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Mechanical properties of hoof horn, sole haemorrhage and lameness in dairy cattle

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MECHANICAL PROPERTIES OF HOOF HORN, SOLE HAEMORRHAGE AND LAMENESS IN DAIRY CATTLE

by

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MECHANICAL PROPERTIES OF HOOF HORN, SOLE HAEMORRHAGE AND LAMENESS IN DAIRY CATTLE

The present study aimed to develop a method to measure changes in the mechanical properties of the sole and white line hoof horn that should be used *in vivo* and to examine the hypothesis that the presence of haemorrhages in the horn would represent a weakening of the structural strength of the horn. Hoof horn samples were collected from the sole and white line areas of the hoof horn of dairy cattle. The maximum punch force, work to fracture, elastic modulus and membrane stress were measured through the use of a punch test and the elastic modulus was measured through the use of a tension test.

The elastic modulus of the diaphragm, membrane stress and elastic modulus measured through tension test of the sole and white line areas were found to increase significantly and exponentially ($P<0.01$, R^2adj. = 0.39 to 0.81) and the punch force and work to fracture increased significantly and linearly ($P<0.01$, R^2adj. = 0.37 to 0.89) in relation to the dry matter content of the hoof horn. Sample thickness accounted for 30 to 40 % of the variation of the punch force and work to fracture results, therefore the thickness of the tested area was measured and included as a covariant in the statistical analysis of those tests.

Punch force of the sole area of the claw horn decreased significantly ($P<0.001$) (8.72, 8.53, 8.06, 7.75, 6.08, 4.99 N, sem 0.078 to 0.460) when haemorrhage levels of the tested area increased (0 to 5). In multiparous cows that had higher scores for lesions of the claw horn when compared to the heifers the punch force decreased at day 160 *postpartum* when the cows had greater lesion scores and was lower in hind claws that had higher lesion scores when compared to the front claws. Lower punch force and elastic modulus were found in heifers with less straight rear legs, lower foot angle, poor HUKI locomotion score, lower scores for the composite trait legs and feet and a higher HUKI final total score. The measurement of the mechanical properties of the hoof horn has contributed to the understanding of the changes occurring during the peripartum period that predispose dairy cows to acquire horn lesions and suffer from lameness and has proven to be a good method for measuring the influence of housing systems and cow conformation on the strength and elasticity of the horn.

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Signed: ......Behina Wil......

Date: ......20/12/05......

FREQUENTLY USED ABREVIATIONS

CP	Crude protein
DM	Dry matter
EM	Elastic modulus
EMd	Elastic modulus of the diaphragm
FM	Fresh matter
HUKI	Holstein UK index
IFM	International foot map
ME	Metabolic energy
NBS	Non biotin supplemented
NDF	Neutral detergent fibre
OM	Organic matter
PR	Punch force
pp	postpartum
RH	Relative humidity
S	Membrane stress
SEM	Scanning electron microscopy
TMR	Total mixed ration
wl	White line
W	Work to fracture
WSC	Water soluble carbohydrates

Chapter 1 - Literature review

1.1 Incidence and prevalence of lameness in the United Kingdom

The incidence of lameness in dairy cattle has increased considerably over the past 20 years (Logue *et al.*, 1999). During this time the dairy industry has undergone considerable changes in the areas of animal breeding, nutrition and housing and some of these changes have contributed to the increase in the incidence of lameness (Clarkson *et al.*, 1996; FAWC, 1997; Shearer and Amstel, 2002). According to several surveys (Clarkson *et al.*, 1996; Hedges *et al.*, 1998; Kossaibati *et al.*, 1999; Whay *et al.*, 2002; Whitaker *et al.*, 2004) the mean incidence of lameness in individual herds in the United Kingdom varied from 22.0 to 55 cases per 100 cows (Table 1.1).

Table 1.1 ***Incidence and prevalence of lameness in dairy herds surveyed in the United Kingdom (1996 to 2004)***

	Clarkson *et al.* (1996)	Weaver (1998)	Hedges (1998)	Kossaibati *et al.* (1999)	Whay *et al.* (2002)	Whitaker *et al.* (2004)
No. of herds	37	55	5	50	53	434
Mean herd size (No of cows)	119	140	139	150	120	148
Incidence	54.6	-	39.4	38.2	-	22.0
Incidence range	11 - 170	-	9 - 79	4 - 144	-	-
Prevalence	21.0	30.3	34.2	26.5	22.1	-
Prevalence range	2 - 54	3 - 100	-	4 - 70	0 - 50	-
Observer	Trained researcher	Farmer	Farmer	Farmer & vet.	Trained researcher	Farmer

The incidence of lameness was defined as the number of lameness cases per 100 cows in a herd, each affected limb during a period of 28 days being regarded a case of lameness. The prevalence of lameness was defined as the number of lame cows in a herd at a single observation. While in all surveys there was a relatively even distribution of farms with low, medium and high incidence of lameness, there was a great variation in the incidence of lameness between farms, which may reflect the complexity of the factors that cause and influence lameness (Logue, 1999).

The different levels of lameness recorded may be related to the location of the farms and the person undertaking the recording (Logue, 1999). In the surveys of Weaver (1998), Hedges *et al.* (1998) and Whitaker *et al.* (2004) the lameness incidence was assessed by the farmer, which could have lead to an underestimation of the level of lameness. It has been found that farmers underestimated the level of lameness when compared with trained observers (Wells *et al.*, 1993; Mill and Ward, 1994; Whay *et al.*, 2002; Whay *et al.*, 2003). Moreover, Whay *et al.* (2003) suggested that the farmers failed to identify three in four cases of lameness. In the survey completed by Clarkson *et al.* (1996), lameness was assessed by a trained observer through the use of locomotion score, i.e. subjective measurement of posture and the reported incidence of lameness was the highest of all surveys.

1.1.1 Lameness scoring systems

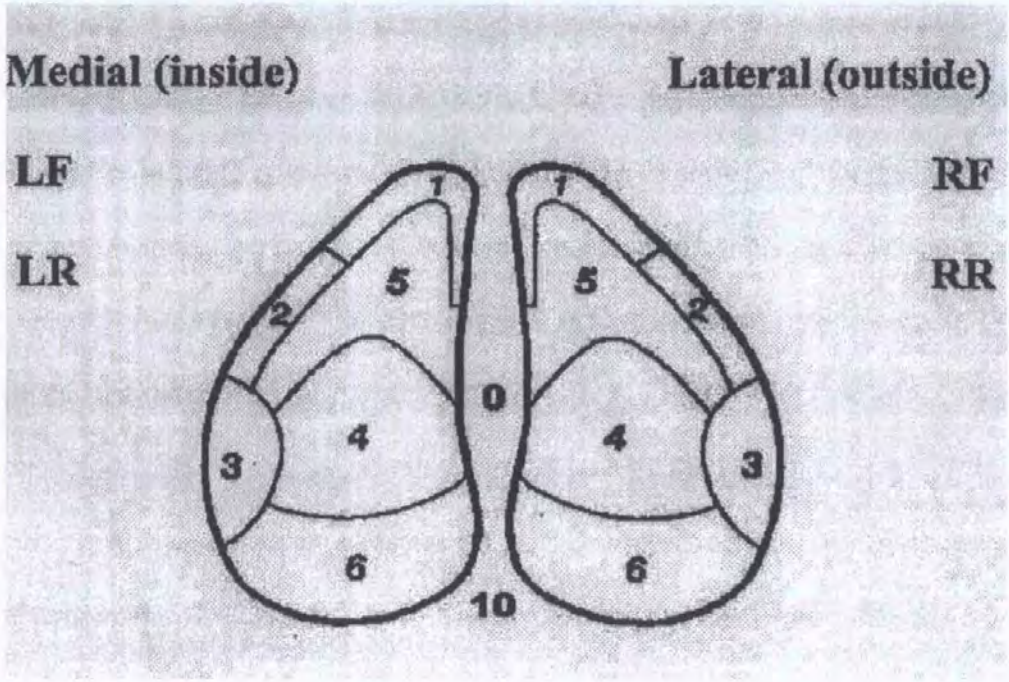
According to Whay (1999) scoring the whole herd for lameness at regular intervals and recording the results would provide information about the lameness prevalence within the herd helping to identify the causes of lameness and to develop a strategy to prevent lameness. This method would also help to evaluate changes in the situation. Several lameness scoring systems (locomotion score) have been developed by different authors. Manson and Leaver (1988) used a system with 5 points and half scores (9 points in total). This system is very detailed with half of the scores dedicated to changes in gaits before animals become clinically lame, making it more difficult to learn. The authors assessed the abduction and adduction of limbs, evenness of gait, tenderness, difficulty in turning, affect on behaviour and difficulty in rising and walking. Tranter and Morris (1991) and Wells *et al.* (1993) designed systems with 5 points and assessed the ease of deciding which leg is affected, the degree and timing of the head movement, the degree of sinking of sound hind quarter, abduction and adduction and changes in stride length. Whay *et al.* (1997) used a 6 point system similar to the one designed by Tranter and Morris (1991), but with an added score for cows walking abnormally but who are not lame. This score was included because many dairy cows appear not to walk with free movements due to a large udder and arthritic conditions under other factors. In addition to the factors assessed by Tranter and Morris (1991), Whay (1999) recommended looking at the arching of the spine and alignment of the pin bone. When locomotion scoring cows the same scoring system should be used each time, the cows should be scored when walking at the same surface and if possible the same person should carry out the scoring (Whay, 1999). Winckler and Willen (2001) and De Rosa *et al.* (2003) found a high inter-observer repeatability of locomotion scores in individual cows. O'Callaghan *et al.* (2002) and Winckler and Willen (2001) found positive correlations between the assessment of behaviour scores and the presence of lesions on the feet ($R = 0.50$, $p=0.0001$). Clinically lame cows were found not to have major pathological changes in the claws, but all cows that had high claw disorder indices were recorded as lame (Winckler and Willen, 2001).

1.1.2 Hoof horn lesion scoring system

Leach *et al.* (1998) designed a scoring system to measure lesions of the claw, sole and white line. In this system, the type and position of lesions such as haemorrhage, yellowing of the horn and sole ulcer are recorded on a map of each claw, corresponding with the 6 zones of the international foot map (Brizzi *et al.*, 1998; Shearer *et al.*, 2002). The international foot map is presented in Figure 1.1. This scoring system gives each lesion a subjective severity score on a scale of 1 to 5, while lesions that develop into a sole ulcer are scored on the scale of 6 to 8. This scoring system has been widely used to investigate the relationship between sole and white line lesions, lesions in different claws and the pattern of the appearance of the sole and white line lesions in relation to several factors associated with lameness (Chaplin et al, 2000; Offer *et al.*, 2000; Le Fevre *et al.* 2001; Midlla *et al.*, 1998). Webster (2001) stated that it is difficult to predict the association with a moderate degree of claw horn lesions with the locomotion score, while claw horn lesions with high scores were more frequently associated to the locomotion score. Whay *et al.* (1997) found that the severity of the lesions had a higher correlation with the locomotion score than the area of the lesion.

1.2. The cost and welfare of lameness

Lameness has been identified as the third most costly disease of dairy cattle, with mastitis and poor fertility being more costly (Kossaibaiti and Esslemont, 1995). Lameness has been found to affect the economic performance of dairy cows in several ways; reduced milk yield, loss of body weight, poor fertility (Gabarino *et al.*, 2001), treatment costs and premature culling (Kossaibaiti and Esslemont, 1995; Warnick *et al.*, 2001; Juarez *et al.*, 2003). The culling rate due to lameness was found to be 3.5 % in the first lactation and rose to 9 % in the seventh lactation (Kossaibaiti and Esslemont, 1995).



LF – left front claw, LR – left hind claw, RF – right front claw, RR – right hind claw
0 – interdigital space, 1, 2 – white line, 5 – sole, 3, 4 – sole-heel junction, 6 - heel

Figure 1.1 ***Zones of the distal surface of the claw (Greenough and Vermut, 1991; Shearer et al., 2002)***

Considering all the direct and indirect costs involved and using data based on 50 herds monitored by the DAISY herd management system, Kossaibati *et al.* (1999) estimated the cost of lameness for a 100 cow herd to be approximately £4,000 per year, ranging from £1,300 for herds with lower levels of lameness to £6,800 for herds with a high incidence of lameness. When the annual incidence of lameness was 38 cases per 100 cows, lameness reduced the annual profit in the herd by £40 per cow. The factors involved in the cost of lameness and the cost of each individual factor are presented in Table 1.2 (Kossaibati *et al.*, 1999).

Table 1.2 ***Factors involved in the cost of lameness and the cost (£) of each individual factor per lameness case***

Factors	Cost (£)
Reduced milk yield	16.5
Increased costs of veterinary treatments	25.4
Discarded milk due to treatment with antibiotics	1.7
Higher herd culling rate and replacement costs	48.3
Higher fertility cost (extra services)	7.8
Increased labour costs (time spent on treatment and attention by the herdsman)	6.1
Extended calving interval	30.5
Total	136.3

Source: Kossaibati *et al.* (1999)

In 1997, the FAWC identified lameness as one of the three main causes of poor welfare in dairy cows in the United Kingdom. Lameness was considered to be one of the most painful conditions to commonly affect cows (Kossaibati *et al.*, 1999; Webster, 2002). Increased levels of plasma cortisol and lactate were found by El-Ghoul and Hofmann (2002) in lame cows that had different claw disorders. Moreover, lame cows have been found to have an increased sensitivity to pain that can persist up to 28 days after the treatment of the cause of lameness (Whay, 1998; Whay, 1999) and show a significant reduction in their daily activity levels when compared with non lame cows (69.6 step/hr moderately lame, 56.9 steps/hr severely lame compared with 82.5 steps/hr non lame cows) (O'Callaghan *et al.*, 2003). The FAWC (1997) concluded that steps should urgently be taken to reduce the incidence of lameness and made recommendations regarding the need to raise awareness of lameness, the inclusion of foot and hind leg conformation in the genetic selection of cows and the improvement of housing conditions.

1.3. Causes of lameness in the dairy cow

There are a large number of conditions that have been found to cause lameness in the dairy cow. Logue (1999) classified lameness cases into four categories based on the pathogenesis of the different lesions namely; claw, interdigital and digital (skin), non-foot (limb/joint) and uncertain causes. The relative proportion of the categories of lameness using data from 18 surveys in different countries, according to Logue (1999), are presented in Table 1.3.

Table 1.3 ***Relative proportion of the categories of lameness***

Categories of lameness	Proportion
Claw	0.73
Interdigital	0.18
Limb	0.05
Uncertain	0.04

Source: Logue (1999)

In the United Kingdom, Kossaibati *et al.* (1999) reported lower figures for claw horn lesions (60.1 %) and higher figures for interdigital/digital lesions (36.3 %). The incidence of factors that cause lameness in the dairy cow is presented in Table 1.4.

Weaver (1998) and Hedges *et al.* (1998) reported similar trends to those found by Kossaibati *et al.* (1999). According to Weaver (1998), the major cause of lameness was sole ulcers, followed by digital dermatitis and then by foreign body penetration of the sole or the white line, that were present in 0.75, 0.53 and 0.20 of the farms respectively.

Table 1.4 ***Incidence of factors that cause lameness in the dairy cow***

Factor	**Incidence of factors that cause lameness**
Claw horn lesions	
Sole ulcers	0.20
Foreign body (abscess and sole penetration)	0.18
White line lesions	0.09
Overgrown horn	0.05
Underun sole	0.04
Bruising of the sole	0.02
Sandcrack	0.01
Heel horn erosion	0.01
Deep sepsis	0.01
Laminitis	0.01
Total of claw horn related lesions	0.60
Interdigital and digital lesions	
Digital dermatitis	0.20
Foul in the foot	0.13
Interdigital hyperplasia	0.02
Interdigital dermatitis	0.01
Total of interdigital and digital lesions	0.36
Total limb/joint lameness	0.02
Uncertain	0.02
Total	1.00

Source: Kossaibati *et al.* (1999)

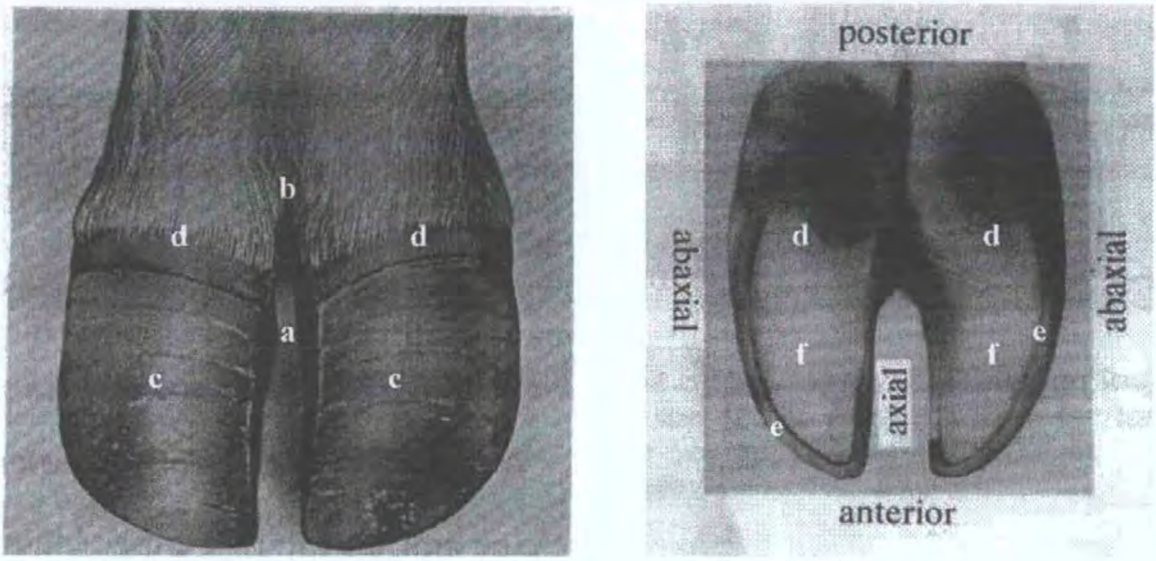
In 1999, Webster considered claw lesions to be the most common and intractable condition in dairy cows. The major problems of the claw horn were sole ulcers and white line lesions and these were recorded at a ratio of 2:1 (Logue, 1999). The major causes of interdigital/digital lesions have been found to be digital dermatitis and foul in the foot, both of which are infectious diseases. The incidence of digital dermatitis was found to be increasing in the United Kingdom (Weaver, 1998; Kossaibati *et al.*, 1999) and the Netherlands (Somers *et al.*, 2002; Somers *et al.*, 2003). Recently, Logue stated that digital dermatitis was going to be the main cause of lameness in dairy cows (2002, personal communication). In many areas it is the most common cause of lameness during the housing period, and has been associated with poor hygienic conditions (Kossaibati *et al.*, 1999). Digital dermatitis was present in 39 of 53 farms in a survey of lameness carried out in the West Country and the Midlands of the United Kingdom (Whay *et al.*, 2002). Infectious claw diseases such as digital dermatitis and interdigital dermatitis, have been found to be very painful for dairy cows and affected animals have been found to change their behaviour to alleviate pain even in early stages of the disease (Berry *et al.*, 1998; Winckler and Willen, 2001).

1.4 Anatomy of the claw and the formation of hoof horn

1.4.1 External structures of the claw

The bovine foot consists of two separate digits, the outer and the inner claws. The interdigital cleft is the space that separates the two claws and is situated along the axis of the foot. The claws are connected in the interdigital space by the interdigital skin (Weaver *et al.*, 1981; Toussaint-Raven, 1985).

The horn of the wall encloses the anterior (dorsal) part of the claw on the axial and abaxial sides. The abaxial side is the visible part of the wall and the axial side is situated in the interdigital space. The dorsal wall of the claw had a greater thickness (4.5 mm) when compared with the wall of the heel area (3.7 mm) and axial part of the claw (3.7 mm) (Leopold and Prietz, 1980). The solear part of the anterior margin is formed by the sole horn. The wall and the sole horn are connected on an area called the white line. The posterior (caudal) part of the claw is enclosed by the horn of the heel that joins the sole horn at the solear area. The horn of the heel is softer and more elastic than that of the sole and the hardest horn is found in the wall. The area of transition between the skin and the horn of the wall and the heel is referred to as the coronary border. This transition area is covered by the soft periople horn (Weaver *et al.*, 1981; Toussaint-Raven, 1985; McCallum, 1999). The external structures of the bovine foot are presented in Figure 1.2.



a – interdigital cleft
b – interdigital skin
c – wall
d – coronary border

e – white line
f – sole horn
g – heel horn

Figure 1.2 ***External structures of the bovine foot,*** source: Toussaint-Raven (1985)

1.4.2 Shape and weight bearing of the bovine claw

The sole of the claw has a hollow shape and its slope becomes steeper towards the interdigital space. The border of the wall, mainly the abaxial side, the white line and the solear surface of the heel form the weight-bearing surface of the claw. On the axial side only the toe area of the wall is part of the weight-bearing surface. At normal gait the bulbs of the heel and the outer wall will make the first contact with the ground and the weight will be distributed equally between inner and outer claw. The weight on the wall will be transferred to the sole due to slight splaying of the claws (Bergsten and Mulling, 2004). Depending on the shape of the claw and the type of floor surface the sole will also be part of the weight-bearing surface. Meyer *et al.* (2004) demonstrated in 13 months old heifers that in the hind limbs the outer claw was always the first one to make contact with the ground, these claws receiving the first impact force. The thickness of the sole is approximately of 7 mm in the toe and heel areas and 5 mm in the middle (Toussaint-Raven, 1985; Scott, 1987; Paulus and Nuss, 2002). Van Amstel *et al.* (2004) reported a mean sole thickness for the hind lateral claws of 4.23 ± 0.18 mm and for the hind medial claws of 5.15 ± 0.18 mm.

The front claws of cows normally have a higher heel and a more horizontal "coronary line" than the hind claws (Toussaint-Raven, 1985). The inner and outer hind claws exhibit a difference in shape that the front claws do not have. The axial part of the heel of the inner hind claw is less developed than the corresponding area of the outer claw. The axial weight-bearing border of the wall on the toe area is shorter in the inner hind claw when compared with the outer claw. As a result, the inner claw has a smaller weight-bearing surface in the axial side than the outer claw and the outer claw bears more weight than the inner claw. The sole of the inner claw is also more concave than the sole of the outer claw (Toussaint-Raven, 1985; Scott, 1987). The outer hind claws and the inner front claws exhibited a 5 % greater wall thickness than the hind inner and front outer claws (Leopold and Prietz, 1980). When the outer hind claw was pared to the same sole thickness as the inner claw, in 18 of 20 specimens the outer claw protruded distally when compared to the inner claw (Paulus and Nuss, 2002). The authors concluded that the lateral (outer) digit is longer than the medial (inner) digit. The weight-bearing area and concavity of the hind and front outer and inner claws are presented in Figure 1.3.

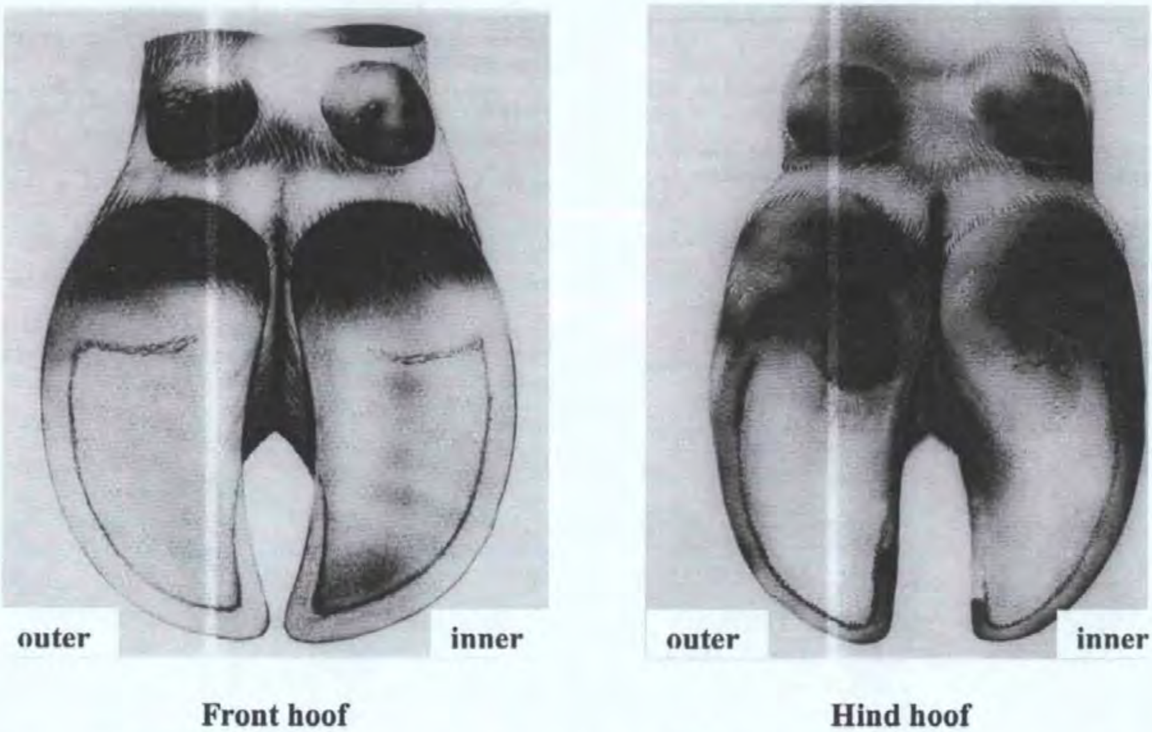
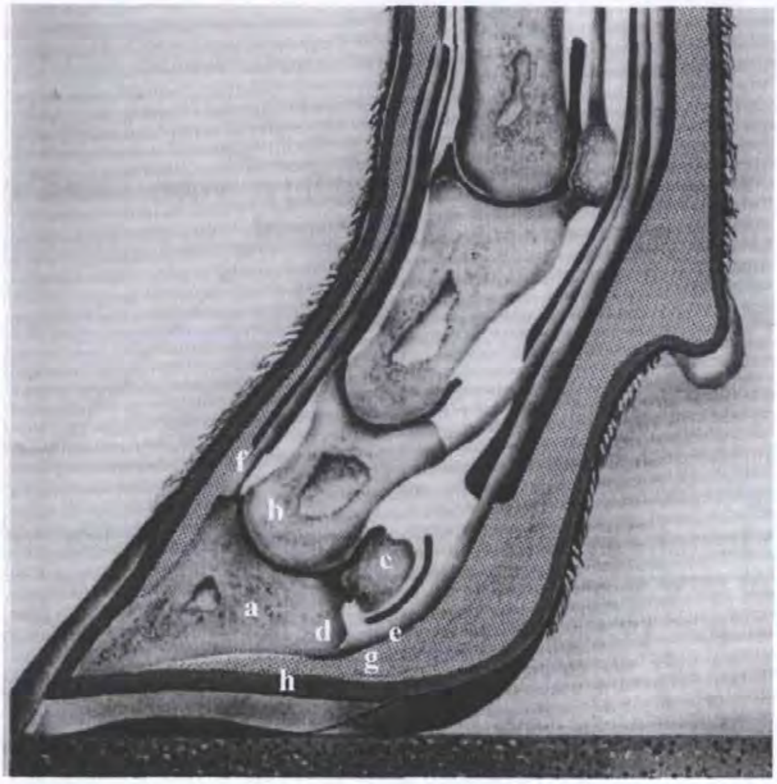


Figure 1.3 ***Weight-bearing area and concavity of the hind and front outer and inner claws,*** source: Toussaint-Raven (1985)

1.4.3 Internal structures of the bovine claw

The distal part of the middle phalanx, the distal sesamoid bone and the distal phalanx (or third phalanx, pedal bone) are the bones contained within the horny capsule. These bones articulate at the distal interphalangeal joint (Leach, 1996). The distal phalanx has a prominent flexor tubercle where the deep flexor tendon is inserted. The deep flexor tendon tenses when the foot bears weight (Manabe *et al.*, 2002). The distal phalanx is joined to the underlying connective tissue and than to the hoof horn through dermal and epidermal folds. In the hoof wall, referred to as parietal horn, these folds are regularly arranged and are called laminae. In the sole they are irregularly arranged and are called papillae. All the structures connecting the surface of the bone with the inner aspect of the horn capsule have been called suspensory apparatus of the coffin bone (Westerfeld *et al.*, 2000). The structures between the surface of the bone and the cornified horn capsule consist of the dermis with a deeper reticular layer and a superficial papillary layer and the non cornified epidermis (Mulling and Lischer, 2002). The internal structures of the bovine hoof are presented in Figure 1.4.



a – distal phalanx
b – middle phalanx
c – sesamoid bone
d – flexor tubercule
e – deep flexor tendon
f – extensor tendon
g – digital cushion
h – dermis

Figure 1.4 ***Internal structures of the bovine hoof***, source: Toussaint-Raven (1985)

Two types of protein fibres are found in the dermis; the collagen and elastin fibres (Mgasa and Kempson, 2002). Very important for the stable attachment of the distal phalanx are the collagen fibre bundles that run from the insertion zone on the bone surface to the basement membrane were they are anchored (Mgasa and Kempson, 2002; Mulling and Lischer, 2002). The orientation and arrangement of the collagen fibre bundles give a strong indication of the tensile forces in the bovine claw. The fibre bundles between the bone surface and the horny capsule are oriented proximo-dorsally, with an angle changing from proximal to distal (Westerfeld *et al.*, 2000; Mgasa and Kempson, 2002). At the apex of the distal phalanx the fibre bundles are of increased thickness indicating high mechanical load in this area (Westerfeld *et al.*, 2000). Three types of forces influence the suspensory apparatus; the pressure exerted by the body weight, the tension of the digital extensor tendon and the tension of the deep flexor tendon. All pressure forces are transformed into tensile forces, therefore only tension is loaded on the structures of the suspensory apparatus of the wall (Westerfeld *et al.*, 2000). In the wall the connective tissue inserts at the distal phalanx in a cartilaginous insertion zone. The appearance and thickness of this insertion zone varies according to the site and the mechanical load. It is well developed in the distal wall and around the apex of the distal phalanx (Westerfeld *et al.*, 2000).

The basement membrane separates the epidermis from the dermis. It is connected by a network of cross-linking fibrils to the connective tissue of the dermis (Hendry *et al.*, 1997). The epidermal cells located near the basement membrane proliferate and have a high metabolic activity. All nutrients required for the epidermal activities have to pass from the dermis into the epidermis, therefore the dermo-epidermal junction is an important structure for the normal function of the claw (Mulling and Lischer, 2002). The dermo-epidermal junction presents microfolds that increase the area of interaction between dermis and epidermis, facilitate the contact of the epidermal cells with the blood vessels of the dermis and optimise its role as a mechanical junction (Frohnes and Budras, 2001; Mulling and Budras, 2002). Epidermal cells are attached via hemidesmosomes to the basal lamina, which in turn is linked by a lamina fibroreticulares to the collagen fibre system of the epidermis (Mulling and Budras, 2002). The corium, or dermis, is divided into the coronary corium, lamellar corium, or parietal corium, and solear corium (Weaver *et al.*, 1981; Toussaint-Raven, 1985; Ossent and Lischer, 1998).

Underneath the distal phalanx the pressure is loaded into the tissue. The subcutaneous connective tissue forms the digital cushion under the pedal bone and at the heel and has a high fat content in these areas (Lischer and Ossent, 2002). In the digital cushion the bundles of collagenous fibres of the dermis enclose cylindrical transverse oriented compartments that are filled by fat tissue (Westerfeld *et al.*, 2000). The digital cushion is divided into the abaxial, middle and axial cushions. The abaxial and middle cushions are thought to protect the insertion of the deep flexor tendon and the axial cushion absorbs peak loads at the area of the axial groove (Maierl *et al.*, 2002a). The digital cushion had a higher fat content in the medial claws when compared to the lateral claws. Areas subjected to high pressure loads situated on the central area of the sole and heel had a lower fat content when compared to lateral areas subjected to low pressure loads (Raber *et al.*, 2002). In cows, the digital cushions had a higher fat content and higher concentration of monounsaturated fatty acids (MUFA) than the digital cushions of heifers (Raber *et al.*, 2002). In the abaxial lower half of the wall, the axial anterior part of the wall and the toe area of the sole the subcutaneous connective tissue is absent and the distal phalanx is firmly attached to the corium. Therefore, the distal phalanx is less attached to the wall at the axial caudal area of the claw. On this area the distal phalanx has the prominent flexor tubercle where the deep flexor tendon is inserted and as a result the pressure on the underlying corium is greater in this area. The axial side of the distal phalanx is supported by the distal interdigital ligaments that are connected to the digital fat cushions (Lischer and Ossent, 2002).

1.4.4 The structure of the hoof horn

The hoof horn consists of three layers, the subcutis, the corium, or dermis, and the epidermis. The epidermis consists of living epidermal cells in the basal and spiny layers and dead epidermal cells in the horny layer. The horn is produced by the programmed death of living epidermal cells. Blood vessels are situated in the corium, which acts as a nutritional and stabilising layer for the epidermis. Epidermal cells receive a supply of nutrients and oxygen via diffusion from the blood vessels in the corium. The living epidermal cells are very susceptible to any disturbance in the circulation within the vessels of the corium (Mulling *et al.*, 1998).

The production of hoof horn in the wall segment of the claw occurs at the coronary border on the proximal part of the wall. The horn of the wall moves continuously downwards and wears off on the weight-bearing border. The white line production occurs in different parts of the wall and has been found to be greatest in the distal cap horn that is situated at the wall-sole border (Budras *et al.*, 1996). The sole horn is produced in the epidermis of the papillae of the sole. The tubules of the hoof horn are formed in the dermal-epidermal folds. The cells grow outward as new cells are formed beneath them. It gives the horn an appearance of flaky layers, which run in the same plane across the material. The intertubular horn has a wave like appearance (Baillie and Fiford, 1996).

The medullar and cortical cells of the tubular horn and the intertubular horn have been found to have differences in their mechanical stability (Budras and Mulling, 1998). Medullar cells have been found to be formed at the tip of the dermal papillae while cortical cells originate over the entire side surface of the dermal papillae and as a consequence, the nutrient supply was found to be better for the cortical cells than for the medullar cells. The lower supply of nutrients has been found to result in incomplete keratinisation of medullar cells, reduced formation of disulphide bonds and compromised production of intercellular cementing substance. Medullar cells were found to be transformed into cell debris that fell out of the centre of the tubules, while the cortical cells underwent a complete keratinisation and were harder and well attached to each other (Kasapi and Gosline, 1997; Budras and Mulling, 1998).

The intertubular horn between the horn tubules was found to be made of many long thin cells and to be of moderate strength. The cells in the intertubular horn were found to run parallel to each other until a tubule was reached, than the cells diverged and converged around it. Cells around the tubules were found to be smaller and to run around the inside of the tubule, forming the wall of the tubule. The wall was found to be made of 3 to 5 layers of cells (Baillie and Fiford, 1996). The presence of the medullar cavity was found to affect the stress concentration within the tubules and to act as stress concentrator within the tubular structure during loading. The increase in the stiffness of the tubular wall was found to reduce the stress concentration (Newlyn *et al.*, 1999). According to Kasapi and Gosline (1998) the medular cavities have a small influence over the increase of the bending stiffness of the hoof wall and may increase the resistance to compressive failure of the wall. Tubules were found to act increasing the fracture toughness of the wall and to act redirecting cracks away from the dermis (Kasapi and Gosline, 1997). The tubules did not affect the hydration of the wall horn through capillary action and did not conduct water vapour down the tubule length. Rather, they facilitated the dehydration of the distal surface of the wall. The most hydrated areas of the wall were found to be adjacent to the dermal tissue (Kasapi and Gosline, 1998). The outer surface of the wall was found to act as a water barrier (Kempson and Campbell, 1998).

A high number of tubules per area and a small ratio between the centre of the tubule and the wall of the tubule were characteristics required for a hoof horn of good quality (Leopold and Prietz, 1980; Voges *et al.*, 2004). The wall horn has been found to contain a greater density of tubules (≈ 55 to 80/mm^2) than the sole and heel horn (≈ 20/mm^2) (Singh *et al.*, 1992; Singh, 1993; Brizzi *et al.*, 1998). Leopold and Prietz (1980) found a significant higher density of tubules in the wall horn of the hind claws (8.7 % more tubules/area) when compared with the front claws. In the bovine wall horn the diameter of the tubules was found to be greater in the dorsal part (0.0403 mm) when compared to the axial (0.0316 mm) and abaxial (0.0379 mm) parts of the wall. The diameter of the tubular medula increased with increasing tubule diameter. The mean diameter of the tubular medula of the wall horn was 12.1 % significantly greater for the hind claws when compared to the front claws (Leopold and Prietz, 1980). Morphological differences between the coronary, sole, heel and terminal horn of the white line are presented in Table 1.5.

In the equine hoof wall the morphology of the tubules, the organisation of the intertubular material and the relationship between both were found to be related to the position through the wall thickness. The shape and cross sectional area of the tubules varied according to this position (Kasapi and Gosline, 1997). Tubules of the inner region of the wall had a smaller medular cavity and were circular in cross-section. Tubules of the outermost region of the wall were elliptical in shape and had a bigger circular cross-section (Kasapi and Gosline, 1997). The density of tubules decreased from the outermost region of the wall to the inner region (Reilly *et al.*, 1996). The tubules of the equine hoof wall were made of different cell layers that varied according to the orientation to the centre of the tubule.

Kasapi and Gosline (1997) distinguished 3 types of cell layers and called them lamellae. The inner lamellae was situated near the centre of the tubule, it was composed of 1 to 2 cell layers and its thickness varied from 5.1 to 7.5 μm depending on the wall region. The middle lamellae did have more cell layers and a mean thickness of 9.9 μm. The outer lamellae consisted of 2 to 3 cell layers and had a thickness of 13.5 μm. The thickness of middle and outer lamellae did not vary between the regions of the wall.

Table 1.5 ***Morphological differences between the coronary, sole, heel and terminal horn of the white line***

	Coronary horn	Sole horn	Heel horn	Terminal horn of white line
Tubule density (numb./mm^2)	55-80	20	20	-
Tubule diameter (μm)	32-40	50-110	120-180	130-350
Medullar area	small	small	wide	wide
Cornification patern	hard	hard	soft	soft
Water uptake (%)	26.5-29.3	-	31.6	-
Hardness (N/mm$^{2)}$	25.7	12.9	6.8-9.3	-
Appearance of cells	Filled with keratin proteins and IFAP	Filled with keratin proteins and IFAP	Keratin material separated by liquid	-
Keratin filaments	Medium-long	Medium-long	short	-
Cross linking of keratin filaments	high	high	low	-

Source: Leopold and Prietz (1980); Mulling *et al.* (1994); Budras and Mulling (1998); Budras and Patan (2003)

In the bovine heel horn the horn tubules were wider (120-180 μm) with thin layers of cortical cells and had greater medullar area. There was also a great amount of intertubular horn. The tubules were found to be arranged in longitudinal spirals. In the sole horn the diameter of the tubules was smaller (50-110 μm), the tubules had an elliptical shape and the medullar area was found to be smaller when compared with the cortical area (Mulling *et al.*, 1994; Budras and Mulling, 1998).

The white line consisted of the cap-horn, the laminar horn leaflets and the terminal horn. The cornification of the cap-horn and the laminar horn was found to occur according to hard cornification patterns and the cornification of the terminal horn according to soft cornification patterns. The laminar horn leaflets were found to be produced in the coronary region and to have long keratin filaments with a high rate of cross-linking, providing structural rigidity to the white line (Hoblet, 2001). The space between cells was found to be small and the intercellular cementing substance was mainly made of glycoproteins. The cap and terminal horn originated from the epidermis covering the distal dermal laminae of the wall. The cells in the cap-horn were filled with small to medium sized keratin filaments and the rate of crosslinking was small. The intercellular space was wider in some areas and glycoproteins constituted a great part of the intercellular cementing substance (Budras *et al.*, 1996). The terminal horn was built by horn tubules that had a wide diameter (130-350 μm) and great medullar areas comprised of cell debris where lipids and glycoproteins accumulated between the cell debris resulting in it having a brittle consistency. It contained also a high amount of intercellular cementing substance (Budras *et al.*, 1996). The formation of the cap-horn and terminal horn allows a more flexible attachment between cells. These morphological differences explain the high incidence of alterations in horn formation that have been found at the white line (Mulling *et al.*, 1994). The outer part of the white line, where the soft horn has bonded to the harder coronary horn, has been found to be especially susceptible to tearing. A high horn production rate at the abaxial termination of the white line has been found to have a negative influence on the horn quality and could be the explanation for increased number of white line lesions. A high horn production rate has been found to lead to incomplete keratinization and production of softer horn. The areas of high horn production have been found to be also more susceptible to vascular disturbances (Budras *et al.*, 1996).

1.4.5 The process of keratinisation

The production of the hoof horn has been found to begin with the division of cells in the basal layer and ends with the death of the cells at the end of their life cycle in the upper spiny layer. The period of differentiation of epidermal cells is called keratinisation and the death of the cells during terminal differentiation is called cornification (Mulling *et al.*, 1998).

It has been found that in the basal layer the keratinising cells start to produce keratin proteins, this production increases in intensity in the Stratum spinosum (spiny cell layer) and Stratum granulosum. There are two different groups of keratins that have been found to be synthesised: the filamentous keratin proteins and intermediate filament associated proteins. The keratin filaments have been found to be synthesised in the Stratum granulosum. The keratin proteins of the bovine epidermis are characterised by their helical structure and have a molecular weight of 41 to 80 kD (Budras *et al.*, 1998). The intermediate filament associated proteins (IFAP) have been found to be synthesised in the spiny cell layer close to the cornified layer, in the case of hard cornification and in the Stratum granulosum in the case of smooth cornification. The main function of the IFAP is to aggregate keratin filaments to larger units by the formation of disulphide bonds. These filament-associated proteins were characterised by a high content in cystine a sulphur containing amino acid. IFAP have a molecular weight of 15 to 30 kD (Budras *et al.*, 1998). The crosslinked keratins formed a stable protein-complex in the cells. In the Stratum corneum the cells were totally cornified, filled with keratin filaments that were linked together by filament-associated proteins. The cells of the stratum spinosum were attached to the cells of the stratum corneum via desmosomes (Bolliger and Geyer, 1992). At areas of cell-to-cell adhesion via desmosomes thick bundles of keratin filaments reinforced the cytoskeleton (Budras *et al.*, 1998). In the Stratum corneum the cell membrane is made rigid through the cross-linking of the cell envelope proteins (Grosenbaugh and Hood, 1993; Budras and Mulling, 1998; Frohnes and Budras, 2001).

Horn from different sites of the claw has been found to vary in the keratin protein patterns. The level of hydration, determined by the keratin material within the cells enables the horn to vary its mechanical properties and in this way the horn is able to adapt to different functions in different parts of the claw. Diffuse water storage takes place at the different horn cell components (Budras and Patan, 2003). The frog epithelium had a greater ability to hydrate (31.6 %) when compared to the dorsal (26.5 %), axial (29.3 %) and abaxial (27.6 %) wall (Leopold and Prietz, 1980). The horn of the wall (25.7 N/mm^2) and sole (12.9 N/mm^2) of the claw has been found to be harder and the heel (bulb) horn (6.8-9.3 N/mm^2) has been found to be softer and to have greater elasticity. The cornification of the heel horn has been found to occur according to soft cornification patterns. In the heel horn, spaces filled with liquid separated the keratin material, fat globules accumulated in the cells, the keratin filaments have been found to be shorter and the rate of cross-linking was small. The total amount of keratin has been found to be lower in the heel horn than in the sole and wall horns (Frohnes and Budras, 2001). In the sole horn filamentous keratin proteins and filament-associated proteins filled the cornified cells and keratin filaments were found to be medium to long and the rate of cross-linking to be high (Bertrand and Gosline, 1987; Mulling *et al.*, 1994; Vermunt and Greenough, 1995: Hendry *et al.*, 1997).

The intercellular cementing substance was found to be produced by the keratinising epidermal cells of the middle spiny layers, in the Golgi apparatus and endoplasmatic reticulum followed by which it was exocytosed into the intercellular space (Mulling *et al.*, 1998). This cementing substance has been found to consist of glycoproteins and complex lipids, such as phosphoglycolipids (Elias, 1981). The main function of the cementing substance is to establish cell to cell adhesion and disturbance in the synthesis of the cementing substance has been found to result in the separation of the cells and the formation of brittle horn (Baillie and Fiford, 1996; Zaun, 1997; Mulling *et al.*, 1998). A high degree of plasma membrane interdigitation has been found to optimise the cell adhesion (Kasapi and Gosline, 1996). In addition, the cementing substance has been found to establish a permeability barrier in the intercellular space and this barrier prevents the passage of aqueous solutions through the horn, protecting the horn cells from loss of water and/or extreme hydration (Mulling and Budras, 1998).

The composition of the cementing substance has been found to vary between the different parts of the hoof (heel, sole and wall) and these differences have been found to be related to the hardness and flexibility of the horn in the different sites (Grosenbaugh and Hood, 1993). The heel horn has been found to have a greater proportion of phosoholipids and lower proportion of glycolipids, which provided an efficient water barrier, while the sole and wall horn had a higher concentration of glycoproteins, which conferred greater cell to cell adhesion (Mulling *et al.*, 1994). The heel horn was found to have a greater proportion of intercellular cementing substance and in some areas the intercellular space was wider, being still filled with intercellular cementing substance (Mulling *et al.*, 1994; Frohnes and Budras, 2001). The horn of the bulb and frog in horses presented a high water uptake capacity and low water release rate, displaying higher water storage capacity when compared with the coronary horn (Patan and Budras, 2003). Offer and Logue (1998) found significant differences in the fatty acid profile in the hoof horn of lame and non-lame cows and considered these changes to be related to changes in the intercellular cementing substance. Lame cows had significantly higher proportions of C18:2, C18:3, C20:3 and C20:4 and significantly lower proportions of C16:0, C16:1, C18:1, C18:3 and C20:0.

1.4.6 Hoof horn growth and wear rates

Claw horn is in a state of continuous turnover (Vermunt and Greenough, 1995). Parturition, diet, season and housing are considered to interact and to affect the growth and wear rates of the claws (Livesey *et al.*, 1998; Offer *et al*, 2000). Chaplin *et al.*, (2000) found a significant lower growth and wear rates in lactating heifers (4.17 and 6.68 mm/m, respectively) when compared to pregnant heifers (5.56 and 8.79 mm/m, respectively) housed in the same conditions over the same period. However, throughout the lactation hoof growth rate has been found to remain at a similar level (2.1 mm/m) and to decline in the dry period (0.17 mm/m) (Livesey *et al.*, 1998). First lactation heifers exhibited higher growth rates than mature cows (Vermunt and Greenough, 1995). Heifers are considered to be more susceptible than cows to the sub-optimal supply of essential nutrients (Livesey *et al.*, 1998).

