Scatterplot; change in the surface area of the weight bearing heel (cm^2) against change in the length of the non-weight bearing heel (cm) for the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 120 and 230 post calving. y= -0.122x - 0.599, R^2=0.25.

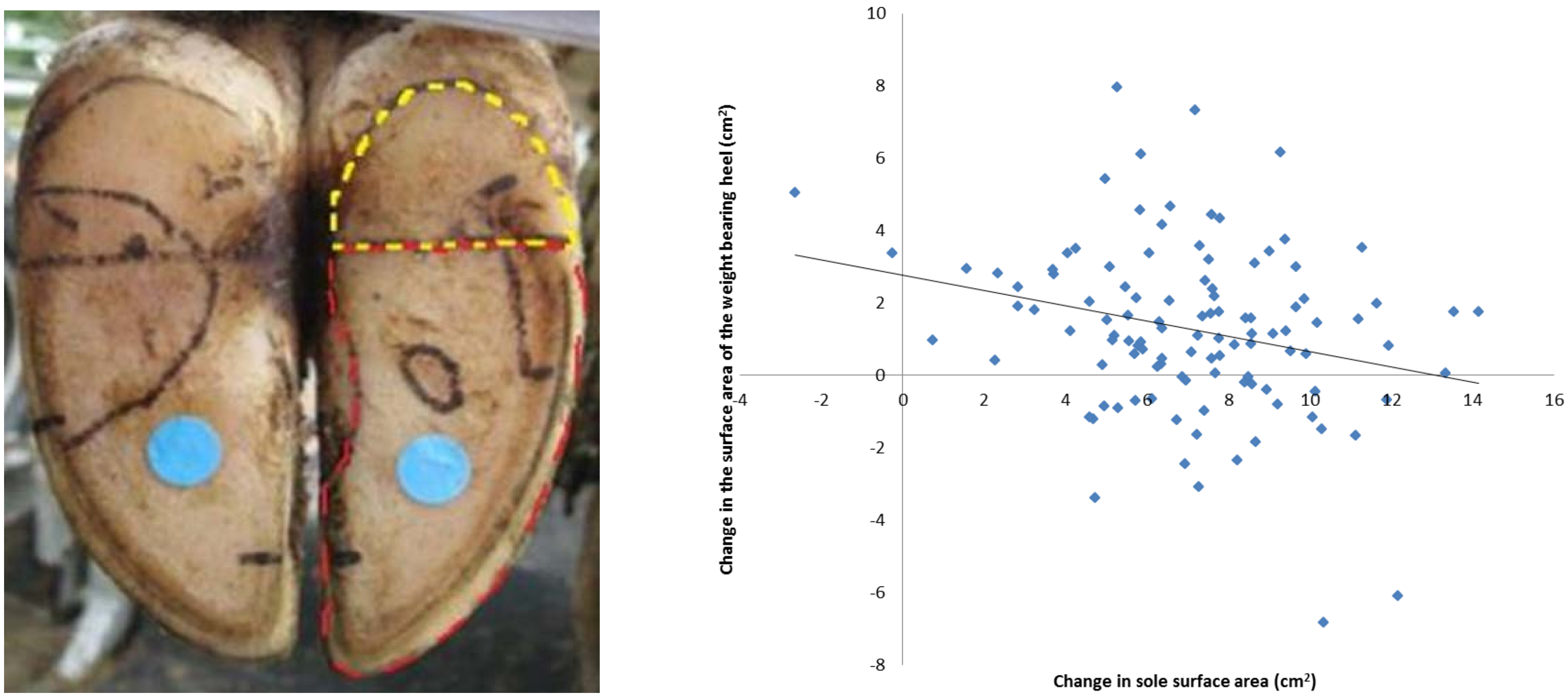

Figure 8.17: Left panel: photograph left hind claw with weight bearing surface area of heel, above line YZ and the sole surface area below line YZ outlined. Right panel: Scatterplot; change in sole surface area (cm^2) against change in the surface area of the weight bearing heel (cm^2) for the medial and lateral claws of both hind limbs of 25 dairy heifers on Days 10, 60, 120 and 230 post calving. y= -0.211x + 2.765, R^2=0.065.

Discussion

This study has demonstrated that, in contrast to heel height and heel angle the weight bearing and non-weight bearing length of the heel are readily estimated in the field situation. Image analysis has allowed the estimates to be related to change in surface area on the ground contact surface of the hoof above the demarcation line (YZ). This means that simple linear measures could be used to reflect heel conformation over time to allow the investigation of environmental impact on the shape of the claw and the development of lesions and lameness. The observation that heels became shallower in year 1 has been borne out by the estimation of non-weight bearing heel length and linked to a simultaneous increase in surface area of the weight bearing heel. In addition, claw depth and sole surface area, two of the common conformational variables described by Vermunt and Greenough (1995), have been assessed under New Zealand pastoral conditions with respect to their worth as monitoring tools.

Claw depth is a trait commonly examined in clinical and conformational assessments of the foot (Vermunt and Greenough 1995). Despite this, research on measurement of claw depth is limited and has mostly been conducted on mortem material (Browne et al., 2007; Nuss et al. 2006, 2011). For the current study a linear measure at an easily located landmark - the distal end of the abaxial groove was employed. This measure of claw depth was easy to obtain with electronic callipers with care taken to avoid creating an unpleasant stimulus to the animal during collection. Ouweltjes et al. (2009) illustrated the method of measurement in live animals, but did not report any data relating to this variable, whilst Somers et al. (2005) demonstrated only a slight change in depth of the right hind lateral claw over a 16 week period (Week 2: 6.4 ± 0.03 cm, Week 18: 6.9 ± 0.04 cm). This result appears to be at variance with the findings of the present study, in which there was a significant effect of time on claw depth. Mean claw depth decreased by ≈4mm over the first 60 days of lactation, before recovering to similar values on Day 230 to those that had been present on Day 10. Results from Chapter 6 have already shown that a lack of horn growth in the period between Days 10 and 60 led to net wall horn wear for these animals in Year 2 and so this may underpin the reduction in claw depth too. Moreover, in the present study there was a significant effect of claw within foot (i.e. lateral *vs.* medial claw, inasmuch as claw depth was similar for medial and lateral claws on Day 10, depth decreased equally in both claws between Days 10 and 60, but thereafter until Day 230 the depth of lateral claws was greater than that of medial claws.

The present study examined claw depth in all four hind claws, but there was no fixed effect of foot in the model. This supports the decision of Somers et al. (2005) to use only one foot. However, closer examination of the difference between claws within the same foot, reveals that left hind medial claws stand out as being significantly smaller in depth than the other three claws. Mean claw depth is the same within left and right hind feet, but the depth difference between the medial and lateral claws of the same foot is larger in the left hind (0.24 cm [95% CI: 0.10 to 0.38] vs. (0.04 cm [95% CI: -0.11 to 0.19]), this was particularly evident at Day 230 Figure 8.5. Although the difference is biologically small, such a finding would support the measurement of not only both claws in a foot but the consideration that both hind feet should be measured so as not to miss the foot exhibiting the most change over time. In the current study this was the left foot, however this could be influenced by animal sidedness (Phillips et al., 1996) or even the handling and management systems in which animals are kept. Further research would establish if the same foot always demonstrated the significant difference and could be targeted to elucidate a relationship between individual animal foot size and ranked claw and the direction of turns for example as cattle leave a rotary platform.