According to Leach *et al.* (1997), Livesey *et al.* (1998) and Offer *et al.* (2001) wear rates increased after parturition and were higher between weeks 4 and 9 after calving (*prepartum* - 4.2mm/m and weeks 4 to 9 *postpartum* - 7.4 mm/m). This difference may occur because of behaviour changes in early lactation (shorter lying time). Wear rates decreased in the dry period (Livesey *et al.*, 1998). Hoof horn growth was influenced by rate of wear (Vokey *et al.*, 2001) and McCallum (1999) found that the pressure on the horn stimulated the hoof horn growth rate to increase.

Season has been found to affect hoof growth and wear rates. An increase in the wear rate of the claw horn has been found to be related to the winter housing of dairy cattle in cubicles, on concrete floors, resulting in a negative net growth rate (Leach *et al.* 1997, Livesey *et al.*, 1998; Whay, 1998; Offer *et al*, 2000; Vokey *et al.*, 2001) (Table 1.6). The overall mean net growth rate was -0.25 mm per month (Leach *et al.*, 1997). However, Offer *et al.* (2000) reported a mean growth rate of the wall horn over 4 lactations of 4.5 mm/month, in animals housed during the winter period and turned out to pasture during the summer. The angle of the dorsal border changes during lactation and has been found to be steeper in the first half of lactation when the claws were shorter (Offer *et al*, 2000). When animals were at pasture the claws were found to be slightly longer and the angles shallower (Boelling and Pollot, 1998; Offer *et al*, 2000). Clark and Rakes (1982) found increased growth rates of the hooves during the spring and the summer periods (6.67-11.08 mm/m) and lower growth rate during autumn and winter periods (4.17-6.49). The authors attributed these differences to changes in the photoperiod.

The hoof wear rate has been found to vary over the sole surface and Tranter and Morris (1992) reported that sole wear occurred more rapidly along the abaxial (outer) edge of the weight-bearing surface (2.5-3.2 weeks for 1.5 mm disappear), was lower on the toe and heel areas (4.3-4.6 weeks for 1.5 mm disappear) and lowest on the mid-sole area (4.8-5.0 weeks for 1.5 mm disappear). The growth rate was greater along the abaxial area and lower in the heel and axial areas, corresponding to the same areas of greater and lesser wear rates. The wear rates of the wall and sole horn were found to be greater on the lateral digits than on the medial digits (Toussaint-Raven, 1985; Offer *et al*, 2000) (Table 1.6). It has been found that it takes approximately 2 months for the newly formed sole and heel horn to reach the weight-bearing surface (Hoblet, 2001). Increased wear rate when compared with growth rate was found to cause the plantar surface of the claw to become flat, which extended the weight bearing surface over the entire sole. This increased pressure on the sole area could lead to increased growth rate of the horn in these areas and the abnormal weight distribution may cause trauma to the corium of the sole (Livesey *et al.*, 1998). Imbalance between growth and wear rates could also lead to the formation of a longer anterior wall and shorter heel, again altering the normal weight distribution of the claw (Toussaint-Raven, 1985).

Clark and Rakes (1982) reported that the hooves of the hind claws grew faster (4.17-11.08 mm/month) than the hooves of the front claws (4.09-8.99 mm/month). On the hind claws the abaxial part of the wall grew faster (5.83-11.08 mm/month) than the dorsal wall (4.17-8.95 mm/month), however, this difference in growth rates was not found on the front hooves.

Nutrition has been found to affect hoof growth rate. Clark and Rakes (1982) and Livesey *et al.* (1998) found an increase in growth rate in response to methionine supplementation (8.95-11.08 mm/month for supplemented cows and 6.03-7.87 mm/month for control cows).

Table 1.6 ***Hoof growth and wear rates of dairy cows reported by different authors***

	Offer *et al.* (2000)	Livesey *et al.* (1998)	Vokey *et al.* (2001)	Offer *et al.* (2001)	Leach *et al.* (1997)	Chaplin *et al.* (2000)
Rates (mm/m)						
Growth medial	5.2	-	5.3-6.6	-	-	-
Wear medial	5.4	-	3.4-4.9	-	-	-
Growth lateral	5.4	2.2	5.6-6.6	5.1/5.7	5.17	4.17
Wear lateral	6.2	1.8	3.7-4.9	5.5/4.8	5.51	6.68
Effects						
Season	autumn	-	-	autumn	autumn	Jun/Jul
Floor surface	concrete	concrete straw	concrete	concrete	concrete	concrete
Housing	cubicles	cubicle straw yard	cubicles	cubicles	cubicles	cubicles
Lactation	first	first	varied	first	first	first

1.5 The development of claw horn lesions

Ossent and Lischer (1998) proposed the existence of a three-stage pathogenesis to explain the process involved in the development of claw horn lesions. In the first stage of this process an unknown factor triggers a pathological response in the blood vessels of the parietal and coronary corium. Vessel paralysis, vasodilatation and opening of arterio-venous shunts reduce the blood supply to the corium. These changes lead to the hypoxia and degeneration of the epidermal basal cells and to separation of the dermal and epidermal layers. This process is called laminitis and is referred to as stage 1. In the second stage, the position of the third phalanx change in relation to the softer tissues, causing pressure induced haemorrhages and necrosis of the corium of the sole. Ishi and Kushiro-chiku (2000) demonstrated that when the difference between the height of the axial point and the abaxial point of the distal phalanx increased and the axial point was lower than the abaxial point, there was a significant increase in the lesion score of the claw horn, probably related to compression of the dermis and epidermis of the sole horn. In the third stage, blood and cell debris appear within the claw horn of the sole and white line. However, it was not demonstrated in the dairy cow that the separation of the dermal-epidermal junction and occurrence of laminitis were responsible for the sinking and rotation of the third phalanx (Lischer and Ossent, 2002). Singh *et al.* (1992) found that dairy cows with overgrown claws had altered horn production in the region of the papillae of the sole, in the form of the presence of non-keratinised horn, and thickening of the arterioles and arteries in the sole corium. However, the dermal epidermal junction (laminae) of the wall horn had non-keratinised horn, but there was no evidence of alteration of the blood vessels or indication of an inflammatory process of the laminae. Kempson and Logue (1998) demonstrated that cows with sole ulcers did not present any changes in the dermal epidermal junction of the wall and cows with laminitis had severe changes in the dermal epidermal junction with no changes in the sole horn.

Vermunt and Greenough (1994), Logue (1999) and Mulling and Lischer (2002) listed nutrition, management, conformation, parturition and age as predisposing factors that may cause vascular changes in the corium, leading to reduced hoof horn growth and the development of sole and white lesions. The combined effect of several factors would be more important than any single effect of one factor, suggesting a multifactorial aetiology. Some authors (Bradley *et al.*, 1989; Vermunt and Greenough, 1994; Greenough and Vermunt, 1996; Ossent and Lischer, 1998; Hirschberg and Mulling, 2002) have postulated the theory that the release of vasoactive substances such as histamine, endotoxins and lactate, that are related to nutritional factors and systemic diseases, may be the causal factor. These substances would cause vasoconstriction and reduce the capillary circulation in the corium. Singh *et al.* (1994) induced vascular changes in the sole, parietal and coronary corium, after infusion of endotoxin. Abnormal keratinization with the presence of densely stained material in the horn tubules, indistinct cell junctions on the stratum spinosum, presence of keratin filaments in the stratum spinosum and presence of degenerated cells in the dermal papillae of the sole and retracted laminae of the wall were observed in heifers after infusion of endotoxins (Singh *et al.*, 1994). However, Webster (1998) and Holah *et al.* (2000) proposed that hormonal changes associated with parturition were a major contributory factor in the development of these lesions. Holah *et al.* (2000) believed that the collagen fibres, that connect the third phalanx to the horn capsule, become softer during the gestation and early lactation period through the action of hormones. This effect was thought to allow the third phalanx to sink and compress the corium. Reduced rigidity and reduced load bearing capacity of the connective tissue suspending the pedal bone has been found in heifers two weeks prior to parturition. These changes progressed until 12 weeks *postpartum* in the hind claws, but were not found in maiden heifers (Tarlton and Webster, 2002). No differences in the force required to cause failure of the suspensory apparatus was noticed between front and hind and inner and outer claws in 20 month old beef bulls (Maierl *et al.*, 2002). Webster (2002) emphasised the importance of the intensity and duration of the mechanical stresses to which the claws are subjected and their effect on the formation of sole and white line haemorrhages.

Ossent and Lischer (2002) considered that the cushioning action of the heel was important to determine the level of compression of the sole and heel areas situated under the pedal bone. In cattle, fat cushions situated in the subcutis act as shock absorbers. These cushions, called digital cushions, have been found to change in consistency and quantity with increasing age (Raber *et al.*, 2002). In claws with sole ulcers the fat in these cushions has been found to be replaced by collagenous connective tissue which has been found to have a lower shock absorbing capacity (Ossent and Lischer, 2002).

Initial molecular changes in the dermis that were followed by functional disturbances of the sole horn have been described and included the activation of matrix-metallonoproteinases (MMPs) (Tarlton and Webster, 2002; Hendry *et al.*, 2002), activation of growth and necrosis factors, molecular alterations in the basement membrane (Hendry *et al.*, 2002) and alterations of capillary walls (Mulling and Lischer, 2002). Elevated levels of MMPs 2 and 9 were observed in ulcerated bovine claw tissue (Hendry *et al.*, 2003). The activation of MMPs has been linked to the degradation of collagen fibres. Mulling *et al.* (2004) demonstrated *in vitro* that the exposure of dermal collagen fibres to MMP-2 and MMP-9 led to a time dependent structural disintegration of the collagen network. The activation of MMP-2 coincided with reduced rigidity and decreased load-bearing capacity of the connective tissue suspending the pedal bone (Tarlton and Webster, 2002). Knott *et al.* (2004) demonstrated an increase in the collagen cross-link ratios around parturition that was associated with tissue repair and remodelling. The damage of a limited number of collagen fibres would lead to microrupture and a slight increase in length of a small fibre bundle. The overall elongation and instability would depend on the number of fibres affected (Mulling *et al.*, 2004). MMP-9 was not found in heifers during the *peripartum* period and in maiden heifers, which indicated that no inflammatory process was involved in the pathology of hoof lesions in the *peripartum* period (Tarlton and Webster, 2002).

Alterations of the circulation included an increase in the capillary pressure and post-capillary resistance and therefore increase of the tissue pressure (Christmann *et al.*, 2002). The constriction of digital veins was thought to be the initial step in these events and no opening of arterio-venous shunts was reported. Some areas of the claw have been found to be supplied by a single branch or very few primary and secondary branches of the terminal arch artery and were found to have a high level of circulatory disturbances. These areas included the distal part of the axial and abaxial coronary segment and of the abaxial and axial wall, the median areas of the sole and of the distal bulb segment (Hirschberg and Mulling, 2002). Dairy cows with overgrown sole presented thickening of the tunica media, intima and adventicia of the wall of the blood vessels of the sole corium. Alterations were observed mainly on the sole ulcer site (transition of zone 3 to 4). The formation of horn was altered on these areas, with a partial or complete disappearance of keratin precursors and formation of fewer disulphide bonds. Abnormal branching of the papillae of the sole and formation of buds and shortening of the laminae of the wall horn was observed. These changes indicate the formation of a horn with compromised structure (Singh *et al.*, 1992).

Terms like laminitis and subclinical laminitis have been used to describe the lesions of the sole and the white line, because the pathogenesis of claw horn lesions in dairy cows was believed to be associated with a laminitic process, inflammatory process of the laminae (Bradley *et al.*, 1989; Vermunt and Greenough, 1994; Greenough and Vermunt, 1996; Ossent and Lischer, 1998). However, the laminitic process theory and the use of these terms has recently been questioned (Webster, 2002; Ossent and Lischer, 2002). Singh *et al.* (1994) and Webster (2001) used the term Pododermatitis aseptica circumscripta and Hoblet (2001) used the term claw horn disruption. In this text the descriptive term claw horn lesions will be used.

1.6 Factors influencing the incidence of lameness and claw horn lesions

Logue (1999) considered parturition and lactation, housing and environment, age and season to be major factors related with the development of claw horn lesions. Other factors that were less strongly correlated with the development of claw horn lesions included: milk yield, genetic merit, nutrition and stockmanship. In a review of published papers Logue (2002) listed the environmental factors associated with a significant increase in lameness. These factors were; hard and abrasive surfaces, wet and dirty surfaces, slatted floors, poor quality cow tracks, inadequate cow housing comfort, larger herds, higher milk yield, buying in replacement animals and therefore increasing the risk of infectious diseases and reduced time at pasture. Mill and Ward (1994) related a low farmer awareness and knowledge of lameness to a higher incidence of lameness. Logue (1999) concluded that the occurrence of claw horn lesions was the result of the interaction of interdependent factors.

1.6.1 Effect of breeding, conformation and genetics on lameness

The increase in levels of lameness in the United Kingdom over the last 20 years may be linked with the 15 % increase in annual milk yield per cow during this period (Logue *et al.*, 1999). While changes in general nutrition and management over this period may also have contributed (Logue *et al.*, 1999) the widespread introduction of the Holstein breed in the United Kingdom has been recognised as a contributory factor in increasing levels of lameness (Veerkamp *et al.*, 1995). Emanuelson (1988), Lyons *et al.* (1991), Pryce *et al.* (1998) and Wassmuth *et al.* (1999) found a negative relationship between milk yield and dairy cow health. Emanuelson (1988) cited several authors and quoted that the genetic correlation between milk yield and clinical mastitis or somatic cell count ranged from -0.10 to 0.66, being generally positive and the genetic correlation between milk yield and ketosis ranged form 0.19 to 0.35. Lyons *et al.* (1991) found that the genetic correlation between milk yield and reproductive, mammary, digestive, locomotive and respiratory problems were of -0.27, 0.18, 0.44, 0.48 and 0.02 respectively. Wassmuth *et al.* (1999) reported genetic correlations between energy corrected milk yield and sole ulcer, calving interval, mastitis and ketosis in Danish Red and Danish Black and White of 0.44 to 0.59, 0.46 to 0.69, 0.32 to 0.44 and 0.30 to 0.39, respectively. Dillon *et al.* (1999) and Pryce and Lovendahl (1999) found that higher milk yield suppressed the onset of cyclicity (more days to first heat and to first service) in dairy cows.

According to Distl (1999) the selection of cows for higher milk yield compromises the soundness of feet and legs. Higher milk yield in the first 5 days of lactation and at day 21 was associated with an increase in clinical lameness at the beginning of lactation and the majority of the lameness was associated with sole and white line lesions (Deluyker *et al.*, 1991). Enevoldsen *et al.* (1991) found that milk yield in early lactation and high milk yield combined with high body weight in the first lactation, were positively correlated with an increase in the levels of sole ulcers. In subsequent lactations, a low milk yield combined with a high body weight was highly positively correlated with an increase in the level of sole ulcers. In addition, greater udder size has been correlated with a high locomotion score as parities increased (Boelling and Pollot, 1998).

The selection of cows for higher milk yield has increased the risk of metabolic stress (Emanuelson, 1988; Pryce and Lovendahl, 1999). Metabolic stress was defined by Knight *et al.* (1999) as the amount of metabolic load that cannot physiologically be sustained. The occurrence of metabolic stress and related production diseases would however depend also on the management of the cows (Nielsen, 1999) and low genetic merit and high genetic merit cows can suffer from metabolic stress (Knight *et al.*, 1999; Logue *et al.*, 1999).

Veerkamp and Koenen (1999) calculated that with a genetic selection for only milk yield, the correlated increase in feed intake was not sufficient to provide the nutrient requirements for the increased yield. In this way the selection for milk yield increased the deficit between energy intake and output during early lactation.

Ingvartsen *et al.* (1999) described the possibility that the potential to mobilise and deposit body reserves is genetically determined. High genetic merit cows have been found to partition a greater level of energy into milk production and significantly less energy to body reserves (Knight *et al.*, 1999; Thomas *et al.*, 1999; Veerkamp and Koenen, 1999). While high genetic merit cows were taller, they had lower scores for body condition than low genetic merit cows (Veerkamp and Koenen, 1999). Fregonesi and Leaver (2001) found that cows with low milk yield (19.1 kg/day) had significantly greater live weight (630 kg vs. 604 kg) and body condition score (2.97 vs. 2.25) than high yielding cows (32.1 kg/day) and a lower dry matter intake (20.4 kg DM/ day vs. 22.4 kg DM/ day). Dillon *et al.* (1999) reported similar results, with high genetic merit cows (PD for fat and protein yield = +16.7kg and +15.2 kg) having significantly greater live weight loss up to week 10 of lactation (- 0.58 kg/day vs. – 0.39 kg/day), lower live weight gain between the tenth week and the end of the lactation (0.38 kg/day vs. 0.45 kg/day) and higher live weight gains during the dry period (1.27 kg/day vs. 1.01 kg/day) compared with medium genetic merit cows (PD for fat and protein yield = +9.7 kg and +7.2 kg).

Genetic correlations with milk yield indicated that a selection for high yield would result in lower live weight gain (-0.08 to -0.84) and greater negative energy balance (-0.40 to -0.84) in duration and magnitude (Veerkamp and Koenen, 1999). The incorporation of a selected number of traits in a selection index, such as live weight change, condition score change and food intake, would allow the use of genetic selection to reduce the risk of excessive mobilisation of body tissue during early lactation and avoid the negative effects of energy balance on health of dairy cows (Pryce and Lovendahl, 1999; Veerkamp and Koenen, 1999).

Genetic correlations between mammary, digestive, locomotive and respiratory problems were high (0.34 – 0.87), indicating that cows that were susceptible to one health problem were likely to be susceptible to other health disorders (Lyons *et al.,* 1991). Lyons *et al.* (1991) found that heritabilities for locomotive traits were high enough for them to be included in a selection program. Heritabilities for locomotive traits are demonstrated in Table 1.7. Significant correlations were obtained between measurements such as, length and width of the claw and circumference of the cannon bone, made on Danish red, Danish Friesian and Jersey bulls at the end of their performance test and the traits hock quality (-0.27 to -0.62) and bone structure (-0.21 to -0.90) of their daughters (Boelling *et al.*, 2001). Kushiro-chiku (1998) demonstrated that the daughters of cows that had laminitis had a significantly higher incidence of laminitis than daughters of cows that did not have laminitis. Veerkamp *et al.* (1995) incorporated 4 linear traits (udder depth, angularity, teat length and foot angle) and 3 milk yield traits in a selection index (ITEM) to increase the longevity, or longer herd life, of cows. Brotherstone and Hill (1991) found high genetic correlations between longer herd life and several linear traits such as fore udder attachment (0.29), rear udder width (0.52), teat position (0.38), teat length (-0.41) and total animal score (0.43). Fore udder attachment, rear udder width, teat length and foot angle were the type traits that showed the highest positive correlations between each lactation. Van Dorp *et al.* (2004) reported that cows with increased udder quality (better attachment and greater depth) had a more favourable locomotion.

Table 1.7 ***Heritabilities for locomotive traits***

	Brotherstone and Hill (1991)	Lyons *et al.* (1991)	Choi and McDaniel (1993)	Boelling and Pollot (1998b)	Boelling *et al.* (2001)	Van Dorp *et al.* (2004)
Locomotion	-	-	-	0.06-0.11	-	0.05-0.07
Locomotive problems	-	0.16	-	-	-	-
Feet and legs score	-	-	-	-	-	0.30
Foot angle - trait	0.13	-	-	0.11-0.17	0.11-0.15	0.10
Bone quality	-	-	-	-	0.19-0.32	0.30
Rear leg set	0.09	-	-	0.11-0.19	0.09-0.24	0.23
Claw angle	-	-	0.02-0.31	-	-	-
Claw length	-	-	0.13-0.58	-	0.07-0.20	-

The outer claw on the hind foot has a significantly greater percentage of lesions and this has been linked to the foot and leg conformation of the cow (FAWC, 1997). While foot and leg conformation are genetically determined, they also are affected by environmental conditions, ageing and disease (Bergsten, 2001). Choi and McDaniel (1993) found a positive genetic correlation between milk yield and hoof length (0.11 to 0.44). However, hoof length was negatively correlated (-0.34) and hoof angle was positively correlated (0.87) with survival. McDaniel (1995) considered that it was the claw diagonal (length between the coronary line at the heel and the tip of the toe) that predicted the longevity of the cow more accurately than other traits. Lower dorsal angle and longer dorsal border of the claw has been found to be correlated to higher incidence of lameness (Manson and Leaver, 1989). A steep foot angle and straight rear legs have been related to less lameness incidence and lower locomotion scores (Boettcher *et al.*, 1998; Boelling and Pollot, 1998b; Van Dorp *et al.*, 2004). Locomotion score had high genetic correlation with the conformation traits total feet and legs score (-0.45), foot angle (-0.76) and rear leg set -0.68) (Boettcher *et al.*, 1998).

Boettcher *et al.* (1998) found a positive correlation between low foot angle (-0.76), wide rumps (0.63), cows that stand with hock pointing inwards and toe pointing outwards (-0.68) and increased levels of lameness. Greater scores for sickled legs were associated with higher scores for locomotion (0.36) (Boelling and Pollott, 1998a). Collard *et al.* (2000) reported that a greater chest width was associated with lower levels of locomotive problems (-0.18). Boettcher *et al.* (1997) reported also a positive genetic correlation between body depth and lameness (0.42), indicating that sires that produced larger daughters had daughters that were more predisposed to lameness. Similarly, Bowell *et al.* (2003) found a positive correlation between body condition score and locomotion (0.43), animals with higher condition score presenting a worsening of the locomotion score. However, Van Dorp *et al.* (2004) found that cows with higher body condition score had genetically better locomotion (-0.27).

Variations of claw shape and incidence of lameness have been found to differ between cattle breeds and between sires within breeds of cattle (Logue *et al.*, 1994; Huang *et al.*, 1995; Vermunt and Greenough, 1995). The contribution of the sire increased with parity and might be important for sole ulcer and white-line disease (Hirst *et al.*, 2002). Laven *et al.* (2004) did not find a significant correlation between the sire foot angle, locomotion score and legs and feet score with the cows hoof lesion assessment. Distl (1999) found, that young bulls of the German Fleckvieh breed had significantly higher heels, greater hoof angle and smaller sole area and their daughters had fewer feet and leg disorders. In the German Holstein breed hoof angle and sole area were negatively correlated with disorders of the hoof in the next generation (Distl, 1999). Holstein and Guernsey cattle have been found to have higher levels of laminitis, sole ulcer, white line separation, heel erosion and interdigital dermatitis when compared with Ayrshire, Jersey and Brown Swiss cattle. While Brown Swiss cattle were found to have a greater incidence of corkscrew claw. However, it was the Jersey cattle breed that had fewest hoof disorders when compared with other dairy breeds (Huang *et al.*, 1995; Boelling *et al.*, 2001).

The colour of the hoof horn was reported to have an influence over the bruising of the horn, with a lower incidence of lameness and less bruising of the sole being found on cows with black hoof pigmentation (Logue *et al.*, 1994). According to Leopold and Prietz (1980), hoof horn with pigmentation presented a lower capacity to absorb water (27.4 vs. 29.0%) and a lower wear rate (38.6 vs. 41.4%) compared to non pigmented horn. However, Hepburn *et al.* (2004) reported that non-pigmented claw wall horn in cattle was significantly harder (46.5 vs. 40.3 nearer the coronary line and 68.5 vs. 64.8 away from the coronary line) in areas up to 4.5 cm under the coronary horn when compared to pigmented horn. No difference was measured in the dry matter of these horn samples.

1.6.2 Effect of parturition and lactation on lameness

Hormonal changes associated with parturition are believed to be the major contributory factor in the development of sole and white line lesions (Holah *et al.*, 1998). Relaxin is one of the hormones known to be particularly active at parturition, increasing the ability of collagen and other connective tissues to deform and allowing the movement of the pelvis during parturition. Therefore relaxin may also loosen the collagen attachments between the distal phalanx and the basement membrane of the laminae, thus increasing the instability of the dermal-epidermal junction (Holah *et al.*, 1998).

Growth hormone (GH), insulin, glucocorticoids and thyroid hormones regulate the adipose tissue metabolism in ruminants (Vernon, 2002). During the peripartal period cows are frequently in the status of negative energy balance and during this period serum concentrations of GH, prolactin and glucocorticoids have been found to increase, while insulin and thyroid hormones levels decrease, resulting in decreased lipogenesis and increased lipolysis (Vernon, 2002). High yielding dairy cows have been found to have higher plasma concentrations of GH and reduced concentrations of insulin growth factor (IGF-1) and insulin (Taylor *et al.*, 2002). The potential regulators of the hoof epidermal keratinization include hydrocortisone, prolactin, thyroid hormones, epidermal growth factor (EGF) and micronutrients such as vitamin D_3 and retinoic acid (Hendry *et al.*, 1997). Receptors for EGF have been found to exist in the bovine hoof horn and EGF has been found to stimulate protein synthesis in hoof tissue explants. Protein synthesis was also influenced by hormone levels. Insulin increased the rate of protein and DNA synthesis and therefore increased the rate of keratinisation. However, increased levels of cortisol and prolactin inhibited protein synthesis. Therefore, decreased levels of insulin during lactation and increased levels of prolactin and glucocorticoids may have a negative effect on keratinisation (Hendry *et al.*, 1997).

Pregnancy, decreased feed intake during late gestation, lactogenesis, and parturition have dramatic effects on metabolism in dairy cows during the transition period from three weeks before calving to three weeks after calving (Grummer, 1995). There is a decrease in the dry matter intake of approximately 10 to 30 % in late pregnancy and early lactation, which is mediated by hormones and neuropeptides and is related to the mobilisation of body reserves (Ingvartsen *et al.*, 1999; Hoblet, 2001; Drackley, 2002). During this time the dairy cows have been found to be in negative energy balance (Goff and Horst, 1997; Pryce and

Lovendahl, 1999; Thomas *et al.*, 1999) and the intake of energy should be maximised without compromising the rumen function. The intake of high quality forage is important during this period and any environmental restrictions limiting feed intake should be avoided (Thomas *et al.*, 1999). The energy balance is a function mainly of dry matter intake, live weight and milk yield over a period of time (Veerkamp and Thompson, 1999). *Prepartum* feed intake is positively correlated with *postpartum* intake so efforts to maximise feed intake should begin before calving. Increasing the nutrient density of the diet during the transition period may enhance feed intake. Feeding more fermentable carbohydrate during the *prepartum* period may adapt the microbial population to lactation diets, promote development of rumen papillae, increase absorptive capacity of the rumen epithelium, and reduce lipolysis by delivering more glucogenic precursors to the liver and enhancing blood insulin (Grummer, 1995). Depending on the magnitude and the duration of the decrease in intake and the negative energy balance the health of the cow could be compromised (Ingvartsen *et al.*, 1999). Minimum daily energy balance and total energy deficit were positively and significantly correlated (residual correlation = -0.20 and -0.25, respectively) with locomotive problems during lactation (Collard *et al.*, 2000).

A major source of energy during the *peripartum* period is fatty acids that are released from the adipose tissue and circulate as nonesterified free fatty acids (NEFA) in the blood. NEFA are taken up by the liver and oxidised to provide energy (Drackley, 2002). Plasma NEFA concentrations are highest at calving and decrease rapidly after calving. Plasma glucose concentration decreases during the transition period except for a transient increase associated with calving. Hepatic glucose is reduced and lipids are increased during the transition phase (Grummer, 1995; Drackley, 1999). Excessive NEFA uptake may lead to the development of fatty liver that impairs the ability of the liver to detoxify ammonia to urea and endotoxins (Drackley, 2002). Therefore, maintaining optimal liver function is important for the health of the cow particularly during the early lactation period (Drackley, 2002). Lischer *et al.* (2003) demonstrated that cows with high levels of plasma liver enzymes activity had a prolonged healing process of sole ulcers. Zaun (1997) related the occurrence of brittle nails in humans to the occurrence of liver diseases, skin diseases und anaemia. High levels of beta-hydroxybutyrate (BHB) (>0.9 mmol/l) were reported in 35 % and elevated urea levels (>5 mmol/l) in 60 % of cows in early lactation (Ward and Parker, 1999). High levels of BHB indicate a negative energy balance while high levels of urea indicate an imbalance between energy and protein in the rumen (Ward and Parker, 1999). Levels of BHB were found to be correlated with the percentage of weight loss and the percentage of body condition score loss (Logue *et al.*, 1999).

In ruminants, body reserves were negatively related with voluntary food intake during the early *postpartum* period. Cows with a higher body condition score at calving had a greater decrease in intake and greater mobilisation of body reserves following parturition and were found to be at a greater risk for developing metabolic disorders (Drackley, 2002). The management of the body condition during late lactation and the dry period has been found to be an important factor in determining the metabolic load of the cow during the *postpartum* period (Olsson *et al.*, 1998; Ingvartsen *et al.*, 1999; Thomas *et al.*, 1999). Thomas *et al.* (1999) found that spring calving cows lost significantly more weight and had a lower condition score than cows calving in the autumn. According to the authors, the availability of pasture at the end of the winter housing period influenced these results and the use of supplementary feeding could be used to avoid great loses of body condition of dairy cows. Drackley (1999) highlighted 5 areas related to the *peripartum* period were better understanding is needed: 1) the control of dry matter intake, 2) quantification of nutrient supply when dry matter intake and gut capacity are changing rapidly, 3) interaction amongst nutrition, metabolism and immune system, 4) metabolic regulation in liver, adipose tissue, muscle and digestive tract in the initiation of lactation and 5) effect of body condition and metabolic responses to different management strategies.

The partitioning of nutrients at parturition and beginning of lactation may have a possible influence on the composition of the horn (Ingvartsen *et al.*, 1999). Energy, protein and calcium have been found not to be obtained in sufficient quantities from the diet during this period and as a consequence these tend to be mobilised from body reserves. Drackley (1999) describes an insufficient supply of amino acids and glucogenic compounds. The shortfall in these nutrients and subsequent deficiencies affect the formation and subsequent hardness of hoof tissue (Leach *et al.*, 1998; Mulling *et al.*, 1999; Mulling, 2000).

An increased incidence of white line and sole lesions has been reported during the *postpartum* period (Enevoldsen *et al.*, 1991; Bergsten and Herlin, 1996; Bergsten and Frank, 1996; Vermunt and Greenough, 1996; Leach *et al.*, 1997; Livesey *et al.*, 1998; Chaplin *et al.*, 2000 and Offer *et al.*, 2000) (Table 1.8). Bell *et al.* (2004) reported that 97 % of heifers that were examined between day 60 and 120 of the first lactation had sole haemorrhages, 27 % had white line disease, 7 % had sole ulcers and 0.4 % had white line abscess. Haemorrhages in the claw horn have been found to be at their greatest levels between 100 and 120 days after parturition and declined in number and severity thereafter (Enevoldsen *et al.*, 1991; Leach *et al.*, 1997; Offer *et al.*, 1998 and 2000). The observed haemorrhages have been found to originate from insults that occurred approximately 4 to 8 weeks earlier, depending on wear and growth rates, because this was the time the newly formed sole horn took to reach the distal surface of the sole (Livesey *et al.*, 1998; Bergsten, 2001). The severity of heel erosions (Enevoldsen *et al.*, 1991; Vermunt and Greenough, 1991; Livesey *et al.*, 1998; Offer *et al.*, 2000) and locomotion score (Whay, 1998) was found also to increase during this period and were positively correlated with the occurrence of sole and white line lesions (Table 1.8).

White line lesions were at their greatest levels at 9 weeks (almost 90 % of claws affected and number of lesions near 300) and sole lesions (70 % of claws affected and number of lesions near 300) at 14 weeks *postpartum* in *primiparous* heifers (Leach *et al.*, 1997) (Table 1.8). The earlier increase in number and severity of white line lesions when compared to the sole lesions was explained through a differing response to the initial insult, differing growth and wear rates of the 2 areas or a differing origin of the lesion (Leach *et al.*, 1997; Le Fevre *et al.*, 2001). White line and sole lesions were found not to be highly correlated (Leach *et al.*, 1997). Offer *et al.* (2000) found that white line and sole lesions appeared at their greatest levels one week apart in first lactation heifers, but this time difference was greater in cows with increasing number of parities. The period of maximum lesion score of the white line became progressively later during subsequent lactations, corresponding with weeks 16, 21, 22 and 27 on lactation 1 to 4, respectively. The period of maximum lesion score of the sole area was at week 16 on the first lactation and at week 20 on subsequent lactations. The difference in the pattern of lesion formation of the sole and white line between the first and subsequent lactations may be related to repeated lesion formation, cumulative damage and to animals calving some time after housing in later lactations, as opposed to simultaneously in the first lactation (Offer *et al.*, 2000). Moreover, Webster (2001) found that the period of maximum lesions formation varied between management systems.

The increase in the frequency of milk fever, mastitis and ketosis occurrence with increasing lactation number is thought to be related to the increase in milk yield between the first and the third lactation (Nielsen, 1999). Logue *et al.* (1994), Huang *et al.* (1995) and Offer *et al.* (2000) considered number of lactations, to be associated with increased levels of lameness. Huang *et al.* (1995) found that the levels of heel erosion, laminitis, sole ulcer and white line separation increased with age. According to Kossaibaiti *et al.* (1999), the incidence of lameness increased significantly from the fifth lactation onwards, while Offer *et al.* (2000) reported higher locomotion scores in lactation 4 when compared with lactations 1 up to 3. When sole ulcers occurred in one lactation, they tended to reappear during subsequent lactations (Enevoldsen *et al.*, 1991; Hirst *et al.*, 2002). First lactation lameness and claw horn lameness during the first lactation increased the hazard (hazard ratio = 2.0 for all types of lameness and of 3.2 for claw horn lameness) for second lactation lameness (Hirst *et al.*, 2002). However, the incidence of acute laminitis was higher in *primiparous* heifers (Bradley *et al.*, 1989) and sole haemorrhages were more severe but subsided more quickly when compared to multiparous cows (Greenough and Vermunt, 1991; Huang *et al.*, 1995; Bergsten and Herlin, 1996). Multiparous cows were more affected by chronic diseases (Singh *et al.*, 1993), sole ulcers and white line disease (Rowlands *et al.*, 1985; Offer *et al.*, 2000). It has been considered that sudden changes in the management and nutrition during the *peripartum* period were probably related to the lesions found in primiparous animals (Enevoldsen *et al.*, 1991; Vermunt and Greenough, 1994). Bargai and Mazrier (2000) found that primiparous animals had greater levels of haemorrhages and white line separation on the front claws and multiparous animals had a greater incidence in the hind claws. The authors related this difference to a shift in the weight distribution from the front claws to the hind claws from the first lactation to subsequent lactations.

Age and increasing parities have been reported to be correlated with a decrease in the claw angle and increase of the dorsal border. Offer *et al.* (2000) found that the length of the dorsal border increased significantly form the first to subsequent lactations. The size of the udder increases constantly with age and older cows had a greater tendency to have sickled hind legs (Boelling and Pollott, 1998). Raber *et al.* (2002) found significant lower levels of fat and greater levels of saturated fatty acids within the digital cushions, which are situated in the subcutis of the claw, than the levels found in multiparous cows. A higher level of monounsaturated fatty acids (MUFA) found in multiparous cows would have the effect of making the digital cushions softer and more resistant to compression.

Haemorrhages of the sole horn have been found in cattle as young as 5 months of age (Bradley *et al.*, 1989) and in 12 month old heifers sole haemorrhages have been found on 0.79 and white line separation on 0.84 of animals (Hoblet *et al.*, 2000). Incidence and severity of heel erosion and sole haemorrhages at 12 months of age influenced incidence rate and severity 1 month *prepartum*. Similarly lesion incidence and severity of heel erosion, wall fissure, sole haemorrhage and digital dermatitis at 1 month *prepartum* influenced their incidence and severity 2 months *post partum* (Drendel *et al.*, 2004). Heifers that had at least 1 claw lesion at 12 months of age had a 27.7 times greater chance of having a claw lesion 2 months *postpartum* (Drendel *et al.*, 2004). The rearing of heifers, their growth rate, feeding and the occurrence of foot disorders were found to influence the overall health of the feet of animals throughout life (Thomas *et al.*, 1999). Rapid growth rate of heifers during the second year led to increased occurrence of sole haemorrhages. In calves, feeding grass silage as opposed to hay resulted in lower horn hardness at the bulb and higher locomotion scores (Offer *et al.*, 2000; Leach *et al.*, 2000). The maternal nutrition has also been found to influence the foetal weight and the measurements of the height, length and diagonal of the claws of the foetus (Ryan *et al.*, 2002). Heifers with good quality horn, demonstrated by a small amount of intercellular material and good cellular structure before parturition, were found not to develop haemorrhages of the sole horn 20 weeks *postpartum*, while heifers with poor quality horn, demonstrated by cells separated by amorphous material and loss of cellular structure, developed moderate to severe haemorrhages (Kempson and Logue, 1993). Vermunt (2004) recommended that heifers should be trained to the system they will join after parturition at the time of breeding preferably in straw bedded cubicles, they should be adapted to the cubicle system prior to joining the main herd and should be introduced to the herd in groups to reduce bullying and their growth rate should be monitored.

Analysing the formation of lesions Bergsten and Herlin (1996), Leach *et al.* (1997), Offer *et al.* (2000) and Le Fevre *et al.* (2001) found that the pattern of development of lesions of the sole and white line were similar for all claws. However, the majority of lesions occurred in the outer hind claws (0.75), while fewer lesions occurred in the inner front claws (0.25). The distribution of lesions between claws is demonstrated in Table 1.8. Lesions in the front claws recovered at week 12 *postpartum*, whereas lesions in the hind claws increased in severity until week 12 *postpartum*. The difference between front and hind claws were reflected also in an increased loosening of the connective tissue of the dermis for a longer time in the hind claws (Tarlton and Webster, 2002). The occurrence of sole lesions on the outer hind claws was greater than the occurrence of white line lesions (Le Fevre *et al.*, 2001). White line lesions were more evenly distributed between the front inner and hind outer claws (Le Fevre *et al.*, 2001). A positive correlation was found between measures of the extent of lesions between all pairs of claws, i.e. hind outer left and right, hind inner left and right, which suggested that lesions tend to occur bilaterally

(Le Fevre *et al.*, 2001; Vokey *et al.*, 2001). The lesion score of the hind and front feet and the total score of all claws for an animal were significantly correlated to the locomotion score of the animals. Furthermore, the locomotion score was significantly correlated to the lesion score of the posterior part of the sole that was the area that presented the highest score for lesion (Table 1.8) (Logue *et al.*, 1994).

Table 1.8 ***Lesion score of the sole and white line area of different claws***

	Hind				Front						
	right	left	inner	outer	right	left	inner	outer	sem	*P*	Source
Mean lesion score week 5-28 *postpartum* (heifers and cows)	-	-	0.56	4.13	-	-	0.54	0.61	0.168	-	Logue *et al.* (1994)
Frequency (%) of sole haem. scores 1-4, heifers housed cubicles	15.16	14.07	10.46	18.77	8.17	7.70	8.99	6.88	-	0.001 *	Vermunt and Greenough (1996)
Mean haem. scores sole, heifers in cubicles, year 1	-	-	0.3	3.9	-	-	1.3	0.3	-	-	Bergsten and Herlin (1996)
Mean haem. scores white line, heifers in cubicles, year 1	-	-	0.8	2.9	-	-	1.8	0.8	-	-	Bergsten and Herlin (1996)
Mean haem. scores sole, cows in cubicles, year 1	-	-	0.2	0.9	-	-	0.6	0.6	-	-	Bergsten and Herlin (1996)
Mean haem. scores white line, cows in cubicles, year 1	-	-	0.8	1.3	-	-	0.5	0.2	-	-	Bergsten and Herlin (1996)
Mean lesion score sole, heifers in cubicles	44	40	4	80	12.5	12	7.5	17	-	0.01	Leach *et al.* (1998)
Mean lesion score white line heifers in cubicles	54	55	30	80	40	34	44	29	-	0.01	Leach *et al.* (1998)
Mean lesion index sole cows	-	-	24.3	36.5	-	-	5.1	3.4	-	-	Le Fevre *et al.* (2001)
Mean lesion index white line cows	-	-	13.3	6.2	-	-	8.7	7.6	-	-	Le Fevre *et al.* (2001)

* - scores for the hind inner and outer claws and the front inner and outer claws were significantly different

Hoof shape, posture and biomechanics determine the weight distribution and the direction of loading of the whole hoof, between and within each claw (Bergsten, 2001). Due to the conformation of the coxo femural joint, that is a typical ball-and-socket joint, as opposed to the flexibility of the scapula and thorax joint, that is a synarcosis where the shoulder blade is held in place by muscles and ligaments, the cow has been found to be more able to relieve weight from the front claws than from the hind claw (Toussaint-Raven, 1985). Although, more than half of the body weight is carried by the front claws (Scott, 1987; Singh, 1993; Nuske *et al.*, 2002; Asleben *et al.*, 2003), the alteration of forces, i.e. the difference of the minimum to the maximum load, rather than the absolute load, was considered to damage to a greater extent the hind outer claws (Toussaint-Raven, 1985; Scott, 1987; Singh *et al.*, 1993). The vertical time-force curves have been found to vary between the front and hind feet, suggesting that the hind feet of cattle are used more for propulsion and the front feet are used to support the front part of the body (Scott, 1987). When measuring the pressure distribution on the claws of dairy cows Toussaint-Raven (1985), Singh (1993) and van der Tol *et al.* (2002) observed that the front inner claws and the hind outer claws were subjected to the greatest pressure. Van der Tol *et al.* (2004) measured on standing cows that 20 % of the load exerted on the hind limb was on the inner claw and 80 % on the outer claw. In the hind claws the greatest pressure was exerted on the area 5 of the sole and in the front claws the greatest pressure was exerted on the area 3 of the white line – heel-sole junction and the area 6 of the heel (van der Tol *et al.*, 2002). The zone 4 of the sole horn was the area that had a higher incidence of lesions and lesions of greater severity (Greenough and Vermunt, 1991; Tranter *et al.*, 1993; Logue *et al.*, 1994; Vermunt and Greenough, 1996; Le Fevre *et al.*, 2001; Offer *et al.*, 2001; Vokey *et al.*, 2001). While, Vermunt and Greenough (1996) found that greater levels of haemorrhages were present at zones 2 and 0 and that these two zones were under greater pressure when an animal made a step. Meyer *et al.* (2004) demonstrated in 13 months old heifers that in the hind limbs the outer claw was always the first one to make contact with the ground, these claws receiving the first impact force. In animals younger than 2 years the lateral claws of the hind hooves had the lowest proportion of high pressures when compared to other claws (Asleben *et al.*, 2003). At 2 months of age dairy heifers showed greater weight load and area of ground surface on the medial claws of the front and hind hooves. At the age of two years the weight load and area of ground surface were similar between the medial and lateral claws of the front hooves, at the hind hooves the lateral claws had to bear more weight and had larger ground surface areas than the medial claws (Asleben *et al.*, 2003). The area of ground surface of the claws seemed to adapt to the weight load resting on it, however the increase in pressure was greater than the increase in ground surface area so pressure increased with age (Asleben *et al.*, 2003). In 9 to 10 month old calves and 15 to 18 month old heifers greater levels of sole lesions on the outer claw of the hind feet were found when compared to other claws (Bradley *et al.*, 1989; Singh *et al.*, 1994). Nuske *et al.* (2002) found in 10 weeks old calves a higher bone mineral density in the hind outer claw when compared to the hind inner claw, in the front inner claw when compared to the front outer claw and the front claws when compared to the hind claws. This indicated that at this age the hind outer and front inner claws bore a greater load than the hind inner and front outer claws. Using the measurement of the thickness of the sole of the outer and inner hind claws Paulus and Nuss (2002) formulated a hypothesis that the outer digit is longer than the inner digit and this was responsible for altering the pressure distribution between both claws.

The conformation of the udder at parturition has been found to force the cow to have a wider stance, causing an increased weight bearing on the outer hind claws (Vermunt and

Greenough, 1996). The resulting overburden of the outer claw of the hind feet can lead to a change in the posture, the abduction of the hind leg and outward rotation of the foot (Toussaint-Raven, 1985). This change in posture alters the pressure distribution on the sole of the outer claw predisposing the claw to the formation of lesions (Vermunt and Greenough, 1996; Webster, 1995; Bergsten, 2001). In the dairy cow it has been found that the overburdened outer claws of the hind feet frequently becomes larger than the inner claws and for this asymmetry to increase with age (Toussaint-Raven, 1985; Offer *et al.*, 2000; Bergsten, 2001). McCallum (1999) found that the pressure on the horn stimulated hoof horn growth rate to increase. Enlarged claws had an increased pressure on the central parts of the sole and caused additional strain on the insertion of the deep flexor tendon and that could lead to the formation of exostosis of the bone (Bergsten, 2001). The exostosis may cause the contusion of the corium and the formation of a sole ulcer. Sole ulcers appear normally on the sole horn located under the plantar process of the pedal bone at the transition of zone 3 and 4 (IFM – Figure 1.1) (Vermunt and Greenough, 1996). In horses the location of the breakeover of the foot can be positioned through shoe placement, shoe shape and trimming and it did have an influence over the strain exerted by the deep flexor tendon and over the pressure on the navicular bone (Page *et al.*, 2002).

Changes in the shape of the claw after the start of the lactation were reported by Offer *et al.* (2000). They found that during lactation, the angle of the dorsal border became smaller while the dorsal wall became longer. These changes were considered to be related to changes in the growth and wear rates due to season, parturition and housing (Offer *et al.*, 2000). Tranter and Moris (1992) found a decrease in the concavity of the sole of the claw in cows following parturition, with a greater decrease in multiparous (2.56 to 1.79 mm) than primiparous (1.81 to 1.68 mm) animals and animals kept on concrete floors than animals kept at pasture. The greatest decrease in sole concavity was found on the outer claws (3.7 weeks to wear) when compared to the inner claws (4.3 weeks to wear). Moreover, digits that had lesions leading to lameness had a lower concavity (0.6 mm) than the non-affected digit of the same leg (1.0 mm), while significantly greater levels of lesions were found to occur on the lateral digits of the hind legs. Differences in concavity between the digits of the same affected legs of lame cows (0.4mm) were greater than differences between the similar digits of non-lame cows (0.2 mm) (Tranter *et al.*, 1993). Van Amstel *et al.* (2004) reported that the moisture content of front claws (31.08 ± 0.9 %) was significantly different to the moisture content of hind claws (33.1 ± 0.9 %).

Chaplin *et al.* (2000) found that lactating heifers performed more lying bouts (9.7 /24 hrs vs. 7.6 /24hrs) of a shorter duration (2.1hrs vs. 3.03hrs) and spent significantly less time lying (39 % vs. 47 % of 24 hrs) when compared with pregnant heifers. It was considered that the discomfort caused by the udder in early lactation may explain the difference in lying patterns between the groups. The total lying time was found to be negatively correlated to the occurrence of higher haemorrhage levels of the claw horn (Colam-Ainsworth *et al.*, 1989).