The findings in the current study reflect a cohort of heifers coming off pasture to enter the milking herd and experience management changes together in contrast to the age/ lactation matched groups of animals studied by Somers et al. (2005). Despite this changes recorded over time for claw depth were small and it is proposed that claw depth is not particularly sensitive to environmental factors but rather tends to slowly increase with the age of the animal provided no extremes in net growth of claw horn are experienced. In summary, this study found claw depth was not particularly valuable as a means to monitor conformation change under New Zealand conditions.

The novel methodology described to assess the non-weight bearing heel length and weight bearing heel length was efficient and easily applied to lifted claws in the field situation. Both the weight-bearing and non-weight bearing heel lengths demonstrated significant change over the course of first lactation, which supported the anecdotal belief that as non-weight bearing heel length falls, weight bearing heel length rises. Specifically, this notion was confirmed by (i) the moderate negative correlation ($r = -0.460$; $P<0.01$) between $\log_{10}$ non-weight bearing heel length and weight bearing heel length and (ii) the association between changes in non-weight bearing heel length and changes in weight bearing heel length ($r = -0.561$; $P< 0.01$) between Days 10 and 60.

For non-weight bearing heel length a significant fall in length was seen between day 10 and day 60. Following an average fall in the region of 0.6 to 0.8 cm the non-weight bearing heel length remained fairly constant. The data fit well with Ahlström et al. (1986) who reported a reduction in heel height between late gestation and first lactation examinations of heifers. A subsequent rise in heel height over time, as animals mature, might then be expected from reports in the literature (e.g. Hahn et al., 1984a; Ahlström et al., 1986) but steady values for non-weight bearing heel length were obtained in the present study for the rest of lactation. The lack of documented change may however, just reflect the extra time needed for heel changes to occur as proposed by Boelling and Pollott (1998) and Kremer et al. (2007). If heifers had been followed for an extended period the expected increase in non-weight bearing heel length may have been seen.

Although the left hind medial claw appears to have the shallowest value for non-weight bearing heel length throughout lactation the claw within foot and foot differences are not significant for this variable, medial and lateral claws were seen to behave in the same way over time. This challenges the finding that lateral claws have greater depth than medial claws (Smit et al., 1986; Vermunt and Greenough, 1996; Nuss and Paulus, 2006; Ouweltjes et al., 2009) particularly in the hind claws (Ahlström et al., 1986) although the difference between lateral and medial claws has been demonstrated to rise with age and lactation number (Andersson and Lundström, 1981; Ahlström et al. 1986; Boelling and Pollott, 1998; Somers et al., 2005).

The symmetry between medial and lateral claws and steady non-weight bearing heel length seen in the current study could relate to the system being studied. The stable pasture /track environment throughout lactation does not appear to represent sufficient environmental challenge to drive heel height change when compared for example to asphalt (Telezhenko et al., 2009) or concrete housing (Boelling and Pollott, 1998). A theory supported by the increased heel height seen for front limb claws when Highland cattle were moved from pasture to concrete floored loose housing (Nuss et al., 2013).

In contrast to the non-weight bearing heel length, there was a gradual increase in weight bearing heel length recorded over lactation claw position playing an important role in the response seen. Confirmed by the moderate negative association between these variables when the changeover lactation was investigated ($r=-0.561$; $P<0.01$). Lateral claws had longer weight bearing heel length than medial claws and the difference between claws was greater in the right hind, which exhibited the greatest weight bearing heel length value of the four claws assessed.

It might have been expected that the claw with the shortest non-weight bearing heel height (left hind medial claw) would exhibit the longest weight bearing heel height (right hind lateral) but this was not the case. There is no direct comparison to this measure in the literature but, Ahlström et al. (1986) and Nuss et al. (2011) reported a distinct pattern of size for each claw position cows; front medial > hind lateral > front lateral > hind medial. Although only consistent for cows studied by Nuss et al. (2011) Ahlström et al. (1986) found this pattern was maintained as heifers matured. While this pattern for total ground surface area does not fit with the outcomes of the current study when the surface area of the weight bearing heel is examined the results do align with the right hind lateral claw returning the largest area.

Overall, the methodology described and applied in the present study was able to successfully document heel conformation change and confirm anecdotal observations regarding change in claw shape. The linear measures therefore represent a useful technique for the assessment of claw heel conformation with the proviso that measures are recorded for an extended period, beyond three months (Boelling and Pollott, 1998) to reflect the length of time required for modification of heel conformation to become apparent.

With respect to monitoring surface areas, the methodology described to mark up and record the surface area above and below the line YZ assess was efficient and easily applied to lifted claws under field conditions. It allowed repeatable estimation of surface areas by image analysis (a random selection of 25 images (10 %), containing 50 claws were reassessed, the coefficient of variation was 4.6 % for sole surface area and 5.6 % for surface area of the weight bearing heel). Both sole surface area below YZ and the area of the weight-bearing heel above the line YZ demonstrated significant changes over the course of first lactation, with a low positive correlation between the two variables (r= 0.217; P<0.01) which could be related to growth of the animals as they mature (Ahlström et al., 1986).

The two areas assessed in this study differ from those previously described in the literature; traditionally total ground surface area is described (Ahlström et al., 1986; Nuss and Paulus, 2006; Nuss et al., 2011) or more recently contact area as assessed by force plate analysis (van der Tol et al., 2003; Carvalho et al., 2005; Telezhenko et al., 2008). The method described in the present study allowed more information to be collected than a simple total area. Division of the claw surface into two regions meant each in turn could be related to other variables assessed e.g. non-weight bearing heel length. Furthermore, an element of functional change could be studied overtime without the need for a force plate and accompanying equipment. Future studies could include the collection of sole profile data to collate with the surface area data (Telezhenko et al., 2009).

Overall, both surface areas assessed were greater in lateral than medial claws, which support studies such as van der Tol et al. (2003) where greater contact surface area was linked to increased lesion development in the hind lateral claws. This asymmetry was also reported for ground surface area and was present in calves from a young age (Schwarzmann et al., 2007). In the present study the difference in sole surface area was greatest in the left hind with the left hind lateral claw being significantly larger in surface area then the other three claws assessed. Although the left hind lateral claw had the greatest sole surface area below the line YZ it did not display the greatest area of the weight bearing heel, above YZ. The right hind lateral claw was significantly greater with respect to surface area of the weight bearing heel. The same result pattern was seen with respect to the weight bearing heel length variable and the two variables exhibited strong correlation when the change between Days 10 and 230 were investigated (r=0.720; *P*<0.01). The association between the change in sole surface area and change in surface area of weight bearing heel between Days 10 and 230 was negative (r= -0.256; P<0.01). Thus, although both area variables increase over time the response is modified depending on the region. With respect to sole surface area all claws steadily increase in value to an end point approximately 30% bigger than the original (mean change [SD]; 7.09 cm^2 [2.82]). While for heel surface area the change seen was less consistent over lactation for some claws the area increased and for others it reduced and the change seen was smaller proportion of the initial heel area (mean change [SD]; 1.27 cm^2 [2.33]).