1.6.3 Effect of housing, environment and season on lameness

Bergsten (2001) and Le Fevre *et al.* (2001) suggested that claw horn lesions are of biomechanical origin, which implies that the hardness of the floor, the shape of the hoof and the loading of the digits are major factors that may predispose animals to the development of sole and white line lesions. Vermunt and Greenough (1996) found that heifers managed in a corral with an earth and gravel surface, had haemorrhages in the white line area near the toe when compared with heifers kept in a cubicle system with concrete floors that developed haemorrhages at the abaxial white line area, sole heel junction and heel, illustrating that different types of floors and management systems may exert different pressure on the hoof. In 1997 the FAWC considered that inappropriate housing, particularly poor cubicle design, lack of hygiene, inappropriate walking surfaces and lack of suitable cow tracks are some of the most important factors predisposing cows to lameness.

Chaplin *et al.* (2000) compared the increase in lesion severity in heifers which were housed in early pregnancy with the increase in lesion severity of heifers following parturition and housed at the same time. Both groups had an increase in lesion severity, however the increase was found to be significantly greater for lactating heifers (9.6 vs. 93.4). These findings indicated that housing had a significant effect on the development of sole and white line lesions. However, the physiological changes occurring during early lactation had a greater influence. Logue (1998) considered that the net hoof wear that occurred during the initial housing period on concrete floors and beginning of parturition was positively correlated with hoof horn lesion scores. Kossaibaiti *et al.* (1999) recommended that cows should be housed in straw yards for approximately 6 weeks following parturition. Similarly, Bergsten (2001) recommended that animals should not be changed from a soft to a hard floor surface during the *peripartum* period and that changes should be made at least one month prior or following parturition.

The properties of floors in terms of hardness, friction and hygiene are of great importance for the health of the feet. A yielding surface has been found to distribute the load more evenly over the weight-bearing surface of the individual claw and between the lateral and medial claw of each limb (Vermunt and Greenough, 1994; Wandel *et al.*, 2002). Smooth floors have been found to increase risk of traumatic injury, while an abrasive surface increased the wear rate of the hooves (Faull and Hughes, 1993; Bergsten, 2001). In 1993 Faull and Hughes classified a third of indoor walking surfaces of 37 dairy herds in England as satisfactory, a third as smooth or very smooth (slippery) and a third as rough or very rough. In the same study, seventy percent of outdoor surfaces were classified as rough; particularly areas around gateways and water troughs were given a poor classification. Herds with smooth floors had worse ranking for lameness than other herds. Bargai (1998) considered some floor types such as floors with grooves deeper than 0.5 cm, old asphalt and the poor cleaning of yards as factors that significantly increased the level of claw horn lesions.

Concrete floors were frequently considered to be too hard to be used as a frequent walking surface for cows and predisposed cows to an increase in the wear rates that generally exceeded the growth rates of the hoof horn (Table 1.9) (Vermunt and Greenough, 1995; Bergsten, 1996; Vermunt and Greenough, 1996; Leach *et al.*, 1997; Berry, 2001; Offer *et al.*, 2001) and to lameness (Table 1.10) (Rowlands *et al.*, 1983; Wells *et al.*, 1995; Galindo and Broom, 2000). However, Webster (2001) did not find differences in the wear and growth rates of hooves of primiparous animals housed in a cubicle system with concrete flooring when compared with those housed in a straw yard (Table 1.9). The greater wear rate of cows housed on concrete floors is associated with the loss of the concavity of the sole (Scott, 1987; Tranter and Moris, 1992). Therefore, the authors recommended keeping cows that were held during lactation on concrete floors, at pasture during the dry period. Excessive wear could result in thin soles and reduced resistance to contusion of the corium (Distl and Schmid, 1994; Bergsten, 2001). Keeping cows in larger groups, more frequent milking, longer feeding time and walking distances were factors that were found to contribute to the overburden and excessive wear of the hooves (Bergsten, 2001). Hood *et al.* (2001) demonstrated the changes that occur in the loading patterns on the solear surface of the foot of horses maintained for 2 weeks on a solid flat concrete surface. An increase of the sole contact area was observed that was related to the erosion of the wall's bearing surface.

The use of rubber mats significantly decreased the incidence of sole haemorrhages on cows kept previously on concrete floors (Bergsten and Frank, 1996) (Table 1.10). Bergsten (2001) recommended the use of rubber mats on top of the concrete in the feeding area and walkways in herds with high incidence of lameness. Benz *et al.* (2002) found significantly larger step length (80 vs.60 cm) and walking speed (99 steps /h vs. 81 steps /h) on slatted floors covered with rubber mats when compared with conventional slatted floors. Telezhenko *et al.* (2004) found that floors covered with elastic rubber mats had significantly higher friction properties (0.602 μ) followed by mastic asphalt (0.479 μ) and different types of concrete (0.404 - 0.415 μ) and when cows walked on floors covered with elastic rubber mats there was an increase in the stride and step length. Phillips and Morris (2001) studied floor surfaces with different types of aggregates embedded in an epoxy resin matrix, the floors with aggregates of 0.5 mm, with a coefficient of friction (μ) of 0.4 to 0.5, proved to be more adequate for the movement of the cows. Animals kept on slatted floors and subsequently on rubber mats improved the horn cell architecture. The number of tubules per field increased from 44 to 46 and the diameter of the tubules decreased from 55 μm to 45 μm (Voges *et al.*, 2004). Cows with healthy horn had small horn tubules with narrow medulla and homogenous intercellular substance. Bergsten and Hultgren (2002) found that rubber coated slatted floors improved the cleanliness and reduced the incidence of digital dermatitis, heel horn erosion and sole lesions of dairy cows.

Table 1.9 ***Effect of different housing systems on the hoof growth and wear rates, hoof measurements and hardness***

	Cubicle concrete	Straw yard	Cubicle rubber alley	se	*P*	Source
Dorsal angle (°)	42	42	-	2.7	NS	Fregonesi and Leaver (2001)
	45.3	45.0	-	2.9	NS	Webster (2001)
	43.0	38.9	-	0.9	0.001	Meyer and Galbraith (1998)
Dorsal wall length (cm)	8.2	7.8	-	0.36	NS	Fregonesi and Leaver (2001)
	8.2	-	9.2	0.2	-	Kremer *et al.* (2004)
Heel depth (cm)	4.5	4.3	-	0.21	NS	Fregonesi and Leaver (2001)
Mean foot area	63.8	67.3	-	-	-	Webster (2001)
Growth rate, hind outer (mm/mo)	5.0	3.3	-	1.1	NS	Meyer and Galbraith (1998)
	6.5	-	6.0	1.0	NS	Vokey *et al.* (2001)
	5.4	5.5	-	2.5	NS	Webster (2001)
Wear rate, hind outer (mm/mo)	3.0	4.6	-	0.35	0.05	Meyer and Galbraith (1998)
	4.8	5.0	-	2.3	NS	Webster (2001)
	4.3	-	4.4	1.3	NS	Vokey *et al.* (2001)
Net growth rate, hind outer (mm/mo)	2.2	-	1.5	1.9	NS	Vokey *et al.* (2001)
Toe length change (mm/mo)	1.35	2.06	-	0.458	NS	Phillips and Schofield (1994)
Heel depth change (mm/mo)	-0.82	1.27	-	0.307	0.01	Phillips and Schofield (1994)
Hardness, sole	86.9	86.6	-	8.3	NS	Webster (2001)
	35.8	-	31.8	1.5	-	Kremer *et al.* (2004)

NS – not statistically significant

Table 1.10 ***Effect of different housing systems on the development of sole and white line haemorrhages and locomotion score***

	Cubicl concre te	Straw yard	Cubicl rubber alley	Tie stalls	se	*P*	Source
Locomotion score change (1-4)	-0.14	-0.06	-	-	0.202	NS	Phillips and Schofield (1994)
Locomotion score (1-5)	1.6	1.6	-	-	0.05	NS	Fregonesi and Leaver (2001)
Mean incidence clinical lameness (%)	19.6	-	10.6	-	-	-	Vokey *et al.* (2001)
Log total lesion score heifer (16 weeks pp)	188.6	9.05	-	-	-	0.001	Webster (2001)
Median change in claw lesion score	2.5*	-	1.3	-	-	-	Vokey *et al.* (2001)
Haem. score sole, hind outer cows, year 3	1.1	-	-	1.4	-	NS	Bergsten and Herlin (1996)
Haem. score sole, hind outer heifers, year 3	2.3	-	-	1.5	-	NS	Bergsten and Herlin (1996)
Median haem. score sole, 13 weeks pp	9.0	-	4.0	-	(0-15)	NS	Bergsten and Frank (1996)
Log haem. score sole (16 weeks pp)	8.45	1.79	-	-	-	0.001	Webster (2001)
Haem. score white line, hind outer cows, year 3	1.4	-	-	0.5	-	0.01	Bergsten and Herlin (1996)
Haem. score white line, hind outer heifers, year 3	2.8	-	-	2.6	-	NS	Bergsten and Herlin (1996)
Log haem. score white line (16 weeks pp)	3.75	0.36	-	-	-	0.001	Webster (2001)

- \- significant increase from beginning of trial

NS – not statistically significant

Wet concrete or concrete floors covered with slurry were found to be more abrasive to hooves than dry concrete floors (Wells *et al.*, 1995; Phillips and Morris, 2000; Webster, 2001). The hoof horn of cows maintained on wet concrete floors and standing in slurry had significantly higher water content (Vermunt and Greenough, 1995; Wells *et al.*, 1995). Offer *et al.* (2001) found softer heel horn and higher incidence of heel horn erosion and Wells *et al.* (1995) found a higher incidence of lameness in similar conditions. Budras and Mulling (1998) and Kempson *et al.* (1998) using morbid samples found that slurry removed the intercellular cementing substance and increased the permeability of the intertubular horn of the sole and the heel. Under the effect of urea the keratin proteins were extracted from the horny mass in the cells (Budràs and Mulling, 1998). The hygiene of the floor has been found to affect the occurrence of infectious diseases and of heel horn erosion (Bergsten and Herlin, 1996; Bergsten, 2001; Somers *et al.*, 2002; Somers *et al.*, 2003). In cubicle systems, the frequency of scraping and the use of slated floors have been found to improve the housing hygiene (Bergsten, 2001).

In cubicle housing systems the cubicle design, size and bedding material are important factors affecting the cows lying comfort (Bergsten, 2001; Vokey *et al.*, 2001). A higher incidence of hock injuries has been found when cows were kept in cubicles with mats when compared with cubicles with mattresses and straw yards (Livesey *et al.*, 2002). However, substituting rubber mats with thicker mattresses filled with chopped rubber did not significantly reduce hoof horn haemorrhages (Laven and Livesey, 2004). Dairy farms that used concrete cubicle bases or cubicle bases with mats or mattresses had a significantly higher incidence of lameness compared with dairy farms that used deep sand as a stall base (Cook, 2002; Cook *et al.*, 2004). Sand was considered to be softer and cows spent a longer time lying in sand based cubicles when compared to bases that were considered harder (Cook, 2002). Concrete floors without bedding were considered to be not acceptable as a cubicle base (FAWC, 1997). A reduction in lying time, due to the lack of cubicle comfort, was considered the major cause contributing to the development of severe claw horn lesions (Colam-Ainsworth *et al.*, 1989; Wierenga and Hopster, 1990; Singh *et al.*, 1993; Leonard *et al.*, 1994). Overcrowding, when the number of animals exceeded the number of cubicles, was reported to reduce the lying time (Wierenga and Hopster, 1990). Bowell *et al.* (2003) found an increase in locomotion score when the number of cubicles per cow decreased.

Housing cows in cubicles resulted in a significantly higher incidence of lameness and claw horn haemorrhages when compared with housing animals in straw yards and tie-stalls (Rowlands *et al.*, 1983; Singh *et al.*, 1993; Bergsten and Herlin, 1996; Livesey *et al.*, 1998; Weaver, 1998; Meyer and Galbraith, 1998; Webster, 2001; Somers *et al.*, 2002; Bowell *et al.*, 2003; Somers *et al.*, 2003; Laven and Livesey, 2004; Whitaker *et al.*, 2004) (Table 1.10). However, animals in straw yards have been found to have a significantly greater incidence of heel horn erosion (Livesey *et al.*, 1998; Webster, 2001). Livesey *et al.* (2000b) found higher white line haemorrhage levels for *primiparous* animals during the first lactation when they were reared from 12 months of age in straw yards as opposed to heifers reared in cubicles. During the second lactation heifers kept in straw yards had slightly lower white line haemorrhage levels (Livesey *et al.*, 2000b). In straw yard systems comfort and animal behaviour observed at pasture could be maintained (Singh *et al.*, 1994; Bergsten, 2001; Fregonesi and Leaver, 2001). Greater total lying time, ruminating time and bed occupation time were found for animals kept in straw yards when compared to animals kept in a cubicle system (Singh *et al.*, 1993; Fregonesi and Leaver, 2001) (Table 1.11). Singh *et al.* (1993b) found similar figures when a cubicle system was compared to animals kept at pasture. At pasture cows spend a longer period of time lying and had fewer changes between lying and standing postures when compared to cows in cubicles (Singh *et al.*, 1993b). These differences may be related to a bigger lying area, when compared to the total area, in the straw yard system (Phillips and Schofield, 1994). In the cubicle system the competition for space was higher. Low ranking cows housed in cubicles stood for a longer time in passageways and half in a cubicle. Less than 40 % of the cows in the lower ranking group avoided lameness during the housing period, when compared to 67 and 82 % of middle-ranking and high ranking cows (Galindo and Broom, 2000).

Table 1.11 ***Effect of different housing systems on the lying behaviour of dairy cows***

	Cubicles concrete	Straw yard	se	*P*	Source
Total lying time (min/24 hrs)	762.5	817.5	20.2	0.01	Fregonesi and Leaver (2001)
Total lying time (min/100min)	34	52	2.8	0.001	Phillips and Schofield (1994)
Total lying time (min)	410	576	(±46)	-	Singh *et al.* (1993)
Maximum lying time (min)	147	237	(±21)	-	Singh *et al.* (1993)

Higher incidence of lameness was found during the winter months (Williams *et al.*, 1986; Enevoldsen *et al.*, 1991). Dairy cows calving in the autumn have been found to have higher levels of claw horn lesions than cows calving in the spring (Bergsten and Frank, 1996; Offer *et al.*, 2000). Cows calving in the autumn experience both calving and housing, while spring-calving animals have the advantage of being out at grass at the time when lesion scores should be increasing. However, nutrition and management factors are different in both seasons (Offer *et al.*, 2000). When studying autumn calving dairy cows it was difficult to separate the effects of changes in housing occurring at parturition, parturition and diet change on the development of claw lesions (Leach *et al.*, 1997; Leach *et al.*, 1998; Offer *et al.*, 2000). Spring calving cows had softer hooves when compared to autumn calving cows, probably because they were housed for a longer time during the *prepartum* period (Berry, 1999). A lower incidence of lameness has been found when cattle were turned out on pasture during the spring and summer (Singh *et al.*, 1993; Kerr, 1998). Meyer and Galbraith (1998) reported greater wear rates of the dorsal border of the hooves after cows were turned out to pasture, this difference probably being related to cows walking on concrete tracks. Ley *et al.* (1998) reported that the season of the year and the nutritional and management differences influenced equine hoof wall elasticity, tensile strength and mineral content. The rate of cell proliferation and keratinisation were lower in hoof horn of cows in the winter when compared to the summer (MacCallum *et al.*, 1998 and 2002). This findings support the lower growth rate of hoof horn during the winter when compared to the summer (Clark and Rakes, 1982; Tranter and Morris, 1992).

Rowlands *et al.* (1983), Tranter *et al.* (1991), Huang *et al.* (1995) and Fitzgerald *et al.* (2000) found seasonal trends for some pathologies. Huang *et al.* (1995) reported greater levels of interdigital dermatitis in March, which was considered the month of the study with the highest rainfall. In addition, greater numbers of cases of sole ulcers and laminitis occurred in November. Tranter *et al.* (1991) reported higher incidence of white line separation and heel horn erosion in the winter months in dairy cows managed at pasture. In this study the winter months were again the months with the highest rainfall. Tranter *et al.* (1993) and Fitzgerald *et al.* (2000) found a significant positive correlation between locomotion score and number of wet days per month. Offer *et al.* (2000) found higher levels of heel horn erosion at the end of the winter and housing period and when animals were turned out to pasture heel horn erosion levels declined. The improvement may be associated with the non-exposure to the concrete and slurry environment of the cubicles. When cows were turned out to pasture the tracks and road surfaces had an important influence over the incidence of lameness. High level of concrete and poor maintenance of roads were positively correlated with incidence of lameness. White line disease was the main lesion affected by road conditions (Leonard *et al.*, 1998).

1.6.4 The influence of foot care on lameness

In a survey conducted in Sweden with 1989 dairy farmers 44 % trimmed the hooves of their cows once a year, 34 % trimmed twice a year and 22 % trimmed 'when necessary' (Hultgren *et al.*, 1998). Blowey (1999) considered hoof trimming to be essential for the prevention of lameness as it reduced the number (7 vs. 11 cases of sole ulcers for trimmed and not trimmed cows) and duration of lameness cases (Manson and Leaver, 1989; Enevoldsen *et al.*, 1991; Manske *et al.*, 2002). Routine foot inspection and necessary trimming has been found to improve the pressure distribution within the claw (Touissaint-Raven, 1987, Singh, 1993; Singh *et al.*, 1993). According to van der Tol *et al.* (2004) after trimming the load on the hind claw changed from 80 % on the outer claw and 20 % in the inner claw to 70 % on the outer claw and 30 % on the inner claw. Kehler and Gerwing (2004) reported a greater reduction of the load bared by the outer hind claw after trimming. In their experiment the weight load distribution between the outer and inner hind claws was 0.68 and 0.32, respectively, before trimming. During the first 6 weeks after trimming the weight load distribution between the claws of the hind limb was almost equal (0.50:0.50). The outer hind claw had a mean maximum pressure load decrease of 9.6 N/cm^2 and the inner hind claw had an increase in pressure load of 9 N/cm^2. Before claw trimming the centre of gravity of the claw was located in the outer weight-bearing surface apical to the site of the typical sole ulcer and after trimming it was shifted towards the tip of the limb. Load distribution on the hind claws improved but was still unbalanced. It was recommended that foot trimming should be carried out at least once (FAWC, 1997) or twice a year (Kehler and Gerwing, 2004), preferably during the housing period. Vermunt and Greenough (1995) found that cows housed on concrete floors required frequent trimming to maintain claw shape. Huber *et al.* (2004) found an improvement in the scores for lesion of claws after trimming in cows housed in cubicles and tied stalls. However, Shearer and Amstel (2002) found that there was a risk of over-trimming when cows were kept on concrete floors. Concrete predisposed the claws to a greater wear rate, which reduced the thickness of the sole. Incorrect trimming could result in thin soles that would offer reduced resistance to contusion of the corium (Enevoldsen *et al.*, 1991; Bergsten, 2001; Shearer and Amstel, 2002). When paring the sole of the outer hind claw to the same level as the sole of the inner hind claw, the sole thickness of the outer claw was frequently thinner than the thickness of sole of the inner claw as a consequence care should be taken not to over-trim the outer claw (Paulus and Nuss, 2002). Trimming significantly reduced the hardness of the centre of the heel (Manson and Leaver, 1988). Enevoldsen *et al.* (1991) found that hoof trimming at 1 to 2 months following parturition reduced the occurrence of sole ulcers. This association was also found to be dependent on milk yield, body weight and season. Huang *et al.* (1995) found that hoof trimming at 4 months intervals were associated with increased laminitis, white line separation, heel erosion and interdigital dermatitis.

The use of footbaths has been found to help to clean feet, reduce pathogen levels and to harden the hoof horn (Bergsten and Herlin, 1996; FAWC, 1997). As a consequence, foot bathing has been found to be an effective treatment for some causes of lameness. The frequency of foot bathing and time of the year has been found to depend on the cause of lameness targeted (Kossaibaiti *et al.*, 1999). Formalin, copper sulphate, zinc sulphate and antibiotics have frequently been used in footbaths for dairy cows (Hoblet, 2002). The exposure of hoof horn to 5 % formalin over a 24 to 48 hours period did not significantly increase the permeability of the horn, but it was found to induce the formation of microcracks parallel to the outer surface of the horn. Footbaths containing formalin have been found to result in harder but less plastic hoof horn, with lower water content, whereas cooper sulphate was found to penetrate the whole extension of the tubules after 24 hours (Vermunt and Greenough, 1995; Kempson *et al.*, 1998).

1.6.5 Nutrition as a factor affecting lameness

A greater incidence of lameness has been found to occur in cattle offered high levels of concentrate feeds in the diet, particularly without gradual introduction to facilitate the adaptation of the rumen micro flora and fauna (Kelly and Leaver, 1990; Singh *et al.*, 1993; Vermunt and Greenough, 1994; Bargai, 1998; Olsson *et al.*, 1998). The subsequent reduction in the pH of the rumen environment has been found to increase the number of gram-positive lactic acid producing bacteria. The increase in the ruminal acidity induces a dysfunction of the ruminal mucosa, which further facilitates the absorption of these products. The lysis of gram-negative bacteria and the subsequent release and absorption of the endotoxins, histamine and lactic acid was found to alter the blood supply to the corium on the dermal-epidermal junction area of the hoof (Vermunt and Greenough, 1994; Ossent and Lischer, 1998). The consequential inflammation of the dermal-epidermal junction and related clinical signs was referred to as laminitis (Nilsson, 1963; Singh *et al.*, 1993; Singh *et al.*, 1994). While horses with laminitis have been found to have severe changes mainly in the dermal-epidermal junction of the wall horn, cows also had severe vascular changes and degeneration of the dermal papillae of the sole horn (Singh *et al.*, 1994). To prevent the development of rumen acidosis Kossaibaiti *et al.* (1999) recommended the inclusion of straw or hay in the diet, the avoidance of a forage to concentrate ration in the diet less than 40:60, avoiding sudden increases in the concentrate levels of the diet during the *postpartum* period, avoiding concentrate feed that contained high levels of starch and are ground finely and to have cows with condition score not over 3 at paturition. Cows with high condition score at parturition were found to have lower forage intake levels and were more likely to develop rumen acidosis (Kossaibaiti *et al.*, 1999).

In many herds in the UK, Ward and Parker (1999) found that there was evidence of poor management of body condition score, sub-optimal intake of macro and trace elements, mainly selenium and magnesium, and that the nutrient levels of grass and maize silage was variable. Protein and energy levels should be balanced throughout the day to avoid imbalances of these nutrients in the rumen and so the provision of a high protein or starch compound feed in the parlour to compensate for low nutrient levels in the forage is not optimal. Similarly, Shaver (2002) considered that subacute rumen acidosis is a common problem in commercial dairy herds in the United States.

Livesey *et al.* (1998) and Bergsten and Frank (1996) found a significant increase in claw horn lesions in heifers receiving high levels of concentrate nutrient sources in their diet. Increased locomotion score and incidence of lameness were found in cows offered high concentrate to silage ratio (Manson and Leaver, 1989) and high concentrate feed levels (Manson and Leaver, 1988). Vermunt and Greenough (1994) recommend that at least one third of the total dry matter intake of a cow should be roughage and that the particle length of the roughage should be of at least 2.5 cm to ensure rumination and saliva flow.

However, Logue *et al.* (1999b) did not find significant differences in lesion score levels between a low input herd with lower milk yield and lower concentrate intake and a high input herd with higher milk yield and higher level of concentrate intake. Greater weight loss, elevated levels of BHB, higher levels of NEFA were found for the herd managed in the low input system when compared to a herd managed to obtain milk yields in accordance with their genetic background. Animals managed in a low input system remained in a negative energy balance for longer in early lactation.

Kelly and Leaver (1990) and Bargai (1998) found that feeding barley was positively correlated with increased incidence and duration of lameness and increased locomotion score. The histidine present in the barley may be metabolised to histamine and this histamine would have an effect of vasoconstriction on the capillary circulation causing lameness (Vermunt and Greenough, 1994).

A sudden increase in the supply of a high protein ration at parturition has been positively correlated with the occurrence of lameness (Bargai, 1998). When feeding a diet with higher crude protein content Manson and Leaver (1988) found a higher incidence and duration of lameness. Pastures in early spring tend to have a high protein and low fibre content and have been positively correlated with increased levels of laminitis (Vermunt and Greenough, 1994; Zadnik *et al.*, 2000).

Offer *et al.* (2000b and 2003) found that the type of forage offered to calves from 3 months of age and for heifers from 15 months until parturition influenced the incidence of lameness during the *postpartum* period. Offering heifers grass silage as opposed to hay resulted in significantly higher lesion scores of the hoof horn (4.42 vs. 2.75) at 20 weeks after parturition, significantly higher heel erosion scores (0.90 vs. 0.73) and significantly lower hoof horn growth (4.9 vs. 5.5mm/mo). In calves, feeding grass silage as opposed to hay resulted in lower horn hardness at the bulb and higher locomotion scores due to claw horn lesions (Leach *et al.*, 2000; Offer *et al.*, 2003). Grass silage with a higher dry matter content and in general dryer diets have been associated with lower incidence of lameness (Clarkson *et al.*, 1993; Wells *et al.*, 1995; Webster, 2001). Offering acidic silage was found to reduce the pH of the rumen and increase the incidence of lameness (Offer *et al.*, 2001). Diets with a large proportion of forage were found to increase the feeding time and consequently the time the cows spent standing on concrete (Offer *et al.*, 2000b; Leach *et al.*, 2000). Moreover, nutrition can affect lameness indirectly by influencing the quantity and quality of the slurry, thus changing the underfoot conditions within the housing system (Leach *et al.*, 2000).

The nutrients required for keratinization to take place are found to be amino acids, the fatty acids, particularly linoleic and arachidonic acid, minerals, mainly calcium, trace elements, particularly zinc and selenium and vitamins A, D, E and biotin (Zaun, 1997; Mulling *et al.*, 1999). The hoof is made up largely of keratin. The strength of the keratin filament bundles is determined by their cross-linking which is done via sulphur containing amino acids, in particular methionine and cysteine (Vermunt and Greenough, 1995; Mulling *et al.*, 1999). Fatty acids are required for the formation of the intercellular cementing substance (Mulling *et al.*, 1999). Adequate levels of calcium were found to be important for the activity of the epidermal transglutaminase, the enzyme responsible for the cross-linking of the cell envelope proteins (Grosenbaugh and Hood, 1993; Mulling *et al.*, 1999). Cholesterol levels are also important for the formation of the cell envelope (Grosenbaugh and Hood, 1993). Biotin has been found to be essential for the synthesis of long chain fatty acids, which are part of the fatty acid composition of the intercellular cementing substance and for keratin protein synthesis (Mulling *et al.*, 1999). All these precursors are important for the formation of a good quality hoof horn (Grosenbaugh and Hood, 1993). The gradient of concentration of these nutrients in the blood vessels and the rate of perfusion determine the efficiency of diffusion into the epidermis (Mulling, 2000).

1.6.5.1 The effect of dietary supplementation of sulphur containing amino acids

Most dairy cattle diets in the UK are silage based and as a consequence it is considered that methionine may be deficient in these diets (Thomas *et al.*, 1980). However, Offer *et al.* (1997) did not find a significant difference in the locomotion score, sole lesion incidence, hoof hardness and production parameters when supplementing a silage based diet with different types of protein sources that provided the animals with two levels of sulphur containing amino acids.

The sulphydryl groups, that are normally provided by sulphur-containing amino acids, when provided in suboptimal levels have been found to be responsible for decreased keratin synthesis, incomplete cross-linking of keratin proteins and structural changes of the cellular envelope (Zaun, 1997; Mulling *et al.*, 1999). Working with horses Ley *et al.* (1998) found a positive correlation between the tensile strength of the hoof horn and its sulphur content. Cattle suffering from laminitis have been found to have reduced levels of methionine and cysteine in the horn and to have softer hooves (Maclean, 1971; Hendry *et al.*, 1997). Cysteine has been found to be more important to the formation of the IFAP than methionine (Grosenbaugh and Hood, 1993). Livesey *et al.* (2000c) observed a decrease in the proportion of cysteine in the sole horn of heifers by week 12 of the first lactation. Galbraith *et al.* (2002) found increased methionine incorporation and DNA synthesis, measured by thymidine incorporation, in tissue explants from the bovine sole that were in medium with increasing concentration of methionine. In a further study looking at cells under two different concentrations of methionine and stained positive for cell proliferation and apoptosis markers Hepburn *et al.* (2004) found greater epidermal cell proliferation and greater number of apoptotic cells at the higher methionine concentration indicating a stimulation of epidermal cell turnover in higher concentrations of methionine. The effect of methionine supplementation on the hoof growth and wear rates and the development of sole and white line lesions are demonstrated in Table 1.12.

Table 1.12 *Effect of methionine supplementation on the hoof growth and wear rates, hardness and the cysteine and methionine content of the horn in dairy cows and goats*

	Suppl.	Control	se	*P*	species	Source
Growth rate, dorsal wall (mm/mo)	8.95	6.67	0.47	0.01	cow	Clark and Rakes (1982)
Growth rate, dorsal wall (cm)	0.58	0.55	0.07	NS	goat	Galbraith *et al.* (1998)
Wear rate, dorsal wall (mm/mo)	4.90	4.60	0.38	NS	cow	Clark and Rakes (1982)
Hardness						
dorsal wall	4.51	4.61	0.04	0.05	cow	Clark and Rakes (1982)
	48.4	45.3	5.79	NS	cow	Galbraith *et al.* (2002b)
sole	22.6	20.4	0.7	0.05	goat	Galbraith *et al.* (1998)
	35.1	35.1	6.51	NS	cow	Galbraith *et al.* (2002b)
sole-heel junction	37.0	37.8	4.51	NS	cow	Galbraith *et al.* (2002b)
Cysteine, abaxial wall (%)	11.3	7.73	1.44	0.05	goat	Galbraith *et al.* (1998)
Cysteine, sole-heel junction (%)	38.7	42.4	15.2	NS	cow	Galbraith *et al.* (2002b)
Methionine, sole-heel junction (%)	12.6	9.89	2.35	NS	cow	Galbraith *et al.* (2002b)

NS – not statistically significant, Suppl. – supplemented animals

The hoof horn of the dairy cows in the experiment of Clark and Rakes (1982) had a lower cysteine and proline content and higher methionine, lysine, tyrosine and glutamic acid than the hooves of control animals. The authors concluded that the lower content of cysteine would result in lower disulphide bonding between keratin filaments and result in the formation of a softer horn. There was no explanation of why methionine was not converted to cysteine.

1.6.5.2 The effect of dietary supplementation of biotin

Biotin is a sulphur containing, water soluble vitamin of the B complex. It is also called vitamin H, this denomination comes from the German word for skin "Haut" and was applied because biotin was first discovered as an essential vitamin for the normal function of the skin (Mulling *et al.*, 1998). The chemical name of biotin is 2-keto23, 4-imadozilido-2-tetrahydrothio-phenevaleric acid.

Biotin has been found to play a critical role in the differentiation of epidermal tissue and in fatty acid and glucose metabolism (Seymour, 1998). Biotin has been found to be essential for the synthesis of long chain fatty acids, which are part of the fatty acid composition of the intercellular cementing substance and for keratin protein synthesis (Mulling *et al.*, 1999; Mulling, 2000).

Biotin functions as an enzyme co-factor to transfer carboxyl groups (Seymour, 1998). Three biotin–dependent carboxylases have been indentified: acetyl-CoA carboxylase, propinyl-CoA carboxylase and pyruvate carboxylase (Girard, 1998). Acetyl-CoA carboxylase acts in the carboxylation of acetyl-coenzyme A in malonyl-coenzyme A, a reaction necessary for the synthesis of long-chain fatty acids. Propinyl-CoA carboxylase catalyses the conversion of propionyl-coenzyme A in methylmalonyl-coenzyme A, that is metabolised subsequently in succinate to enter the Krebs cycle. Pyruvate carboxylase acts in the conversion of pyruvate in oxaloacetate. This metabolic pathway allows the entry of some amino acids (alanine and glycine) and lactic acid into the Krebs cycle (Girard, 1998). These 3 enzymes are important in the *peripartum* period when gluconeogenesis is at its maximum (Girard, 1998). Biotin is also required for the formation of adenine and guanine and the conversion of ornithine to citruline in the urea cycle, having an important role in cell growth and differentiation and in protein synthesis (Seymour, 1998). Keratinocytes treated with biotin exhibited a higher production of cytokeratins when compared with untreated keratinocytes. No difference of the growth rate of treated and untreated cells was noticed (Higuchi *et al.*, 2002). Fritsche *et al.* (1991) observed a specific increase in cytokeratins in keratinocytes treated with biotin that would normally occur in the terminal differentiation of epidermal cells, indicating that biotin stimulates the differentiation of epidermal cells.

Qualitative and quantitative changes in the synthesis of keratin filaments, interruption of the co-ordination of keratinisation and cornification, alteration of the structure of the cellular envelope and increased production and intercellular cementing substance of poor quality, was found to occur in the hoof horn of biotin deficient calves. The hoof horn of these calves had a brittle and crumbly consistency with decreased cell-to-cell adhesion and increased permeability of the epidermis (Mulling *et al.*, 1999). Biotin deficiency in goats has been found to result in reduction of the hoof horn growth rate and in a decrease of the concentration of cysteine in the hoof wall (Galbraith *et al.*, 1998). In pigs and poultry deficiency resulted in dermatitis and in cracks and fissures in the feet and toes (Seymour, 1998).

Grains are a poor source of biotin and forages when consumed in great quantities provide a better source (Girard, 1998). Biotin is synthesised in the rumen and is required for the cellulolytic activity of ruminal microorganism and propionic acid production in bacteria. The main cellulolytic organisms in the rumen, *Bacteroides succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens*, have an absolute requirement for biotin (Baldwin and Allison, 1983). High concentrations of biotin in the rumen has been observed when diets rich in fermentable carbohydrates, such as starch, were supplemented with urea (Girard, 1998). The net output of biotin by microbial metabolism decreases in low roughage - high concentrate diets (Abel *et al.*, 2001).

The supplementation of diets with biotin has been found to significantly increase hoof hardness and reduce lameness and hoof pathologies of pigs, horses and cows (Table 1.13). In horses the supplementation should be carried out for 6 to 9 months. Because the majority of hoof horn problems occurred on the bearing border of the wall improvements were observed only after 8 to 15 months of supplementation (Geyer and Schulze, 1994). The addition of 10 – 30 mg a day was found to increase the hoof quality of horses that had previously had crumbly hoof horn and sand cracks (Comben *et al.*, 1984; Kempson, 1990; Geyer and Schulze, 1994; Zenker *et al.*, 1995). The tensile strength of the coronary horn increased after 15 and 19 months of supplementation (Geyer and Schulze, 1994; Zenker *et al.*, 1995) and the hoof horn quality decreased after discontinuation of the supplementation with biotin (Geyer and Schulze, 1994).

Table 1.13 Lameness incidence, locomotion score and development of sole and white line lesions of biotin supplemented and non-supplemented animals

	biotin	control	se	*P*	animals	Quant.	Source
No. toe lesions/ gilt	4.8	5.3	0.1	0.01	Gilts	220 µg/kg	Bryant *et al.*(1985)
Total toe lesion severity score/ gilt	9.1	9.9	0.3	0.05	Gilts	220 µg/kg	Bryant *et al.*(1985)
Frequency heel crack (%)	12.9	15.6	0.98	0.05	Gilts	220 µg/kg	Bryant *et al.*(1985)
Histological evaluation score, coronary and white line horn (0-3)[a]	1.52	1.76	0.35	0.05	Horse	20 mg/day	Zenker *et al.*(1995)
Incidence of lameness (%)	66.4	71.2	-	NS	Cow	20 mg/day	Hedges *et al.* (2001)
No. hooves with moderate to severe damage,hind left	21	101	-	0.01	Cow	20 mg/day	Fitzgerald *et al.* (2000)
No. affected digits, outer	53	140	-	0.05	Cow	20 mg/day	Fitzgerald *et al.* (2000)
Prevalence of vertical fissures, 24 mo supp. (%)	16	33	-	0.05	Beef cattle	10 mg/day	Campbell *et al.* (2000)
Histological evaluation score, coronary horn (0-3)[a]	0.31[b]	0.60	0.25	0.001	Cow	20 mg/day	Schmid (1995)
No. animals with sole haem. (%)	24	50	-	0.01	Cow	20 mg/day	Bergsten *et al.* (2003)
Mean total sole haem. score, hind outer	0.22	0.21	0.21	NS	Cow	20 mg/day	Midla *et al.* (1998)
Healing of sole ulcer, horning over time (days)	18.3	13.5	±8.6	NS	Cow	20 mg/day	Lischer *et al.*(2002b)
Incidence of white line separation (%)	10.0	15.4	-	0.01	Cow	20 mg/day	Hedges *et al.* (2001)
Prevalence of white line separation, hind outer (%)	10.4	18.0	-	NS	Cow	20 mg/day	Midla *et al.*(1998)
White line separation score, 120 days pp (0-4)[c]	0.94	1.31	-	NS	Cow	20 mg/day	Hoblet *et al.*(2002)
Histological evaluation score, heel horn (0-3)[a]	0.33[b]	0.58	0.32	0.01	Cow	20 mg/day	Schmid (1995)

[a] - 0= unchanged, 3= severe changes
[b]- same animals as control cows, however after biotin supplementation
[c]- 0= normal, 4= complete separation of white line
Quant. = quantity

Increase in the claw angle, the height of the heel, the diagonal of the claw and the weight bearing area of the claw were reported in biotin supplemented when compared to non-supplemented cows (Distl and Schmid, 1994). These changes in claw dimensions are probably related to the greater horn hardness and changes in the wear and growth rates (Table 1.15). Greater density of tubules in the horn of the wall and greater integrity of intertubular horn of weaner pigs supplemented with biotin were reported by Kempson *et al.* (1989). Greater density of tubules could be related to the increased hardness (Distl and Schmid, 1994; Schmid, 1995 and Campbell *et al.*, 2000) and compressive strength of the wall horn found in biotin supplemented animals (Webb *et al.*, 1984) (Table 1.14). However, no morphologic differences in the density of horn tubules and the intertubular horn were found between biotin supplemented and control cows (Higuchi *et al.*, 2004). Increased integrity of the intercellular substance due to greater cell adhesion and absence of microcracks in biotin supplemented when compared to non-supplemented cows was reported by Koster *et al.* (2002). The biotin supplemented cows had higher proportions of stearic and oleic fatty acids (C18:0 and C18:1 respectively) in the hoof horn indicating a difference in the composition of the intercellular cementing substance (Koster *et al.*, 2002). Similarly, Meyer *et al.* (2002) reported a higher proportion of long chain fatty acids in the hoof horn of biotin supplemented cows and Higuchi *et al.* (2004) reported a significantly higher total lipid content of the sole horn of supplemented cows when compared to control cows. Lower plasma concentration of biotin were found on cows presenting clinical symptoms of laminitis when compared to sound herdmates (Higuchi and Nagahata, 2001) and on cows that had a moderate to bad healing process of sole ulcers when compared to cows with a good healing process (Lischer *et al.*, 2001).

Table 1.14 Tensile and yield strength, moisture content and hoof horn hardness of biotin supplemented and non-supplemented animals

	biotin	control	se	*P*	species	quantity	Source
Yield strength, compression, abaxial wall, inner front claw (10^6 x N/m^2)	12.0	9.3	1.0	0.05	Pig	1 mg/kg feed	Webb *et al.* (1984)
Tensile strength coronary horn, border (N/mm^2), after 33 mo	44.1	34.8	9.1	0.05	Horse	20 mg/day	Zenker *et al.* (1995)
Tensile strength, coronary horn (N/mm^2)	73.24[a]	67.73	7.40	0.05	Cow	20 mg/day	Schmid (1995)
Tensile strength, sole horn (N/mm^2)	62.84[a]	58.59	8.06	0.05	Cow	20 mg/day	Schmid (1995)
Tensile strength, heel horn (N/mm^2)	54.19[a]	41.88	7.20	0.05	Cow	20 mg/day	Schmid (1995)
Tensile strength white line horn (N/mm^2)	5.70	5.51	0.40	NS	Cow	20 mg/day	Collis *et al.* (2004)
Hardness, abaxial wall, front claw (Shore A)	97.0	95.8	0.6	0.05	Pig	1 mg/kg food	Webb *et al.* (1984)
Hardness, dorsal wall, front claw (Shore D)	81.4	77.5	3.00	0.001	Beef cattle	10 mg/day	Campbell *et al.* (2000)
Hardness,wall coronary border (Shore D)	81.20	79.60	-	NS	Cow	20 mg/day	Brooks *et al.* (1997)
Hardness coronary horn (Shore D)	76.4[a]	75.21	2.50	NS	Cow	20 mg/day	Schmid (1995)
Hardness sole horn, (Shore D)	47.95[a]	48.30	7.69	NS	Cow	20 mg/day	Schmid (1995)
Moisture, coronary horn (%)	11.89[a]	12.43	2.90	NS	Cow	20 mg/day	Schmid (1995)
Moisture, sole horn (%)	22.21[a]	22.42	3.25	NS	Cow	20 mg/day	Schmid (1995)

[a]- same animals as control cows, however after biotin supplementation
NS – not statistically significant

Table 1.15 Claw measurements and hoof growth and wear rates of biotin supplemented and non-supplemented animals

	biotin	control	se	*P*	species	quantity	Source
Growth rate anterior wall, hind claw (mm/28 days)	10.44	10.75	-	NS	Pig	1mg/ kg food	Johnston and Penny (1989)
Wear rate anterior wall, hind claw (mm/28 days)	6.44	6.64	-	NS	Pig	1mg/ kg food	Johnston and Penny (1989)
Hoof horn growth (mm/ 187 days)	35.34	30.69	-	0.05	Horse	0.12mg/ kg BW	Reilly *et al.* (1998)
Growth rate lateral wall, hind outer claw (mm/ day)	0.213	0.171	0.011 (sed)	0.01	Goat	100mg/ kg DM	Galbraith *et al.* (1998)
Growth rate, hind, heifers (mm/mo)	30	31	4.3	NS	Cow	20 mg/day	Bergsten *et al.* (2003)
Growth rate, hind, cows (mm/mo)	28	22	3.5	NS	Cow	20 mg/day	Bergsten *et al.* (2003)
Heel depth, front claw, 293 days (mm)	44.0	47.1	5.8	0.01	Cow	20 mg/day	Midla *et al.* (1998)
Change in heel depth, front claw, 275 days (mm)	-4.9	-2.5	6.0	0.05	Cow	20 mg/day	Midla *et al.* (1998)

[a]- same animals as control cows, however after biotin supplementation
NS – not statistically significant

1.6.5.3 The effect of dietary supplementation of zinc

Zinc deficiency causes alterations such as parakeratosis and hyperkeratosis in the epidermis of the skin and hoof (Stern *et al.*, 1998). Working with horses Coenen and Spitzlei (1996) found a positive correlation between visual scoring of hoof quality, hoof horn hardness and zinc concentration of the sole and wall horn. Hooves with horn alterations had significantly greater wall horn hardness (58.0 ± 2.3) and zinc content (195.3 ± 22.6 mg/kg) when compared to hooves with alterations of the horn (51.8 ± 7.8 and 170.7 ± 29.5). After feeding zinc to the horses with inferior hooves an increase in the concentration of zinc in the hoof wall horn was established (Coenen and Spitzlei, 1996).

In beef cattle, animals supplemented with a zinc polysaccharide complex and a zinc amino acid/ peptide chelate presented significantly better scores for clinical status of the claw horn (0.57± 0.39, 1.01± 0.40 and 1.08± 0.31, respectively), better quality of the coronary horn as evaluated through the microscope (0.74± 0.25, 0.87± 0.36 and 0.89± 0.38, respectively), when compared to animals receiving placebo (no zinc) and zinc oxide, but no significant difference in the tensile strength of the coronary horn was measured (58.37± 5.98, 57.00± 7.16, 56.11± 7.85, respectively). Coronary horn alterations observed at microscopy were the enlargement of the centre (marrow) of the tubules. Better responses were attributed to a higher bioavailability and higher absorption of the organic zinc compounds when compared to the inorganic form (Stern *et al.*, 1998). Dairy cows fed 200 mg of zinc methionine daily during 12 month had fewer cases of foot rot, heel horn erosions, interdigital dermatitis and laminitis and lower incidence of sole ulcers and white line lesions (Moore *et al.*, 1988).

The supplementation of dairy cows with an organic mineral complex containing 360 mg of zinc methionine, 125 mg of copper lysine, 200 mg of manganese methionine and 25 mg of cobalt glucoheptonate per cow daily reduced the incidence of double sole, digital dermatitis, white line separation, sole haemorrhages and sole ulcers. However, the study was conducted over a period of 2 years, year 1 without supplementation and year 2 with supplementation and differences that occurred between years could confound the results

(Nocek *et al.*, 2000). Lower incidence of claw disorders and lower scores for heel erosion, white line and sole haemorrhages between cows supplemented with the same organic mineral complex and cows supplemented with an inorganic mineral complex that contained the same concentration of minerals were reported by Ballantine *et al.* (2002). Uchida *et al.* (2001) did not find difference in the incidence of lameness, measured through locomotion score, in dairy cows supplemented with an organic mineral complex (360 mg of zinc methionine, 125 mg of copper lysine, 200 mg of manganese methionine and 25 mg of cobalt glucoheptonate per cow daily) and cows receiving inorganic minerals.

1.7 The mechanical properties of the hoof horn

The hoof horn is a natural biological composite composed of keratinised material that must be capable of accommodating and resisting high loads without excessive deformation or catastrophic failure (Newlyn *et al.*, 1999). The hoof wall archives its requirements through a compromise between the need to accommodate forces, minimise crack propagation and avoid excessive deformation (Reilly *et al.*, 1998). During normal weight bearing the hoof deforms in a consistent pattern, generating internal forces to counter the applied load (Douglas *et al.*, 1996). The dermo-epidermal junction in the laminae and papillae areas is subjected to load by pressure and tensile forces (Mulling and Budras, 2002). The hoof wall strain in horses was influenced by the riding style, by the rider, by shoeing and by the walking surface (Summerley *et al.*, 1998; Thomason, 1998).

According to Vincent (1992) the morphology of the material or structure being tested is at least as important as its mechanical properties. Keratin is modelled as a fibre reinforced composite material consisting of microfibrils embedded in an amorphous, non-fibrous matrix, formed by globular proteins (Baillie and Fiford, 1996). The matrix usually binds fibres and transfers stresses to them, helps in withstanding compressive forces and inhibits cracks from propagating through fibres (Kasapi and Gosline, 1999). Microfibrils are built by bundles of protofibrils that are formed by an α-helix of amino acids wound together. The keratinized cells are further organised into either tubular structures or intertubular material, forming a macroscale composite and the hoof wall is considered a multi-level or hierarchical composite (Kasapi and Gosline, 1997). According to Baillie and Fiford (1996) the structure of the hoof horn with its overlapping fibre-reinforced flakes can be compared also to an engineered laminated composite.

1.7.1 Factors that influence hoof horn quality

The structure and the biomechanical properties of the hoof horn have been found to be determined by factors that were classified into internal and external factors. The internal factors include the structure, composition and chemical bonding of keratin proteins, keratin filaments and filament-associated proteins, the structure, composition and amount of the intercellular cementing substance and the architecture of the horn, i.e. the arrangement of horn tubules and intertubular space (Pellman *et al.*, 1993; Zaun, 1997; Mulling *et al.*, 1998; Patan and Budras, 2003). The quality of keratinisation has been found to be dependent on the appropriate supply of nutrients and oxygen. The strength of the keratin proteins was found to be determined by the amount of their disulphide bonds (Mulling *et al.*, 1994).

The external factors that affected the structure of the hoof horn were humidity, chemical and microbiological factors. The extent of the effect of the external factors was found to be determined by the existent horn structure (Budras and Mulling, 1998). The hydration of the hoof horn is likely to be controlled by the tubules (Baillie and Fiford, 1996). Nutritional factors have been considered to be an internal and external factor. External factors are often referred to as environmental factors. These environmental factors are related to housing hygiene, such as the level of urine and manure, and reduce the quality of healthy horn. Urea has the capability to extract keratin from the hoof horn and manure acts by removing the intercellular substance. The tubular medulla that is of poorer quality is dissolved first and consequently the susceptibility of hoof horn to bacterial invasion increases (Budras and Mulling, 1998; Kempson *et al.*, 1998). Kung *et al.* (1991) reported that the incubation of equine hoof horn in slurry and urine reduced the tensile strength of hoof horn. Kempson *et al.* (1998) found that the heel horn was more susceptible to the influence of chemical factors than the horn on other parts of the hoof. In another study, Langridge *et al.* (1998), using water-soluble tracers, found that the heel horn had a greater degree of permeability when compared with the wall and sole horn. This permeability may explain the susceptibility of the heel to horn erosion. Wagner and Hood (2002) demonstrated that the fully cornified epithelial cells of the outer stratum medium, frog and sole of the equine hoof exchange water and crystalloids (Na^+, Cl^- and K^+) but not proteins with the environment, in response to diffusion. Beside the crystalloids, lipids were also eluted from the frog epithelium (Wagner and Hood, 2002). The frog epithelium had a greater ability to hydrate (31.6 %) when compared to the dorsal (26.5 %), axial (29.3 %) and abaxial (27.6 %) wall (Leopold and Prietz, 1980). A low water binding capacity of the nails in humans is related to the occurrence of brittle nails (Zaun, 1997).

1.7.2 Methods for testing the mechanical properties of the hoof horn

The stiffness of a material can be determined from the elastic modulus or Young's modulus. Several tests, such as tensile tests, bending tests and compression tests are used for the measurement of the elastic modulus. In a fibre composite material the Young's modulus parallel to the fibres is given by the Young's modulus of the fibres multiplied by the volume fraction of the fibres added to the Young's modulus of the matrix multiplied by the volume fraction of the matrix. This equation is known as the Voigt estimate (Kitchener and Vincent, 1987). This equation assumes that the fibrous and matrix phases are linearly elastic and that the fibres are uniform in properties and shape and similarly aligned. The fibrous phase must also have equal properties in all directions. These criteria are violated in most biological materials and their mechanical properties are dependent on where and on what direction a load is applied (Aranwela *et al.*, 1999; Kasapi and Gosline, 1999).

The Poisson's ratio (v), the ratio of the lateral strain to the axial strain, of the hoof horn was estimated to be 0.4 (range of 0.38 to 0.46) by Kasapi and Gosline (1996).

1.7.2.1 Hardness

Hardness is defined as the resistance of a material to penetration by a harder object. The harder the material, the smaller the degree of penetration of the indenter and the smaller the size of the indentation that remains (Vincent, 1992). Wear can be regarded as a process of microfracture and so part of the resistance to wear is the toughness of the material. Increased resistance to wear is archieved by increasing hardness (Vincent, 1992). Two different techniques have been used for measuring hardness of equine and bovine hoof horn: the Shore durometer (Manson, 1986; Distl and Schmid, 1994; Coenen and Spitzlei, 1996; Zoscher *et al.*, 2000) and the ball indentation method (Pellman *et al.*, 1993; Mulling *et al.*, 1994; Zoscher *et al.*, 2000). The Shore durometer, once placed flat on the materials surface, presses a spring-loaded probe into the surface with a constant force. The penetration depth decreases with increasing hardness (Vermunt and Greenough, 1995). The Shore durometer has fine needle-like tips and small inhomogenities can lead to false results and a high variation in the results (Budras *et al.*, 1998; Hochstetter, 1998).

A high correlation has been found between the measurement of mechanical strength, through the ball impact method (DIN 53456) and histometric, histochemical and immuno-histochemical methods (Pellman *et al.*, 1993; Mulling *et al.*, 1994). Measurements of hoof hardness have been found to be affected by the moisture content of the hoof horn, the hardness decreases with increasing moisture content (Collins *et al.*, 1998; Hinterhofer *et al.*, 1998). The moisture content has been found to be affected by the micro-architecture and biochemical composition of the horn (Vermunt and Greenough, 1995: Hendry *et al.*, 1997).

Hardness measured through the ball impact method and Shore D meter in cattle decreased significantly from the dorsal wall towards the heel and from the coronary border of the wall towards the weight bearing border. Hardness of the sole horn was significantly lower than hardness of wall horn samples when measured through Shore D and ball impact methods (Table 1.16). The lower hardness values could indicate that the sole horn is more prone to injuries due to compressive pressures (Berry, 1999; Galbraith *et al.*, 2002; Van der Tol *et al.*, 2002). Within the sole the hardness decreased from the sole towards the heel (Table 1.16). Frohnes and Budras (2001) reported in horses lower hardness values for the bulb horn when compared with the sole horn. Areas that presented lower hardness values presented also lower dry matter values (Zoscher *et al.*, 2000). Heel horn hardness was higher in 3 month old calves when compared with older animals (Offer *et al.*, 2000).

The Shore D hardness of the dorsal wall horn and the sole horn of front claws of dairy cows was significantly higher than the hardness of the dorsal wall horn and sole horn of the hind claws (Table 1.17). The water content of the dorsal wall horn and sole horn of the front hooves was lower than the water content of the dorsal wall horn and sole horn of the hind hooves (Table 1.17) (Schmid, 1995).

Table 1.16 ***Hardness, tensile strength, elastic modulus and moisture content of the coronary, sole, heel and white line horn of cattle***

	Abaxial wall	Sole	Heel	White line	se	Source
Hardness (Shore A)	Toe 89.5 Mid 88.0	Toe 84.7 Mid 75.1	40.0	-	1.1	Manson and Leaver (1988)
Hardness (Shore A)	Toe 87.6 Mid 86.9	Toe 84.0 Mid 76.6	39.4	-	1.1	Manson and Leaver (1989)
Hardness, ball impact (N/mm^2)	25.7	12.9	6.8 9.3[b]	Cap 6.9 Term 5.1[c]	-	Mulling *et al.* (1994)
Hardness, ball impact (N/mm^2)	Toe 22.2-17.6[d] Lat 17.2-15.8[d]	10.9	Wall 15.7-11.2[d]	-	-	Zoscher *et al.* (2000)
Hardness (Shore D)	Toe 78.4 Mid 77.3	48.7	43.0[b]	Toe 70.4 Abax. 64.7	9.5	Borderas *et al.* (2004)
Hardness	-	86.7	70.8	-	9.8	Webster (2001)
Hardness (Shore D)	Toe 63.2-62.0[d] Lat 60.6-56.2[d]	49.3	58.5-52.5[d]	-	-	Zoscher *et al.* (2000)
Abrasion resisting (%/ 10s)[a]	Toe 35.1 Mid 40.3	-	wall 47.4	-	-	Leopold and Prietz (1980)
Tensile strength (N/mm^2), 65%RH	67.73	58.59	41.88	-	8.6	Schmid (1995)
Elastic modulus, tension (N/mm^2)	Toe 613.5 Lat 375.3	134.9	-	-	14 7.2	Zoscher *et al.* (2000)
Dry matter (%)	Toe 74.6 Lat 73.4	68.8	-	-	2.7	Zoscher *et al.* (2000)
Max. water uptake capacity (%)	Toe 26.5 Mid 27.6	-	wall 31.6	-	-	Leopold and Prietz (1980)

[a] – fully hydrated samples
[b] - sole-heel junction
[c] – cap = cap horn, term = terminal horn
[d] – values form coronary band to border

Table 1.17 ***Hardness, tensile strength, elastic modulus and moisture content of the hoof horn of front and hind claws of cattle***

	Hind	Front	se	*P*	Source
Hardness, wall toe	4.91	4.56	-	-	Clark and Rakes (1982)
Hardness, coronary horn (Shore D)	64.5	65.9	8.0	-	Distl and Schmid (1994)
Hardness, coronary horn (Shore D)	74.1	76.4	2.0	0.001	Schmid (1995)
Hardness, sole horn (Shore D)	44.1	48.3	8.6	0.001	Schmid (1995)
Tensile strength, coronary horn (N/mm^2), 65% RH	61.34	67.33	9.45	0.05	Schmid (1995)
Tensile strength, sole horn (N/mm^2), 65% RH	51.98	53.08	9.34	NS	Schmid (1995)
Elastic modulus, tension (N/mm^2)	349.5	405.5	247.0	-	Zoscher *et al.* (2000)
Moisture content, coronary horn (%)	14.73	10.26	2.3	0.001	Schmid (1995)
Moisture content, sole horn (%)	25.03	19.80	2.50	0.001	Schmid (1995)
Moisture content (%)	28.7	26.9	3.7	-	Zoscher *et al.* (2000)
Moisture content (%)	36.8	34.1		0.01	Van Amstel *et al.* (2004)

Mulling *et al.* (1994) found a high negative correlation between hardness and horn structure and the sites of predilection for occurrence of lesions in the ground surface of the hoof. Borderas *et al.* (2004) found a negative correlation between claw hardness and the severity of claw lesions, indicating that softer claw horn was related to increased severity of claw lesions. Manson (1986) found a significant negative correlation between the sole mid hardness and the locomotion score. Sole, heel and dorsal wall hardness were significantly lower in the affected digits of lame cows (47.6±6.3, 36.4±6.0 and 70.0±5.0, respectively) when compared with the non-affected digit on the same leg (50.6±5.0, 37.8±5.4 and 71.1±4.2, respectively). Sole hardness and sole and heel moisture (47.6±6.3, 30.7±2.6 % and 35.8±4.9 %, respectively) were also lower in the digits of lame cows when compared with the same digit of non-lame cows (49.2±3.8, 31.5±2.3 % and 38.6±5.0 %, respectively) (Tranter *et al.*, 1993). Lesion scores and heel horn erosion were significantly and negatively related to heel horn hardness (Offer *et al.*, 2001).

The hardness of the horn of the abaxial wall was significantly higher in biotin supplemented when compared to non-supplemented animals (Table 1.14) (Webb *et al.*, 1984; Distl and Schmid, 1994; Brooks *et al.*, 1997 and Campbell *et al.*, 2000). However, Hedges *et al.* (2002) did not find differences in the hardness of the sole horn between biotin supplemented and non-supplemented dairy cows, when using a Vickers hardness test.

In calves, feeding grass silage as opposed to hay resulted in lower horn hardness at the bulb (Offer *et al.*, 2000b). However, Webster (2001) did not find significant differences between the hardness of the sole and heel horn of heifers fed with a low or high dry matter forage diet. Feeding high concentrate ratios caused also a decrease in the hoof horn hardness (Manson and Leaver, 1989). Nutrition can influence lameness indirectly by influencing the quantity and quality of the slurry and changing by this way the underfoot conditions within the housing system (Offer, 2000b). Hoof horn hardness was highly related to the moisture of the environment (Tranter *et al.*, 1993). Animals kept on slatted floors covered with rubber mats presented lower hardness of the sole and white line area at the tip of the toe (56 and 31.5) measured through a Shore D hardness testing set when compared to animals kept on concrete slatted floors (61 and 35.5) (Kremer *et al.*, 2004). Those differences in horn hardness are probably related to a lower wear rate of the claws of cows kept on floors covered with rubber mats.

According to Clark and Rakes (1982) hoof horn pigmentation did not affect hoof horn hardness. However, Hepburn *et al.* (2004) reported that non-pigmented claw wall horn in cattle was significantly harder (46.5 vs. 40.3 nearer the coronary line and 68.5 vs. 64.8 away from the coronary line) in areas up to 4.5 cm under the coronary horn when compared to pigmented horn.

1.7.2.2 Tensile tests

Tensile tests proved to be optimal for the testing of the stability of the intercellular connections of the horny cells (Budras *et al.*, 1998). The tensile strength is the force (N/mm^2) required to tear the horn sample in two pieces (Geyer and Schulze, 1994). Specimens for tensile tests have normally a 'dog bone shape', with a rectangular cross-section notched to different depths. To avoid the occurrence of end effects Aranwela *et al.* (1999) recommend the use of test specimens that have a length to width ratio greater than ten.

The hoof horn behaves as a linear viscoelastic material (Vincent, 1992; Collins *et al.*, 1998). In viscoelastic polymeric materials the strain rate influences the measured values of elastic modulus (Aranwela *et al.*, 1999; Baillie *et al.*, 2000) and with increasing strain rates a transition from ductile to brittle behaviour can occur (Kasapi and Gosline, 1996). The testing speed used by different authors varied between 1 and 5mm/min (Baillie *et al.*, 2000; Douglas *et al.*, 1996; Hinterhofer *et al.*, 1998). Kasapi and Gosline (1996) tested 4 different strain rates (0.0016/s, 0.032/s, 0.33/s and 70/s, corresponding to tests speeds of 5 mm/min, 102 mm/min, 1020 mm/min and 234,000 mm/min respectively) when performing tensile tests on horse wall horn samples. Initial elastic modulus, maximum stress and total energy showed significant increase with increasing strain rates. The hoof wall became stiffer with increasing loading rate, being more capable of absorbing more energy before failure. However, no transition to brittle behaviour occurred.

The elastic modulus of the wall horn of the toe area of the claws of dairy cows was found to be higher than the elastic modulus of the lateral wall horn and the elastic modulus of the sole horn (Table 1.16). Higher values were also reported for the front claws when compared to the hind claws (Table 1.17) (Zoscher *et al.*, 2000). Dry matter of these areas and claws were significantly different being higher in areas that presented higher values of elastic modulus (Zoscher *et al.*, 2000). The tensile strength differed significantly also between the dorsal wall horn, the sole horn and the heel horn of the front hooves of dairy cows, incubated at 65 % relative humidity, when compared with the dorsal wall horn, the sole horn and the heel horn of the hind hooves (Table 1.17) (Schmid, 1995).

In horses Zenker *et al.* (1995) found tensile strength values of 36 N/mm^2 for the medial and lateral walls and of 46 N/mm^2 for the dorsal wall. The samples were incubated at 65 % relative humidity before testing. Hinterhofer *et al.* (1998) tested the elastic modulus of equine hoof samples at physiological moisture levels and obtained the values of 735 ± 289.5 N/mm^2 for wall samples, 230 ± 92.4 N/mm^2 for sole samples and 9.9 ± 0.6 N/mm^2 for frog samples. The physiological moisture content of the wall, sole and frog samples were 22.7 ± 3.4 %, 31.5 ± 3.1 % and 34.6 ± 3.3 % respectively. The differences were statistically significant ($p<0.05$). No difference was obtained between the elastic modulus of dorsal wall samples and lateral wall samples and of the moisture content of these samples (761.8 and 708.0 N/mm^2, respectively). However, Douglas *et al.* (1996) obtained significant differences between the dorsal outer wall samples of equine hooves with physiological moisture content and samples obtained form the medial and lateral wall areas

(955 ± 199 Mpa for the dorsal wall and 607 ± 100 Mpa for both lateral and medial wall areas). The lateral and medial hoof wall is thinner than the dorsal wall and therefore samples include horn from closer to the dermis. Dorsal outer wall samples were significantly different from dorsal inner wall samples (955 ± 199 Mpa and 502 ± 98 Mpa respectively). The dorsal inner wall samples had significantly higher moisture content (35.5 %) than the outer wall samples (27.9 %). There was a significant negative correlation between the moisture content of the outer wall samples and their elastic modulus (Douglas *et al.*, 1996). A gradient of stiffness between the rigid horn of the outer wall and the soft tissues of the dermis will help to reduce the stress at the interface between epidermis and dermis (Douglas *et al.*, 1996; Wagner *et al.*, 2001). Kasapi and Gosline (1997) reported that the initial elastic modulus increased from 300 to 560 Mpa from the inner to the outer regions of the equine hoof wall. Hinterhofer *et al.* (2002) found an increase in the concentration of carbon and sulphur from the *stratum externum* inwards of the equine hoof wall. These differences could explain the higher mechanical resistance of the *stratum externum*.

The initial elastic modulus of the tubules of the hoof wall of horses varied through the wall thickness and were related to the orientation of the intermediate keratin filaments in the cells in the different areas. The highest initial elastic modulus of the tubules was obtained on the inner third of the wall (470 Mpa, ranging from 120 to 1140 MPa). This value was 6 times higher than the value (80 Mpa, ranging from 40 to 120 MPa) obtained for the intertubular material adjacent to the tubules. Mid-wall tubules exhibited an elastic modulus of 290 Mpa (ranging from 140 to 570 Mpa). On this area the elastic modulus of the intertubular material was 140 Mpa (ranging from 40 to 270 Mpa). Yielding stresses of inner and mid-wall tubules and of inner and mid-wall intertubular material were 4.2, 4.8, 4.1 and 4.1 Mpa respectively. Ultimate stress was dependent on specimen type and maximum strain was independent. When testing the elastic modulus of keratin strands the initial elastic modulus was higher along than across fibres. The tubules in the inner wall region are the high longitudinal stiffness element of this region and having a less important role in the mid-wall region. The volume fraction of intermediate fibres was significantly lower (23 %) in the inner wall region, when compared with the mid-wall (31 %) and outer wall (30 %) regions. The reduction of the volume fraction of intermediate fibres could explain the difference in stiffness between the inner, mid and outer walls. The mechanical properties of the equine hoof wall are modulated by varying the intermediary filament orientation, the intermediary filament volume fraction and the water content (Kasapi and Gosline, 1999).

The orientation of the samples of the hoof wall in relation to the tubules has an influence over the test results. Dorsal wall samples, stressed parallel to the tubule orientation, were significantly stiffer (998 ± 242 Mpa) than samples stressed perpendicular to the tubules (912 ± 143 Mpa) (Douglas *et al.*, 1996). Through finite element analysis Newlyn *et al.* (1999) obtained an axial to lateral modulus ratio lower than 2. This ratio is small when compared to synthetic composites, but is related to the tubular and intertubular horn being the same cellular material arranged in a different way. The tubule volume fraction is also lower in the hoof wall when compared with synthetic composites (Newlyn *et al.*, 1999).

Tensile strength of the white line of the claws of dairy cows varied between 3.8 and 6.5 Mpa on the medial claw and from 3.1 to 4.7 Mpa on the lateral claw and differences were statistically significant. White line with structural damage had a significantly lower tensile strength (2.4 Mpa) than not damaged white line (4.5 Mpa) (Hedges *et al.*, 2002; Collis *et* *al.*, 2004). Geyer and Schulze (1994) reported a tensile strength of 60 N/mm^2 for unaltered wall horn in horses and of 20 to 28 N/mm^2 for horn with alteration of the intertubular horn. The samples were incubated at 65 % relative humidity before testing.

When Hinterhofer *et al.* (1998) conditioned wall and sole samples at a relative humidity of 65 % the elastic modulus of conditioned samples was 1802.3 ± 700 N/mm^2 for wall samples and 1673.8 ± 557.8 N/mm^2 for sole samples and differences were not statistically significant as they were in non conditioned samples. The moisture content of the conditioned samples was of 15.9 ± 1.2 % for wall samples and 16.2 ± 0.9 % for sole samples. The elastic modulus of dry samples ranged between 1,636 and 8,650 N/mm^2. Hinterhofer *et al.* (1998) and Douglas *et al.* (1996) pointed out the importance of testing the samples at physiological moisture levels to represent the *in vivo* situation. Bertram and Gosline (1987) reported that the elastic modulus from the hoof wall horn of horses increased from 410 Mpa at 100 % relative hydration to 14,600 Mpa at 0 % relative hydration. Kitchener and Vincent (1987) reported a tensile strength of 137 Mpa for dry oryx horn, 122 Mpa for oryx horn with 80 % dry matter and 56 Mpa for oryx horn with 60 % dry matter.

After the supplementation of dairy cows with biotin there was a significant increase of the tensile strength of the dorsal wall horn, the sole and the heel horns (Table 1.14). The increase in tensile strength was observed after 10 months in the dorsal wall horn and after 4.5 months in the sole horn and heel horn (Schmid, 1995). A significant increase in the tensile strength occurred after supplementing horses during 33 months with biotin (39.4 ± 11.8 to 44.1 ± 6.6 N/mm^2) (Zenker *et al.*, 1995).

No significant difference was obtained between the elastic modulus of pigmented and non-pigmented hoof horn samples of horses (Douglas *et al.*, 1996; Hinterhofer *et al.*, 1998; Ley *et al.*, 1998).

1.7.2.3 Compression tests

Douglas *et al.* (1996) reported that the elastic modulus of equine hoof wall samples measured in compression was significantly higher than the elastic modulus measured in tension. However, a strong linear relationship was obtained between both tests. The mean modulus of elasticity of dorsal outer wall samples obtained in compression tests were 1004 ± 198 Mpa and they were significantly higher than the mudulus of elasticity of the inner wall samples, that presented an elastic modulus of 523 ± 91 Mpa. Dorsal inner wall samples had a significantly higher moisture content (35.5 %) than outer wall samples (27.9 %) (Douglas *et al.*, 1996).

The supplementation of pigs with biotin increased significantly the compressive strength of the abaxial sidewall horn from 9.3 (± 0.5) x 10^6 N/mm^2 to 12.0 (± 0.5) x 10^6 N/mm^2 in samples with physiological moisture content (Webb *et al.*, 1984).

1.7.2.4 Bending tests

The bending stiffness is the resistance to bend of a beam under constant load. Baillie *et al.* (2000) reported bending stiffness values for bovine hoof wall horn that ranged from 8,000 Mpa at 0 % moisture content to approximately 400 Mpa at 36 % moisture content. The bending stiffness decreases with increasing moisture content and when the moisture content was higher than 10 % there was a transition from a more brittle behaviour to a more ductile behaviour. Moisture content and temperature can cause this transition to occur (Baillie *et al.*, 2000). Collins *et al.* (1998) and Kitchener and Vincent (1987) reported also increasing bending stiffness with increasing dry matter of donkey hoof wall horn and oryx horn. The bending stiffness of dry hoof wall horn of donkeys was 10 to 15 times higher than the bending stiffness of samples with physiological moisture content (Collins *et al.*, 1998).

Wagner *et al.* (2001) reported significantly higher bending modulus for the outer stratum medium of the equine hoof wall (187.6 ± 41.3 Mpa) when compared with the bending modulus of the stratum medium zona alba (dermo-epidermal junction) (98.2 ± 36.8 Mpa). Yield strength was also higher for the outer stratum medium (19.4 ± 2.6 Mpa) than for the stratum medium zona alba (5.6 ± 1.7 Mpa). The yield point is the point at which the force-displacement curve becomes non-linear (Wagner *et al.*, 2001). The bending modulus values of the stratum medium zona alba were between the bending modulus values from the outer stratum medium and the laminar corium, supporting the concept of a gradient in stiffness between the outer and inner components of the wall of the hoof (Wagner *et al.*, 2001).

The freezing and dehydration and rehydration of equine hoof wall samples did not have an effect over the bending modulus of the samples. Samples were frozen at –10 °C for 10 min (Kasapi and Gosline, 1999).

1.7.2.5 Fracture toughness and crack propagation

The fracture toughness is a measure of the energy required to produce a new surface area through cracking at a critical point (Clark and Lyall, 2000). A crack must first be initiated and the initiation of a crack depends on strength. The fracture of the molecular bonds of the tip of the crack depends on the strength of these bonds and on the shape of the crack tip (Vincent, 1992). Brittle materials require relatively little energy for fracture and the fracture surface is relatively smooth (Vincent, 1992). Clark and Lyall (2000) shaped the specimens in a rectangular shape (10 x 10 x 3 mm), then two holes were drilled in specific areas and a notch of known length was cut into the specimen. Notches were aligned with the orientation of the horn tubules. Fully hydrated specimens were loaded perpendicularly and the energy required to advance the crack at the critical point of failure was determined.

The stratum medium of the equine hoof wall is highly resistant to crack propagation. The value for equine hoof wall horn was determined to be approximately 11,000 Jm^{-2} for fully hydrated samples, this value is equivalent to values obtained for most man made fibre reinforced composites (Bertram and Gosline, 1986). Kasapi and Gosline (1996) obtained a similar value of 12,000 $Jm^{-2} \pm 300$ for the mean fracture toughness of fully hydrated samples of the equine hoof wall. The maximum fracture toughness of the equine hoof wall horn was 22,800 Jm^{-2} and was obtained at 75 % relative hydration, being higher than values for fully hydrated and dry samples (Bertram and Gosline, 1987). This level of hydration is within the range that can be found *in vivo*. However, Collins *et al.* (1998) reported that the *in vivo* moisture content of donkey hoof horn corresponded to a relative humidity environment of 96 %. Kasapi and Gosline (1996) tested different strain rates (0.017 mm/s, 1.7 mm/s, 17 mm/s and 2500 mm/s) that should represent strain rates observed *in vivo* and the fracture toughness was not affected by strain rate. The values determined for the wall horn of bovine claws was approximately 2/3 of the value determined for the wall horn of horses.

Clark and Lyall (2000) reported fracture toughness values of 8182 and 8483 Jm^{-2} for fully hydrated horn of the abaxial wall of mature beef cows with and without vertical fissures of the claws. No significant difference was obtained between both groups.

According to Bertram and Gosline (1986) the weak plane of the equine hoof wall keratin is parallel to the sole of the hoof. The tubules are oriented perpendicular to this weak plane and act by reinforcing the intertubular material. Even with the presence of the reinforcing tubules Baillie and Fiford (1996) reported that the fracture toughness of the bovine hoof wall was 3 times higher in the plane parallel to the tubules when compared with the plane perpendicular to the tubules. Baillie *et al.* (2000) reported similar results. Kasapi and Gosline (1997) reported that in the regions of the equine hoof wall with a high proportion of tubules (inner and outer regions) the fracture path followed the tubules. However, in regions dominated by intertubular material (middle region) the fracture path followed the intertubular intermediary filament plane. These authors also reported a significantly higher toughness for the inner region of the hoof wall when compared with the middle and outer regions. The differences in toughness may not exist at *in vivo* hydration levels (Kasapi and Gosline, 1997). Cracks on dehydrated samples of bovine hoof horn that were perpendicularly orientated in relation to the horn tubules failed in a brittle manner. In dehydrated samples were the crack was orientated parallel to the horn tubules the crack failure path was more difficult and deviated from the original plane (Baillie *et al.*, 2000). Any crack that would appear in the weight-bearing border of the hoof would propagate parallel to the weight bearing border. The wavy nature of the intertubular material would also help to stop cracks from propagating. In the hoof horn of horses the crack propagation occurred between the cells and parallel to the tubules (Bertram and Gosline, 1986). However, the bovine hoof horn behaved as a fibrous composite and the crack propagated parallel to the keratin fibres and not between cells. In such situations the intercellular bond was stronger than the bond between the keratin fibres and the matrix. A pullout of keratin fibres was noticed in the failure surfaces (Baillie and Fiford, 1996).

Storing equine hoof wall horn samples at 4 °C in distilled water with 0.02 % sodium azide to prevent bacterial growth, for the period of 3 months did not compromise the fracture toughness of the samples (Kasapi and Gosline, 1997).

1.7.2.6 Punch test

The punch test has not been used previously to assess the physical properties of hoof horn. However, the punch tests has been used to determine the shear and creep behaviour and the deformation and failure properties of metals, polymers and composites, such as, ultra-high molecular weight polyethylene (UHMWPE), low-alloy steel, polymethyl methacrylate, leafs, dental restorative materials and in the food industry (Aranwela *et al.*, 1999; Satapathy and Bless, 2000; Dobes and Miicka, 2001; Nomoto *et al.*, 2001; Kurtz *et al.*, 2002; Lewis, 2002). Punch tests are frequently used to determine mechanical properties of small or miniature specimens (Husain *et al.*, 2002; Lewis, 2002). The basic elements of the technique are a punch and a specimen holder, or die. According to the geometry of the specimen different shapes and dimensions of the punches and dies have been used (Husain *et al.*, 2002). Tensile properties are determined with a hemispherical or ball punch end and shear properties with a flat-headed punch end (Dobes and Milicka, 2001; Lewis, 2002). Samples can or not be clamped in place and are called respectively disc-bend and bulge tests (Dobes and Milicka, 2001; Husain *et al.*, 2002). Roessig and Mason (1998) considered that the two-dimensional punch test was more suitable to test shear dominated failure, because of the high stress intensity factor (specimen partially supported by a die).

However, Liu and Piggot (1998) observed that most polymers appear to be failing in tension when tested in the punch tests. Kurtz *et al.* (2002) described a transition from a shear deformation mode to a tensile deformation mode in UHMWPE. Aranwela *et al.* (1999) considered that the primary mode of failure in a shear punch test performed on leaves is shearing, although tensile and compressional failure are also involved.

When results of the small punch test where compared to result of standard ASTM tension tests the variation between results was of 2.5 to 25 % (Lewis, 2002). Liu and Piggot (1998) reported a good correlation between the tensile yield strength obtained in standard tensile tests carried out on dog bone shaped specimens (ASTM D638) and the punch shear strength when testing different polymers. Results of both tests only varied more than 15 % when more brittle materials, such as epoxy and PVC, were studied. Dobes and Milicka (2001) found a similar pattern between the time to rupture in small punch test and the time to fracture in a traditional creep test. The calculation of the bulk material properties, such as elastic modulus and ultimate tensile strength, when using the small punch test is very complicated due to the contact mechanics involved (Aranwela *et al.*, 1999; Lewis, 2002). In a shear punch test the stress and strain distribution is not homogenous throughout the sample and direct comparisons to tests with constant strain rate are not appropriate (Kurtz *et al.*, 2002). According to Lewis (2002), although several relationships have been established (Dobes and Milicka, 2001), the source of variation in the data has not been well characterized and a large volume of data from small punch tests should be compared with standard tests. According to Kurtz *et al.* (2002) the load-displacement curves from shear punch tests exhibited similar distinctive features such as, initial stiffness, transition load, hardening stiffness and peak load and were highly reproducible. The transition load reflected the occurrence of a zone with plastic deformation. The load-displacement curves from small punch tests resembled that of conventional creep tests when testing low-alloy-steel, however, the curves of the small punch test had a significant greater primary stage (Dobes and Milicka, 2001). Finite element analysis has been used to compare elastic modulus obtained from standard tests and the initial slope of the force-displacement curve of small punch tests, the initial slope being linearly related to the elastic modulus (Kurtz *et al.*, 1999; Kurtz *et al.*, 2002). However, the small punch test has been widely used in parametric studies, where relative changes in one parameter have been studied, while the other parameters remained unchanged, (Lewis, 2002).

Work to failure, the area under the force displacement curve up to the ultimate punch displacement, has been considered a test parameter with strong discriminating and predictive potential and was inversely related to the wear rate of resins retrieved from tibial inserts (Lewis, 2002). However, the same test parameter presented inconsistent results when the treatment of the resin, such as sterilization method, was studied (Lewis, 2002). Liu and Piggot (1998) considered the ultimate shear strength, associated with the peak load, an important parameter for the understanding of interface strengths (between the fibres and the polymer) of fibre-reinforced composites. Nomoto *et al.* (2001) found significant differences in the shear punch strength when testing different dental restorative materials. The rank order according to shear strength of these materials was consistent with their clinical performance. Aranwela *et al.* (1999) considered that the shear punch test detected variations that correlated to physical aspects of leaf biology. Kurtz *et al.* (2002) found that the shear punch test discriminated well between shelf aged and virgin UHMWPE. Shelf aged material exhibited significantly greater initial stiffness and transition loads and lower peak load and hardening stiffness. Dobes and Milicka (2001) reported a decrease in the ultimate punch displacement with the increase in the test temperature when testing low-alloy-steel. Reppond *et al.* (1995) and Reppond and Babbit (1997) observed a linear decrease in the ultimate punch force with increasing moisture content when surimi made from different types of fish was tested.

Satapathy and Bless (2000) reported a higher slope of the force-displacement curve and higher peak loads in deep punch tests when hemispherical punches were used compared to conical punches. Aranwela *et al.* (1999) did not find significant differences in the strength of leaves when sharp and curved punches were used, however work to fracture was significantly reduced when sharp edged punches were used and the authors concluded that the sharp edge may have behaved like a wedge in the tissue. Force and work to fracture increased linearly with the increase in the punch size (Aranwela *et al.*, 1999).

The restraining method, not restrained and restrained with a screw clamp, had a significant effect over the shear punch strength of dental restorative material. Shear strength was greater for specimens restrained with a screw clamp (Nomoto *et al.*, 2001).

Punch displacement rate (0.5 to 2.5 mm/min) in the small punch test had a significant effect over the ultimate peak punch load and the ultimate punch displacement, but did not influence the work to failure, of ultra-high-molecular-weight polyethylene (Kurtz *et al.*, 1999). Nomoto *et al.* (2001) failed to find any effect of test speeds varying from 0.25 to 2.0 mm/min on the shear punch strength of polycarboxylate cement. Aranwela *et al.* (1999) found a significant interaction between the test speed and the clearance gap, this is the gap between the punch and the die, for the strength of leaves measured through a punch test. At low speeds of 0.84 mm/s the punch strength was significantly greater when the test was performed with a clearance gap of 0.05 mm when compared to a clearance gap of 0.15

mm. The authors concluded that at small clearances the die supported the tissues more, reducing its capacity to flex and enhancing its stiffness. At high speeds the material could not respond rapidly enough to the load. High test speeds used were of 2.98 mm/s and the authors considered test speeds that ranged between 0.25 and 1.0 mm/s suitable for using in punch tests. Work to fracture increased with greater clearance gaps. As the clearance increases and the tissue flexes more, the displacement required to fracture the material also increases and as work is the product of force and displacement it should increase with greater clearance gaps.

Nomoto *et al.* (2001) did not record any significant difference when testing polycarboxylate cement with a specimen thickness of 0.5 and 1.0 mm in a shear punch test.

1.8 Rationale of the research program

The mean incidence of lameness in individual herds in the United Kingdom varied from 22.0 to 55 cases per 100 cows (Clarkson *et al.*, 1996; Hedges *et al.*, 1998; Kossaibati *et al.*, 1999; Whay *et al.*, 2002; Whitaker *et al.*, 2004) and lameness was considered to be one of the most painful conditions to commonly affect cows (Kossaibati *et al.*, 1999; Webster, 2002). Claw horn lameness has been found to cause 60 to 73 % of lameness cases in the UK (Kossaibati *et al.*, 1999; Logue, 1999). The major problems of the claw horn were sole ulcers and white line lesions (Logue, 1999). The occurrence of haemorrhages in the sole and white line horn of dairy cows was associated to the development of more severe lesions and the severity of the lesions had a correlation with the locomotion score of the cows (Whay *et al.*, 1997). The occurrence of haemorrhages in the claw horn in dairy cows was to proven to be related to physiological changes in the *peripartum* period (Holah *et al.*, 1998), housing systems, mainly the exposure of the hoof to moisture and slurry (Budras and Mulling, 1998; Kempson *et al.*, 1998) and to the animal conformation (Boettcher *et al.*, 1998; Boelling and Pollot, 1998b).

The biomechanical properties of the hoof horn should reflect the horn structure that is determined by the composition and chemical bonding of keratin proteins, keratin filaments and filament-associated proteins, the structure, composition and amount of the intercellular cementing substance and the architecture of the horn, i.e. the arrangement of horn tubules and intertubular space (Pellman *et al.*, 1993; Mulling *et al.*, 1998). The quality of keratinisation has been found to be dependent on the appropriate supply of nutrients and oxygen. The living epidermal cells are very susceptible to any disturbance in the circulation within the vessels of the corium (Mulling *et al.*, 1998). The structure of the hoof horn is affected also by external factors such as, humidity, chemical and microbiological factors (Budras and Mulling, 1998).

The present study aimed to test and develop a method to measure changes in the mechanical properties of the sole and white line hoof horn that should be used *in vivo* and to examine the hypothesis that the presence of haemorrhages in the horn would represent a weakening of the structural strength of the horn. Experiments were set up also to measure the influence of factors that affect the occurrence of claw horn haemorrhages over the mechanical properties of the hoof horn such as:

- Changes during the lactation period,
- Supplementation of diets with biotin,
- Heifer conformation,
- Claw measurements and horn growth and wear rates.

Chapter 2

Experiment 1 - The effect of housing and days in lactation on the mechanical properties of the hoof horn

2.1 Introduction

The distal part of the hoof horn that encompasses the sole, white line and heel areas, is the source of the majority of the lameness problems in dairy cows (Logue, 1999; Kossaibati *et al.,* 1999). The levels of haemorrhage in the sole and white line areas of the claw horn have been found to increase during the *postpartum* period (Enevoldsen *et al.,* 1991; Leach *et al.* 1997 and Offer *et al.,* 1998 and 2000), under some management and housing conditions (Chaplin et al, 2000; Bergsten, 2001 and Webster, 2001) and have been found to be greater in the hind outer claws when compared to other claws (Bergsten and Herlin, 1996; Leach *et al.,* 1997; Offer *et al.,* 2000 and Le Fevre *et al.,* 2001).

Research into the mechanical properties of equine and bovine hoof horn has been undertaken by several authors (Bertram and Gosline, 1986; Bertram and Gosline, 1987; Baillie and Fiford, 1996; Douglas *et al.,* 1996; Kasapi and Gosline, 1997; Kasapi and Gosline, 1999 and Baillie *et al.,* 2000). However, these studies were carried out on morbid samples and did not measure possible changes that may occur throughout the lactation and housing period in dairy cows.

As a consequence, the objective of the present study was to develop methods to measure changes in the mechanical properties that occur in the distal part of the hoof horn throughout the lactation period. The chosen material was clean hoof slivers from the sole and white line areas that can easily be obtained from routine hoof trimming. Due to the constraints of sample dimensions and the necessity for testing small areas a small punch test was selected for testing the hoof horn samples. This test also simulated the stresses the hoof horn was subjected to when a cow stood on a sharp object (i.e. stone) and was considered important due to the potencial incidence of stone punctures in the Seale Hayne herd in the summer months. Punch tests are frequently used to determine mechanical properties of small or miniature specimens (Husain *et al.*, 2002; Lewis, 2002) and have been used to successfully determine the shear and creep behaviour and the deformation and failure properties of a wide variety of materials (Aranwela *et al.*, 1999; Satapathy and Bless, 2000; Dobes and Miicka, 2001; Nomoto *et al.*, 2001; Kurtz *et al.*, 2002; Lewis, 2002).

2.2 Material and methods

2.2.1 Experimental design

The experiment was undertaken at the Bradmoors dairy unit at the University of Plymouth, Seale-Hayne campus, Newton Abbot, Devon from September 1999 until August 2000 (320 days). The experimental design was a completely randomised design with 36 replicates compared through time. A total of 36 cows were randomly selected from the herd according to the calving date. Cows entered the experiment at 25 (days ± 5) *prepartum*. The variables measured were compared through changes in time.

The cows were Holstein-Friesian dairy cows, with average milk yield of 8500 l per 305 days lactation and were in the first to seventh lactation (7 cows were on lactation 5 or over). The cows calved form end of September 1999 until January 2000.

2.2.2. Diet

During the *prepartum* period a total mixed ration (TMR) was fed, which consisted of grass silage, maize silage (approx. 80:20 ratio) and 2.0 kg of barley straw per head per day. The components of the *pre-partum* diet are presented in Table 2.1. The composition of the dry cow mineral is presented in Table 2.2.

Table 2.1 ***Components of the prepartum diets (kg/head/day)***

Components	**Quantity**
Feeds	(kg DM/head/day)
Grass silage	6.0
Maize silage	2.0
Barley straw	2.5
Dry cow mineral	0.1
Composition (kg DM)	
Metabolisable energy (MJ/kg)	10.5
Crude protein (g/kg)	140.0

All the cows were fed the same TMR following calving. The components and composition of the lactating cow diet are presented in Table 2.3. This diet consisted of 50:50 maize and grass silage dry matter based, 2 kg rolled wheat, 2 kg molasses, 2.5 kg of 38 % crude protein (CP) compound with bioplex minerals (FM) per day. The TMR was offered once daily at 09.30 hrs. In addition, cows were offered compound feed according to yield at 0.4 kg per litre to a maximum of 8 kg (FM) of a 22 % CP compound with bioplex minerals in parlour daily.

Table 2.2 *Composition of the dry cow mineral supplement /kg*

Element	%	mg	iu
Phosphorus	12.0		
Calcium	1.5		
Sodium	10.8		
Magnesium	10.0		
Copper		1500	
Manganese		5000	
Cobalt		120	
Zinc		5000	
Iodine		500	
Selenium		20	
Vitamin A			400000
Vitamin D3			80000
Vitamin E			500

The composition of the components of the lactating cow diet (kg) supplemented with compound feed in parlour is presented in Table 2.4.

At the end of April the cows were turned out to pasture and brought in twice a day for milking. They received a 16 % CP compound with bioplex minerals, according to milk yield, at 0.4 kg per litre to a maximum of 8 kg (FM) in parlour daily. The cows were grazed out at pasture until September - October 2000.

Table 2.3 ***Components and composition of the lactating cow diet (kg DM /head /day)***

Components	Quantity
Feeds	(kg DM/head/day)
Grass silage	20.00
Maize silage	17.40
Compound (38 % CP) plus bioplex minerals	2.50
Wheat	4.00
Minerals	0.08
Dairy compound added to diet (22 % CP)	2.00
Parlour dairy compound (22 % CP)	≤ 8.00
Composition (/kg DM)	
Dry matter (%)	40.00
Metabolisable energy (MJ/kg)	11.30
Crude protein (g/kg)	17.30
NDF (g/kg)	8202.30
Starch (g/kg)	79.30
Sugar (g/kg)	30.0
CAD balance (meq)	396.20

2.2.2.1 Analysis of feed samples

Samples of each feed were collected and analysed weekly for dry matter and proximate nutrient composition.

Table 2.4 ***Composition of the components of the diet (kg DM) supplemented with concentrated feed in parlour***

	Grass silage	Maize silage	38 % CP Compound	Wheat	Dairy Compound (22% CP)	Dairy Compound (16% CP)
Amount fed (kg DM/head/day)	20	17.0	2.5	4.0	<8	<8
Dry matter (g/kg Fresh weight)	283	422	862	857	870	870
Ash (g/kg DM)	8.0	-	8.4	-	9.0	9.0
NDF (g/kg DM)	555	421	225	123	200	375
Starch (g/kg DM)	-	301	80	670	160	75
Water soluble carbohydrate (g/kg DM)	25.67	26.0	70	26	70	60
ME (MJ / kg DM)	10.6	12.0	12.3	13.7	12.9	12.5
Oil (g/kg DM)	5	4	4.5	2.1	5	6
Crude protein (g/kg DM)	174	89.8	436	127	252	183
Ammonia N (g/kg total N)	<0.2	-	-	-	-	-
Lactic acid	187	-	-	-	-	-

2.2.3 Housing and husbandry

The Bradmoors dairy unit consists of a purpose built 'umbrella' building built in 1998. The cows were housed in a straw bedded area for dry cows, individual calving boxes, three areas of cubicle housing for lactating cows, individual feeding facilities, weighing and handling area, collecting yard and milking parlour.

The 36 cows were housed in 42 cubicles from September 1999 until April 2000. They had access to automatic identification and feed measurement equipment. The cubicles were fitted with cow mats and were bedded with clean sawdust twice a day. In addition, cows were allocated a loafing area adjacent to the cubicles, based on 10 % of the size of lying area within the recommended DEFRA (2003) - welfare codes of practice. In April 2000, the cows were kept at pasture during the day and in cubicles during the night and from May onwards the cows were kept only at pasture.

During the *peripartum* period cows were moved from the cubicles and housed in individual calving yards and fed experimental diets from individual feed intake bins. Intake was measured manually. Feed and bedding was provided twice daily.

The water supply comprised a high-pressure mains connection, supplying water into water troughs via a standard ball-cock arrangement. The number of water points per cow was as recommended by BS 5052, 1990 and all housing conform to BS 5502:part 40, 1990.

The passageways and cubicles were cleaned twice a day with a scraper pulled by a tractor. The cows were milked twice a day through an Alfa-Laval 8 x 8 automatic tandem parlour with automatic cluster removal, milk yield and compound feed allocation and recording.

2.2.4 Measurements

2.2.4.1 Collection of hoof horn samples

Samples of hoof sole tissue were collected from all the claws of all cows at 30 (4-50), 60 (51 -75), 160 (145-220) and 270 (250-330) days *postpartum*. The first outer layer of horn (1 mm) was discarded and a sample of 0.1 to 2.5 mm thickness was taken. Samples were collected with hoof trimming knives and these samples were taken parallel to the ground surface from zones 1, 2, 3, 4 and 5 of the solear surface of the hoof, according to the International Foot Map (Figure 1.1) (Greenough and Vermut, 1991; Shearer, 2002) corresponding with the sole and white line areas. Samples of each claw were collected in a separate clean bucket, transferred immediately to sealed plastic bags to avoid moisture loss and stored in a refrigerator at a temperature of 2 °C until analysis (Hinterhofer *et al*, 1998; Douglas *et al*, 1996). Samples were analysed as soon as possible following collection. The time until analysis was recorded and following analysis the horn samples were stored frozen at - 20° C in sealed plastic bags.

2.2.4.2 The testing of the punch resistance of the hoof horn

Hoof horn samples were analysed for punch resistance using a P/2N needle probe on a TA.XT2i Texture Analyser with a 25 kg load cell (Stable Micro-Systems, Vienna Court, Lammas Road, Godalming, Surrey, GU7 1YL, UK). Dimensions and shape of the P/2N needle probe are demonstrated in Figure 2.1. Samples were held in place between 2 metal plates that had a hole in the centre. Both plates were screwed together and to the base of the texture analyser (see Figure 2.2). The metal plates had a thickness of 15 mm and the hole had a diameter of 10 mm. The test-mode was to measure force in compression. The test probe travelled at a speed of 1.0 mm/sec, which was a speed that enabled the material to adapt to the load (Aranwela *et al.*, 1999). When reaching the sample and the trigger force of 5 g, the force-displacement curve was recorded. When the distance of 12.0 mm was reached the probe returned to the initial position. This distance was high enough to permit the puncture of the samples so that the reading of the maximum force at puncturing was obtained. Maximum punch force values (N) were obtained from the force-displacement curve (Figure 2.3).