The heel bulb, is designed to function in impact and load transference rather than in load bearing, the horn in this region is softer than that of the sole or wall and more compressible than wall horn (van der Tol et al., 2003). Therefore, recruitment of this softer horn region for weight bearing (even if accompanied by an overall decrease in loading pressure) may in turn lead to increased claw horn lesions related to mechanical trauma in this region particularly on the lateral hind claw (van der Tol et al., 2003). The current study does not address loading pressures however; the ability to document both conformational change in the heel region (incorporating heel height and alterations in heel bulb dimensions) and lesion development in animals at pasture could be of great use in ascertaining the ideal surfaces, walking distances and optimal conformation for New Zealand conditions.

Conclusion

Claw depth was not particularly valuable as a means to monitor conformation change under New Zealand conditions. However, the initial hypothesis was borne out, in that the methodology for monitoring change in heel conformation described and applied in the present study was able to successfully document that heifers lost non-weight bearing heel length in their first lactation. Furthermore weight bearing heel length and the surface area of the weight bearing heel both increased over the course of lactation. These linear measures represent a useful technique for monitoring purposes but monitoring should be employed over at least three to four months to account for the relatively slow response of the heel compared to other claw variables.

In addition image analysis revealed that both sole surface area (below line YZ) and the weight bearing surface area of the heel (above line YZ) increased in size over the study and there was a small, negative relationship between the two variables.

There is value in monitoring not just two claws in one hind foot, but all four hind claws when looking to monitor modification of conformation in animals at pasture.

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Chapter nine: General Discussion

This thesis aimed to examine of the feasibility of using common anatomical traits as a means of assessing conformational changes in the claw over the duration of the first lactation of New Zealand dairy heifers that are managed at pasture. Post mortem material was used for the development and validation of techniques for estimating individual variables (chiefly claw volume and use of a portable ultrasound machine), so that these could be assessed *in vivo* using readily-available ultrasonography equipment. The hypothesis that underpinned these studies was that, given that changes which occur during the first lactation are of significance for the remainder of the animal's life (Offer et al., 2000), a means of accurately modelling these changes *in vivo* would allow for better understanding of the intrinsic and extrinsic factors that underpin them.

Morphometry

During the validation process, the use of post mortem material that was derived from dairy animals of a wide age range was unavoidable. Nonetheless, in addition to the development of methods that would later be applied *in vivo*, this post mortem material also allowed the development of histological and morphometric methods that could be applied to relate vascular change to underlying aetiology (trauma vs. systemic disease) of claw horn haemorrhage. The morphometrical techniques described in this thesis (Chapter 5) were able to distinguish between two sites within the same claw of a non-lame animal.

While the sites at which vascular changes can be recognised histologically is quite well established, the cause of them is not; inasmuch as similar vascular changes have been described for animals with acute laminitis, chronic laminitis, sole ulcer, sole haemorrhage, overgrown claws and, to a lesser extent, even normal control animals. Hirschberg et al. (2001) and Hirschberg and Plendl (2005) demonstrated, by means of elegant micro-perfusion casts, that local pododermal angio-architecture is finely tuned by region to reflect the forces acting upon it. These studies demonstrated links between weight bearing and live weight with the development of the vasculature and that areas which experience mechanical compression or elongation contained regional modifications to the morphology of the papillae. However, it was noted that anatomical morphometry cannot be considered alone, as there are tight physiological controls over the functional microcirculation to the structures of the dermis. Thus, the dynamic nature of the microcirculation complicates the relationship between structure, function and the development of lesions and lameness (Hirschberg et al., 2001).

In the light of these results, the finding of the current study as regards vessel size differing with location would appear to represent a normal physiological state rather than being related to remodelling. Were this study to have used either treatment or affected animal groups, the morphometric studies might have been able to substantiate broader conclusions about the aetiology or pathogenesis of vascular changes. Nevertheless, the hypothesis upon which the current study was based had some merit, as shown by Hirschberg and Plendl (2005) who compared normal and diseased (laminitis complex, digital dermatitis) claws. They described changes in the weight bearing regions such as the cap and terminal papillae of the wall region, the sole and bulbar papillae of diseased claws compared to normal claws. Among their findings they reported that angiogenic remodelling of the subepidermal capillary network within the papillae of weight-bearing (traumatised) vs. non weight bearing regions. This supports the decision to look for vascular changes in dynamically active regions of the claw such as Site 2.

The use of vascular morphometry to assess slides from animals in affected groups compared to a normal control group might have helped to clarify whether the effect of Site on the ratio of lumen area to the total area (L/V) in the present study was purely related to physiological and/or functional effects or whether pathophysiological changes related to a defined diseased state were also involved. Such techniques have been applied to assess the response to an insult or treatment: for example Hart (1980) examined the morphometry of brain parenchymal vessels after subarachnoid haemorrhage in cats, Amann et al. (2002) examined the effect of DL-α-tocopherol treatment on vessel structure in the cardiovascular system in renal failure in rats, and Akinaga et al. (2003) examined the response of vessels to chronic exposure to air pollutants in mice.

Further benefits from morphometry could be demonstrated if it is used in conjunction with histopathological scoring (Boosman et al., 1989; Thoefner et al., 2005; Mendes et al., 2013). Those authors created schemas which included histopathological factors relating to the presence of tissue oedema, hyperaemia, haemorrhage, cellular infiltrates or fibrosis, and vascular changes (arteriosclerosis, arterio-venous shunt formation) alongside the morphology of the lamellae and their constituent cell layers, changes or pathology within those cells or the basement membrane. Boosman et al. (1989) for example, who scored areas that were equivalent to the current study's Site 2, concluded that laminitis may be due to haemodynamic disruption, but that histopathological changes were not disease-specific. Similarly, Thoefner et al. (2005) described changes related to the early stages of induced acute laminitic disease which highlighted the involvement of the basement membrane of the dermoepidermal junction. Mendes et al. (2013) combined such histopathological techniques with morphometry to describe changes in the appearance of the lamellar basement membrane between cull dairy animals with and without signs of laminitic disease.

Therefore, despite this recent work there is still no clear set of signs and locations that allow the distinction of acute from chronic laminitic disease, let alone the subclinical lesions thought to arise from subacute ruminal acidosis. Although histopathological changes related to laminitic disease have been described, each group of workers has different definitions for normal vs. abnormal and the categories of laminitis studied (acute vs. chronic). Pathological changes appear to be on a continuum and future work should be aimed at placing clinical findings alongside both observed histopathological change and morphometric evaluation along this continuum. In this way, criteria that best describe the essentially normal animal vs. animals with acute, chronic or subclinical disease could be developed. This would require strict definitions of disease category including how long the animal had been affected, alongside the assessment of multiple regions within the claw.