A total of 15 to 20 tests were completed on different areas of each hoof in accordance with the number of tests performed by Aranwela *et al.* (1999), Nomoto *et al.* (2001) and Kurtz *et al.* (2002) and this was considered to be sufficient to detect test variations. The data collected from sole and white line areas were recorded separately. Each sample was scored visually for level of haemorrhage, using a scale of 0 to 5 (0- no haemorrhage, 1- diffuse red in horn, 2- stronger red colouration than 1, 3- deep dense red, 4- port colouration and 5- red and raw). The thickness of the sample on the tested area was recorded simultaneously by using a micrometer with a resolution of 0.01 mm. The punch resistance of areas scored 1 to 5 for haemorrhage, in the same hoof, were compared to measure the effect of haemorrhage level on the tests. Mean data from each cow was used to compare the effect of collection period and in each period individual claws of each cow were compared.

2.2.4.3 Dry matter analysis of hoof horn slivers

The dry matter content of the hoof horn samples of every claw at day 160 *postpartum* was analysed through oven drying at 100 °C for 72 hrs, immediately after the mechanical tests were completed. Samples were dried to a constant weight. The drying time required was tested in hoof slivers before the beginning of the experiment.

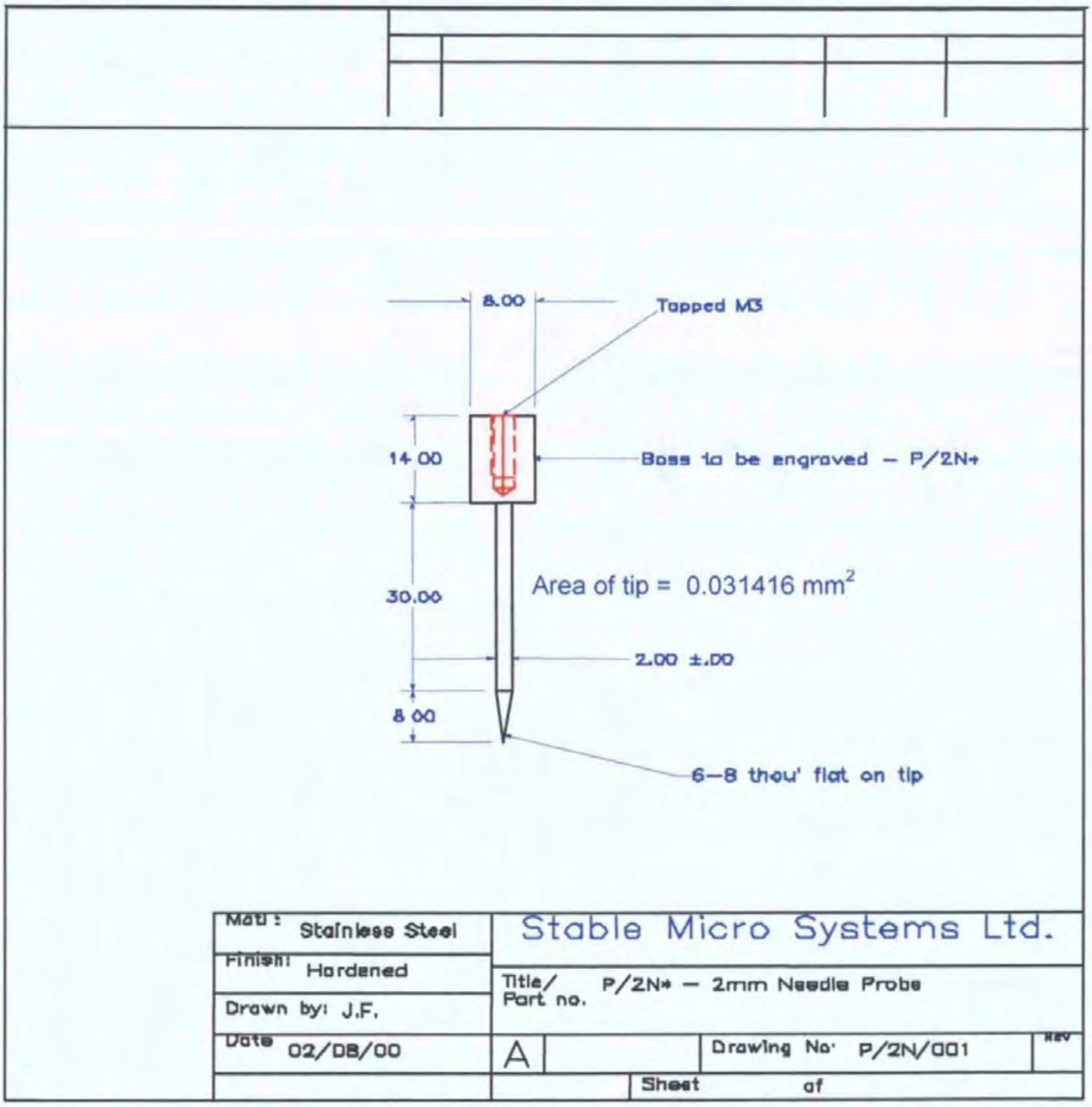


Figure 2.1 *Dimensions and shape of the P/2N needle probe (Stable Micro-Systems) used in the punch test, source: Stable Micro-Systems (2000)*

Figure 2.2 ***Experimental equipment for the punch test using a P/2N needle probe on a TA.XT2i Texture Analyser (Stable Micro-Systems) with a 25 kg load cell. Samples were held in place between 2 metal plates with a hole in their centre and results were recorded in a computer as a force-displacement curve.***

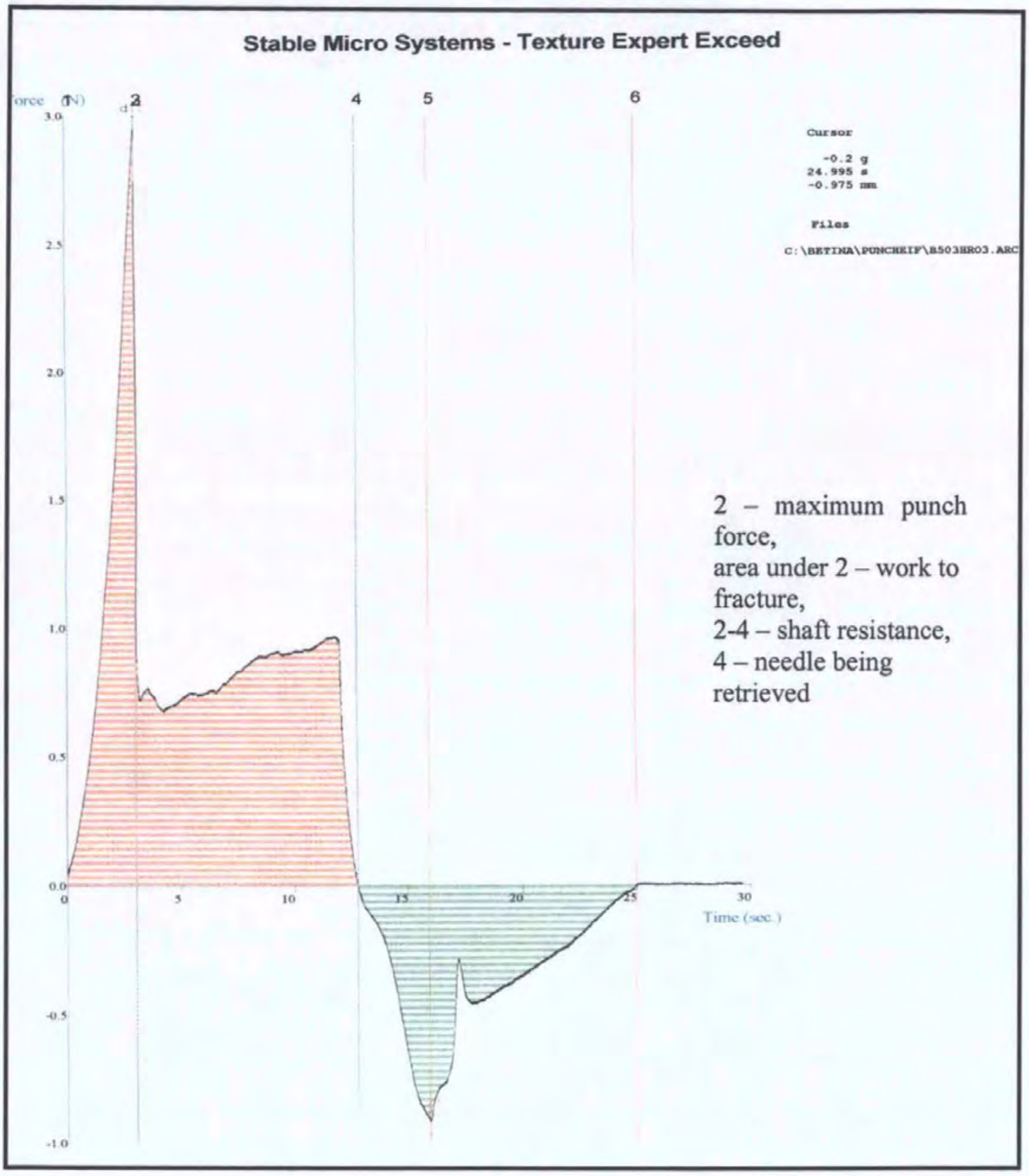


Figure 2.3 ***Force-displacement curve with Maximum punch force recorded***

2.2.4.4 Hoof lesion scoring

The cows had all claws assessed for sole ulcers, sole haemorrhage and heel erosion, at mean 30 (sd. 12.76), 60 (sd. 7.22), 160 (sd. 26.30) and 270 (sd. 23.85) days *postpartum*, at the same time when the hoof horn samples were collected. Any other alteration that was found during examination, such as *Digital dermatitis,* was recorded. The lesions on each foot were scored according to Leach *et al.* (1998). This method involved visual scoring of the quantity and severity of white line bruising and sole bruising of each hoof. Haemorrhages were scored from 0 to 5, zero being a horn with no presence of haemorrhage and 5 being a horn with presence of severe haemorrhage. Sole ulcers were graded from 6 to 8, depending on the exposure of the corium and the presence of infections. The definition of severity scores is represented in Table 2.5. The scores for haemorrhage and ulcers were transformed according to Leach *et al.* (1998) into geometric scores to better represent the severity of lesions, scores for 0, 1, 2 3, 4, 5 and 6 being respectively transformed into 0, 1, 2, 4, 8, 16 and 32. The affected areas of the sole and white line areas of the claws were delineated with a marker pen and thereafter the hoof was photographed with a digital camera. A map of each claw with marked areas and corresponding scores was recorded on a sheet of paper. The images were analysed on an image analysis program (Scion Corporation) in order to measure the affected claw areas. Each claw received a score that was calculated by multiplying the size of the affected area, as a percentage of the total area, with the geometric scores for haemorrhage or ulcer. Scores for each affected area were summed to obtain the total score for each claw. White line and sole areas were scored separately for each claw. A total score for each hoof and a total score for the cow were calculated from the scores of the claws.

2.2.4.5 Growth rate and hoof measurements

The growth and wear rates were measured on the lateral wall of the right front and rear outer claws at 60, 160 and 270 days *postpartum.* A mark was made with a soldering iron on the hoof wall 2 cm below the coronary border (starting at day 30 *postpartum*). The distances from the coronary border to the mark and from the mark to the distal end of the wall were measured. A new mark was made 2 cm below the coronary border every time the hooves were measured. A monthly growth and wear rate was estimated from these measurements. This procedure was described by Clark and Rakes (1982).

Table 2.5 ***Severity scores used in the scoring for claw horn lesions***

Visual appearance of lesion	Arithmetic score	Geometric score
Haemorrhage		
Diffuse red in horn	1	1
Stronger red colouration	2	2
Deep dense red	3	4
Port colouration	4	8
Red raw, possibly fresh blood	5	16
Sole ulcer		
Corium exposed	6	32
Severe sole ulcer, major loss of horn	7	64
Infected sole ulcer	8	128

Source: Leach *et al.* (1998)

The hoof angle, length of the dorsal hoof wall and height of the heel were measured on the right front and rear outer claws using the procedure described by Boelling and Pollott (1998). The recording times were days 160 and 270 *postpartum.* Hoof angle was measured in degrees with a protractor. The length of the dorsal wall was measured as the distance between the dorsal hairline and the tip of the toe (mm). The height of the heel was measured as the distance between the floor surface and the hairline at the rear of the claw.

2.2.4.6 Locomotion scoring

The cows were assessed for locomotion score twice weekly. The scoring system had 5 points and was designed by Tranter and Morris (1991), being presented in Table 2.6. The factors assessed when scoring cows for lameness were the ease of assessing which leg was affected, the degree and timing of the head movement, the degree of sinking of the sound quarter, the changes in the stride length and the abduction or adduction of the hind legs. Lame cows were examined for cause of lameness and the development of each case of lameness was monitored. During the time at pasture the claws of lame cows were examined to assess the incidence of stone puncture.

Table 2.6 ***Grades used in the locomotion scoring system***

Grades	Description
0	No abnormality of gait
1	Tenderness, lameness hardly noticeable
2	Slightly lame
3	Markedly lame
4	Affected limb not weight bearing

Source: Tranter and Morris (1991)

2.2.4.7 Veterinary records of lameness of the Seale-Hayne herd

Veterinary records for the years 1998, 1999 and 2000 were analysed to assess the typical causes of lameness in the herd.

2.2.4.8 Body condition score

Body condition score was measured weekly, using a scale from 1 to 5 with half points (Lowman *et al.,* 1976). The scoring of body condition was completed by the same person throughout the experiment.

2.2.4.9 Individual milk yield

Previous lactation yield and number of lactations were recorded for each cow. Individual milk yield was recorded daily.

2.2.5 Statistical analysis

The experiment was a repeated measures design, using individual cows as observations. The milk yield, live weight, condition score, locomotion score, lesion score and punch resistance data were all found to be normally distributed. The parameters were compared by collection period and claws, using general linear modelling analysis of variance (ANOVA) (Minitab 12.0). The thickness of the samples was used as a covariate using ANCOVA when punch resistance data were analysed. The comparison of means was completed using the Tukeys test. Haemorrhage level data of the horn samples were not normally distributed and were analysed by the nonparametric Kruskal-Wallis test. Regression analysis was used to assess the effect of sample thickness on punch resistance, days *postpartum* on lesion scores of the sole and white line, number of lactations on locomotion score and days *postpartum* on locomotion score data. Multivariate stepwise regression was used to evaluate the parameters that influenced the variation in lesion score and punch resistance data. Correlation analysis between the measured parameters was done using Pearsons correlation.

2.3. RESULTS

2.3.1. Incidence of lameness and causes of lameness on the Seale-Hayne dairy herd

The causes of lameness related to the claws in the Seale-Hayne dairy herd in the years 1998, 1999 and 2000 are presented in Table 2.7.

Table 2.7 ***Claw horn and interdigital lesion cases in the Seale-Hayne dairy herd during 1998, 1999 and 2000 treated by the veterinary surgeon***

Diagnosed cause	1998 (n)	1999 (n)	2000 (Jan-July) (n)	Frequency of factor in 3 years (proportion)
Super foul	2	1	-	0.05
Digital dermatitis	3	2	3	0.13
Inter digital growth or sore	3	3	1	0.11
Laminitis	-	1	-	0.02
Stone punctures	2	1	-	0.06
White line lesion	5	7	7	0.30
White line or sole abscess (not specified)	-	7	3	0.16
Solear abscess (underun sole)	1	-	-	0.02
Severe bruising of sole	2	1		0.05
Sole ulcer	6	3	3	0.19
Cows presenting claw horn and interdigital lesions in the herd	0.17	0.22	0.16[4]	-
Culling due to lameness	2[1]	5[2]	2[3]	-

note: in some cases one cow had more than one type of injury.
[1] Traumatic injury, claw horn lesion
[2] Heart murmur and swollen leg (1), claw horn lesions (4)
[3] Claw horn lesions
[4] One case was a joint injury – not described

2.3.2. Locomotion Score

Amongst the 34 cows that completed the experiment, 4 cows (proportion 0.12) never became lame, exhibiting locomotion score between 1 and 2, nine cows (proportion 0.26) had a maximum locomotion score of 3, indicating only slight lameness and 21 cows (proportion 0.63) were severely lame, corresponding to locomotion scores of 4 to 5. Clinical and subclinical alterations observed in these animals during this period are detailed in Table 2.8.

Table 2.8 ***Clinical and subclinical alterations observed on animals with different locomotion scores (1 to 5)***

Locomotion score	**Clinical and subclinical alterations**	**number of cows**
1	- digital dermatitis	1
2	- digital dermatitis	1
	- large area of sole haemorrhage	1
	- no visible alterations	1
3	- digital dermatitis	3
	- large area of sole haemorrhage	2
	- digital dermatitis and large area of sole haemorrhage	2
	- no visible alterations	2
4	- sole ulcers (2 presented a high locomotion score before the sole ulcer was visible)	4
	- large area of sole haemorrhage	1
	- a large area of sole haemorrhage and digital dermatitis	4
	- digital dermatitis	1
	- no visible alterations (older cows and high scores for short time)	4
5	- lameness causes not related to the claw horn (interdigital growth, heart murmur with swollen front legs, lesion on udder, joint injury)	4
	- white line abscess and sole ulcer	2
	- laminitis because of sudden diet change	1

The effect of number of lactations on locomotion score was analysed at day 90 *postpartum* that proved to be the period were the cows exhibited the highest mean locomotion scores (Figure 2.6). Number of lactations had a significant (P <0.05) but low effect (R^2adj.= 18.9%) on locomotion score at day 90 *postpartum* (Figure 2.4). The low fit of the trendline was related to a greater variation in the locomotion score of younger animals and the few numbers of older animals.

Locomotion score at day 90 *postpartum* = 2.0545 + 0.2854 number of lactations, R^2 adj. = 18.9%, $P < 0.05$.

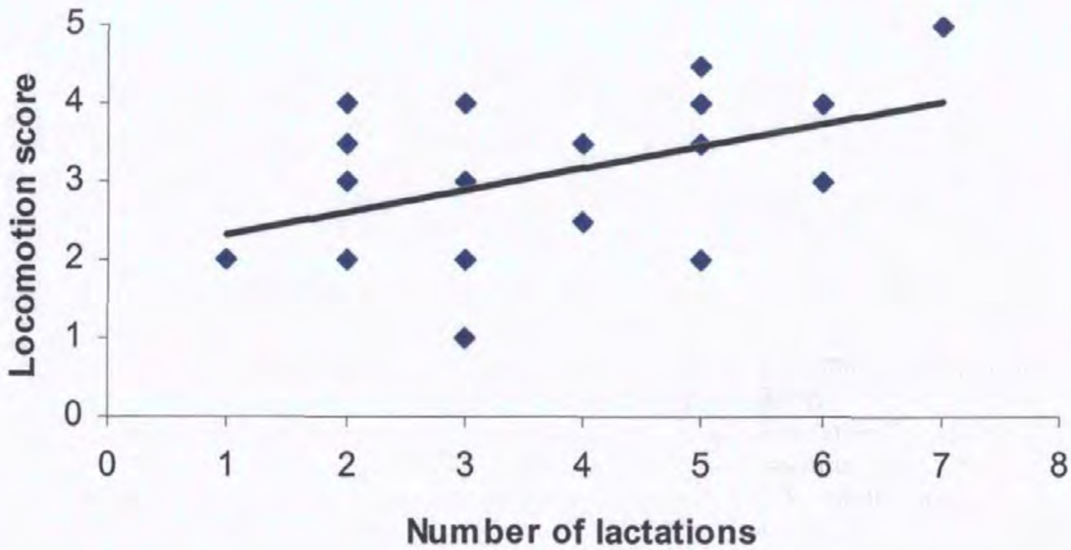


Figure 2.4 ***Effect of number of lactations on the locomotion score at day 90 postpartum***

Locomotion score was found to have a significant ($P<0.001$) quadratic relationship with days in lactation (Figure 2.5). Locomotion score increased as the lactation progressed up to 90 days *postpartum*, being significantly higher between days 30 and 90 *postpartum* when compared with locomotion score at parturition. After day 120 *postpartum* locomotion score started to decline.

2.3.3 Lesion score

Lesion scores of sole and white line areas were calculated separately, because the pattern of lesion formation was considered to be different in these two areas (Leach *et al.*, 1998). The sole lesion score was found to be correlated to the lesion score of the white line ($P<0.001$) (r = 0.325).

Locomotion score = - 0.0001 days in lactation^2 + 0.0285 days in lactation + 1.3159, R^2 adj. =0.8452, $P = 0.001$

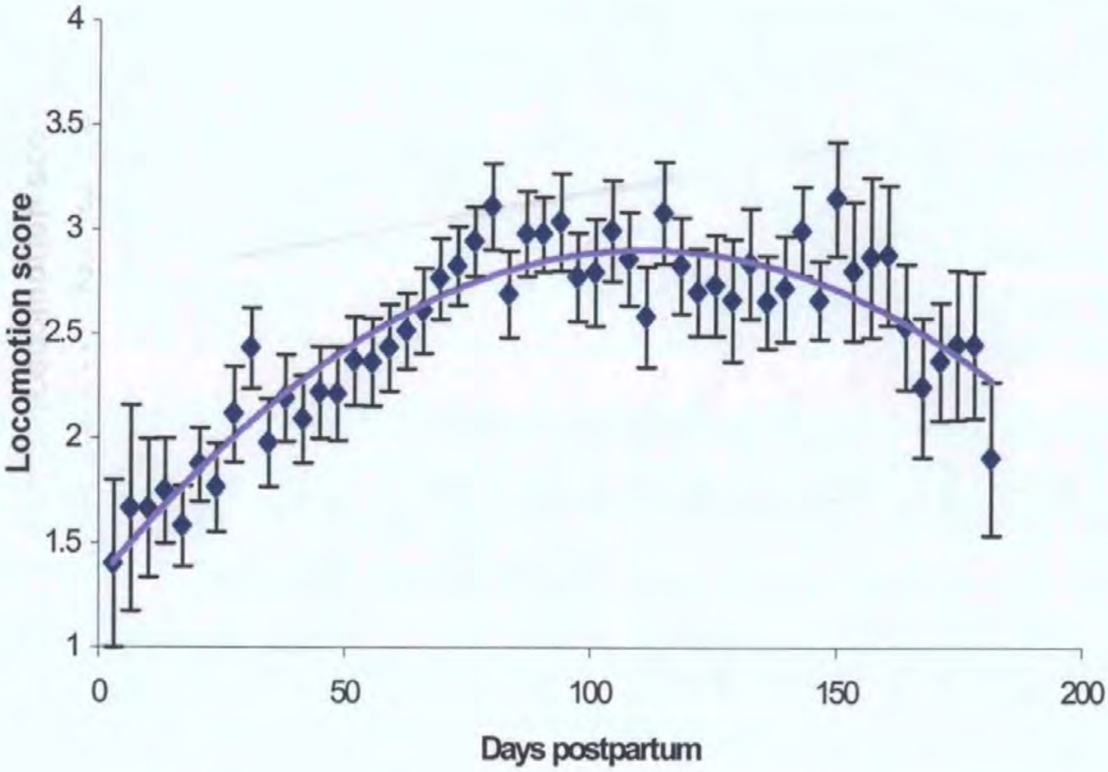
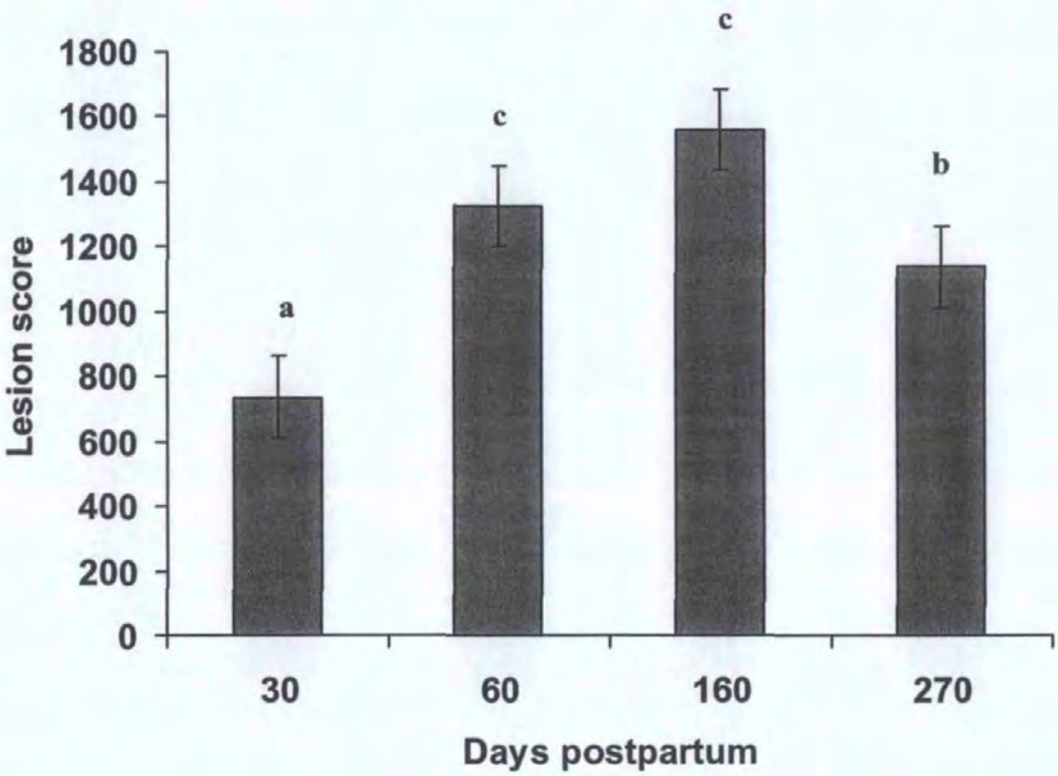


Figure 2.5 *Mean locomotion score from* ***days 0 to 120 postpartum***

2.3.3.1. Lesion score of the sole area

Regression analysis between total lesion score of the sole area of the animal (total lesion score = 568.09 + 11.75 days – 0.036 days^2), lesion score of the hind right outer (lesion score sole hind right outer claw = 102.49 + 3.78 days – 0.012 days^2) and hind left outer claws (lesion score sole hind left outer claw = 119.95 + 3.30 days – 0.011 days^2) and days *postpartum* were significant ($P < 0.001$), but R^2 adjusted values were very low (R^2adj. = 11.5, 12.9 and 13.1, respectively). The differences between the data collection periods in the *postpartum* were analysed through ANOVA - GLM. The total lesion score of the sole horn on days 30, 60, 160 and 270 of the *postpartum* period are presented in Figure 2.6. The total lesion score of the sole increased significantly ($P<0.01$) between days 30 and 60 *postpartum*, but decreased after day 160 *postpartum* when animals were at pasture.



a, b, c – mean data labelled with different letters differ significantly

Figure 2.6 ***Total lesion score of the sole horn during the postpartum period***

The regression analysis between total lesion score of the animal for the sole and white line areas and number of lactations were not significant. The total lesion score of the sole was found to be significantly affected by cow and claws (Table 2.9). The hind outer claws were found to have significantly (P<0.01) higher lesion scores compared to other claws at days 60, 160 and 270 *postpartum* (Table 2.9).

Table 2.9 ***Lesion score of the sole horn of front and hind, inner and outer and left and right claws on days 30, 60 and 160 of the postpartum period***

	Front				Hind					
	Left		Right		Left		Right			
Day	Inner	Outer	Inner	Outer	Inner	Outer	Inner	Outer	sem	*P*
30	67.5 [ab]	34.9 [b]	66.4 [ab]	77.4 [ab]	100.3 [ab]	153.4 [a]	72.2 [ab]	164.5 [a]	24.37	0.01
60	141.1 [b]	60.5 [b]	133.9 [b]	79.5 [b]	98.4 [b]	390.3 [a]	71.6 [b]	365.1 [a]	35.00	0.001
160	137.2 [b]	113.3 [b]	182.7 [b]	94.5 [b]	128.3 [b]	361.2 [a]	158.8 [b]	382.1 [a]	27.31	0.001
270	128.1 [b]	118.7 [b]	127.0 [b]	92.0 [b]	119.0 [b]	225.1 [a]	90.6 [b]	236.8 [a]	20.92	0.001

[a, b] – different letters in the same row indicate values differ significantly

The proportion of haemorrhage of the sole horn on days 30, 60 and 160 of lactation is presented in Table 2.10 and the proportion of haemorrhage of the sole horn in claws hind right, hind left, front left and front right and total of all claws is presented in Table 2.11. The area of claw with haemorrhage scored 0 decreased in the *postpartum* period and the areas with scores of severity 2, 3 and 4 increased. The hind claws had larger areas with higher scores when compared to the front claws ($P<0.001$).

Table 2.10 ***Median haemorrhage level of the sole horn on days 30, 60 and 160 postpartum***

		Days			
Haem. level	30	60	160	sdev	*P*
0	0.839 [a]	0.618 [b]	0.434 [c]	0.230	0.001
1	0.00	0.00	0.00	0.115	NS
2	0.006 [b]	0.091 [a]	0.170 [a]	0.180	0.001
3	0.00 [b]	0.145 [a]	0.091 [a]	0.175	0.001
4	0.00 [b]	0.023 [a]	0.00 [b]	0.099	0.001
5	0.00	0.00	0.00	0.021	NS

[a, b, c] – different letters in the same row indicate values that differ significantly
NS – not statistically significant

Table 2.11 ***Median proportion of haemorrhage of the sole horn in claws hind right, hind left, front left and front right and total of all claws***

Haemorrhage level	Front Left	Front Right	Hind Left	Hind Right	Median of claws	sdev	*P*
0	0.639 [a]	0.721 [a]	0.548 [b]	0.609[b]	0.623	0.275	0.05
1	0.00 [b]	0.028 [a]	0.00 [b]	0.00[b]	0.00	0.121	0.001
2	0.055	0.053	0.089	0.047	0.055	0.200	NS
3	0.00 [b]	0.00 [b]	0.143 [a]	0.098 [a]	0.049	0.165	0.001
4	0.00 [b]	0.00 [b]	0.019 [a]	0.016 [a]	0.00	0.091	0.001
5	0.00	0.00	0.00	0.00	0.00	0.021	NS

[a, b] – different letters in the same row indicate values differ significantly,
NS – not statistically significant

The lesion scores of the hind left outer and hind right outer claw and the total lesion score for the cow on day 160 were significantly ($P<0.05$) correlated with locomotion score on day 270 (r = 0.46, 0.47 and 0.44, respectively). The lesion scores of the hind left outer claw was also correlated with locomotion score on day 160 (r = 0.39). In cows presenting lameness cases related to the claw horn, all the lesion scores of the hind left and right inner and outer claws and the total score for the hind left and right claws, were significantly correlated to the locomotion score on day 160 (r = 0.48 to 0.58).

2.3.3.2. Lesion score of the white line area

The *postpartum* period was found to have no significant effect on lesion score of the white line when analysed through regression analysis and also through ANOVA – GLM using collection periods as variables. The total lesion scores of the white line horn on days 30, 60, 160 and 270 *postpartum* are presented in Figure 2.7 and the total lesion scores of the white line horn of front and hind claws are presented in Table 2.12.

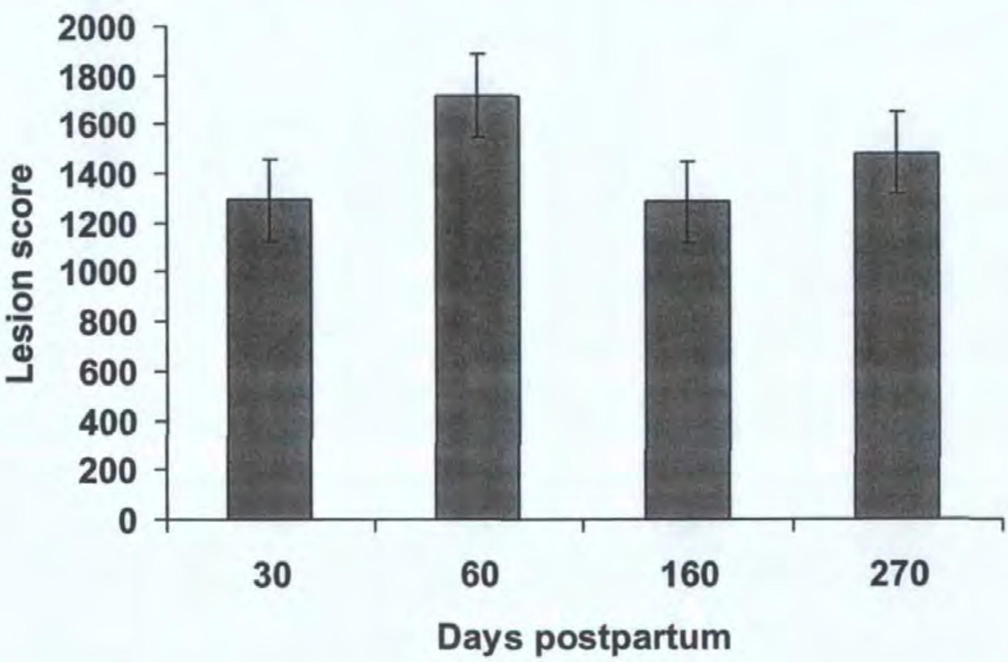


Figure 2.7 ***Total lesion score of the white line horn at days 30, 60, 160 and 270 of the postpartum period***

Table 2.12 ***Lesion score of the white line horn of front and hind, inner and outer and left and right claws on days 30, 60 and 160 of the postpartum period***

	Front				Hind					
	Left		Right		Left		Right			
Day	Inner	Outer	Inner	Outer	Inner	Outer	Inner	Outer	sem	*P*
30	127.4	127.3	199.3	122.4	189.2	203.5	145.1	168.2	27.43	NS
60	193.1 [ab]	154.9 [b]	170.0 [b]	147.8 [b]	201.6 [ab]	326.7 [a]	236.5 [ab]	278.4 [ab]	32.22	0.001
160	150.1 [b]	130.7 [b]	140.8 [b]	119.8 [b]	140.2 [b]	254.6 [a]	126.6 [b]	219.8 [ab]	23.65	0.001
270	146.2 [b]	165.4 [b]	144.2 [b]	147.7 [b]	144.3 [b]	361.4 [a]	129.2 [b]	244.6 [ab]	43.68	0.01

[a, b] – different letters in the same row indicate values that differ significantly,
NS – not significantly different

Lesion score of the white line was significantly ($P<0.001$) affected by cow and claw when analysed though GLM - ANOVA, with the hind left outer claws having significantly ($P<0.001$) greater lesion scores after day 60 *postpartum* compared with front claws. The hind right outer claw presented consistently higher scores than the front claws but the difference was not significant. The hind left outer claw presented significantly ($P<0.001$) higher lesion scores on days 160 and 270 *postpartum* when compared with the hind inner claws.

2.3.4 Analysis of hoof horn structural strength

2.3.4.1 Punch resistance of the sole horn

When testing by multiple regression analysis for the predictors that had a significant effect on the punch resistance of the sole horn, claw number, thickness of the sample and haemorrhage level of the tested area were highly significant ($P<0.001$). Days *postpartum*, cow number and days to analysis were found not to have a significant effect. The regression equation for punch force of the sole horn was:

Punch force of the sole horn = 6.02 + 0.321 claw number + 5.92 thickness - 0.373 Haemorrhage, R^2adj. = 0.36, $P<0.001$.

The influence of sample thickness over the punch force of the sole horn was measured through regression analysis. There was a significant ($P<0.001$) positive linear relationship between sample thickness and punch force (Figure 2.8), with thicker samples requiring a greater force to be punched. The variability of results at all thickness levels were apparently similar, there being no indication that a specific thickness was less variable.

Punch force of the sole horn (N) = 6.05 + 6.34 sample thickness (mm), R^2adj. = 0.31, $P<0.001$

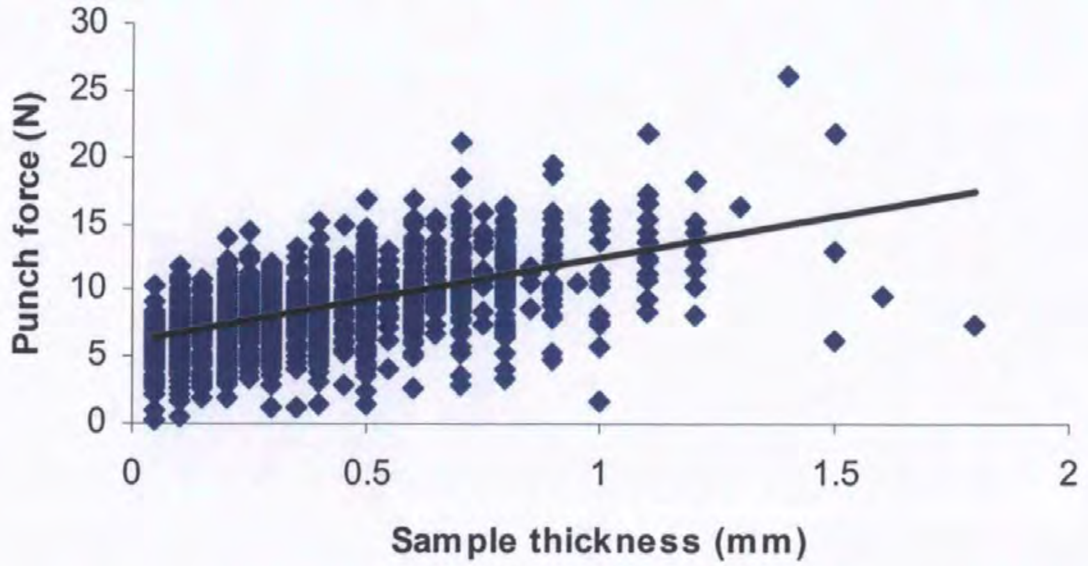


Figure 2.8 *Effect of sample thickness on punch force of the sole horn*

Sample thickness was used as a covariate when the effect of sample haemorrhage level, days *postpartum*, claw and cow on punch force of the sole horn were analysed. When analysed through GLM - ANCOVA using the sample thickness as a covariate, the factors sample haemorrhage, cow, claw and *postpartum* period all had a significant ($P<0.001$) effect on maximum punch force of the sole horn. The mean force required to punch the sole horn tissue with differing haemorrhage scores are presented in Table 2.13. The significantly ($P<0.001$) greatest mean force required to punch the sole horn sample was observed on samples that did not present haemorrhage (score 0) and mean punch force declined significantly at every increase in haemorrhage level.

Table 2.13 ***Effect of haemorrhage score of the sole horn tissue on punch force***

Haemorrhage score	0	1	2	3	4	5
Punch force (N)	8.72 [a]	8.53 [b]	8.06 [c]	7.75 [d]	6.08 [e]	4.99 [f]
n. observations	1130	546	401	304	74	23
sem	0.078	0.104	0.125	0.151	0.331	0.460

[a, b, c, d, e, f] – different letters in the same row indicate values that differ significantly

The maximum punch force of the sole horn was significantly ($P<0.001$) lower at day 160 *postpartum* compared with days 30 and 270 *postpartum* and the highest punch force was measured at day 270 *postpartum* when the animals were at pasture. The total lesion score of the sole area was significantly higher ($P<0.01$) at days 60 and 160 *postpartum* compared to days 30 and 270 *postpartum* and were lowest at day 30 *postpartum* (Table 2.14). The maximum punch force of the sole horn was significantly ($P<0.001$) negatively correlated to the total lesion score of the sole area (r = - 0.24), the lowest values of punch force being measured at the periods were the total lesion score for the sole area were at its highest. The difference in lesion score of the sole area between collection periods was negatively but significantly ($P<0.001$) correlated with the difference in punch force of the sole horn between the same collection periods (r = - 0.263), when the lesion score increased the punch force decreased.

Table 2.14 ***Total lesion score and maximum punch force (N) of the sole and white line (wl) horns on days 30, 60, 160 and 270 postpartum***

	Days postpartum					
	30	60	160	270	sem	*P*
Total lesion score sole	735 [c]	1326 [a]	1560 [a]	1138 [b]	125.0	0.01
Total lesion score wl	1292	1716	1286	1483	167.1	NS
Sole punch force	8.543 [b]	8.345 [bc]	7.755 [c]	8.983 [a]	0.150	0.001
wl punch force	7.390 [b]	7.238 [b]	6.569 [c]	7.754 [a]	0.222	0.001

[a, b, c] – different letters in the same row indicate values that differ significantly

The maximum punch force of the sole horn at days 30 and 60 *postpartum* was significantly (P<0.01 and 0.05) greater in the front right claws compared with the hind left claws. At days 160 and 270 *postpartum* the maximum punch force of the sole horn was significantly (P<0.001 and 0.01) greater in the front claws compared with the hind claws (Table 2.15).

2.3.4.2 Punch resistance of the white line horn

When testing by multiple regression analysis for the predictors that had a significant influence over the punch force of the white line, cow, claw, sample thickness and haemorrhage level of the tested area were significant (P < 0.05, 0.05, 0.01 and 0.001, respectively) and days *postpartum* and days to analysis were not significant. The regression equation for punch force of the white line horn was:

Punch force of the white line horn = 4.53 - 0.0170 cow + 0.158 claw + 7.92 Width - 0.315 Haemor, R^2adj. = 0.39, P <0.001.

Table 2.15 ***Punch force of the sole and white line (wl) horn of front and hind claws at day 30, 60, 160 and 270 postpartum (pp) and dry matter (DM) on day 160 postpartum***

	Front		Hind			
	Left	Right	Left	Right	sem	*P*
Sole						
day 30 pp	9.12 ^{b}	9.24 ^{a}	8.16 ^{c}	8.33 ^{bc}	0.233	0.01
day 60 pp	8.57 ^{ab}	9.10 ^{a}	7.86 ^{b}	8.03 ^{ab}	0.297	0.05
day 160 pp	8.74 ^{a}	8.71 ^{a}	7.50 ^{b}	7.27 ^{b}	0.230	0.001
day 270 pp	9.20 ^{a}	9.33 ^{a}	9.00 ^{ab}	8.39 ^{b}	0.205	0.01
White line						
day 30 pp	7.89	7.86	6.84	7.24	0.360	NS
day 60 pp	7.46	7.32	7.18	6.95	0.389	NS
day 160 pp	6.97	7.08	6.82	6.24	0.293	NS
day 270 pp	7.58 ^{b}	7.71 ^{ab}	8.92 ^{a}	7.28 ^{b}	0.344	0.01
DM (%) day 160	65.62	65.45	64.01	64.25	0.561	NS

a, b, c – different letters in the same row indicate values that differ significantly,
NS – not significantly different

When analysed through ANCOVA using sample thickness as a covariate the maximum punch force of the white line horn was significantly lower (P<0.001) at day 160 *postpartum* compared with days 30, 60 and 270 *postpartum* and the highest value was measured at day 270 *postpartum* when animals were at pasture. No significant difference in the lesion score of the white line area was measured between the measurement periods (Table 2.14).

The maximum punch force of the white line horn of the different claws was not significantly different at days 30, 60 and 160 *postpartum*, however was significantly ($P<0.01$) higher for the hind left claws compared with the front and hind right claws at day 270 *postpartum* (Table 2.15).

The maximum force required to punch the white line horn had a significant ($P < 0.001$) and linear relationship with sample thickness (Figure 2.9).

Punch force of the white line horn = 3.72640 + 8.64740 sample thickness, R^2adj. = 0.40, $P<0.001$

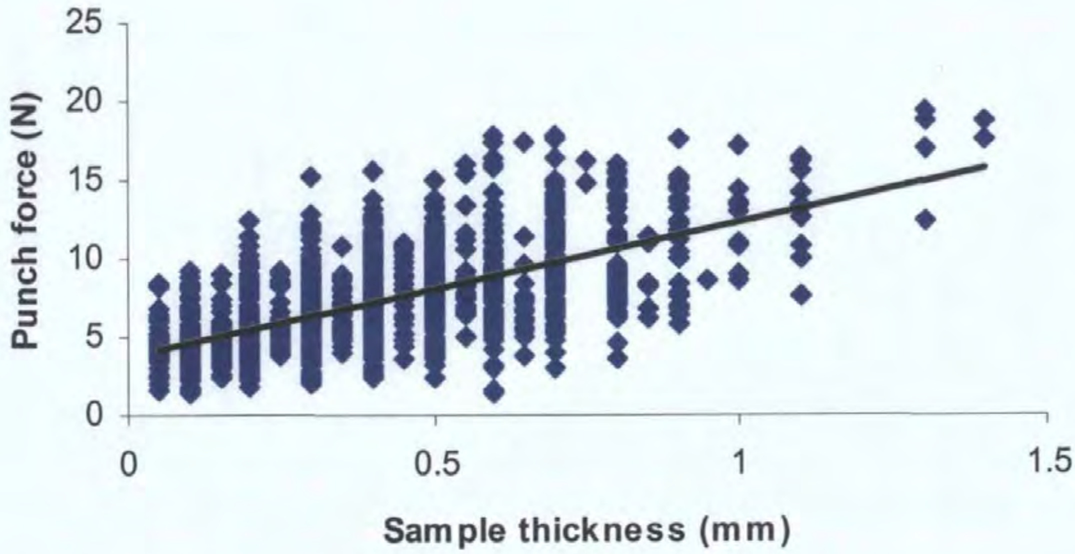


Figure 2.9 ***Effect of sample thickness on punch force of the white line horn***

2.3.5 Dry matter of the hoof horn

The dry matter of the hoof horn was measured at 160 days *postpartum*. Dry matter did not differ significantly between front and hind claws (Table 2.15). The dry matter was significantly ($P<0.001$) and positively correlated to the punch force of the sole horn (r = 0.83).

2.3.6 Hoof growth and hoof measurements

The mean values of the claw measurements taken at day 160 and 270 *postpartum* are presented in Table 2.16. There was a significant ($P<0.001$) difference between the measurement length, height of the heel and growth rate of the front right claw and of the hind right claw that were taken at day 160 *postpartum* when compared with the measurement made at day 270 *postpartum* when the cows were at pasture. The dorsal length of the claw increased, the heel height decreased and the growth rate increased for both claws when animals were at pasture.

Table 2.16 ***Mean values for dorsal length, heel height, lateral height, growth and wear rates taken at days 160 and 270 postpartum***

Measurements	160 dpp Housed	270 dpp Pasture	sem	*P*
Front right				
Dorsal length (cm)	7.25	7.77	0.11	0.001
Lateral height (cm)	7.02	7.05	0.15	NS
Heel height (cm)	4.94	3.81	0.16	0.001
Growth rate (cm/month)	0.58	0.97	0.04	0.001
Wear rate (cm/month)	0.76	0.67	0.10	NS
Hind right				
Dorsal length (cm)	7.30	7.84	0.07	0.001
Lateral height (cm)	6.66	6.42	0.12	NS
Heel height (cm)	4.20	2.90	0.13	0.001
Growth rate (cm/month)	0.71	0.78	0.05	0.001
Wear rate (cm/month)	0.54	0.72	0.04	0.001

NS – not statistically significant

The measurements of front and hind claws are presented in Table 2.17. The front claws had a significantly ($P<0.001$) higher heel height, lateral height and claw angle than the hind claws. Growth rate was significantly ($P<0.01$) greater for the hind claws when animals were housed and significantly ($P<0.05$) lower when animals were at pasture compared with front claws. Wear rates were significantly ($P<0.001$) greater for the front claws when animals were housed and did not differ between front and hind claws when animals were at pasture.

Table 2.17 ***Mean values for dorsal length, heel height, lateral height, claw angle, growth and wear rates of front and hind right claws***

Measurements	Front claw	Hind claw	s.e.m.	*P*
Day 160 postpartum				
Dorsal length (cm)	7.25	7.25	0.115	NS
Lateral height (cm)	7.05	6.66	0.138	0.05
Heel height (cm)	5.20	4.49	0.211	0.01
Growth rate (cm/month)	0.69	0.84	0.043	0.01
Wear rate (cm/month)	0.90	0.66	0.051	0.001
Day 270 postpartum				
Dorsal length (cm)	7.77	7.90	0.139	NS
Lateral height (cm)	7.05	6.42	0.162	0.001
Heel height (cm)	4.25	3.11	0.213	0.001
Growth rate (cm/month)	0.95	0.75	0.075	0.05
Wear rate (cm/month)	0.63	0.69	0.062	NS
Claw angle (^{o})	44.15	43.04	0.434	0.05

NS – not statistically significant

The length of the hind right claw measured at 160 and 270 days *postpartum* was significantly ($P<0.05$) positively correlated to locomotion score at the same periods (r =

0.41 and 0.46, respectively). The height of the heel and the wear rate of the front claw measured at 160 days *postpartum* were negatively correlated to the punch force of the front right claw measured at 40 days *postpartum* (r = - 0.46, - 0.47). Similarly, the height of the heel of the front and hind claws measured at 160 days *postpartum* were negatively correlated to the punch force of the front and hind right claws measured at 160 days *postpartum*. The lateral height of the hind right claw measured at 160 days *postpartum* was positively correlated to the punch force of the hind right claw measured at 160 and 270 days *postpartum*.

2.4 Discussion

2.4.1 Incidence of lameness in the Seale-Hayne herd

The incidence of lameness in the research group of cows (proportion of 0.63 becoming severely lame during experimental time) was similar with the level found in the survey of Clarkson *et al.* (1996) of proportion of 0.55 and is considered to be high (Kossaibati *et al.*, 1999). The incidence of claw horn disorders was high in the herd (proportion 0.16 - 0.22), considering that only severe cases were reported, and in the research group (0.47). A third of the cows in the research group had *Digital dermatitis,* which probably enhanced the lameness caused by claw horn lesions. These figures agreed with the figure of 0.40 proportion of affected animals presented in the survey of Kossaibati *et al.* (1999). The high incidence of claw horn lesions was probably related to older cows presenting recurrent lesions, such as sole ulcers, in subsequent lactations and to the housing on concrete floors during the winter months. The recent introduction of the heifer comfort group in the straw yard should help to reduce these figures in future years. The Seale-Hayne herd had a high incidence of white line disorders (proportion 0.30) (Table 2.7) that could be related to rough walking surfaces when animals were at pasture. The ratio of sole and white line problems was 1:2, being the opposite as the ratio presented by Logue (1999).

The significant ($P<0.01$) increase in lesion score of the sole horn from day 30 *postpartum* to day 60 *postpartum* as the lactation progressed and as the cows were housed for longer, followed by a subsequent decrease in lesion score were similar to the results reported by Enevoldsen *et al.* (1991), Leach *et al.* (1997) and Offer *et al.* (1998 and 2000), that reported an increase in the lesion score of the sole horn until days 100 to 120 *postpartum*. The hormonal changes and the consequent softening of the collagen fibres that connect the third phalanx to the horn capsule, associated with parturition, are believed to be the major contributory factor in the development of hoof lesions in the *postpartum* period (Webster, 1998; Holah *et al.*, 2000; Tralton and Webster, 2002). The increase and subsequent reduction in the locomotion score during the *postpartum* period was best described by a quadratic equation and Whay (1998) described a similar increase of the locomotion score in the *postpartum* period.

The lesion scores of the sole horn decreased at day 270 *postpartum,* this decrease probably being related to the cows being in late lactation and also the cows being at pasture during this period. A yielding surface, such as grass has been found to distribute the load more evenly over the weight-bearing surface of the individual claw and between the lateral and medial claw of each limb (Vermunt and Greenough, 1994; Wandel *et al.*, 2002). Concrete floors were frequently considered to be too hard to be used as a frequent walking surface for cows and predisposed cows to lameness (Rowlands *et al.,* 1983; Wells *et al.*, 1995; Galindo and Broom, 2000). At pasture also cows spend a longer period of time lying and had fewer changes between lying and standing postures when compared to cows in cubicles and increased lying time was related to lower lameness incidence (Singh *et al.*, 1993b, Kerr, 1998).

Lesion scores of the sole area were significantly higher in the hind outer claws after day 60 *postpartum* compared to hind inner and front claws. Locomotion score and lesion score of the hind left outer claws at day 160 postpartum were significantly ($P<0.05$) and positively correlated (R = 0.40 to 0.58). Bergsten and Herlin (1996), Leach *et al.* (1997), Offer *et al.* (2000) and Le Fevre *et al.* (2001) reported also that the majority of lesions (0.75) occurred in the hind outer claws. Although, more than half of the body weight is carried by the front claws (Scott, 1987; Singh, 1993; Nuske *et al.*, 2002; Asleben *et al.*, 2003), the alteration of forces, i.e. the difference of the minimum to the maximum load, rather than the absolute load, was considered to damage to a greater extent the hind outer claws (Toussaint-Raven, 1985; Scott, 1987; Singh *et al.*, 1993). Another factor that increased the pressure on the hind outer claws was the conformation of the udder at parturition that has been found to force the cow to have a wider stance (Vermunt and Greenough, 1996). White line lesion scores were significantly higher for hind left outer claw when compared with front claws and this difference was not observed for hind right outer claw. The higher scores presented by the hind left outer claw may potentially be related to the left hand turn that cows have to make when leaving the parlour in the housing system at the Seale-Hayne farm. Lesion scores of the white line area did not differ significantly between measurement periods and this maybe related to the consistently high lesion scores measured for the white line area since the beginning of the *postpartum* period.

The length, the height of the heel and growth of the front and hind claws and the wear of the hind claws differed significantly between days 160 and 270 *postpartum*. The claws were longer (7.25 vs. 7.77 cm for front claws and 7.30 vs. 7.84 cm for hind claws) and the height of the heel shorter (4.94 vs. 3.81 cm for front claws and 4.20 vs. 2.90 cm for hind claws) when cows were at pasture (day 270 *postpartum*), this could be due to the higher growth rate during this period (0.58 vs. 0.97 cm/month for front claws and 0.71 vs. 0.78 cm/month for hind claws). Offer *et al.* (2000) reported smaller claw angles and longer claws when animals were out at pasture when compared with the winter housing period. Increase in the wear rate of hind claws when animals were at pasture compared to the housing period (0.54 vs. 0.72 cm/month) were probably related to increased walking distances and rough walking surfaces. Meyer and Galbraith (1998) reported greater wear rates of the dorsal border of the hooves after cows were turned out to pasture, this difference probably being related to cows walking on concrete tracks. Increase in growth rate when at pasture was probably the combination of several factors such as the longer exposure to daylight, a reaction to the increase in wear rate, changes in the underfoot environment from the concrete flooring to pasture and diet changes (Tranter and Morris, 1992; MacCallum *et al.* 1998 and 2002). The shorter height of the heel was probably related to a change in the foot angle due to the longer claw and a change in the pressure distribution and wear rates of the heel area.

2.4.2 Hoof horn structural strength

The ultimate punch force associated with the peak load used as a test parameter in the present experiment was considered an important test parameter according to Liu and Piggot (1998) and was used for comparative purposes by Reppond *et al.* (1995), Reppond and Babbit (1997), Dobes and Milicka (2001) and Nomoto *et al.* (2001). The force-displacement curves of the punch tests exhibited similar distinctive features and were highly reproducible. Initially, the curves displayed a nonlinear toe-in region followed by a linear stiffness and a peak load. Due to the high radial clearance (5mm) and low sample thickness (< 0.4mm) bending effects did probably occur and forces occurred mainly in tension and not in shear. As a consequence, the force-displacement curves differed from the curves described by Kurtz *et al.* (2002) and Lewis (2002) for shear punch tests. The shear punch test force-displacement curves were bilinear presenting initial stiffness, transition load and hardening stiffness.

In this experiment the punch test was found to be sufficiently sensitive to measure significant differences in the structural strength of hoof horn with varying levels of haemorrhage, of different claws and areas of the claw and in different stages of the lactation period. Hedges *et al.* (2002) reported a significantly lower tensile strength in horn of the white line area that had structural damage when compared with non-damaged white line horn. However, the authors did not explain whether the white line that had structural damage had haemorrhaged areas.

The maximum punch force of the sole horn was significantly (P<0.05 to 0.001) lower for the claws that had higher lesion scores and was significantly (P<0.001) lower at day 160 *postpartum*, which was the period were the cows were housed for longer and when the mean lesion scores were higher. This indicated that the increase in haemorrhage levels of the hoof horn was clearly related with the production of a horn of significantly lower structural strength. This relationship is presented in Table 2.13 were the punch force of the sole horn with no haemorrhage was 8.72 N and decreased progressively with increasing haemorrhage levels being 4.99 N when the haemorrhage score was highest (5). However, the lower structural strength of the horn at day 160 *postpartum* could also be related to the length of time the animals were housed and potentially the longer time hooves were exposed to slurry and concrete surfaces. Chaplin *et al.* (2000) found an increase in the severity of lesions in housed animals, however the increase in the severity of lesions was grater in animals that were housed and were in lactation. The significantly ($P<0.001$) greater maximum punch force of the sole and white line horns measured at day 270 *postpartum* may be related to the change in the housing conditions, as cows were grazed at pasture at this point as opposed to housed indoors in cubicles, and the possible change in the moisture content and haemorrhage level of the hoof horn.

The punch force of the hoof tissue was significantly and positively ($P<0.001$) affected by the dry matter content of the hoof horn (R^2adj.= 0.83). Several authors have demonstrated that the moisture content of the hoof horn had a great influence over its mechanical properties. Measurements of hoof wall hardness (Collins *et al.*, 1998), elastic modulus of the sole horn (Bertram and Gosline, 1987; Hinterhofer *et al.*, 1998) and bending stiffness of oryx, donkey and cattle hoof wall horn (Kitchener and Vincent, 1987; Collins *et al.*, 1998; Baillie *et al.*, 2000) decreased significantly with the increasing moisture content of the horn. The hoof horn of cows maintained on wet concrete floors and standing in slurry had significantly higher water content (Vermunt and Greenough, 1995; Wells *et al.*, 1995). Budras and Mulling (1998) and Kempson *et al.* (1998) using morbid samples found that slurry removed the intercellular cementing substance and increased the permeability of the intertubular horn of the sole and the heel and urea removed keratin proteins from the horny mass in the cells. Therefore the mechanical properties of the hoof horn of cows standing in slurry for prolonged time could not only be influenced by an increase in the water content of the horn but also by a decrease in the quality of the horn.

The white line horn had a lower maximum punch force when compared to the sole horn, indicating the weaker structural strength of this area. Similar results were reported by Budras *et al.* (1996) and Mulling *et al.* (1994). Budras *et al.* (1996) found average hardness values obtained with the use of the ball-impact method of 5.1 N/mm^2 for the cap-horn area and 6.9 N/mm^2 for the terminal horn area of the white line, which were significantly lower than the values for the sole horn (12.9 N/mm^2) and for the wall horn (27.5 N/mm^2).

2.5 Conclusion

The testing of the maximum punch force of hoof horn slivers of the sole and white line areas proved to be an appropriate method to measure changes occurring in the hoof horn of cows during the lactation period. The maximum force required to punch the hoof horn samples was clearly related to the occurrence of haemorrhages on the horn. A decrease in the maximum punch force of the sole horn was observed at 160 days *postpartum* and on hind outer claws when the sole area had the highest scores for lesion. Other factors found to affect the force required to punch the hoof horn were the dry matter of the sample and the sample thickness. The effect of sample thickness was accounted for in the statistical model.

Chapter 3

Experiment 2 – Testing of factors affecting mechanical testing of hoof horn

3.1 Introduction

A number of factors have been found to affect the mechanical properties of the hoof horn (Experiment 1). The effect of the moisture content on the mechanical properties of the hoof horn is well documented in the literature and it has been found to affect hoof horn hardness, elastic modulus, bending stiffness and fracture toughness (Bertram and Gosline, 1987; Kitchener and Vincent, 1987; Kung *et al.*, 1991; Douglas *et al.*, 1996; Collins *et al.*, 1998; Hinterhofer *et al.*, 1998; Baillie *et al.*; 2000, Dyer *et al.*, 2004). Hinterhofer *et al.* (1998) and Douglas *et al.* (1996) found that in order to represent the *in vivo* situation, hoof horn samples should be tested at their physiological moisture content. Therefore, it was important to avoid loss of moisture from hoof horn following sample collection. In order to prevent moisture loss from hoof samples, Collins *et al.* (1998) wrapped the samples in 3 layers of Parafilm and Hinterhofer *et al.* (1998) and Kasapi and Gosline (1999) stored the samples in resealable plastic bags, at 4°C, until analysis.

Different time periods between sample collection and analysis have been used in experimental work reported in the literature. Hoof horn samples were tested 4 hours after collection by Wagner *et al.* (2001) and 48 hours after collection by van Amstel *et al.* (2004). However, the majority of authors did not state the exact time from collection to sample analysis (Hinterhofer *et al.*, 1998; Douglas *et al.*, 1996) and this may affect the mechanical properties of the hoof horn samples.

The freezing of horn tissue samples may represent an alternative method of storage. However, only Kasapi and Gosline (1999) tested the effect of freezing on the bending modulus of equine hoof wall samples. While these authors found that bending modulus was not affected by freezing, the samples were frozen for only for 10 minutes in their experiment.

The variation in sample thickness was found to affect the punch resistance (Experiment 1). However, when collecting samples from live animals it was difficult to standardize the sample thickness and as a consequence sample thickness was used as a covariate when analysing punch resistance.

As a consequence, the aim of this experiment was to measure the effect of storage methods, time between sample collection and analysis, the moisture content and sample thickness on the punch resistance and elastic modulus of the sole and white line areas of the bovine sole horn to access and potentially reduce the effect of these factors on the measurement of mechanical properties of the hoof horn.

In addition, a tension test was included in this experiment to allow the comparison of the results with results found in the literature. According to Kasapi and Gosline (1996), the hoof tissue fails in tension and therefore the measurement of the tensile properties of the hoof horn is of primary importance for evaluating hoof performance.

3.2 Material and methods

3.2.1 Sample collection, preparation and mechanical tests

The hooves of beef cattle, aged from 12 to 18 months, were obtained from a local abattoir. Hooves were randomly selected. The animals were slaughtered for reasons other than the completion of this study. All hooves exhibited normal physiological aspects and showed no visible pathological changes to the wall and sole areas. The hooves were attached to the distal portions of the limb. On the same day of collection the hooves were placed in a vice and the distal 1mm surface of the sole horn was removed using a wood plain, in order to reduce variation in sample thickness. Samples were taken from the clean sole and white line horn areas of each claw (zones 2 and 5 of the IFM, Figure 2.1), labelled and stored in sealed plastic bags in the refrigerator (4°C) until analysis on the same day. All samples of each hoof were tested for punch resistance and tension testing following collection.

3.2.1.1 Punch resistance test

The testing method used for the punch test was described in Experiment 1. The values of maximum force at punch (N) and the work to fracture (energy expenditure, J) were obtained from the force-displacement curve produced. The work required to fracture the sample was calculated by measuring the area of the force-displacement curve under the maximum peak force. Considering the test piece to be a circular plate clamped around its circumference (fixed edges) and with a small thickness (membrane) where the deflection of the plate exceeds the magnitude of the plate thickness, membrane stresses at the centre of the plate and elastic modulus of the membrane were calculated according to the method used by Blake (1982) from the equation for maximum lateral deflections of circular plates:

$$\delta/t + 0.583\ (\delta/t)^3 = 0.1576\ q/E\ (R_0/t)^4$$

where:

δ = maximum deflection of plate

t = plate (membrane) thickness

q = transverse pressure = punch force/ outer radius of plate^2

E = elastic modulus

R_0 = outer radius of plate

and the equation for the tensile membrane stresses at the centre of the plate:

$$S_t = 0.423\ (Eq^2R_0^2/t^2)^{1/3}$$

where:

S_t = tensile stress at the centre of the plate (N/mm^2)

E = elastic modulus

q = transverse pressure = punch force/ outer radius of plate^2

R_0 = outer radius of plate

t = plate (membrane) thickness

A total of 10 to 12 tests were completed on areas 2 and 5, according to the IFM (Figure 1.4). The measurements were taken from sole and white line areas and were recorded separately.

3.2.1.2 Tension test

The tension tests were completed using a TA.HDi texture analyser equipped with a 100 kg load cell (Stable Micro Systems). The samples were shaped in a 'dog bone shape' and had the following measurements in the central area: 2 mm x 20 mm x thickness (0.05-0.3 mm). The sample shape and dimensions are presented in Figure 3.1. Hoof horn samples were held in place with self-tightening roller grips. The test-mode used was to measure force in tension and the trigger force was 5g. During the test the grips were moved apart at a speed of 1mm/sec until reaching the distance of 30 mm, when they were returned to the start position. This crosshead speed generated a medium to high strain rate according to Kasapi and Gosline (1996). At the distance of 30 mm the tests were conducted to failure, the grips were moved apart until the samples were split in two and the force-displacement curve was recorded. When failure occurred near the grips the results were discarded. The force-displacement curve presented a linear elastic region followed by a region of non-elastic deformation. The elastic modulus was calculated from the linear region, by dividing the difference in the force increase from two different times in the force-displacement curve by the difference in the length of the sample in the same times. The tension tests were completed twice on the samples collected from the sole and white line areas of each claw.

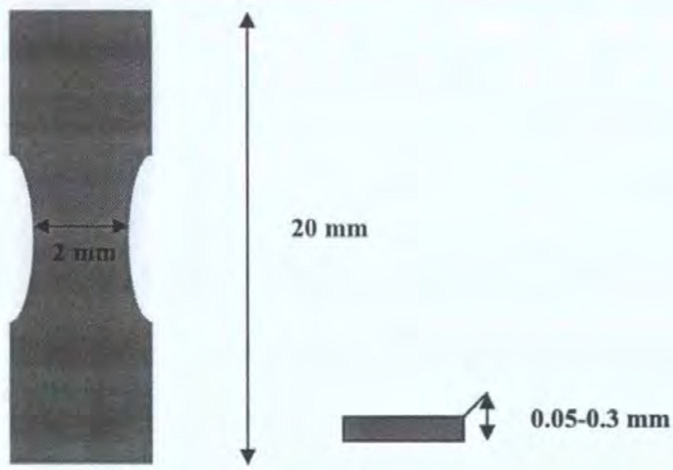


Figure 3.1 ***Dimensions of sample for the tension test***

3.2.2 Effect of moisture content on the mechanical properties of the bovine hoof horn

Samples, with a thickness of 0.2 mm, were collected from six claws from areas corresponding to 2 (white line) and 5 (sole) of the International Foot Map (IFM) (Figure 1.4) (Leach *et al.*, 1998; Shearer *et al.*, 2002).

3.2.2.1 Testing the effect of moisture using relative humidity chambers

The hoof tissue samples from the 6 different claws were placed in 5 environments containing relative humidities (RH) corresponding to 11, 33, 58, 75 and 97 %. The relative humidities were achieved by suspending the samples over solutions of specified salts: lithium chloride (11 % RH), magnesium chloride (33 % RH), sodium bromide (58 % RH), sodium chloride (75 % RH) and potassium sulphate (97 % RH). Samples were left in these environments for 7 days and then tested for moisture content using a moisturemeter (Thermoconstanter RTD-33, TH-2 – Novasina, calibrated for 25 °C). Immediately following the measurement of the moisture, the punch resistance and tension tests were completed (Collins *et al.*, 1998) and after the completion of the tests the samples were weighed and placed in the oven (100 °C for 72 hrs) for the determination of the dry matter.

3.2.2.2 Effect of soaking the samples in distilled water for different lengths of time

Hoof tissue samples of the sole and white line horn of the same areas (2 and 5, according to the IFM, Figure 1.4) of 5 different hooves were tested for punch resistance and tension testing after collection and then placed in distilled water. Thirty samples, six of each hoof were placed in distilled water. One sample of each hoof was taken out of the distilled water at 3, 6, 12, 24, 48 and 72 hours and tested again for punch resistance and tension testing. After testing the samples were weighed and put in the oven (100 °C for 72 hrs) for determination of the dry matter.

3.2.3 Effect of number of days kept in plastic bags on the mechanical properties of the bovine hoof horn

Hoof tissue samples, with a thickness of 0.2 mm, were collected from the areas corresponding to areas 2 and 5 of the International Foot Map (IFM) (Figure 1.4) (Leach *et* *al.*, 1998; Shearer *et al.*, 2002) of four different claws. Six samples of each claw were placed in self-sealing plastic bags and stored at a temperature of 2°C. Before testing the samples were left at room temperature in the plastic bags for 1 hour. Samples were tested for punch resistance and elastic modulus (tension test) at 0, 48, 96, 144 and 192 hours. At zero hour, when samples were put in the plastic bags and after completing the tests the samples were weighed and than placed in an oven (100 °C) for 72 hrs for the determination of the dry matter (Hinterhofer *et al.*, 1998).

3.2.4 Effect of freezing on the mechanical properties of the bovine hoof horn

Hoof tissue samples, with a thickness of 0.2 mm, were collected from the areas corresponding to areas 2 and 5 of the IFM (Figure 1.4) (Leach *et al.*, 1998; Shearer *et al.*, 2002) of six different hooves and kept in sealed plastic bags. Samples were tested for punch resistance and tension tests on the day of collection (not frozen) following which, the samples were stored in a freezer (-20 ^{0}C) in 3 separate plastic bags. The first bag was taken out of the freezer on the following day (day 1) and left to defrost at room temperature for 4 hrs. Punch resistance and tension tests were then completed. After being stored for one week (day 7) and for one month, the second and third bags were taken out of the freezer, left to defrost at room temperature for 4 hrs and punch resistance and tension tests were completed.

3.2.5 Effect of sample thickness on the mechanical properties of the bovine hoof horn

Areas 2 and 5 of the IFM (Figure 1.4) (Leach *et al.*, 1998; Shearer *et al.*, 2002) of six different hooves were cut into different thicknesses: 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 mm, using a plane. Samples were placed in plastic bags and tested immediately for punch resistance and elastic modulus.

3.2.6 Statistical analysis

The data collected were found to be normally distributed and analysed using GLM - ANOVA (Minitab 13.0). The membrane stress data were log 10 transformed before analysis. The data from the time kept in distilled water, time stored in plastic bags and freezing were analysed for the effect of treatment and means were compared using the Tukeys test (95 % confidence interval). Sample thickness was entered as a covariate, because even when thickness was standardised (collected with the settings on the plane were the same) some variation did occur. The data from relative humidity and sample thickness tests were analysed using regression analysis.

3.3 Results

Tension tests were conducted to failure and force-displacement curves presented a linear elastic region followed by a region of non-elastic deformation. Webb *et al.* (1984) reported a force-displacement curve with a linear elastic region followed by a region of non-elastic deformation when performing compression tests on hoof wall horn specimens.

3.3.1 The effect of the dry matter content of the hoof horn on the maximum punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus of the tension test of the horn of the sole and white line areas

3.3.1.1 The effect of sample thickness when hoof samples were tested for the effect of moisture content

Sample thickness varied between 0.03 and 0.20 mm (mean = 0.076 mm) when hoof samples were tested for the effect of moisture content and had a significant ($P < 0.001$) linear effect on the punch resistance (Punch force of the sole horn (N) = 4.359 + 39.608

thickness (mm), R^2 adj.= 0.73) and work to fracture (work of the sole horn (kJ) = 4.552 + 50.378 thickness (mm), R^2 adj.= 0.62) and a quadratic effect on the elastic modulus of the diaphragm (emod diafrag (N/mm^2) = 71.089 - 771.887 thickness (mm) + 2458.19 thickness^2 (mm), R^2 adj.= 0.47) and membrane stress of the sole horn (membr stress (N/mm^2) = 44980.7 - 723088 thickness (mm) + 2596845 thickness^2 (mm), R^2 adj.= 0.64).

When the white line area of the hoof horn was tested for the effect of the moisture content on the mechanical properties the sample thickness varied between 0.03 and 0.20 mm (mean = 0.073 mm) and had a significant ($P < 0.001$) linear effect on the punch resistance (Punch force of the white line (N) = 1.537 + 28.760 thickness (mm), R^2 adj.= 0.67), work to fracture (work to fracture of the white line (kJ) = 0.630 + 36.176 thickness (mm), R^2 adj.= 0.68) and elastic modulus of the diaphragm (emod diafrag of the white line = 66.117 - 322.084 thickness (mm), R^2 adj.= 0.26) and a quadratic effect on membrane stress of the horn of the white line area (membr stress white line = 23958.5 - 363227 thickness (mm) + 1289444 thickness^2 (mm), R^2 adj.= 0.54).

3.3.1.2 The effect of the time period that samples were soaked in distilled water on the dry matter, maximum punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus of the sole and white line areas of the hoof horn

The effect of the time period that samples were soaked in distilled water on the dry matter, punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus of the sole and white line areas of the hoof horn was analysed through ANCOVA using sample thickness as a covariate. The effect of the time soaked in distilled water on the sample dry matter and elastic modulus measured through tension were also analysed using regression analysis. The time that the hoof samples were soaked in distilled water had no significant effect on the elastic modulus of the samples, but did have a significant ($P < 0.05$) quadratic but low effect on the dry matter of the samples:

Dry matter (%) = 67.142 – 0.1575 time + 0.00179 $time^2$, R^2adj. = 0.13

The dry matter, maximum punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus measured through a tension test of the sole and white line horn of samples soaked in distilled water for different periods of time are presented in Table 3.1.

The moisture content of the hoof samples increased significantly ($P<0.001$) after 3 hours of soaking in distilled water. The maximum punch resistance of the sole area of the hoof horn decreased after 6 hours of soaking in water, but this decrease was only significant after 72 hours of soaking ($P<0.05$). The maximum punch resistance of the white line area, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus of the sole and the white line areas were not affected by the period of time hoof samples were soaked in water.

3.3.1.3 The effect of decreasing levels of humidity on the dry matter, maximum punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus of the sole and white line areas of the hoof horn

Placing samples in chambers with decreasing levels of relative humidity for 7 days, resulted in a significant increase ($P<0.001$) in the dry matter of the samples. The relationship between the relative humidity of the environment and the dry matter of the hoof samples is presented in Figure 3.2. The results for the mechanical tests for the samples that were placed in the environment with 11% RH were not presented. These samples were very brittle and fractured when attached to the roller grips or placed under the plates on the tension and punch tests and the few results that were obtained were very variable.

Table 3.1 Dry matter (DM), maximum punch resistance (PR), work to fracture (W), elastic modulus of the diaphragm (EMd), membrane stress (S) and elastic modulus (EM) of the sole and white line (wl) areas of the hoof horn, of samples soaked in distilled water for different periods of time (hrs)

Time (hrs)	0	3	6	12	24	48	72	sem	*P*
DM (%)	70.95[a]	64.85[b]	64.48[b]	64.10[b]	64.86[b]	65.08[b]	65.50[b]	0.737	0.001
PR sole (N)	7.46[ab]	8.56[a]	6.79[ab]	6.75[ab]	7.71[ab]	7.65[ab]	6.58[b]	0.447	0.05
PR wl (N)	3.69	4.28	3.82	3.35	4.15	3.31	3.49	0.24	NS
W sole (kJ)	8.23	10.04	7.51	8.02	8.66	8.84	7.26	0.447	NS
W wl (kJ)	3.95	3.74	3.12	2.94	3.00	2.66	2.78	0.361	NS
EMd sole (N/mm^2)	38.26	33.37	30.77	26.97	32.68	33.07	26.42	7.475	NS
EMd wl (N/mm^2)	25.16	32.32	50.78	44.11	50.85	49.99	44.22	8.73	NS
S sole (N/mm^2)	12093	7912	10199	8496	13591	11029	3902	4795	NS
S wl (N/mm^2)	5020	3782	8612	6710	7551	6215	4469	2018	NS
EM sole (N/mm^2)	116.9	221.6	232.7	206.7	243.1	274.4	188.7	39.49	NS
EM wl (N/mm^2)	-	274.3	250.2	174.1	251.0	304.8	101.8	61.42	NS

[a,b] different letters indicate values that differ significantly, NS – not significantly different

The increase in the dry matter observed in environments with lower relative humidity resulted in a significant increase (P<0.001) in the punch resistance of the sole and the white line areas of the hoof horn, which is presented in Figures 3.3 and 3.4.

Dry matter (%) = 95.907 – 0.1566 relative humidity (%), R^2 adj. = 0.87, P<0.001

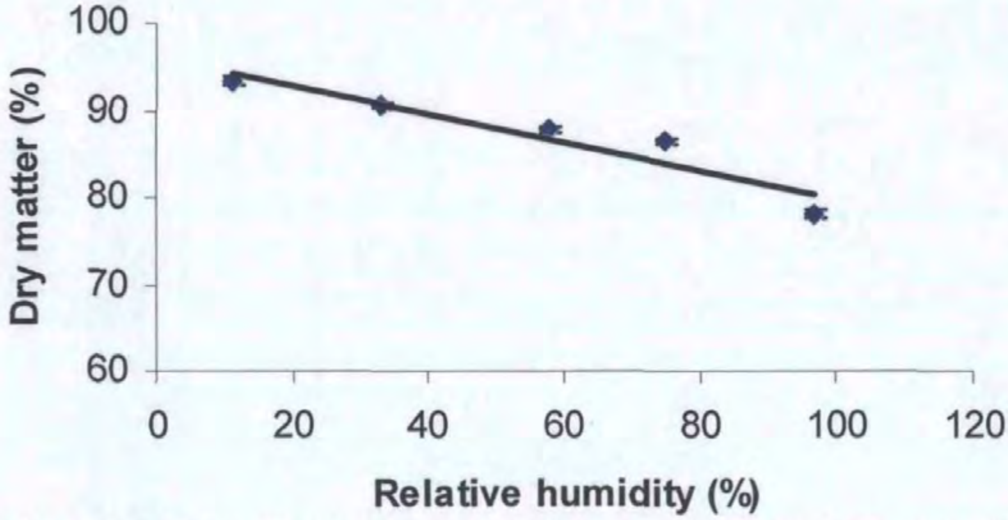


Figure 3.2 ***Effect of environments with different relative humidity on the dry matter of the samples***

Maximum punch force of the sole area (N) = 0.4273 dry matter (%) – 19.952, R^2 adj. = 0.65, P<0.001

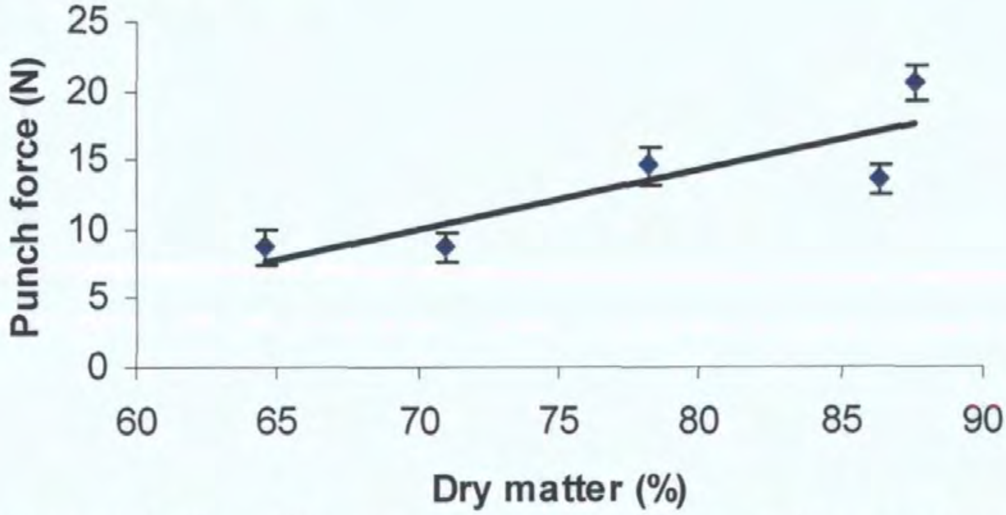


Figure 3.3 ***Effect of dry matter on the maximum punch force of sole area of the hoof horn***

Maximum punch force of the white line area (N) = 0.3621 dry matter (%) – 19.787, R^2 adj. = 0.89, $P<0.001$

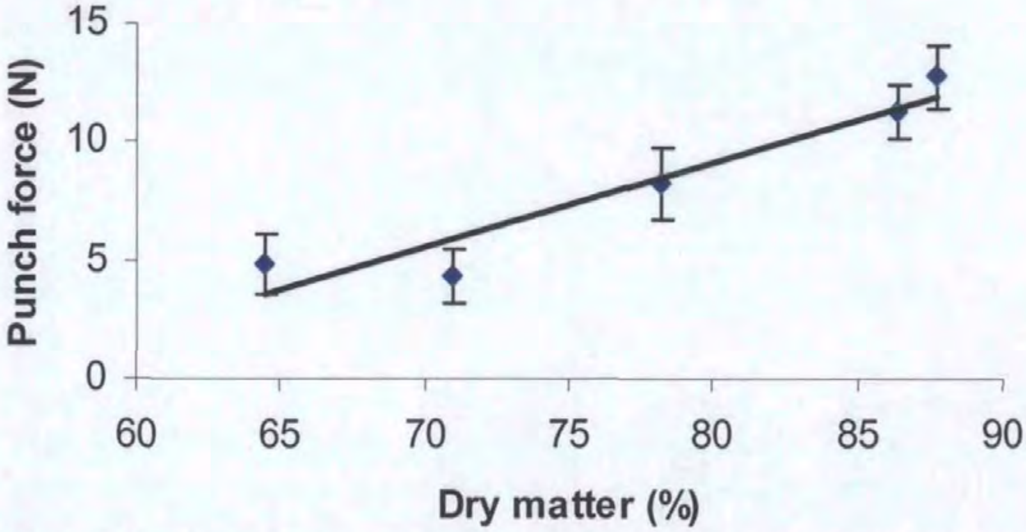


Figure 3.4 ***Effect dry matter on the maximum punch force of white line area of the hoof horn***

The work to fracture of the sole and white line horns also increased significantly and linearly ($P<0.01$, 0.001) with increasing dry matter (Figures 3.5 and 3.6).

Work to fracture the sole area (kJ) = 0.1771 dry matter (%) – 2.2304, R^2adj. = 0.37, $P<0.01$

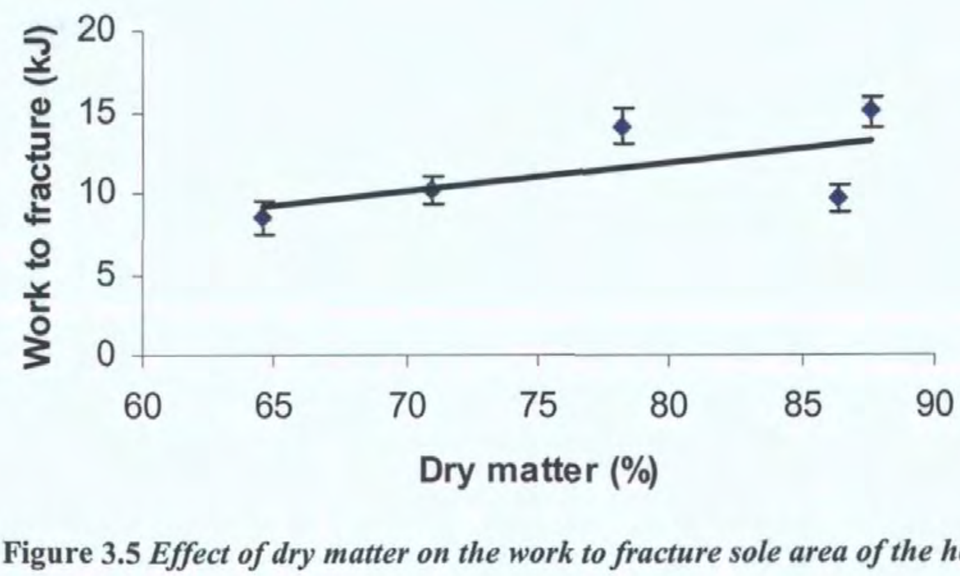


Figure 3.5 ***Effect of dry matter on the work to fracture sole area of the hoof horn***

Work to fracture the white line area (kJ) = 0.2901 dry matter (%) – 14.867,

R^2 adj. = 0.74, P<0.001

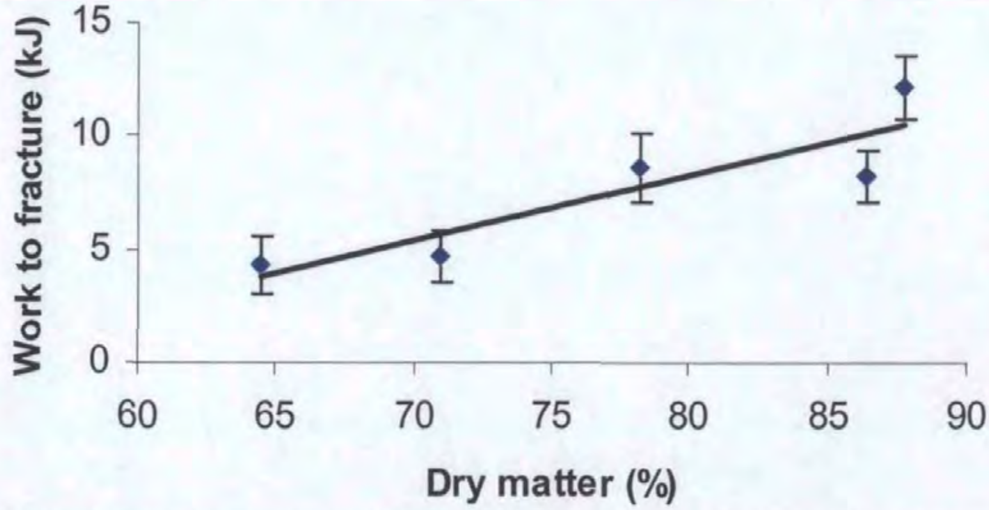


Figure 3.6 ***Effect of dry matter on the work to fracture the white line area of the hoof horn***

The increasing of dry matter resulted in significant (P<0.001) quadratic increase in the elastic modulus of the diaphragm of the sole (Figure 3.7) and white line areas (Figure 3.8) and membrane stress of the white line area of the hoof horn (Figures 3.9).

Emod diafrag sole area (N/mm^2) = 2241.68 - 69.1383 DM (%)+ 0.538140 DM^2(%),
R^2adj. = 0.55, P<0.001

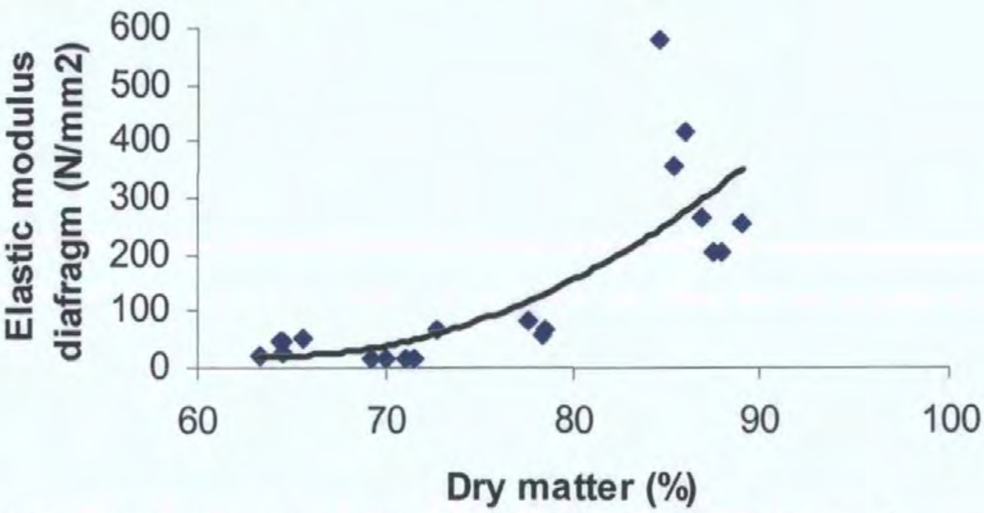


Figure 3.7 ***Effect of dry matter on the elastic modulus of the diaphragm of the sole area of the hoof horn***

Emod diafrag white line area (N/mm^2) = 4159.94 - 121.559 DM (%) + 0.894768 DM^2 (%), R^2 adj. = 0.39, P< 0.01

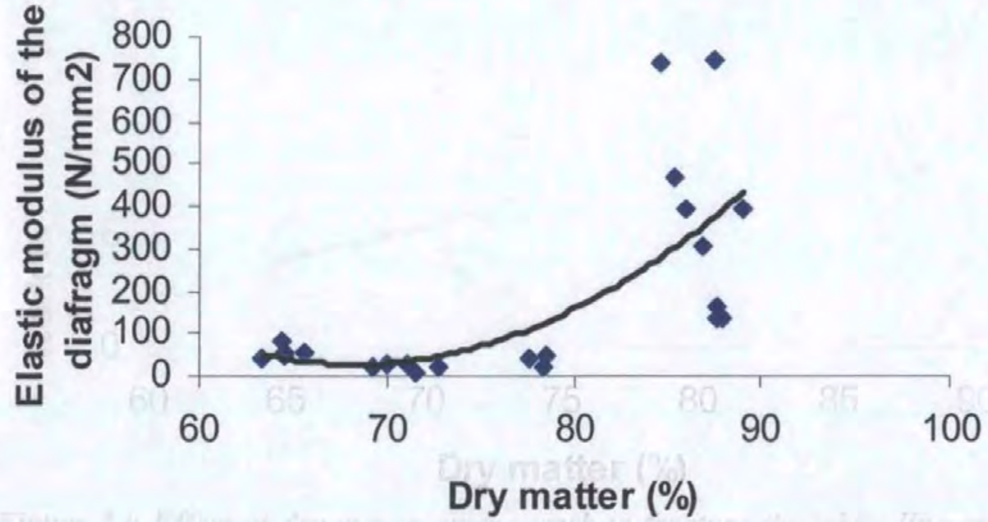


Figure 3.8 ***Effect of dry matter on the elastic modulus of the diaphragm of the white line area of the hoof horn***

Membrane stress white line area (N/mm^2) = 998048 - 28038.7 Dm (%) + 197.255 DM^2 (%), R^2adj. = 0.39, P <0.01

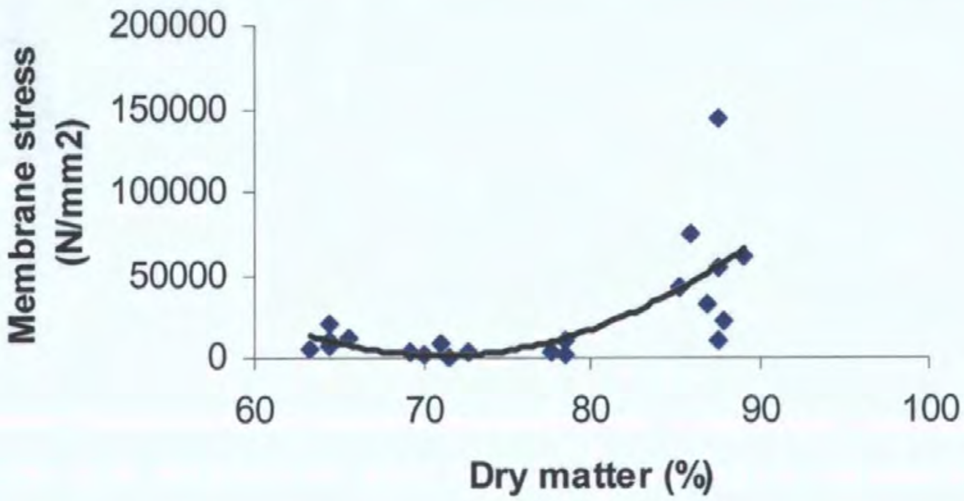


Figure 3.9 ***Effect of dry matter on the membrane stress of the diaphragm of the white line area of the hoof horn***

Dry matter content had no significant effect on the membrane stress of the diaphragm of the sole area of the hoof horn. The elastic modulus measured through tension tests was positively and exponentially related to the dry matter of the sole horn tissue (Figure 3.10).

Elastic modulus of the sole horn (N/mm^2) = $0.0602e^{0.1012DM}$, R^2 adj. = 0.81, $P<0.001$

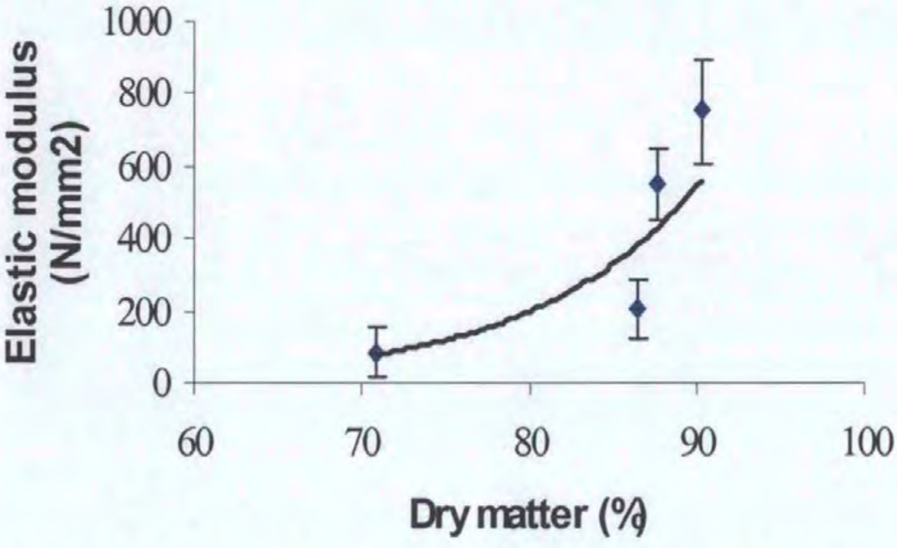


Figure 3.10 ***Effect of dry matter on the elastic modulus of sole horn samples***

3.3.2 Effect of days taken until analysis of the samples on the maximum punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress, elastic modulus measured through tension and dry matter of the sole and white line areas of the hoof horn

3.3.2.1 The effect of sample thickness when days taken until analysis was tested

The sample thickness varied between 0.03 and 0.20 mm (mean = 0.086 mm) for sole horn samples and between 0.05 and 0.19 mm (mean = 0.11 mm) for white line samples when the effect of days taken until analysis was tested and had a significant ($P<0.01$ and 0.001) linear effect over the punch resistance (Punch force sole area (N) thickness (mm), R^2 adj.= 0.24; work white line area (kJ) = 1.738 + 48.295 thickness (mm), R^2 adj.= 0.44) and elastic modulus of the diaphragm (Emod diafr sole area = 46.00 - 215.083 thickness (mm), R^2 adj.= 0.33; Emod diafr white line area = 28.587 - 76.776 thickness (mm), R^2 adj.= 0.10) and a quadratic effect over membrane stress of the sole (memb. Stress sole area= 59825.5 - 898537 thickness (mm) + 3135633 thickness^2 (mm), R^2 adj.= 0.57) and white line horns (membr stress white line area = 11664.9 - 148182 thickness (mm) + 485117 thickness^2 (mm), R^2 adj.= 0.77).