Foot volume

Moving on to look at the role assessment of conformation plays in monitoring the health of animals, a relatively straight forward equation to predict foot volume from easily-collected data was introduced by Scott et al. (1999) on the basis of post mortem material collected from beef animals after slaughter. Vermunt (1999) commented on the potential value of volume estimation in lameness research however, before the equation was used in this thesis to predict foot volume it was validated on data collected from the feet of New

Zealand cull dairy cows (Chapter 2). The predicted values obtained from the New Zealand data with this equation correlated well to measured claw volume (estimated by water displacement), but exhibited poor agreement with the predicted volumes from the model developed by Scott et al. (1999). Therefore, a new model was developed and simultaneously the option of measuring individual claw volume explored to expand the information that could be collected and applied in a research environment.

Initially, multiple conformational variables were used to develop the best agreement between measured volume and predicted volumes in the post mortem material. In common with Scott et al. (1999) the objective of the study was the field application of the equation developed. Hence, the model for prediction was modified to exclude the more subjective variables, such as claw width and length, and maximised fit to the New Zealand claw data.

The developed model was successfully applied to data collected in two consecutive years to allow the effect of time foot and claw to be investigated. The *in vivo* assessment of hind claw volume described in this chapter is the first time volume has been reported in relation to time. The study demonstrated that lateral claws had greater volume than medial claws and that claws behaved in the same way over time irrespective of claw position. In both years of the study the change in the proximal coronary band length variable was highly correlated to the change in volume and indicated that proximal coronary band length could potentially be recorded as a proxy for claw volume. However, in Year 2 abaxial groove length exhibited played a greater role in the modification of claw volume so more work is needed to look at the utility of proximal coronary band length vs. predicted volume estimation as a measure of claw size.

The next stage in the development of a model to assess foot volume would be to further test the developed formula on another population of hooves to ensure it can be applied to the wider New Zealand dairy cow population. This would be of particular importance in assessing the generalizability of the formula to cows of a wider age band than were used in the present study. Indeed Scott et al. (1999) proposed that the equation which they had derived was likely to be specific to the age and type of animal that they had studied. In agreement with this assertion, the model developed in the present study included age.

Further work on volume estimation might thereafter include examination of breed effects (e.g. Friesian, Jersey or a “Kiwi” cross).

The major limitation to the development of a model for estimation of foot volume is that its validation relies upon establishing a measured volume for comparison. In other words, this either requires a wide range of post mortem material or an alternative to water displacement which allows volume estimation in the live animal. Alternatives to water displacement include three-dimensional imaging modalities such as computer tomography (Labens et al., 2013) but expense and access are likely to preclude this for cattle. Likewise, Labens et al. (2013) also described a 360° image analysis technique for volume estimation on the hooves of horses. They obtained promising results, but concluded their photogrammetric technique was not sufficiently precise to pick up the small volume changes that resulted from corrective trimming of the equine hoof. Use of such methods in artiodactyls would have the extra challenge related to the second digit.

Nonetheless, the stable relationship between claw volume and time that has been described in Chapter 6 could make claw volume a useful baseline to examine against lesion and lameness development. For example, claw volume estimation could be used to investigate position-related size-order of claws and laterality (sidedness) in ungulates, as discussed by Phillips et al. (1996). This coupled with investigation of how the contact surface area of the sole and heel bulb change with respect to the size order of claws, might aid understanding of lesion distribution on the claw ground surface.

Conformation variables

Measurements of many of the conformation traits described by Vermunt and Greenough (1995) have appeared only sparingly in the literature and their use has trailed off as a result of: (1) difficulty of obtaining the measure in the live animal and (2) inconsistent results. Claw width and length in particular appear seldom in the modern literature, probably because of the degree of subjectivity in their determination. In the present study, these traits were applied to post mortem material to assess ease of measurement and test the volume estimation equation of Scott et al. (1999) in New Zealand material (Chapter 2) but were subsequently dropped in favour of a more feasible method for field application.

With respect to the most commonly used conformational variables discussed by Vermunt and Greenough (1995), i.e., dorsal border length and toe angle, this thesis has inspected their use under a pasture based system for two consecutive years (Chapter 6). An effect of time has been demonstrated for both variables studied with medial and lateral claws exhibiting different behaviour over time. However, the pattern of change at pasture has been shown to vary with animal cohort. Consequently, the study supports the measurement of both claws and both feet as additional information regarding the size of the difference between claws of a foot was obtained.

Initial values for conformational variables that were measured at the start of the studies appear to have had a significant bearing upon the modifications which took place in the first 60 to 120 days of lactation. Thus, better understanding of factors which influence conformational traits would help ensure that animals were in the best possible situation at the start of their productive life, perhaps by undertaking breed and genetic manipulations to create a claw type most suitable for the animals' future environment. Although the study has shown that net wear impacts on claw conformation, it has not shown that this necessarily has a negative impact on the animal (i.e. in terms of the risk of lesion development or worsening mobility).

Although the present observations did not incorporate an intervention study, it is nonetheless clear that the effects of any intervention would have to exceed the impact of the background environment. Comparing the results of this thesis with those in the literature, it appears that the impact of pastoral management of dairy heifers upon toe angle and dorsal border length falls between that of rubber flooring where shallow toe angle with increased dorsal border length is seen to develop and that of concrete or asphalt, where shortened claws with steeper toe angles are described (Vokey et al., 2001; Telezhenko et al., 2009; Fjeldaas et al., 2011).

Data on claw height has been provided by some authors (for example Vermunt and Greenough, 1996; Somers et al., 2005) but has not been considered as particularly useful information. This was borne out in the present study, in which claw depth was found to undergo very little alteration over the course of a production year. This study is the first to assess all the linear measures described by Scott et al. (1999) (i.e. proximal coronary band length, distal wall length and abaxial groove length) in all four hind claws, over a complete lactation. While proximal coronary band length and distal wall length displayed little change over the course of first lactation, abaxial groove length was potentially more useful. The groove itself was a useful landmark for siting observations (Anderson and Lundström, 1981) and abaxial groove length behaved in a similar way to dorsal border length. Moreover, whilst dorsal border can become concave or deviated at the toe (Murray et al., 1994) in response to claw overgrowth, abaxial groove length would be more robust. In contrast to dorsal border length, there is no published data for change in abaxial groove length over time within other systems so further work is required to evaluate its utility in describing conformational change.

Interestingly, this study has demonstrated that claw position within foot has a significant effect upon almost all parameters of foot conformation. This finding, however, complicates the analysis, inasmuch as the clustered nature of the data needs to be taken into account. Thus, within an animal, the two hind limbs are not independent of each other, nor are the two claws within a foot. This means that the number of observations must be adjusted accordingly: 10 cows does not equate to 20 independent hind feet or 40 independent claws. Furthermore, overall assessment of all four claws gives extra information for the majority of the conformational variables as there appears to be a claw order in operation between left and right feet; an observation that is perhaps related to the ‘diagonal sidedness’ proposed by Phillips et al. (1996) (i.e. that many cattle exhibit unevenness between the two front and two hind limbs). It would be interesting to determine whether this claw order was itself affected by system, farm or cow.

The concept of laterality or sidedness featured in Chapters 6 and 8, as a potential explanation for the differences between the claws of left and right hind feet. Phillips et al. (1996) demonstrated that 26/30 animals exhibited diagonal laterality with respect to foot size. Previous studies have shown that laterality develops at an early age in calves (Phillips et al., 2003) and foals (van Heel et al., 2006). Furthermore, Phillips et al. (2003) demonstrated that the level of intensification on a farm could drive the distribution of behavioural laterality (e.g. rumination, tongue protrusion, favourite font limb to initiate a step and also favoured side on the milking platform or a track) in a herd. From such data, the suggestion has been made that space restriction, trackways and their management, and animal stress as a result of intensification caused animals to develop a bias towards a favourite side. Furthermore, laterality has been linked to conformational change in Warmblood foals (van Heel et al., 2006). This study used a preference test to demonstrate that where grazing foals protracted one forelimb in preference to the other, uneven hoof conformation and pressure loading developed by 55 weeks of age. When foals were reassessed as three year olds, the uneven conformation was still present in 24% of the animals (van Heel et al., 2010). Taken together, these data could be interpreted to suggest that some animals are more suited to a given system than others, and that those that are less well suited may suffer a higher incidence of claw horn lesions or lameness if relocated to a different system. Therefore, when looking to describe the optimal claw conformation for a given managed environment, this concept should be considered. Matching heifer rearing environment to the system in which the animals will be milked after calving for the first time may reduce the impact of environmental change upon individual animals.

Sole thickness

Chapters 3 and 7 describe the validation and application of portable ultrasound studies and together confirmed the value of measuring the distance to distal phalanx at Site 1 (the tip of the distal phalanx) as a monitoring tool in first lactation heifers. It appears that both claws of one hind foot should be assessed as a minimum, although it is possible that this could be refined to one claw with repetition of the study.

Ultrasonography has already proved its worth in the investigation of lameness from both clinical and research perspectives. There have been several reports of the influence of soft tissue elements within the claw in relation to lesion development and lameness in dairy animals (Bicalho et al., 2009; Machado et al., 2011; Toholj et al., 2014). In particular, Toholj et al. (2014) used multiple examinations of individual live animals to predict the future occurrence of sole ulcers in that cow. As in the present study, Toholj et al. (2014) reported that soft tissue dimensions at different sites within the claw (in their case, at the tip of the distal phalanx and below the flexor tuberosity) were strongly correlated to each other.

The distance to distal phalanx was measured in order to try to predict the presence of thin soles on Day 110 of lactation. Individuals that were categorised as having thin soles on Day 10 were 3 times more likely to have thin soles on Day 110, but the confidence interval was wide (0.75 to 12), which meant that the measure was not a useful predictor. It appears that the use of distance to distal phalanx Day 10 as a predictor of sole thickness on Day 110 was thwarted by the dynamic response of the sole horn to balance wear with growth, and thereby to maintain an optimal sole thickness. At Site 1, there is only a small amount of soft tissue between the sole horn and the distal phalanx and no digital cushion (Räber et al., 2004) in contrast to Site 2. This would suggest that the changes in distance to distal phalanx seen at Site 1, under New Zealand conditions relate to the modification of sole horn thickness rather than the soft tissue components that comprise that depth.

Current literature points firmly to poor body condition score being related to reduced dimensions of the digital cushion (Bicalho et al., 2009; Machado et al. 2011), so this possibility is not to be dismissed lightly in pasture fed cows whose condition scores tend to be low when grass growth is restricted. Further research could focus on the relationship between distance to distal phalanx and sole horn haemorrhage and the development of a range for the optimal sole thickness for a given animal type in a given management system.

Heel conformation, sole thickness and wear

Chapter 8 of this thesis describes a novel method that was developed to capture changes in heel height and heel bulb conformation that occur during first lactation. In addition the relationship between heel conformation and the ground contact surface area of the claw was investigated.

Changes in heel conformation appeared to take longer to become evident than do the modifications to toe angle and dorsal border length that were reported in earlier chapters. To that extent, the present study largely agreed with the conclusions of previous workers (Boelling and Pollott, 1998; Kremer et al., 2007), although it was notable that non-weight bearing heel length demonstrated a rapid change in early lactation. Although there are inconsistent reports in the literature regarding the consistency and/or difficulty of measuring heel height and angle, the present method allowed decreased heel height coupled with increased ground contact surface area in the heel region to be clearly recognised.

Chapter 8 indicates there has been recruitment of the soft heel bulb horn to the ground contact area, coupled with the development of lower heels. Some of this change will reflect maturation of the animal but, for some individuals, there was an increase in the area of the heel (i.e. the area above the line perpendicular to the tangent of the abaxial groove: 'Line YZ') with a concurrent decrease in the length of the non-weight bearing heel. In other words, the heels became shallower and the area of the sole at the heel bulb in contact with the ground increased. This important because the heel bulb region is not designed to weight bear, but to cushion impact and facilitate load transfer during locomotion (as reflected by the softer more compressible horn found in this region: van der Tol et al., 2003). The lateral heel bulb is the area of first impact during locomotion, with the centre of pressure passing from outer heel bulb to the toe tip of the claw as stride progresses (van der Tol et al., 2003). Greater loading of this softer horn may result in a predisposition to the development of lesions and lameness.

The lateral hind claw is at particular risk of injury, because the medial hind claw plays a lesser role in weight bearing (van der Tol et al., 2003; Carvalho et al., 2005; Meyer et al., 2007; Schmid et al., 2009). Even though the bulb and sole of the medial claw both become involved in weight-bearing, the medial claw is loaded after the lateral claw (Meyer et al., 2007; Schmid et al., 2009) in what appears to be an 'involuntary' fashion compared to the deliberate placement of the lateral claw (Meyer et al., 2007). Moreover, horn in the heel bulb region has different biomechanical attributes to wall horn (Franck et al., 2006), whereby different arrangements of tubules and cortex horn result in greater elasticity of bulb horn compared to that of either dorsal or axial heel horn. In consequence, loading pressures upon the bulb which are comparable to those experienced in the live animal can exceed the force required for horn to fail (van der Tol et al., 2003; Franck and De Belie, 2006); with the consequence that environmental factors such as very hard or rough floors have the potential to induce horn injury and damage to underlying tissues. Importantly, in terms of the present results, Franck and De Belie (2006) established that claw contact surface area initially increased in response to loading, but as loading further increased the contact surface area was not able to keep pace. Likewise, van der Tol et al. (2003) also concluded that maximum pressures experienced on the lateral heel bulb of the hind claw could cause damage to the horn or underlying tissues should any reduction in contact area occur (for example by an uneven or unsupportive floor surface). Extrapolating from these results to the present studies, the conformational changes documented in Chapter 8 are likely to plateau, which if the claw continues to be challenged, would then be followed by the development of lesions and lameness.