3.3.2.2 Effect of days taken until analysis of the samples

The effect of the days from sample collection to analysis on the hoof sample dry matter content, punch resistance, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas of the hoof horn were analysed using ANCOVA with sample thickness as a covariate. The effect of days from sample collection to analysis of the samples on the elastic modulus of the sole and white line areas measured through tension tests were analysed using also regression analysis and did not have a significant effect on the elastic modulus of the sole and white line areas of the hoof horn samples.

The maximum punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress, elastic modulus measured through tension and dry matter of the sole and white line horn on days 0, 2, 4, 6, and 8 after the collection of the samples are presented in Table 3.2. The number of days from sample collection to analysis of the hoof samples, when analysed through ANCOVA, had no significant effect on the maximum punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress, elastic modulus measured through tension and dry matter of the sole and white line horns.

However, the elastic modulus was significantly and positively correlated to the dry matter ($P<0.05$, $r = 0.48$). The punch resistance of the sole and the white line were significantly and positively correlated ($P<0.001$, $r = 0.69$).

Table 3.2 ***Effect of days to analysis on the dry matter (DM),maximum punch resistance (PR), work to fracture (W), elastic modulus of the diaphragm (EMd), membrane stress (S) and elastic modulus (EM) of the sole and white line (wl) area of the hoof horn***

	Days to analysis						
	0	2	4	6	8	sem	*P*
DM (%)	72.54	72.05	72.88	71.25	71.11	0.568	NS
PR sole (N)	8.78	10.40	10.13	9.81	9.61	0.762	NS
PR wl (N)	4.95	6.40	6.08	6.51	6.90	0.662	NS
W sole (kJ)	10.22	11.54	11.29	10.64	10.11	0.812	NS
W wl (kJ)	4.73	7.12	6.67	7.20	7.89	1.021	NS
EMd sole (N/mm^2)	28.75	29.96	28.25	31.08	29.74	5.379	NS
EMd wl (N/mm^2)	26.86	19.87	24.01	21.07	15.37	3.494	NS
S sole (N/mm^2)	14702	7656	5458	6415	17833	5780	NS
S wl (N/mm^2)	2043	2515	3192	1915	1463	536.3	NS
E mod sole (N/mm^2)	158.2	148.1	226.3	125.8	175.5	43.36	NS
E mod wl (N/mm^2)	91.8	159.5	218.9	143.3	194.1	37.27	NS

NS – not significantly different

3.3.3 The effect of the number of days frozen on the maximum punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress, elastic modulus measured through tension and dry matter of the sole and white line horns

3.3.3.1 The effect of sample thickness when days frozen was tested

The hoof horn sample thickness varied between 0.05 and 0.14 mm (mean = 0.079 mm) for the sole area samples and between 0.05 and 0.15 mm (mean = 0.10 mm) for the white line area samples. Thickness had a significant ($P < 0.05$ to 0.001) linear effect over the punch resistance (Punch force of the sole area (N) = 4.300 + 59.5290 thickness (mm), R^2 adj.= 0.48), work to fracture (work to fracture of the sole area (kJ) = 4.508 + 63.847 thickness (mm), R^2 adj.= 0.46) membrane stress (membrane stress of the sole horn (N/mm^2) = 22779.2 - 171907 thickness (mm), R^2 adj.= 0.52) of the sole horn and the punch resistance (Punch force of the white line area (N) = 4.378 + 24.582 thickness (mm), R^2 adj.= 0.13), elastic modulus of the diaphragm (emod diafrag of the white line area (N/mm^2) = 54.602 - 185.354 thickness (mm), R^2 adj.=0.17) and membrane stress (memb stress of the white line area (N/mm^2) = 16251.4 - 113896 thickness (mm), R^2 adj.= 0.59) of the white line area of the hoof horn.

3.3.3.2 The effect of days frozen over the dry matter, punch resistance, work to fracture and membrane stress of the sole and white line areas of the hoof horn

The effect of the number of days the samples were frozen for on the dry matter content, punch resistance, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas of the hoof horn were analysed using ANCOVA with sample thickness as a covariate. The effect of the number of days the samples were frozen on the sample dry matter content and elastic modulus measured through tension test were analysed using regression analysis. The effect of the number of days the samples were frozen had no significant effect on the dry matter content of the samples, but did have a significant (*P*<0.001 and 0.05) linear effect over the elastic modulus of the sole and white line areas of the hoof horn samples:

EM sole area = 197.560 + 6.942 day, R^2adj. = 0.43;

EM white line area = 158.168 + 6.417 day, R^2adj. = 0.22.

The effect of the number of days the samples were frozen on the maximum punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress, elastic modulus measured using tension tests and dry matter content of the sole and white line horns are presented in Table 3.3.

The number of days the hoof horn samples were frozen did not significantly affect the maximum punch resistance, work to fracture, elastic modulus of the diaphragm, membrane stress, dry matter content of the sole and white line horns and elastic modulus measured through tension tests of the white line horn. However, there was a significant increase (*P* <0.001) in the elastic modulus of the sole horn when samples were frozen for 30 days compared to non frozen samples.

Table 3.3 ***Effect of the number of days the samples were frozen on the dry matter content (DM), maximum punch resistance (PR), work to fracture (W), elastic modulus of the diaphragm (EMd), membrane stress (S) and elastic modulus (EM) of the sole and white line (wl) areas of the hoof horn***

	Days					
	0	1	7	30	sem	*P*
DM (%)	72.34	-	-	74.22	0.779	NS
PR sole (N)	8.34	8.72	9.86	9.10	0.660	NS
PR wl (N)	6.21	6.63	7.44	7.10	0.701	NS
W sole (kJ)	8.49	9.36	10.76	9.61	0.691	NS
W wl (kJ)	6.73	6.31	7.41	7.13	0.882	NS
EMd sole (N/mm^2)	33.75	35.63	36.91	34.47	5.100	NS
EMd wl (N/mm^2)	29.80	36.78	40.63	36.75	4.844	NS
S sole (N/mm^2)	9075	8611	10371	8106	1913	NS
S wl (N/mm^2)	5884	4157	4606	4511	1183	NS
Emod sole (N/mm^2)	144.4 ^{b}	268.3 ^{ab}	251.1 ^{ab}	403.1 ^{a}	42.53	0.001
Emod wl (N/mm^2)	116.6	136.5	292.7	330.7	67.21	NS

^{a,b} different letters indicate values that differ significantly,
NS – not significantly different

3.3.4 The effect of thickness on the punch resistance, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas of the hoof horn samples

The hoof sample thickness of the tested sole areas varied between 0.05 to 0.20 mm. The thickness of the tested areas of horn samples had a significant linear effect ($P<0.001$) on the maximum punch force and work to fracture of the sole area of the hoof horn. The hoof horn. The regressions of thickness on punch force and work to fracture of the sole area of the hoof horn are presented in Figures 3.11 and 3.12.

Maximum punch force of the sole area of the hoof horn (N) = 5.567 + 48.337 thickness (mm), R^2 adj. = 0.76, $P<0.001$

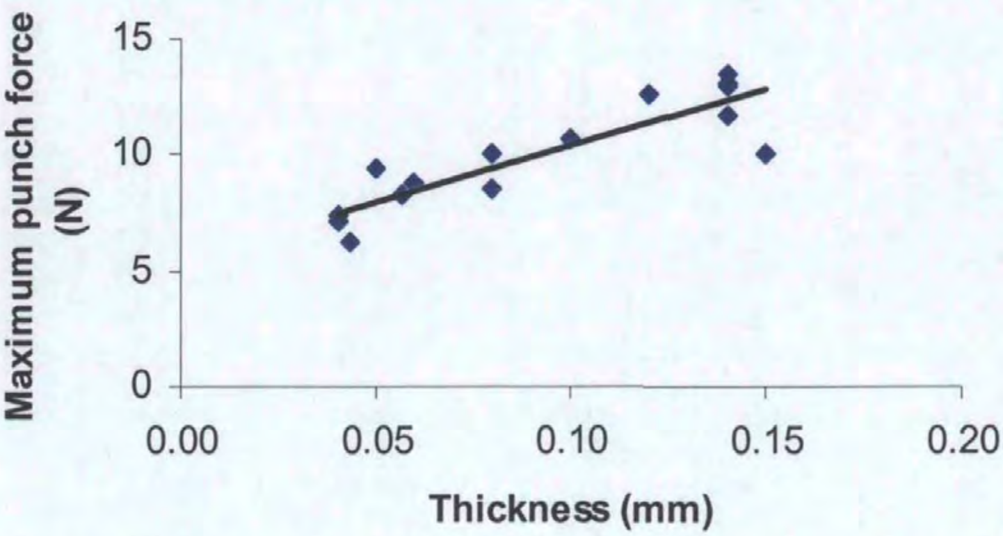


Figure 3.11 ***Effect of thickness of the sample on the punch force of the sole area of the hoof horn, n = 18***

Work to fracture sole (kJ) = 7.261 + 42.537 thickness (mm), R^2adj. =0.61, $P<0.001$

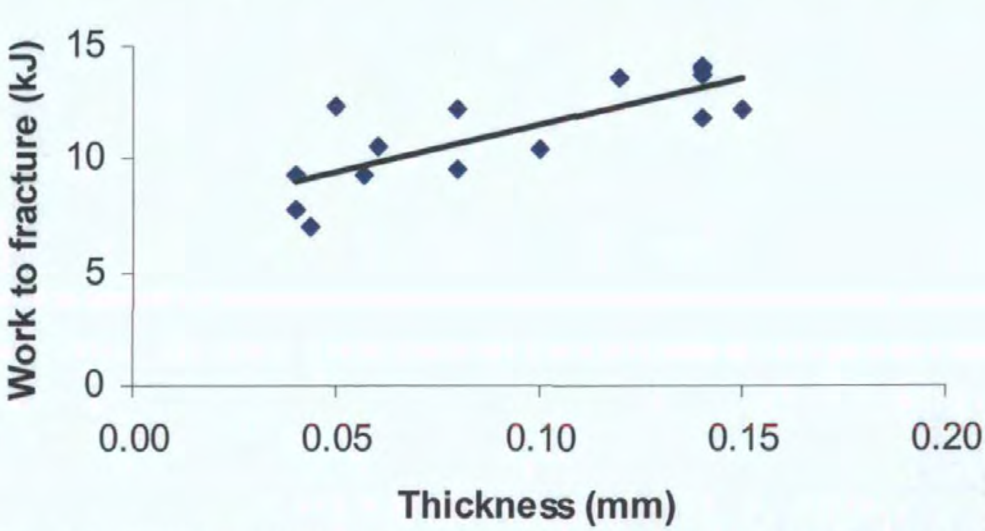


Figure 3.12 ***Effect of thickness of the sample on the work to fracture of the sole area of the hoof horn, n = 18.***

The thickness of the tested white line areas varied between 0.03 and 0.35 mm. The increase in thickness resulted in a significant ($P<0.001$) and linear increase in the maximum punch resistance and work to fracture the white line horn. The regression of the thickness on the maximum punch force and work to fracture of the white line area of the hoof horn are presented in Figures 3.13 and 3.14. The maximum punch force and work to fracture of the hoof horn tissue of the white line area increased significantly ($P<0.001$) with increasing sample thickness.

Maximum punch force of the white line area (N) = 3.813 + 29.639 thickness (mm), R^2adj. = 0.80, $P<0.001$

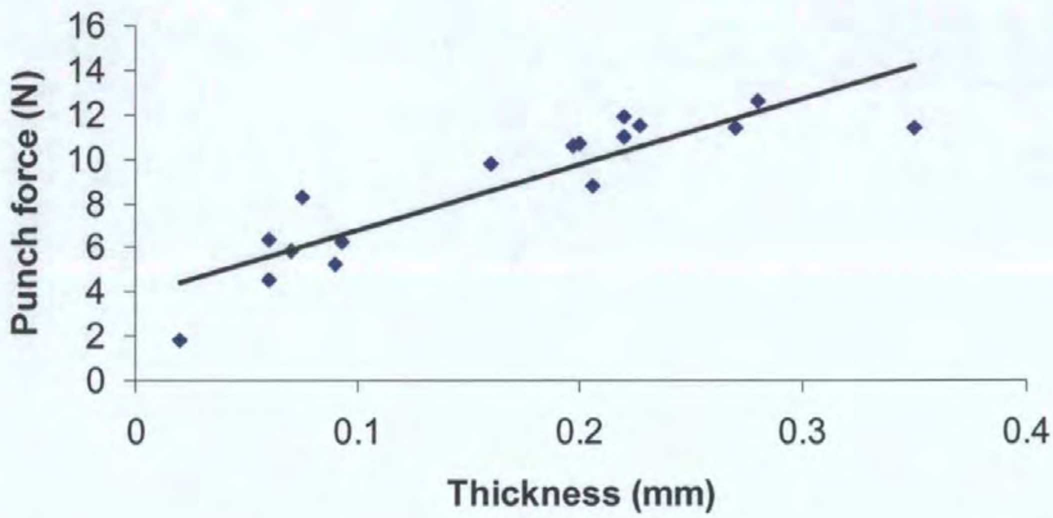


Figure 3.13 ***Effect of thickness of the sample on maximum punch force of the white line area of the hoof horn, n = 18.***

Work to fracture white line area (kJ) = 3.170 + 40.381 thickness (mm), R^2 adj. = 0.75, P<0.001

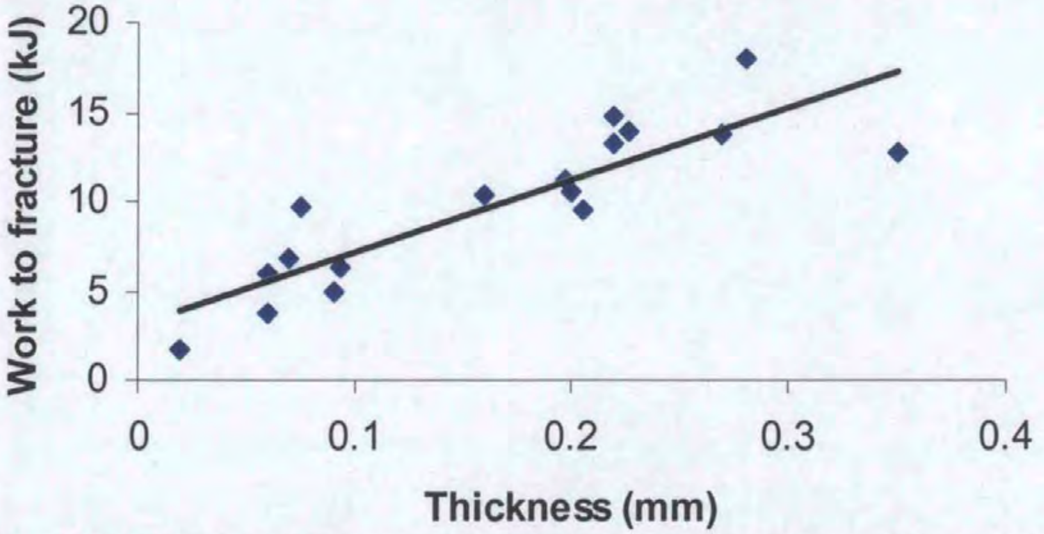


Figure 3.14 ***Effect of thickness of the sample on work to fracture of the white line area of the hoof horn, n = 18.***

The membrane stress of the sole and white line areas of the hoof horn decreased significantly (P<0.001) and exponentially as the thickness of the hoof horn samples increased and these equations are presented in Figure 3.15 and 3.16.

Membrane stress (N/mm^2) = $41884e^{-22.838 \text{ thickness (mm)}}$, R^2adj. = 0.74, P<0.001

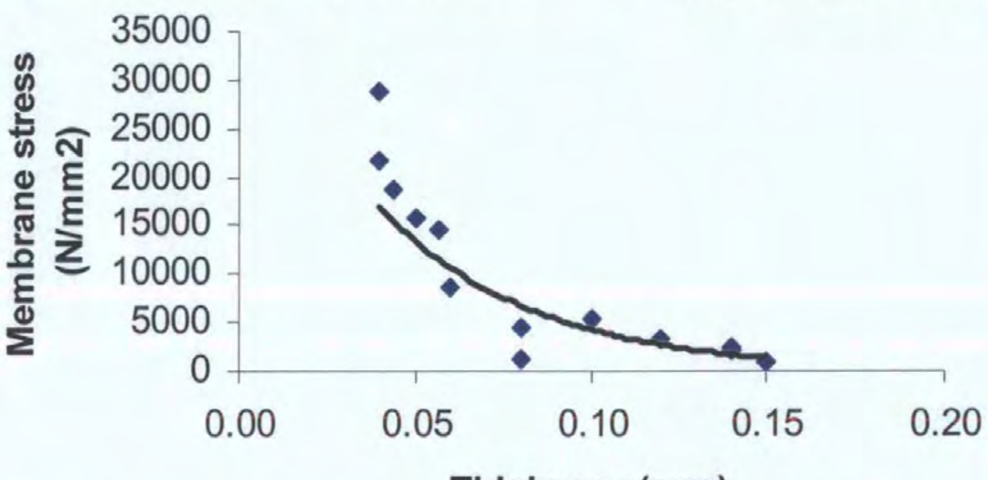


Figure 3.15 ***Effect of thickness of the sample on the membrane stress of the sole area of hoof horn, n = 18.***

Membrane stress (N/mm^2) = $18364e^{-16.256 thickness (mm)}$, R^2 adj = 0.87, P<0.001

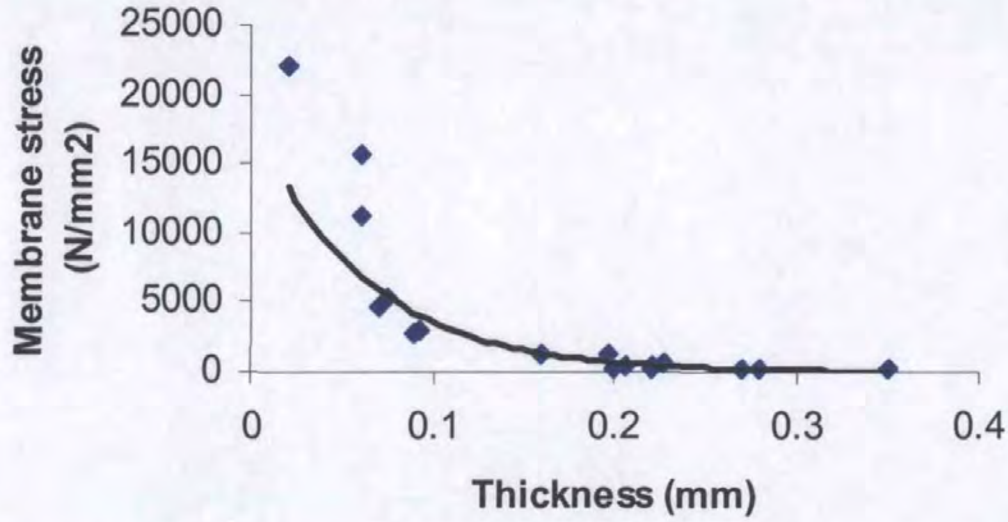


Figure 3.16 ***Effect of thickness of the sample on the membrane stress of the white line area of the hoof horn, n = 18.***

Differing sample thickness did not have a significant effect on the elastic modulus of the diaphragm of samples of the sole and white line areas.

3.4 Discussion

3.4.1 Effect of sample moisture content on the mechanical properties of the bovine hoof horn

Kitchener and Vincent (1987) found that the mechanical properties of keratinous materials are strongly influenced by their state of hydration. The moisture content of hoof horn has been found to affect the measurements of hardness, elastic modulus, bending stiffness and fracture toughness of the hoof horn (Bertram and Gosline, 1987; Douglas *et al.*, 1996; Collins *et al.*, 1998; Hinterhofer *et al.*, 1998; Baillie *et al.*; 2000, Dyer *et al.*, 2004). In this experiment the maximum punch force, work to fracture, membrane stress and elastic modulus measured through tension tests of the sole and white line areas of the hoof horn were significantly and strongly influenced by the moisture content of the hoof horn.

The increase in the elastic modulus of the diaphragm, membrane stress and elastic modulus measured through tension of the sole and white line horn were found to be exponential in relation to the dry matter content of the hoof horn. The punch resistance and work to fracture increased significantly and linearly with increasing dry matter content of the hoof horn samples. Douglas *et al.* (1996) found a significant ($P<0.05$) linear increase in the elastic modulus measured through tension ($R^2 = 0.781$) and compression ($R^2 = 0.771$) tests with decreasing moisture content of equine hoof outer wall horn, however, samples were only tested at physiological moisture levels and moisture content variation was 27.9 ± 1.7 %. At this range of moisture levels the relationship of elastic modulus and moisture content would have been probably linear too at the present experiment. Collins *et al.* (1998) measured the elastic modulus of donkey hoof wall through bending tests and the samples had a similar range of moisture contents (2, 18, 33 and 34 %) as hoof horn samples in the present experiment (8 to 36 %). Those authors did not fit a regression curve on their data, but the mean elastic modulus for samples with 34, 18 and 2 % moisture content was 145.3, 853.0 and 2167.6 N/mm^2, respectively. Kitchener and Vincent (1987) reported a mean bending stiffness of oryx horn with 0, 20 and 40 % moisture content of 6100 (s.e. 160), 4300 (s.e. 90) and 1800 N/mm^2 (s.e. 90), respectively. Baillie *et al.* (2000) measured the elastic modulus of bovine hoof wall through bending tests on samples that had a variation in the moisture content of 0 to 35 %, but again did not fit a regression curve on their data. However, the elastic modulus was under 1000 N/mm^2 when the moisture content varied between 8 and 36 %, between 1000 and 2000 N/mm^2 when moisture levels varied between

3 and 8 % and between 2000 and 8000 N/mm^2 on dry samples (0% moisture level). Bertram and Gosline (1987) tested the elastic modulus of the equine hoof wall horn of fully hydrated and dry samples, the elastic modulus being 410 N/mm^2 and 14,600 N/mm^2, respectively. The mean elastic modulus of the sole horn in the present experiment varied from 85.5 to 751.88 N/mm^2 when the moisture contents of the horn were of 29.2 and 9.7 %, respectively. Collins *et al.* (1998), Baillie *et al.* (2000) and Bertram and Gosline (1987) tested horn with 0% moisture content and therefore found higher values of elastic modulus when compared to the present experiment were the lowest mean moisture content was 9.7 %. Collins *et al.* (1998) and Bertram and Gosline (1987) found higher values of elastic modulus for fully hydrated samples (145.3 and 410 N/mm^2) when compared to the elastic modulus of hydrated samples in the present experiment (85.5 N/mm^2) and the differences may be related to the area tested. The wall horn that was tested by these authors had a greater elastic modulus compared with the horn of the sole area that was tested in the present experiment (Hinterhofer *et al.*, 1998). The elastic modulus of the bovine sole and white line areas of the hoof horn were not tested before on horn samples with the range of moisture levels tested in the present experiment. The punch force and work to fracture did increase linearly with the decrease in the moisture content and not exponentially as the elastic modulus and as the punch force was not tested before on hoof horn samples it was important to determine how they were affected by moisture levels of the horn.

Baillie *et al.* (2000) found that when the moisture content of the bovine hoof wall was higher than 10 % (dry matter lower than 90 %), there was a transition from a more brittle behaviour to a more ductile behaviour. The hoof horn became less stiff and tougher with increased hydration levels. The authors assumed that the physiological moisture content would be the optimum moisture level for the toughness of the hoof horn. A similar transition to a brittle behaviour was found in this experiment when the dry matter content was over 85 %. Kasapi and Gosline (1996) suggested that the hydration-dependence of the fracture toughness of the equine hoof horn was a manifestation of the viscoelastic nature of the material.

The dry matter content of the bovine toe, lateral wall, sole horn area and front and hind claws have been found to be 74.6, 73.4, 68.8, 73.1 and 71.3 %, respectively (Zoscher *et al.*, 2000). These differences in dry matter content were found to be significant and the areas that had the higher dry matter contents had higher values of modulus of elasticity; 613.5 (sd. 203.6), 375.3 (sd. 133.8), 134.9 (sd. 104.1), 405.5 (sd. 251.0) and 349.5 N/mm^2 (sd. 242.5) for toe, lateral wall, sole horn area and front and hind claws. Mulling *et al.* (1994) reported similar results, the bovine wall horn presenting higher hardness values (25.7 N/mm^2), measured through the ball impact method, compared to the sole horn (12.9 N/mm^2), heel horn (6.8 N/mm^2) and cap (6.9 N/mm^2) and terminal horn (5.1 N/mm^2) of the white line area. The moisture content has been found to affect the mechanical properties of the areas of the equine hoof (Douglas *et al.*, 1996; Hinterhofer *et al.*, 1998; Kasapi and Gosline, 1999). The mean DM content of bovine sole horn presented by Van Amstel *et al.* (2004) and Zoscher *et al.* (2000) of 67.9 and 68.8 %, respectively, is very similar to the mean dry matter for the sole horn of 70.9 (± 0.7) % obtained in this experiment. The mean elastic modulus of the sole area of the hoof horn with physiological moisture content was 116.9 N/mm^2 (sem. 39.5) in the present experiment and is similar to the elastic modulus of the sole horn area with physiological moisture content presented by Zoscher *et al.* (2000) of 134.9 N/mm^2 (sd. 104.1). In both experiments the elastic modulus was measured through tension tests.

Hinterhofer *et al.* (1998) reported that when wall and sole samples were conditioned at a relative humidity of 65 % (15.9 % and 16.2 % moisture content for wall and sole samples), the elastic modulus was 1802.3 ± 700 N/mm^2 and 1673.8 ± 557.8 N/mm^2, respectively and differences were not statistically significant. However, non conditioned wall and sole samples had a moisture content of 22.7 % and 31.5 % and elastic modulus of 735 ± 289.5 and 230 ± 92.4 N/mm^2, the differences being statistically significant. These authors and Douglas *et al.* (1996) pointed out that only samples tested at physiological moisture levels represent the *in vivo* situation.

Collins *et al.* (1998) reported no significant differences in the elastic modulus of fresh (187.4 N/mm^2 and 67% DM) and hydrated (145.3 N/mm^2) donkey wall horn samples. However, the dry matter content of the hydrated samples was not stated. In the present experiment the DM of the bovine sole horn decreased from 71 % to 65 % after 3 hours of soaking in distilled water and again no differences in the punch force of the sole (7.46 and 8.56 N) and white line areas (3.69 and 4.28 N) and elastic modulus of the sole area (116.9 and 221.6 N/mm^2) between fresh and hydrated samples was found. The dry matter did not change significantly from 3 hours up to 72 hours of soaking. Kitchener and Vincent (1987) reported that oryx horn samples had undergone complete saturation (could not absorb more water) when immersed in distilled water for 3 days, while Wagner and Hood (2002) reported a significant increase in the mass of sole, frog and wall horn after 24 hours immersion in distilled water. The mean water uptake of the wall, sole and frog samples was of 3.97, 4.09 and 35.20 %, respectively. Samples in the experiment of Kitchener and Vincent (1987) had greater dimensions (80 mm x 15 mm x 10 mm) when compared to sample dimensions of the tension test in the present experiment (20 mm x 2 mm x 0.05 mm). According to Zaun (1997) human nails can absorb up to 30 % moisture and the capacity to bind with the water was related to chemical components of the keratin. The movement of water in the stratum corneum is considered to be transcellular rather than extracellular, the intracellular compartment being hydrophilic embedded in a hydrophobic extracellular matrix (Wagner and Hood, 2002).

Collins *et al.* (1998) reported that the dry matter of donkey wall horn ranged from 65 to 75 % and that drying methods had a significant effect on the results. Drying at 100^{0}C for 24 hrs resulted in an intermediate dry matter of 68 %. The level of moisture extraction increased significantly at higher temperatures (105 to 120^{0}C) and the authors concluded that the volatilization of other substances was occurring. These authors also obtained intermediate dry matters by freeze drying and drying over P_2O_5 and found that when drying at room temperature for several days, vacuum drying and drying at 90^{0}C not all moisture was extracted, resulting in significantly higher false dry matter values. Oven drying was used by Van Amstel *et al.* (2004); Douglas *et al.* (1996); Schnid (1995); Hinterhofer *et al.* (1998) and Kitchener and Vincent (1987); however, different temperatures and times were used, 43.5^{0}C for 50 hours, 103.5^{0}C, 105^{0}C for 48 hours, 110^{0}C for 50 hours and 130^{0}C for 24 hours, respectively. There were several authors that did not state the method used to dry the sample and measure the moisture content of the horn (Baillie *et al.*, 2000; Frohnes and Budras, 2001; Wagner *et al.*, 2001) or did not measure the moisture content (Webb *et al.*, 1984; Bertram and Gosline, 1987; Dyer *et al.*, 2004). It can be clearly seen that differing drying methods affect the measured dry matter content and consequently the mechanical properties of the hoof horn. Satisfactory results can be obtained with oven drying at 100^{o}C the method used in the present experiment.

The moisture content of the hoof horn has been found to be affected by the micro-architecture and biochemical composition of the horn (Vermunt and Greenough, 1995: Hendry *et al.*, 1997) and by external factors (Budras and Mulling, 1998; Kempson *et al.*, 1998). The external factors that were found to affect the structure of the hoof horn were humidity, chemicals and microbiological factors (Budras and Mulling, 1998) and were often referred to as environmental factors. Environmental factors, such as the level of urine and manure, have been found to be related to the housing hygiene and reduce the quality of healthy horn. A good management of these factors contribute effectively to better hoof horn quality.

The hoof horn of cows maintained on concrete floors and standing in slurry have been found to have significantly higher moisture content (Vermunt and Greenough, 1995; Wells *et al.*, 1995). Offer *et al.* (2001) found a softer horn of the heel and higher incidence of heel horn erosion in heifers and Wells *et al.* (1995) found a higher incidence of lameness in dairy cows kept in similar conditions. Budras and Mulling (1998) and Kempson *et al.* (1998) found that slurry reduced the level of intercellular cementing substance and increased the permeability of the intertubular horn of the sole and the heel in morbid samples. Similarly, Kung *et al.* (1991) reported that the incubation of equine hoof horn in slurry and urine reduced the tensile strength of hoof horn.

3.4.2 Preparation of samples – time from sample collection to sample analysis

According to Wagner *et al.* (2001) both the period of time between sample collection and analysis and the method of tissue storage played a major role in the hydration status and the subsequent determination of the elastic modulus.

In the short term, moisture loss is the factor that is likely to have the greatest effect on the mechanical properties of hoof horn samples. To prevent moisture loss Collins *et al.* (1998) wrapped the samples in 3 layers of Parafilm and stored the samples at 4^0C, Hinterhofer *et al.* (1998) and Kasapi and Gosline (1999) kept the samples in resealable plastic bags at 4^0C until analysis. However, different periods of time between sample collection and analysis were used in the literature. Wagner *et al.* (2001) tested hoof horn samples 4 hours after collection and Van Amstel *et al.* (2004) 48 hours after collection. However, the majority of authors did not state the period of time from sample collection to analysis (Hinterhofer *et al.*, 1998; Douglas *et al.*, 1996). In this study, keeping the samples in plastic bags and at 4^0C for up to 8 days did not result in significant changes of the moisture content of the samples, the dry matter being 72.54 % at day 0 and 71.11 % at day 8, demonstrating that this method of sample storage prevented sample dehydration. As a consequence no significant changes of the punch force and elastic modulus of the sole and white line areas of the hoof horn between the day the samples were collected and 8 days of storage were found. The punch force of the sole area was 8.78 N on day zero and 9.61 N on day 8 and of the white line area was 4.95 N on day zero and 6.90 N on day 8. The elastic modulus of the sole area was 158.2 N/mm^2 on day zero and 175.5 N/mm^2 after 8 days of storage and of the white line area 91.8 N/mm^2 on day zero and 194.1 N/mm^2 after 8 days. Differing periods between sample collection and analysis for up to 8 days would be unlikely to affect the results of mechanical properties tests.

3.4.3 Freezing of hoof horn samples as a method of storage

In this experiment freezing significantly affected the elastic modulus of the sole horn samples when samples were frozen for 30 days (144.4 N/mm^2 on the day of sample collection and 403.1 N/mm^2 after 30 days frozen) (P<0.001). While the punch resistance of the sole (8.34 N not frozen and 9.10 N frozen for 30 days) and white line areas (6.21 N not frozen and 7.10 N frozen for 30 days) were not significantly affected by freezing and there was no significant increase in the dry matter content (72.34 % not frozen and 74.22 % frozen for 30 days) of hoof horn samples when non frozen samples were compared with samples that were frozen for 30 days. Kasapi and Gosline (1999) did not find a significant effect of freezing, dehydration and rehydration of equine hoof wall samples on the bending modulus of the samples. In their experiment samples were frozen at -10° C for 10 min and similarly, in the present experiment, freezing for shorter periods of 1 and 7 days had no significant effect on punch force, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus measured through tension tests of the sole and white line areas. Thus short term freezing was found not to affect the mechanical properties of hoof horn samples. However, long term freezing, i.e. 30 days, was found to significantly increase the elastic modulus of the horn but had no effect on punch resistance.

3.4.4 Thickness of hoof horn samples

The British Standard (BS) 2782: Part 3: Method 340A that determines the shear strength of moulding material uses a disc as test piece with the dimensions of 25.3 ± 0.1 mm diameter and 1.6 ± 0.1 mm thickness. A variation of 6 % of the sample thickness is allowed. The thickness has been used in the calculation of the shear strength and the thickness was also used in the calculation of the deflection of circular plates. Aranwela *et al.* (1999) when measuring the fracture properties of leaves through a shear punch test did not measure the thickness of the leaves and thickness was not considered when comparing the results. However, Dobes and Milicka (2001), Husain *et al.* (2002) and Kurtz *et al.* (2002) used specimens with standardized thickness for small punch and small shear punch tests. While, Nomoto *et al.* (2001) did not record any significant difference on shear punch strength when testing polycarboxylate cement with a specimen thickness of 0.5 and 1.0 mm in a shear punch test. In the present experiment the thickness of the hoof horn sample was found to affect significantly (P<0.001) the punch force of the sole and white line areas of the horn samples. The thickness of samples varied from 0.05 to 0.2 mm and this variation in thickness contributed to a variation between 0.39 and 2.33 (proportion) of the punch force results of the white line area of the horn and to a variation between 0.25 and 1.03 (proportion) of the variation of the punch resistance results of the sole horn samples. These results indicated that there was a need to control the thickness of the hoof horn samples and to measure the thickness of tested area when testing the punch resistance of the sole and white line horn. This measurement of thickness should be included in the analysis of punch resistance tests from hoof horn samples and the thickness of the sample should be included as a covariant in that analysis. The other option would also be to reduce the variation in the thickness of the hoof horn samples but this has proven to be difficult mainly when collecting the samples from live animals.

3.5 Conclusions

The moisture of the samples was found to be a factor that had a great influence over the mechanical properties of hoof horn tissue. Thus when testing the mechanical properties of hoof horn tissue at physiological moisture levels it is important to prevent the dehydration of the samples and control and measurement of sample moisture should always be included in a testing protocol. Keeping samples in sealed plastic bags in the refrigerator for up to one week or freezing samples in plastic bags for a short time prevented effectively the dehydration of the hoof horn tissue. The reduction in the variation of the sample thickness could be achieved by collecting samples with a plain and this enabled a degree control over the thickness of the samples which was important for reducing the intra test variation of results in the punch test. Some variation still occurred and thus the sample thickness should always be measured and included as a covariate in the analysis of punch force data.

Chapter 4

Experiment 3 - The effect of biotin supplementation on hoof characteristics in Holstein-Friesian dairy cows

4.1 Introduction

Biotin has been found to be a cofactor for enzymes used in a number of metabolic pathways, such as amino-acid metabolism, cellular respiration, gluconeogenesis and lipogenesis (Seymour, 1998; Tomlinson *et al,* 2004). The three biotin–dependent carboxylases that have been indentified are of clear importance in the *peripartum* period of dairy cattle when gluconeogenesis is at its maximum (Girard, 1998). Biotin is also required for the formation of adenine and guanine and the conversion of ornithine to citruline in the urea cycle, having an important role in cell growth and differentiation and protein synthesis (Seymour, 1998). Fritsche *et al.* (1991) observed a specific increase in cytokeratins in keratinocytes treated with biotin which would normally occur in the terminal differentiation of epidermal cells.

Biotin has been found to be essential for the synthesis of long chain fatty acids, which are part of the fatty acid composition of the intercellular cementing substance of the hoof horn (Mulling *et al.*, 1999; Mulling, 2000; Scaife, 2000). Meyer *et al.* (2002) reported a higher proportion of long chain fatty acids and Koster *et al.* (2002a) a difference in the composition of the intercellular cementing substance in the hoof horn of biotin supplemented cows. The intercellular cementing substance of the hoof horn is impermeable for aqueous substances, which regulates the hydration level of the hoof horn and plays a role in the regulation of the mechanical properties of the hoof horn (Mulling *et al.*, 1999).

Qualitative and quantitative changes in the synthesis of keratin filaments, alteration of the structure of the cellular envelope and increased production and intercellular cementing substance of poor quality, have been found to occur in the hoof horn of biotin deficient calves. The hoof horn of these calves had a brittle and crumbly consistency with decreased cell-to-cell adhesion and increased permeability of the epidermis (Hochstetter, 1998; Mulling *et al.*, 1999). Increased integrity of the intercellular substance due to greater cell adhesion and absence of microcracks in biotin supplemented when compared to non-supplemented cows was reported by Koster *et al.* (2002). These reported changes in the intercellular substance may have resulted in greater claw horn hardness that was measured by Brooks *et al.* (1997), Distl and Schmid (1994), Schmid (1995) and Campbell *et al.* (2000) in supplemented dairy cows.

In cows supplemented with biotin Distl and Schmid (1994) and Schmid (1995) found a lower incidence of sole ulcer and heel horn erosion, Campbell *et al.* (2000) a lower incidence of vertical fissure, Bergsten *et al.* (2002) a lower incidence of sole haemorrhages and Hedges *et al.* (2001), Midla *et al.* (1998) and Hoblet *et al.* (2002) a lower risk and incidence of white line separation.

The aim of the present experiment was to compare the mechanical properties of the hoof horn of biotin supplemented and non-supplemented dairy cows. The measurements of the mechanical properties were compared with the incidence of lesions in the sole and white line areas and with the lameness incidence.

4.2 Material and methods

4.2.1 Experimental design

The effect of biotin hoof horn quality and characteristics was measured using 18 cows that received supplemental biotin and 18 that were not supplemented with biotin. The experiment was designed as a randomised block, with 2 dietary treatments replicated 18 times (36 cows). The cows in each treatment were paired for previous lactation milk yield, parity, lameness history and the scoring for sole bruising.

4.2.2 Housing, husbandry and animals

The cows were housed in the same dairy unit described in Experiment 1. They were Holstein-Friesian dairy cows, with average milk yield of 8096 l per 305 days lactation (6326 – 11618 l per 305 days, sem = 155), being in the second to fifth lactation. They were kept in straw yards *prepartum*, introduced into a cubicle system *postpartum*. The cows calved between end of September and December 2001 and the experiment ended in May 2001.

4.2.3 Dietary Treatments

The cows were given the treatment diets at 25 (± 5 days) days *prepartum* up until 160 day *postpartum*. The cows were allocated to one of the 2 dietary treatments:

Biotin: 18 cows received a dry cow diet *prepartum* and a lactating cow diet *postpartum* with supplementation of 22 mg (± 2mg) biotin per head per day.

Non biotin supplemented (NBS): 18 cows received a dry cow diet *prepartum* and a lactating cow diet *postpartum* with the supplementation of 2 kg of the same compound feed but with no additional biotin.

The biotin (Rovimix H-2, Roche) was added to 2 kg of a dry cow supplement in the *prepartum* period and in 2 kg of a compound supplement which was fed out of parlour in the *postpartum* period.

4.2.3.1 Dry cow diet

During the *prepartum* period the cows received a diet of barley straw, grass silage and 2 kg of a dry cow supplement or supplement with biotin (11 mg/kg). The composition of the dry cow diet is presented in Table 4.1. The composition of the components of the diets analysed according to MAFF (1989) is presented in Table 4.3.

Table 4.1 Components of the dry cow diet (kg DM)

Components	**Quantity (kg DM/day)**
Barley straw	3.1
Grass silage	1.2
Dry cow supplement	2.0
Composition (/kg DM)	
Dry matter (%)	66.8
Metabolisable energy (MJ/kg)	8.95
Crude protein (g/kg)	65.2
NDF (g/kg)	508.1

4.2.3.2 Lactating cow diet

All the cows received the same diet consisting of a total mixed ration (TMR) on an *ad libitum* basis. This ration contained grass and maize silage (50:50 ratio), 2.5 kg of a 38 %

crude protein (CP) compound and 2 kg of rolled wheat and minerals. In addition, cows received a maximum of 8 kg of dairy compound feed (22 % CP) in the parlour offered twice daily. Non-biotin supplemented cows received 2 kg dairy compound feed from out of parlour feeders. Supplemented animals received 2 kg of the same compound added with 22 mg (± 2 mg) of biotin. The lactating cow diet, analysed according to MAFF (1989), is presented in Table 4.2 and the composition of the components of the diets is presented in Table 4.3

Table 4.2 ***Components of the lactating cow diet (kg DM/day)***

Components	Quantity
Feeds (kg DM)	
Grass silage	3.3
Maize silage	5.1
Protein compound (38 % CP)	2.2
Rolled wheat	1.7
Minerals (C_aCo_3)	0.08
Parlour dairy compound (22 % CP)	≤ 7.0
Composition (/kg DM)	
Dry matter (%)	41.1
Metabolisable energy (MJ/kg)	11.4
Crude protein (g/kg)	17.6
NDF (g/kg)	35.6
Starch (%)	20.5

Table 4.3 ***Nutrient value of feedstuffs included in the pre and postpartum diets (kg DM)***

	Grass silage	Maize silage	Barley straw	38% protein compound	Wheat	Dairy compound	Dry cow supplement
Dry matter (g/kg Fresh weight)	166	289	870	862	857	870	680
Ash (g/kg DM)	96.0	45.0	-	84.0	-	90.0	110
NDF (g/kg DM)	588	421	809	225	123	200	-
Starch (g/kg DM)	-	364	-	80	670	160	-
Water soluble carbohydrate (g/kg DM)	18.0	26.0	-	70	26	70	-
ME (MJ / kg DM)	10.6	11.1	6.5	12.3	13.7	12.9	12.1
Oil (g/kg DM)	22	-	-	45	21	50	-
Crude protein (g/kg DM)	98	68	43	436	127	252	90

4.2.3.3 Analysis of the lactating cow diet

The components of the lactating cow diet were collected weekly, bulked and analysed monthly for nutrient composition and dry matter content, according to MAFF (1989). A dry matter analysis of the TMR was completed weekly by toluene distillation, according to the method detailed in MAFF (1989).

4.2.4 Measurements

4.2.4.1 Locomotion score

All cows in the experiment were locomotion scored twice weekly during the *postpartum* period. The scoring system used had 5 points, being designed by Tranter and Morris (1991) and was described in Experiment 1.

4.2.4.2 The scoring of hoof lesions

The cows had all of their feet assessed for sole ulcer, sole haemorrhage and heel erosion, before calving (day 0 – prior to supplementation) and at 40, 100 and 150 days *postpartum*. The lesions on each claw were scored according to Leach *et al.* (1998). The soles of the cows feet were photographed with a digital camera and the images were analysed on an image analysis program (Scion Corporation) (this methodology was described in Experiment 1). Considering the growth rate of the coronary horn observed in Experiment 1 during the housing period of 6.9 to 8.4 mm per month, the possible changes in the scoring of lesions and mechanical properties of the sole horn after supplementation with biotin would be observed at the measuring period of 100 and 150 days *postpartum*.

4.2.4.3 Growth rate and hoof measurements

The growth and wear rates were measured on the right front and rear hooves at 40, 10O and 150 days *postpartum*. The hoof angle, dorsal length, lateral length and height of the heel were measured at the same time that the hoof growth and wear were measured (this procedure was described in Experiment 1).

4.2.4.4 The testing of mechanical properties of the hoof horn

Samples of sole and white line tissue were collected from all claws of all experimental cows at 40, 100 and 150 days *postpartum*. To achieve this the first outer layer of horn (1 mm) was discarded and a horn sample with 0.05 to 0.2 mm thickness was taken with a plane (Experiment 2) for the measurement of its mechanical properties.

The hoof samples were taken from zones 2, 4 and 5 of the sole according to the IFM (see Figure 1.4) (Shearer *et al.*, 2002). These hoof samples were kept in sealed plastic bags to avoid the loss of moisture and stored in a refrigerator at a temperature of 2 °C until analysis (Hinterhofer *et al*, 1998; Douglas *et al*, 1996) and were analysed for punch resistance and elastic modulus at physiological moisture levels according to Hinterhofer *et al.* (1998) and Douglas *et al.* (1996). The punch resistance test used was described in Experiment 1, while the equation for large deflection of circular plates and the calculation of elastic modulus of the diaphragm and membrane stress and the tension test used for the measurement of the elastic modulus were described in Experiment 2.

4.2.4.5 Dry matter content of the hoof horn

Following the completion of the mechanical tests, the hoof horn samples were weighed, placed in an oven (100°C) for 72 hrs and reweighed to determine their dry matter content.

4.2.4.6 Body condition score

The body condition score of the animals was estimated every other week, starting at the first week *postpartum* using a 1 to 5 scale with half points, according to Lowman *et al.* (1976).

4.2.4.7 Milk biotin concentration

Milk biotin concentration was measured from 8 cows on each treatment (Biotin, NBS) at day 120 of the experiment. Milk samples were collected from the afternoon and the morning milking and sent frozen in dry ice to Roche Vitamins Ltd., Basel, Switzerland for analysis. The biotin concentration was determined by microbiological assay using *Lactobacillus plantarum* ATCC 8014 (AOAC, 1996) (VFHA, Roche Vitamins Ltd - Internal analytical method: Microbiological assay of biotin in milk, version 1.0).