Furthermore, Telezhenko et al. (2008) demonstrated that exposure to substrate of differing hardness and abrasive properties led to changes in claw conformation and weight distribution in the left hind claws of housed dairy cows. Heifers started the study with more even weight distribution across claws than cows, exhibiting greater pressure on both the medial claw and medial bulb than older cows. Increased contact area reported over the course of the study was associated with a fall in contact pressure While the zones assessed by Telezhenko et al. (2008) were not the same as the current study, the images for animals on abrasive mastic-asphalt floors demonstrated increased weight bearing surface area in a region that aligns with the weight bearing heel surface area (i.e. above line YZ) for the current study. In other words, the changes of the heel of animals managed at pasture have occurred in an area similar to those related to management on asphalt flooring (Telezhenko et al., 2008), perhaps indicating that abrasion plays a hitherto-unforeseen role in the conformational changes seen at pasture.

As claw horn is a visco-elastic material, moisture content should play a role in its biomechanical strength (Franck et al., 2006). Several studies have therefore shown that there is an inter-relationship between the hydration status of the horn and the wear it experiences in contact with different floor surfaces (Greenough and Vermunt, 1994; Bonser et al., 2003; van Amstel et al., 2004; Borderas et al., 2004; Franck et al., 2007). Horn becomes softer with hydration and harder with drying, it erodes more rapidly on some surfaces than others (smooth vs. rough). Reduced horn hardness is related to worsened claw health score and thin soled cows have higher sole horn moisture content than cows with normal sole thickness. In addition, the puncture resistance of horn is reduced with increased moisture content (Winckler and Margerison, 2012).

Moreover, the nature of the substrate on which cattle are kept has important implication for horn integrity, claw conformation and health. For example: soft floors allow increased contact surface area between claw and floor (Hinterhofer et al., 2005), and offer more claw support and increased cow comfort, particularly when lesions (sole ulcer) are present (Flower et al., 2007). Conversely, hard floors focus pressure onto smaller areas of contact, particularly in the bulbar and dorsolateral regions of the claw (Hinterhofer et al., 2005), while slats put claws under mechanical stress (Hinterhofer et al., 2006). Aggregate size and prominence is related to reduced claw: floor contact area and, hence, higher pressures on discrete regions (Franck and De Belie, 2006, whilst Phillips and Morris (2001) reported that the abrasive properties of a surface increased with aggregate size (no aggregate, 1.2 mg/min: 0.5 mm aggregate, 32.5 mm/min; 1.2 mm aggregate 52.9 mm/min and 2.5 mm aggregate; 67.2 mg/min).

The importance of wear in determining claw conformation was also indicated by the ultrasound estimates of distance to distal phalanx in Chapter 7. Tranter and Morris (1992) demonstrated sole wear was greatest in early lactation in New Zealand dairy cows therefore it might expected that excessively thin soles might be develop in heifers in response to this increased wear but this was not the case. Instead, the sole horn appeared to respond in a dynamic fashion to maintain optimal sole thickness in response to the challenge of environmental wear.

In relation to the New Zealand dairy cow, therefore vigilance is needed when lactating animals are managed on hard or rough surfaces. Even though dairy cattle are not often housed in New Zealand, they do have to walk long distances to milking on a daily basis. For example, the changes seen in Year 2 of this study could be driven by exposure to rough, uneven track surfaces which constitute a daily challenge to the sole horn. Although sole horn seems to have the capacity to respond to wear in a dynamic fashion, as seen by the measurement of distance to distal phalanx in Year 1, a flattening of the heel and increase in the surface area above the line YZ was noted. In Year 2, there was also a concurrent change in claw conformation, particularly at the heel. Daily abrasion coupled with repeated deformation of the claws while walking and resultant loss of sole concavity (Tranter and Morris, 1992) could be driving the conformational changes, in an attempt to optimise pressure distribution across the claws by increasing the ground contact surface area to reduce maximum pressures on the claw. Such a notion would be in line with the conclusions of Telezhenko et al. (2008). Eventually, as indicated by the work of Franck and De Belie (2006), modification of claw conformation could be expected to reach a point where it could no longer keep pace with the demands on the claw, at which point injuries could be expected, particularly if surfaces were poorly designed or maintained (van der Tol et al., 2003).

This conclusion would further concur with a recent Danish study which reported a link between severity of lameness in a herd and the quality of the tracks on which the herd walks (Burow et al., 2014): there was increased odds for lameness when tracks were not completely surfaced with a material such as asphalt, gravel, concrete and/or rubber along their length or had a soil or grass surface, compared to a continuously finished track. Barker et al. (2009) also reported that hard track surfaces (e.g. concrete or tarmac) were associated with increased risk of sole ulcer in UK cows. The distance walked by animals did not feature as a significant risk factor in the results of Burow et al. (2014) however; the distances were low compared to those covered by dairy cows in New Zealand (700 m compared to 2.8 km for a typical New Zealand farm: Tranter and Morris, 1992). The majority of New Zealand tracks would fall into the part or unprepared track categories; and, when this fact is considered alongside the work of Tarlton et al. (2002) and Knott et al. (2007) that suggests first lactation heifers are at particular risk of injury as a result of the physiological changes related to parturition (increased laxity of the suspensory apparatus within the claw). In addition, compared to cows, heifers are potentially at risk due to their lower social status in the herd. Less dominant animals spend more time in less desirable conditions such as in the alleyways of barns (Galindo and Broom, 2000) or the back of the feed pad (Mason et al., 2011) and negative social interaction can lead to unusual loading stress on their claws during manoeuvres to avoid older more dominant animals (Chesterton et al., 2004).

The availability of image analysis software has made assessment of surface areas much more accessible and means that changes can be assessed with minimal equipment. Although the findings do not extend to loading pressures it still provides a relatively cheap and feasible way of assessing management interventions to reduce wear such as, track maintenance, comparison of track surfaces, restriction of the distance walked by animals or the use of rubber mats to reduce concrete exposure. In addition, it may reveal whether routine attention to claw conformation in the form of foot trimming, as commonly practiced in the Northern Hemisphere, has any advantage for cows under New Zealand conditions. The addition of sole profile monitoring would add another dimension to this assessment.

Tranter and Morris (1992) have already demonstrated loss of concavity and there is a suggestion that maintaining this would result in fewer lesions. Trimming can restore concavity, but perhaps management interventions could prevent its loss in the first place.

The techniques developed in this thesis could be a of great value in confirming the positive influence of management interventions such as track maintenance and attention to surface preparation, especially if performed in conjunction with mobility and lesion scoring.

Conclusions

This thesis provides a detailed description of claw conformation with respect to time (spanning the first lactation from Days 10 to 220 plus) and claw position (medial and lateral claws of both hind feet) for the pasture-based New Zealand dairy heifer over two consecutive years. While traditional variables such as toe angle and dorsal border length were included less well known features of claw conformation such as predicted claw volume, proximal coronary band length, distal wall length, abaxial groove length and distance to distal phalanx have also been monitored to establish how these variables were modified over time and whether there was an effect of claw position.

The study has developed several new techniques to aid the study of claw conformation and lameness, which include: The application of vascular morphometry to histological sections of bovine claw material. The validation and subsequent field use of a portable ultrasound machine to: (1) follow distance to distal phalanx in a group of first lactation heifers and (2) establish the predictive value of distance to distal phalanx in identifying thin soles at Day 110. In addition, a novel method to assess heel conformation in combination with image analysis to track the surface area of the claw in ground contact was trialled in the live animal.

A key finding of this study (Chapter 7) was that rather than soles wearing to become excessively thin in response to the long walking distances faced in a New Zealand pasture-based dairy system, the sole horn exhibited a dynamic response with thin soles tended to become thicker, while thick soles wore to become thinner. This concurs with the suggestion by van Amstel et al. (2003) that an optimal sole thickness exists for a given system.

In addition to this other key findings include:

Confirmation of the need to: (1) refine volume prediction equations to align with animal production type as well as age and (2) assess agreement as well as correlation in such studies (Chapter 2).

The description of an effect of site within the claw on the ratio of vessel lumen area to lumen plus tunica intima and media area for hoof blood vessels in non-lame New Zealand dairy cows (Chapter 5).

The successful application of simple linear measures to monitor heel conformation and the demonstration of a reduction in non-weight bearing heel length accompanied by an increase in the surface area of the weight bearing heel above the line YZ (Chapter 8).

And finally that evaluation of both hind feet and all claws is advisable to capture maximum conformational information as the difference between the two claws of a foot can differ in size between the left and right hind feet (Chapter 6 and 8).

In summary this study has extended the current knowledge regarding the measurement and monitoring of conformation in the New Zealand dairy heifer at pasture. Furthermore, it has provided additional tools to allow the impact of a management intervention to be studied for both the study of lameness and the investigation of clinical lameness cases suspected to be associated with thin soles. In addition conformational data has been generated for New Zealand conditions which have allowed some of the conformational traits assessed to be examined in the light of the impact of the environment by which they are modified.

From the overarching hypothesis, it was expected that the modification of claw traits at pasture would be mid-spectrum falling between the extremes described in the literature for animals housed on concrete or asphalt versus those housed on straw or with rubber flooring. However, this thesis has established that some traits (dorsal border length and toe angle) fluctuate with year on year influences even on the same farm-unit, while other traits such as volume are more stable. Where data are available for comparison to other systems, findings have been interesting and challenging. In particular, the heel region of the pasture-based animals studied appears to experience modification over time in line, with extreme wear associated with the abrasive properties of asphalt (Telezhenko et al. 2008).

As a whole, this thesis indicates that the impact of the New Zealand pasture-based system on claw conformation needs to studied and demonstrated as an entity in itself, rather than via extrapolated from the existing literature. The reality is not as simple as placing pasture along an expected spectrum between straw and asphalt, inasmuch as claw traits are finely tuned by the environment in an attempt to realise an optimum claw shape for a given situation. The pastoral system in New Zealand incorporates many underfoot conditions which are modified between years on the same farm and therefore the outcome for each variable is complex.

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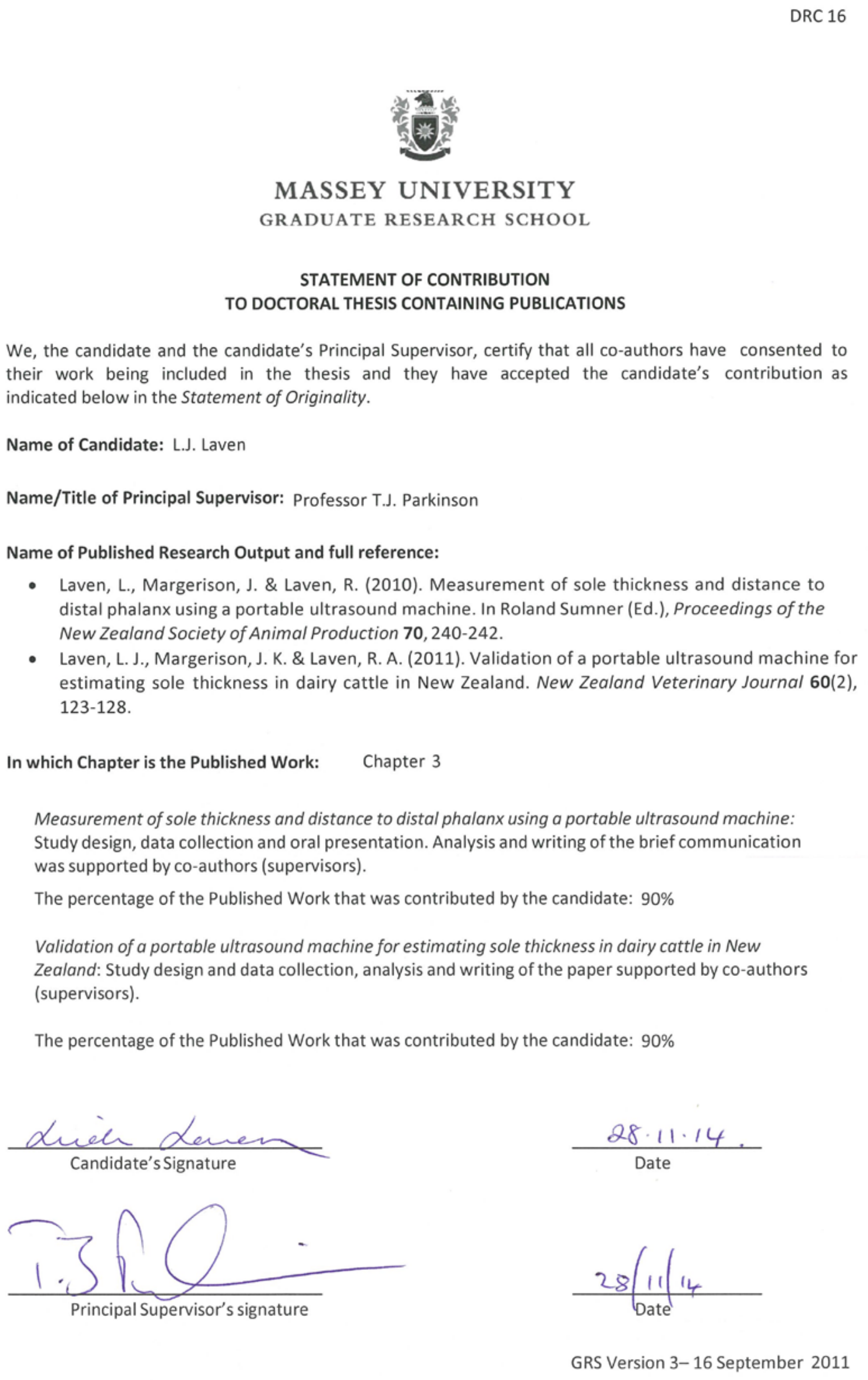
Appendix:



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STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: L.J. Laven

Name/Title of Principal Supervisor: Professor T.J. Parkinson

Name of Published Research Output and full reference:

- Laven, L., Margerison, J. & Laven, R. (2010). Measurement of sole thickness and distance to distal phalanx using a portable ultrasound machine. In Roland Sumner (Ed.), *Proceedings of the New Zealand Society of Animal Production* **70**, 240-242.
- Laven, L. J., Margerison, J. K. & Laven, R. A. (2011). Validation of a portable ultrasound machine for estimating sole thickness in dairy cattle in New Zealand. *New Zealand Veterinary Journal* **60**(2), 123-128.

In which Chapter is the Published Work: Chapter 3

Measurement of sole thickness and distance to distal phalanx using a portable ultrasound machine: Study design, data collection and oral presentation. Analysis and writing of the brief communication was supported by co-authors (supervisors).

The percentage of the Published Work that was contributed by the candidate: 90%

Validation of a portable ultrasound machine for estimating sole thickness in dairy cattle in New Zealand: Study design and data collection, analysis and writing of the paper supported by co-authors (supervisors).

The percentage of the Published Work that was contributed by the candidate: 90%

Candidate's Signature

28.11.14.
Date

Principal Supervisor's signature

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Name of Candidate: L.J. Laven

Name/Title of Principal Supervisor: Professor T.J. Parkinson

Name of Published Research Output and full reference:

- Laven LJ, Laven RA, Parkinson TJ, Lopez-Villalobos N, Margerison JK (2012). An evaluation of the changes in distance from the external sole surface to the distal phalanx in heifers in their first lactation. *Veterinary Journal* **193**(3):639-643.

In which Chapter is the Published Work: **Chapter 7**

The Candidate was primarily responsible for the study design related to this output, performed all of the data collection. The chapter contains considerably more data than were reported in the published paper, and, in consequence, also contains an entirely separate data analysis. Data analysis and writing of final paper was supported by co-authors (supervisors).
Some of the data in the chapter were also reported in an oral presentation by the Candidate at the International Ruminant Lameness Symposium, Rotorua, New Zealand, 2011.

The percentage of the Published Work that was contributed by the candidate: 80%

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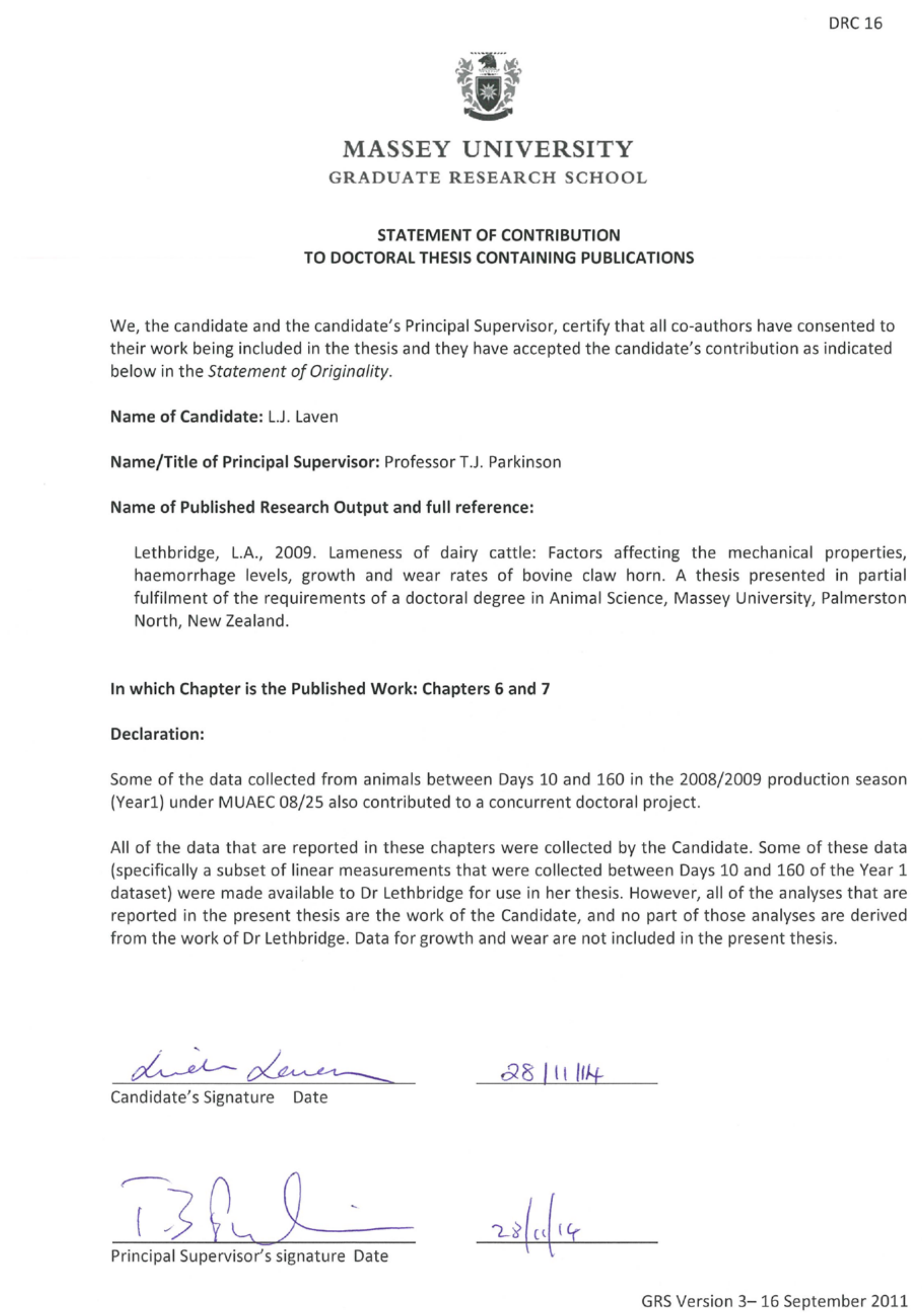

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Name of Candidate: L.J. Laven

Name/Title of Principal Supervisor: Professor T.J. Parkinson

Name of Published Research Output and full reference:

Lethbridge, L.A., 2009. Lameness of dairy cattle: Factors affecting the mechanical properties, haemorrhage levels, growth and wear rates of bovine claw horn. A thesis presented in partial fulfilment of the requirements of a doctoral degree in Animal Science, Massey University, Palmerston North, New Zealand.

In which Chapter is the Published Work: Chapters 6 and 7

Declaration:

Some of the data collected from animals between Days 10 and 160 in the 2008/2009 production season (Year1) under MUAEC 08/25 also contributed to a concurrent doctoral project.

All of the data that are reported in these chapters were collected by the Candidate. Some of these data (specifically a subset of linear measurements that were collected between Days 10 and 160 of the Year 1 dataset) were made available to Dr Lethbridge for use in her thesis. However, all of the analyses that are reported in the present thesis are the work of the Candidate, and no part of those analyses are derived from the work of Dr Lethbridge. Data for growth and wear are not included in the present thesis.

Linda Laven 28/11/14
Candidate's Signature Date

28/11/14
Principal Supervisor's signature Date

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