4.2.5 Statistical analysis

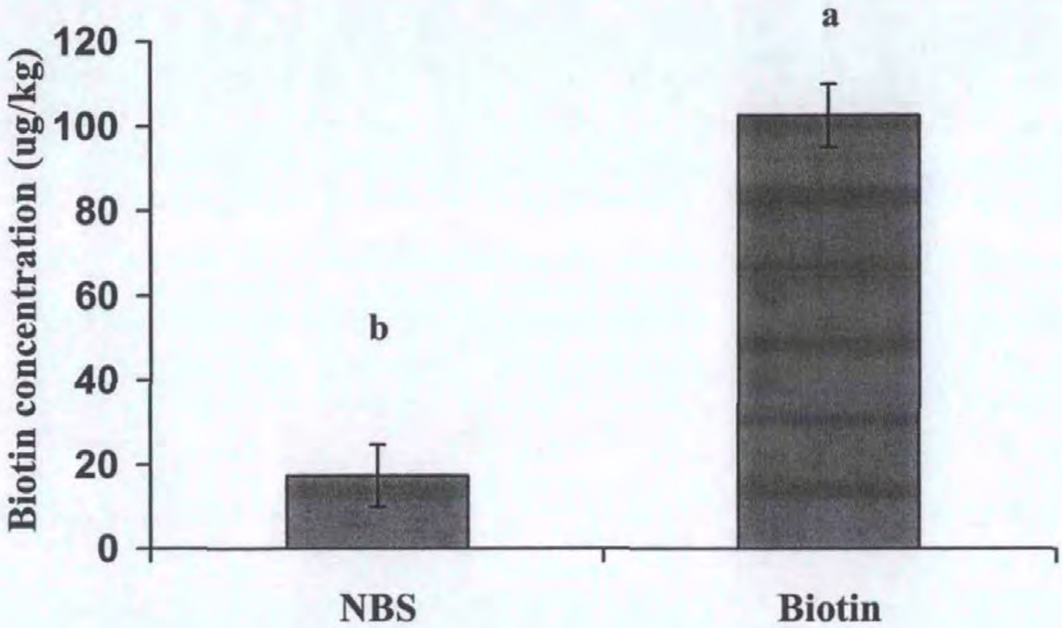
The experiment was a randomised block design, using individual cows as observations. The condition score, locomotion score, measurements of the claw, dry matter of the hoof horn, elastic modulus, punch force, work to fracture, elastic modulus of the diaphragm and membrane stress were found to be normally distributed. The lesion score data were log 10 transformed before analysis. The condition score, locomotion score, lesion score, measurements of the claw, dry matter of the hoof horn and elastic modulus, were compared by treatment, using analysis of variance (ANOVA), general linear modelling command (Minitab 12.0). ANOVA was used also to compare differences of lesion score, locomotion score, punch force and elastic modulus between collection periods and between claws. Hoof horn sample thickness was used as a covariate when punch force, work to fracture, elastic modulus of the diaphragm and membrane stress data were analysed. ANCOVA, general linear modelling command (Minitab 12.0) was used to compare these parameters by treatment and to compare differences between collection periods and between claws. The comparison of means was completed using the Tukeys test. Multiple regression analysis was used to test for the predictors that had a significant influence over the total lesion score, the maximum punch force, the elastic modulus of the diaphragm, membrane stress and elastic modulus measured through tension of the sole and white line horn. Regression analysis was completed to test the effect of days in supplementation on lesion score of the sole and white line horn and of sample thickness on punch force. Correlation analysis was completed using Pearson's correlation.

4.3 RESULTS

4.3.1 Effect of biotin supplementation on locomotion score, lesion score of the sole and white line areas, claw measurements, punch force, work to fracture, elastic modulus of the diaphragm, membrane stress, elastic modulus measured through tension test, dry matter content of the hoof horn, body condition score and milk biotin concentration

4.3.1.1 Milk biotin concentration

Using the microbiological assay method with *Lactobacillus plantarum* ATCC 8014 (AOAC, 1996) the cows supplemented with 20 mg biotin had a significantly ($P<0.001$) higher milk biotin concentration (102.6 µg/kg ± 7.7) at day 120 *postpartum* compared with non biotin supplemented cows (17.3 µg/kg ± 7.2) (Figure 4.1).



a, b – different letters indicate mean values that differ significantly

Figure 4.1 ***Concentration of biotin in milk of biotin supplemented and non supplemented (NBS) cows***

4.3.1.2 Locomotion score of biotin supplemented and non-supplemented cows

There were no significant differences in locomotion score on day 30, 60, 90, 120 and 150 *postpartum* between biotin supplemented and non biotin supplemented cows (Table 4.5).

Table 4.5 ***Locomotion score at days 30, 60, 90, 120 and 150 postpartum of cows supplemented and not supplemented with biotin (NBS)***

Locomotion score	Biotin	NBS	sem	*P*
Day 30	1.58	1.57	0.17	NS
Day 60	1.64	1.95	0.18	NS
Day 90	1.65	1.96	0.19	NS
Day 120	1.97	2.34	0.24	NS
Day 150	2.34	2.50	0.26	NS

NS – not significantly different

4.3.1.3 Lesion score of the sole and white line areas of the claw horn of biotin supplemented and non-supplemented cows

The total lesion score of the sole and white line areas at days 50, 100 and 160 *postpartum* did not differ significantly between biotin supplemented and non-supplemented cows (Table 4.6). At 160 days *postpartum* biotin supplemented cows did have a significantly (P<0.05) lower lesion score of the sole area of the hind left medial claw. The lesion score of the sole and white line areas of all claws of biotin supplemented and non-supplemented cows at 160 days *postpartum* are presented in Table 4.7.

Table 4.6 ***Mean total lesion score of the sole and white line (wl) areas of the claw horn at days 50, 100 and 160 postpartum (pp) of cows supplemented (biotin) and not supplemented with biotin (NBS)***

Area and measurement period	Biotin	NBS	sem	*P*
Sole				
day 50 pp	653.9	837.0	115.5	NS
day 100 pp	1209.2	1380.5	116.8	NS
day 160 pp	1615.9	1739.1	101.2	NS
wl				
day 50 pp	1054.0	1031.0	116.7	NS
day 100 pp	1593.5	1303.0	137.3	NS
day 160 pp	1672.8	1751.5	112.7	NS

NS – not significantly different

Table 4.7 ***Mean lesion score of the sole and white line (wl) areas of front and hind, left and right, inner and outer claws of biotin supplemented (biotin) and non-supplemented (NBS) cows at 160 days postpartum***

Areas and claws	Biotin	NBS	sem	*P*
Sole area				
Front left inner	169.6	165.8	13.50	NS
Front left outer	160.2	165.1	12.62	NS
Front right inner	148.4	164.4	13.15	NS
Front right outer	146.6	152.5	15.45	NS
Hind left inner	155.6	199.3	17.7	0.05
Hind left outer	336.4	336.5	23.19	NS
Hind right inner	172.2	196.9	19.22	NS
Hind right outer	328.8	352.2	25.62	NS
wl area				
Front left inner	194.2	209.7	19.82	NS
Front left outer	162.6	177.4	21.67	NS
Front right inner	181.9	174.7	18.20	NS
Front right outer	165.7	171.4	19.40	NS
Hind left inner	217.4	241.7	19.98	NS
Hind left outer	254.0	274.0	19.57	NS
Hind right inner	233.9	236.3	19.18	NS
Hind right outer	278.6	284.1	21.92	NS

NS – not significantly different

4.3.1.4 Growth rate, wear rate, claw angle, dorsal length, lateral length and heel height of biotin supplemented and non-supplemented cows

There were no significant differences between the growth rate, wear rate, claw angle, dorsal length, lateral length and heel height between cows supplemented and non-supplemented with biotin when measured at 100 and 160 days *postpartum* (Table 4.8). Only the result for the 160 days *postpartum* measurement point was presented because at this time the biotin supplementation was more likely to have affected the measurements, considering the time the hoof horn takes to reach the weight bearing surface after formation.

4.3.1.5 Punch resistance, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line horn of biotin supplemented and non supplemented cows

There were no significant differences in punch resistance, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole area of the hoof horn between biotin supplemented and non supplemented cows (Table 4.9).

Table 4.8 ***Mean dorsal length, lateral height, heel height, hoof angle, hoof growth and wear of front and hind lateral claws of biotin supplemented (biotin) and non-supplemented (NBS) cows at 160 days postpartum***

Measurements	Biotin	NBS	sem	*P*
Front claw				
Dorsal length (cm)	7.78	7.96	0.129	NS
Lateral height front (cm)	7.84	8.13	0.156	NS
Heel height front (cm)	5.91	5.73	0.194	NS
Angle front (cm)	51.25	50.43	1.019	NS
Growth front (cm/month)	0.361	0.369	0.029	NS
Wear front (cm/month)	0.273	0.237	0.043	NS
Hind claw				
Dorsal length (cm)	7.95	8.20	0.321	NS
Lateral height hind (cm)	7.51	8.01	0.183	NS
Heel height hind (cm)	4.43	4.72	0.200	NS
Angle hind (cm)	52.63	51.61	0.704	NS
Growth hind (cm/month)	0.491	0.507	0.026	NS
Wear hind (cm/month)	0.384	0.307	0.064	NS

NS – not significantly different

Table 4.9 ***Punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the for the sole area of the hoof horn at days 50, 100 and 160 postpartum (pp) of biotin supplemented (biotin) and non supplemented (NBS) cows***

Measurements	Biotin	NBS	sem	*P*
Punch force (N)				
day 50 pp	8.85	9.06	0.332	NS
day 100 pp	8.71	8.66	0.356	NS
day 160 pp	8.12	8.16	0.214	NS
Work to fracture (kJ)				
day 50 pp	10.12	10.02	0.307	NS
day 100 pp	11.27	11.28	0.387	NS
day 160 pp	10.42	10.53	0.232	NS
Elastic modulus diaphragm (N/mm^2)				
day 50 pp	53.70	43.50	14.95	NS
day 100 pp	16.83	18.53	3.42	NS
day 160 pp	18.15	18.16	1.65	NS
Membrane stress (N/mm^2)				
day 50 pp	19992.1	11512.1	7320.6	NS
day 100 pp	2156.1	3672.1	1352.5	NS
day 160 pp	2762.9	3038.9	630.2	NS

NS – not significantly different

There were no significant differences in punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the white line area of the hoof horn of biotin supplemented compared with non supplemented cows at days 50, 100 and 160 *postpartum* (Table 4.10).

Table 4.10 ***Punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the for the white line horn at days 50, 100 and 160 postpartum of biotin supplemented (biotin) and non supplemented (NBS) cows***

Measurements	Biotin	NBS	sem	*P*
Punch force (N)				
day 50 pp	7.15	6.99	0.639	NS
day 100 pp	6.19	6.42	0.467	NS
day 160 pp	5.25	5.80	0.293	NS
Work to fracture (kJ)				
day 50 pp	7.74	7.41	0.629	NS
day 100 pp	7.16	7.54	0.576	NS
day 160 pp	5.94	6.44	0.379	NS
Elastic modulus diaphragm (N/mm^2)				
day 50 pp	32.11	32.27	6.96	NS
day 100 pp	18.61	17.12	3.55	NS
day 160 pp	19.85	19.63	1.27	NS
Membrane stress (N/mm^2)				
day 50 pp	5880.7	4395.5	1731.9	NS
day 100 pp	2664.6	1350.9	1125.1	NS
day 160 pp	1692.0	1347.2	238.6	NS

NS – not significantly different

4.3.1.6 Elastic modulus of the sole horn of front and hind claws of biotin supplemented and non supplemented cows

There were no significant differences between the elastic modulus of the tension test of the sole area of the hoof horn of front and hind claws of biotin supplemented compared with non supplemented cows at 100 and 160 days *postpartum* (Table 4.11).

Table 4.11 ***Elastic modulus of the tension test of the sole horn of front and hind claws at 100 and 160 days postpartum (pp) and dry matter at days 50 and 100 postpartum of biotin supplemented (biotin) and non supplemented (NBS) cows***

Measurements	Biotin	NBS	sem	*P*
Dry matter (%)				
day 50 pp	72.84	74.10	0.635	NS
day 100 pp	73.97	73.75	0.515	NS
Elastic modulus (N/mm^2)				
front claw, day 100 pp	69.33	58.29	12.10	NS
hind claw, day 100 pp	61.98	87.56	14.66	NS
front claw, day 160 pp	130.30	103.20	23.5	NS
hind claw, day 160 pp	106.30	112.00	25.8	NS

NS – not significantly different

4.3.1.7 Dry matter of the hoof horn of biotin supplemented cows and non supplemented cows

There were no significant differences in the dry matter of the sole horn of biotin supplemented compared with non supplemented cows at days 50 and 100 *postpartum*. (Table 4.11).

4.3.1.8 Body condition score of biotin supplemented and non-supplemented cows

The body condition score and condition score change did not differ significantly between biotin supplemented compared with non supplemented cows. The condition score and difference in condition score of biotin supplemented and non supplemented cows at different days in the *postpartum* period are presented in Table 4.12.

Table 4.12 ***Body condition score of biotin supplemented (biotin) and non biotin supplemented (NBS) cows at days 12, 52, 97, 152 and 194 of the postpartum period and change in the condition score between days 150 and 100, 150 and 50 and 100 and 50 of the postpartum period***

Days	Biotin	NBS	sem	*P*
12 pp	2.47	2.77	0.235	NS
Day 52 pp	2.58	3.03	0.166	NS
Day 97 pp	2.62	3.13	0.184	NS
Day 152 pp	2.69	3.27	0.203	NS
Day 194 pp	2.75	3.38	0.254	NS
Change 50-100 dpp	0.143	0.300	0.0737	NS
Change 50-150 dpp	0.212	0.375	0.1070	NS
Change 100-150 dpp	0.077	0.125	0.0621	NS

NS – not significantly different

4.3.2 Effect of days postpartum and claws locomotion score, lesion score of the sole and white line areas, claw measurements, punch force, work to fracture, elastic modulus of the diaphragm, membrane stress, elastic modulus measured through tension test, dry matter content of the hoof horn and body condition score

In this section the data of biotin supplemented and non supplemented cows were combined for the analysis of the effect of days *postpartum* and claws on locomotion and lesion score, claw measurements and mechanical tests, because except for the lesion score of the sole area of the hind left inner claw there was no significant difference on other parameters between the groups. The lesion score for the sole area of hind claws was analysed also separately for biotin supplemented and non supplemented cows.

4.3.2.1 Locomotion score in the postpartum period

The locomotion score for all animals increased significantly ($P<0.001$) and linearly during the *postpartum* period. The regression line for locomotion score and days *postpartum* is presented in Figure 4.2.

The locomotion score did not vary significantly between the months of the experiment (November 2000 to April 2001) and the mean monthly locomotion scores are presented in Figure 4.3.

Locomotion score = 0.0063 Days + 1.4604, R^2adj.= 0.84, $P<0.001$

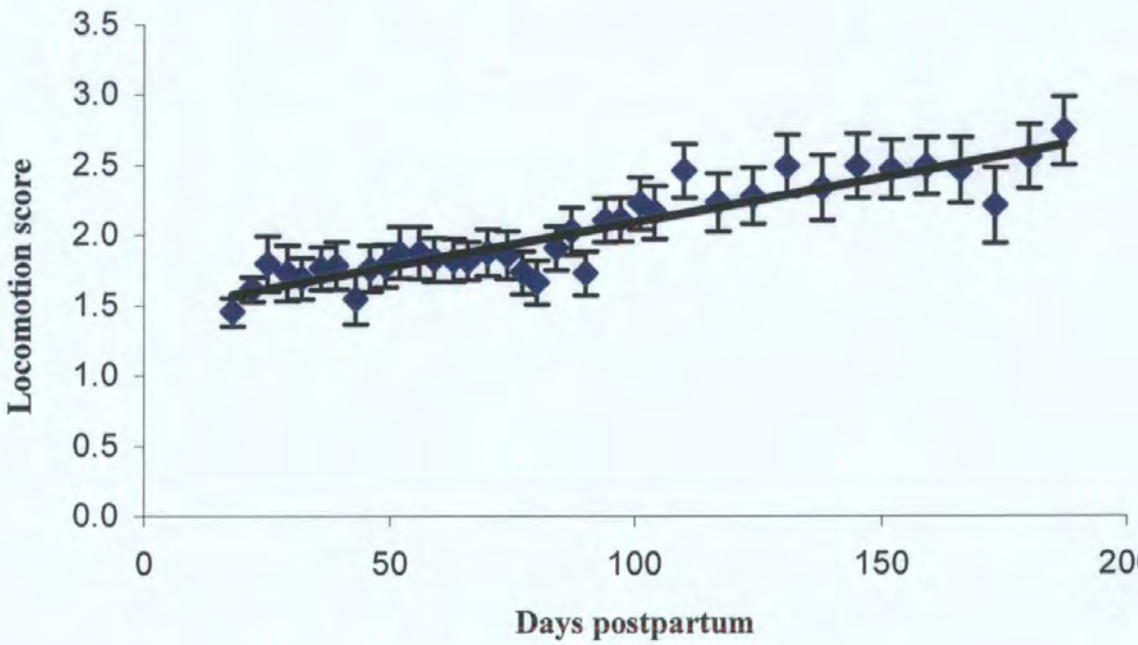


Figure 4.2 ***Locomotion score over the postpartum period***

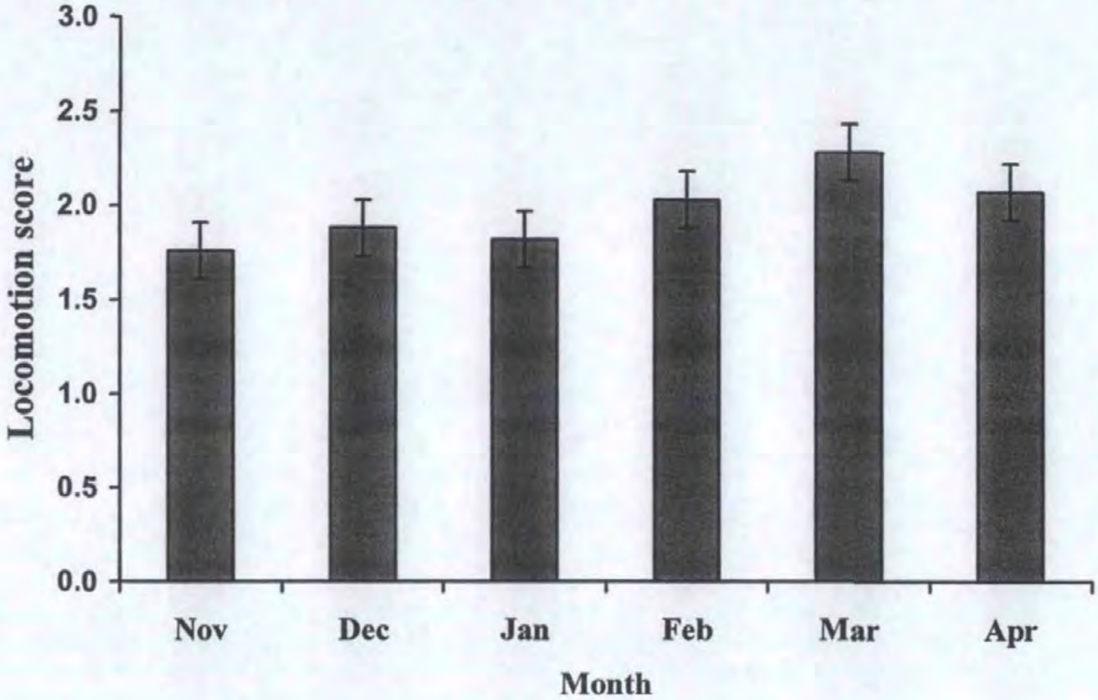


Figure 4.3 ***Effect of calendar months on the mean locomotion score***

4.3.2.2 Lesion score of the sole and white line areas of the claw horn during the postpartum period

Using multiple regression analysis to assess the factors that had a significant effect on the total lesion score of the sole horn, the number of days *postpartum*, parity and cow were found to be significant factors (*P* <0.001, <0.01 and <0.05) and diet and lesion score measured *prepartum* were found to be not significant. The regression equation for total lesion score of the sole horn was:

Total lesion score of the sole horn = 215 + 104 lactation No. - 0.411 cow + 7.19 days *postpartum*, R^2adj. = 0.54, *P* <0.001

Using multiple regression analysis to assess the factors that had a significant effect on the total lesion score of the white line horn, the number of days *postpartum* and parity were found to be significant factors (P <0.001, <0.05). Diet and *prepartum* lesion score were found not to have a significant effect. The multiple regression equation for the lesion score of the white line horn was:

Total lesion score of the white line horn = 692 + 4.69 days *postpartum* + 58.8 lactation No., R^2adj. = 0.26, P <0.001

The total lesion score of the sole and white line horns were found to be significantly positively and linearly related to the number of days the animals were in lactation (P<0.001) (Figure 4.4 and 4.5, respectively).

Total lesion score of the sole horn = 491.872 + 7.07852 days postpartum, R^2adj. = 0.50, P <0.001

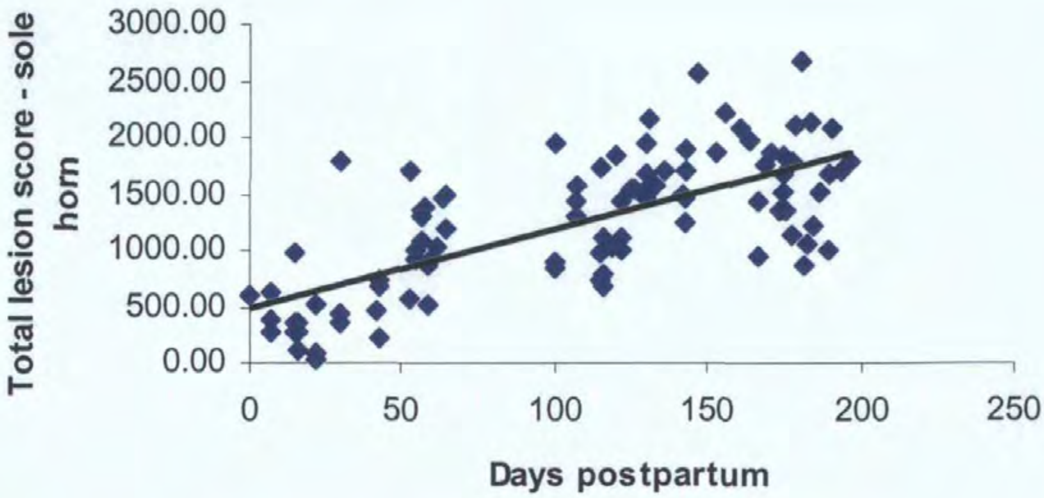


Figure 4.4 ***Total lesion score of the sole horn and days postpartum***

Total lesion score of the white line horn = 910.283 + 4.64033 days postpartum, R^2adj. = 0.25, P <0.001

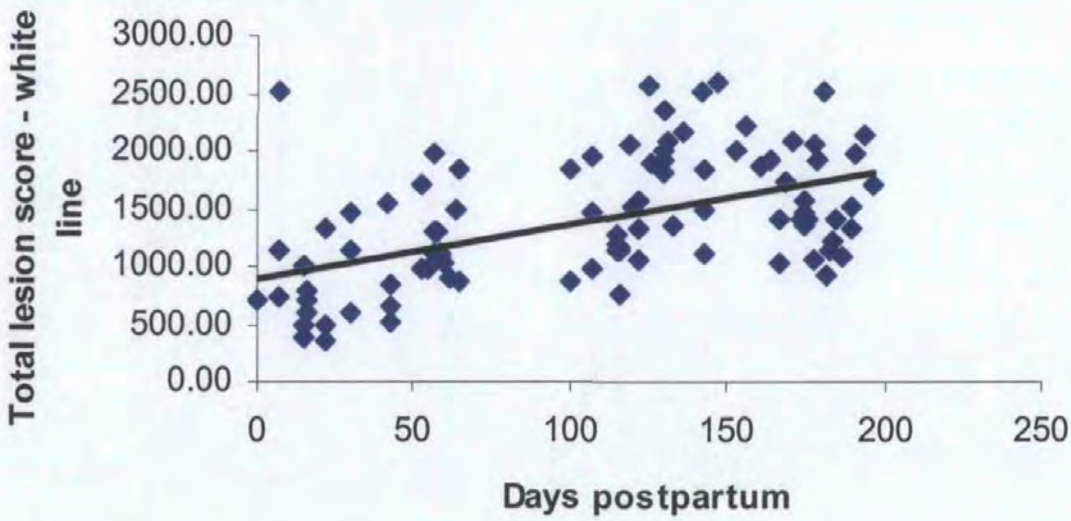
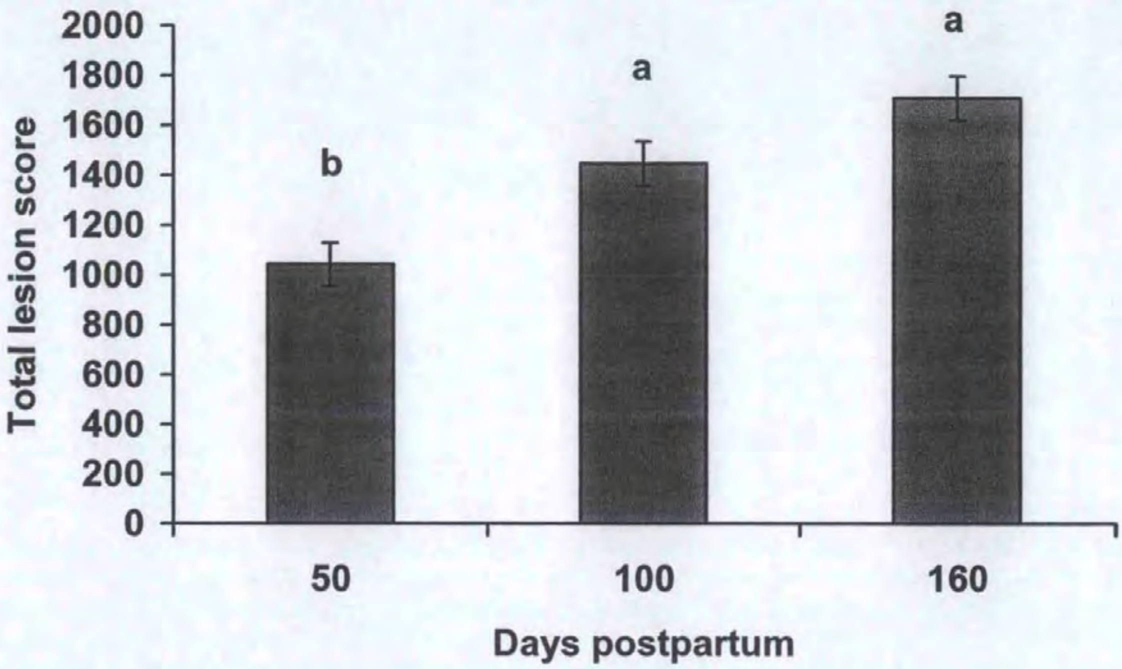


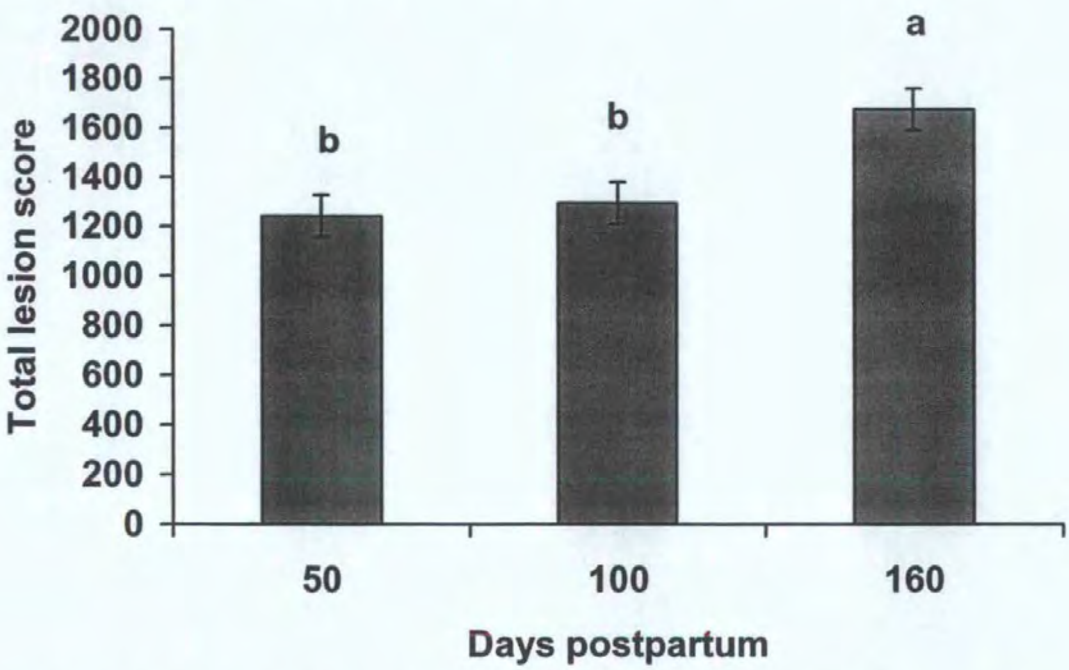
Figure 4.5 ***Total lesion score of the white line horn and days postpartum***

A comparison of the collection periods using ANOVA showed that the mean total lesion score of the white line and sole areas of all claws and cows increased significantly (P<0.001) during the *postpartum* period. The mean total lesion score of the white line and sole areas are presented in Figure 4.6 and Figure 4.7, respectively. The mean total lesion score of the white line area of all claws increased significantly between 50 and 100 days *postpartum*. The lesion score of the sole area increased significantly between 100 and 160 days *postpartum*.



a, b – different letters indicate mean values that differ significantly

Figure 4.6 ***Mean total lesion score of the white line area of the claw horn of all claws at 50, 100 and 150 days postpartum***



a, b – different letters indicate mean values that differ significantly

Figure 4.7 ***Mean total lesion score of the sole area of all claws of all cows at 50, 100 and 150 days postpartum***

4.3.2.3 Lesion score of the sole and white line areas of front and hind, left and right and inner and outer claws of all cows

The lesion scores of sole and white line areas of all claws in the *postpartum* period are presented in Table 4.13. The lesion score of the sole area of hind left and right outer claws were significantly greater at 50, 100 and 160 days *postpartum* when compared with the lesion score of the hind left and right inner and all front claws (P<0.001). The lesion scores of the white line area at days 50, 100 and 160 *postpartum* were significantly greater for the hind claws when compared to the front claws (P<0.001).

4.3.2.4 Lesion score of the sole area of hind claws of biotin supplemented and not supplemented cows during the postpartum period

The lesion scores of sole area of hind claws of biotin supplemented and non-biotin supplemented cows in the *postpartum* period are presented in Table 4.14. The lesion scores of the hind claws increased significantly (P<0.05 to 0.001) in the *postpartum* period. The non supplemented cows had a significant (P<0.01) increase in the lesion score of the hind right medial and hind left lateral claws from day 50 *postpartum* and in the biotin supplemented cows this increase occurred later form day 100 *postpartum*. The lesion scores of the hind lateral claws increased significantly (P<0.01) for all cows after day 100 *postpartum* and the lesion score of the left medial claws increased significantly (P<0.001) for all cows after day 50 *postpartum*.

Table 4.13 ***Mean lesion score of sole and white line areas of front and hind, left and right, inner and outer claws at 50, 100 and 160 days postpartum (pp)***

	Front				Hind					
	Left		Right		Left		Right			
Area and days (pp)	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	sem	*P*
Sole										
50 pp	50.0 [b]	55.9 [b]	52.8 [b]	58.0 [b]	169.3 [a]	94.6 [b]	176.0 [a]	95.5 [b]	13.8	0.001
100 pp	114.2 [c]	128.1 [c]	118.6 [c]	130.3 [c]	262.3 [a]	168.2 [bc]	224.5 [ab]	148.8 [c]	15.2	0.001
160 pp	162.6 [b]	167.7 [b]	149.4 [b]	156.2 [b]	336.4 [a]	176.9 [b]	340.2 [a]	184.2 [b]	12.8	0.001
White line										
50 pp	94.4 [c]	92.5 [c]	96.1 [c]	102.4 [bc]	169.9 [a]	161.6 [ab]	162.7 [ab]	152.5 [abc]	14.9	0.001
100 pp	135.6 [c]	155.3 [bc]	132.3 [c]	159.8 [bc]	214.0 [ab]	236.2 [a]	213.6 [ab]	218.2 [ab]	17.4	0.001
160 pp	169.6 [bc]	201.5 [bc]	168.4 [c]	178.5 [bc]	263.5 [a]	228.9 [ab]	281.2 [a]	235.0 [ab]	14.0	0.001

[a, b, c] – different letters in the same row indicate values that differ significantly

Table 4.14 ***Lesion scores of the sole area of hind claws of biotin supplemented and not supplemented cows at days 50, 100 and 160 postpartum***

	Days				
	50	100	160	sem	*P*
Not supplemented					
Hind left medial claw	113.2^{b}	169.8^{a}	199.3^{a}	17.0	0.001
Hind left lateral claw	171.2^{b}	292.1^{a}	336.5^{a}	21.7	0.001
Hind right medial claw	106.9^{b}	159.2^{a}	196.9^{a}	18.2	0.01
Hind right lateral claw	191.1^{b}	231.6^{b}	352.2^{a}	23.1	0.01
Biotin supplemented					
Hind left medial claw	74.9^{b}	166.5^{a}	155.6^{a}	24.4	0.05
Hind left lateral claw	167.3^{b}	232.6^{b}	336.4^{a}	27.7	0.001
Hind right medial claw	82.7^{b}	138.4^{b}	172.2^{a}	27.5	0.05
Hind right lateral claw	159.1^{b}	217.3^{b}	328.8^{a}	28.4	0.001

$^{a, b}$ – different letters in the same row indicate values that differ significantly

4.3.2.5 Measurements of front and hind claws and hoof horn growth and wear

The mean lateral height of front and hind claws, heel height of front claws and claw angle of hind claws increased significantly (P<0.01) throughout the *postpartum* period. A significant (P<0.01) increase was found between 100 and 160 days *postpartum* for the lateral height and the heel height of the front hooves and the lateral height of the hind hooves. The claw angle of the hind claws increased between days 50 and 160 *postpartum* (Table 4.15).

Table 4.15 ***Mean values for measurements of dorsal length, lateral height, heel height and claw angle of lateral front and hind right claws at days 50, 100 and 160 postpartum***

Measurements	Days 50	100	160	sem	*P*
Front					
Dorsal length, (cm)	7.89	7.67	7.87	0.094	NS
Lateral height (cm)	7.44 ^{b}	7.51 ^{b}	7.98 ^{a}	0.124	0.01
Heel height (cm)	5.02 ^{b}	5.31 ^{b}	5.81 ^{a}	0.136	0.001
Claw angle (°)	49.50	49.93	50.87	0.815	NS
Hind					
Dorsal length (cm)	7.89	7.80	8.07	0.092	NS
Lateral height (cm)	6.96 ^{b}	7.01 ^{b}	7.75 ^{a}	0.151	0.001
Heel height (cm)	4.16	4.28	4.57	0.147	NS
Claw angle (cm)	49.03 ^{b}	51.41 ^{ab}	52.14 ^{a}	0.678	0.01

$^{a, b}$ – different letters in the same row indicate values that differ significantly,
NS – not significantly different

The mean growth rates of the hind lateral claws during the *postpartum* period were 0.499 cm/ month (sem 0.018) and of front lateral claws 0.365 cm/ month (sem 0.020). The mean wear rates in the *postpartum* period were of 0.328 cm/ month (sem 0.045) for the lateral hind claws and of 0.256 cm/ month (sem 0.030) for lateral front claws. The net growth rate was positive for front and hind claws (0.109 and 0.171 cm/month, respectively) being higher for hind claws than front claws.

4.3.2.6 Punch resistance, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas of the hoof horn

Using multiple regression analysis to test for factors that had a significant effect on the punch force of the sole horn; sample thickness, sample haemorrhage level, days *postpartum* and days from sample collection to analysis were significant (P <0.001 and <0.05), while diet, cow and claw were found not to be significant. The regression equation for punch resistance of the sole horn was:

Punch resistance of the sole horn (N) = 6.36 + 16.9 thickness + 0.0894 days to test - 0.00142 days postpartum - 0.307 haemorrhage, R^2adj. = 0.31, P <0.001.

The factors that had a significant effect on the elastic modulus of the diaphragm and membrane stress of the sole horn were; sample thickness, sample haemorrhage level, days *postpartum* and claw (P <0.001 and <0.05), while diet, cow and days from sample collection to analysis were found not to be significant. The regression equation for elastic modulus of the diaphragm of the sole horn was:

Elastic modulus of the diaphragm (N/mm^2) = 25.8 - 70.8 thickness - 2.69 haemorrhage + 0.0497 days postpartum - 0.603 claw (1-8), R^2adj. = 0.16, P <0.001.

The regression equation for membrane stress of the sole horn was:

Membrane stress (N/mm^2) = 8230 - 42349 thickness - 799 haemorrhage + 13.5 days postpartum - 206 claw (1-8), R^2adj. = 0.25, P <0.001.

The sample thickness had a significant (P < 0.001) positive and linear relationship with the punch force of the sole horn (Figure 4.8) and was used as a covariate when punch force measurements of the sole area of the horn was analysed.

Maximum punch resistance of the sole horn (N) = 5.69010 + 20.9025 sample thickness, R^2adj. = 0.33, P <0.001

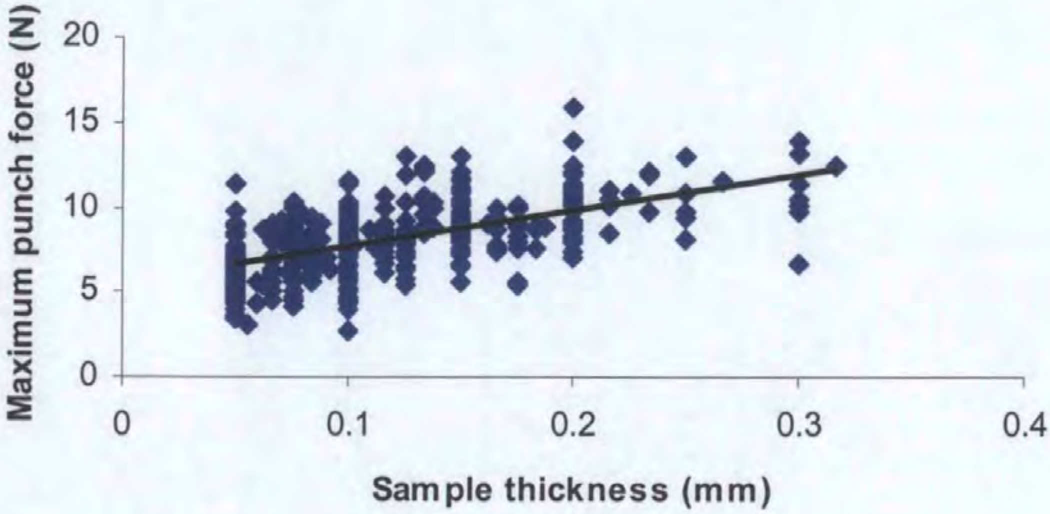


Figure 4.8 ***Influence of sample thickness on maximum punch force of the sole area of the horn***

Using multiple regression analysis to test for the factors that had a significant effect on the punch force of the white line area of the horn; sample thickness, sample haemorrhage level, days *postpartum,* days from sample collection to analysis and claw number were found to be significant (P<0.001 and <0.05), while diet and cow were found not to be significant. The regression equation for punch resistance of the white line horn was:

Punch resistance of the white line horn (N) = 3.38 + 14.2 thickness + 0.148 days to test - 0.00189 days postpartum - 0.362 haemorrhage + 0.191 claw number (1-4), R^2adj. = 0.38, P <0.001.

The factors that had a significant effect on the elastic modulus of the diaphragm and membrane stress of the sole horn were; sample thickness, sample haemorrhage level, days *postpartum* and days from sample collection to analysis (P <0.001 and <0.01), while diet, cow and claw were found not to be significant. The regression equation for elastic modulus of the sole horn was:

Elastic modulus of the diaphragm of the white line horn (N/mm^2) = 35.2 - 123 thickness + 2.32 days to test - 0.0280 days postpartum - 4.81 haemorrhage, R^2adj. = 0.45, $P < 0.001$.

The regression equation for membrane stress of the sole horn was:

Membrane stress of the white line horn (N/mm^2) = 10009 - 50168 thickness + 815 days to test - 22.3 days postpartum - 1720 haemorrhage, R^2adj. = 0.24, $P < 0.001$.

The sample thickness had a significant ($P < 0.001$) positive and linear relationship to the punch force of the white line area of the horn (Figure 4.9) and as a consequence was used as a covariate when punch test measurements of the white line horn were analysed.

Maximum punch force of the white line (N) = 3.49552 + 16.4527 sample thickness, R^2adj. = 0.32, $P < 0.001$

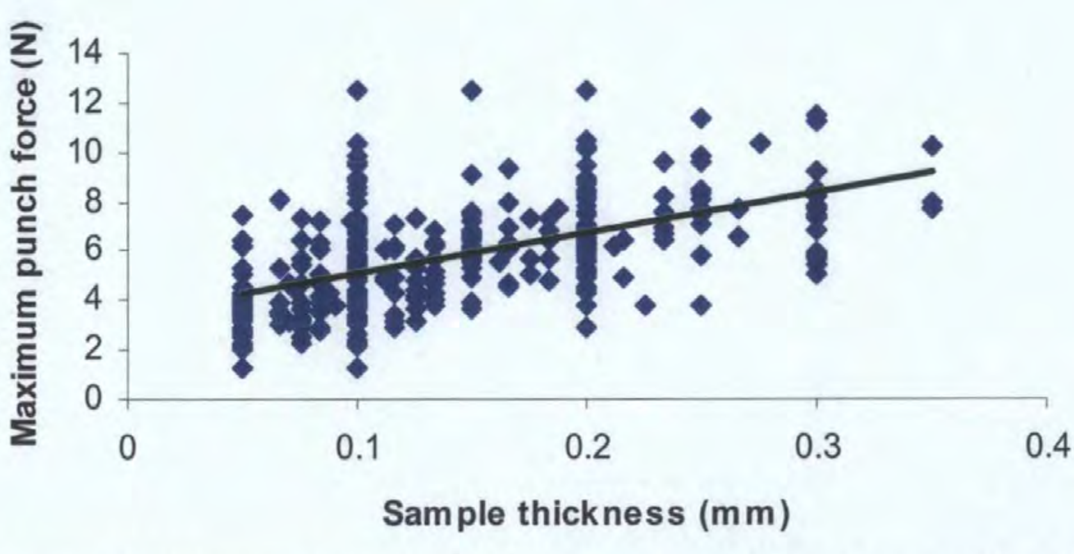


Figure 4.9 ***Influence of sample thickness on maximum punch force of the white line area of the horn***

4.3.2.6.1 Punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas of the hoof horn during the postpartum period

The punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line area of the horn at 50, 100 and 160 days *postpartum* analysed through ANCOVA are presented in Table 4.16. There was a significant (P<0.05 and 0.01) decrease in punch force of the sole and white line areas and work to fracture of the white line area of the horn between day 50 and 160 *postpartum*. The elastic modulus of the diaphragm and membrane stress of the sole and white line area of the horn decreased significantly (P<0.05 to 0.001) between day 50 and 100 *postpartum*. The work to fracture of the sole area of the horn increased significantly (P<0.05) between day 50 and 100 *postpartum*.

4.3.2.6.2 Punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas of the hoof horn of front and hind, left and right and inner and outer claws

The punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line horn of all claws at 100 and 160 days *postpartum* are presented in Table 4.17. At day 100 *postpartum* the punch force of the sole area of the horn in hind outer claws was significantly (P<0.01) lower when compared with the hind right inner claw. At day 160 *postpartum* no significant difference was measured in the punch force of the sole area between claws. The punch force of the white line area of the hoof horn of hind left claws at day 160 *postpartum* were significantly (P<0.01) lower when compared with the front right inner claw. At day 100 *postpartum* no significant difference was measured in the punch force of the white line area of the hoof horn between claws. The work to fracture of the sole area of the horn of the hind left inner claw at day 100 *postpartum* was significantly (P<0.05) lower when compared with the hind right inner and front left inner claws. The work to fracture of the sole horn area of the hind left outer claw at day 160 *postpartum* was significantly ($P<0.05$) lower when compared with the front right outer claw. The work to fracture of the white line area of the hoof horn of the front right outer and hind left outer claws at day 160 *postpartum* were significantly ($P<0.01$) lower when compared with the front right inner claw. No significant differences were measured between claws in the work to fracture of the white line area of the horn at day 100 *postpartum*, in the elastic modulus of the diaphragm and membrane stress of the sole and white line areas at days 100 and 160 *postpartum*.

Table 4.16 ***Punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line (wl) areas at 50, 100 and 160 days postpartum***

	Day postpartum				
Measurement	50	100	160	sem	*P*
Punch resistance (N)					
sole	8.98 [a]	8.59 [ab]	8.18 [b]	0.219	0.05
wl	7.10 [a]	6.17 [ab]	5.58 [b]	0.350	0.01
Work to fracture (kJ)					
sole	10.12 [b]	11.10 [a]	10.54 [ab]	0.219	0.05
wl	7.70 [a]	6.90 [ab]	6.38 [b]	0.377	0.05
Elastic modulus (N/mm^2)					
sole	48.80 [a]	17.70 [b]	18.20 [b]	7.10	0.001
wl	32.19 [a]	17.83 [b]	19.74 [b]	3.34	0.01
Membrane stress (N/mm^2)					
sole	15873.2 [a]	2947.0 [b]	2900.9 [b]	3482.0	0.01
wl	5138.1 [a]	1979.2 [b]	1519.6 [b]	905.8	0.05

[a, b] – different letters in the same row indicate mean values that differ significantly

Table 4.17 ***Punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line horn of front and hind, right and left and inner and outer claws at 100 and 160 days postpartum (dpp)***

	Front				Hind					
	Left		Right		Left		Right			
Measurement	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	sem	*P*
Punch resistance (N)										
Sole 100 dpp	8.34 ^{ab}	8.07 ^{ab}	8.76 ^{ab}	8.61 ^{ab}	7.30 ^{b}	7.94 ^{ab}	7.12 ^{b}	9.20 ^{a}	0.413	0.01
Sole 160 dpp	8.39	7.97	8.32	7.96	7.36	8.13	7.49	8.37	0.275	NS
White line 100 dpp	6.40	7.07	6.55	6.99	6.09	6.10	5.68	5.68	0.520	NS
White line 160 dpp	5.13 ^{ab}	5.92 ^{ab}	5.31 ^{ab}	6.20 ^{a}	4.82 ^{b}	4.92 ^{b}	5.59 ^{ab}	5.41 ^{ab}	0.274	0.01
Work to fracture (kJ)										
Sole 100 dpp	10.47 ^{ab}	11.72 ^{a}	11.65 ^{ab}	11.08 ^{ab}	9.74 ^{ab}	9.70 ^{b}	10.08 ^{ab}	11.74 ^{a}	0.552	0.05
Sole 160 dpp	10.83 ^{ab}	10.58 ^{ab}	10.92 ^{a}	10.32 ^{ab}	9.33 ^{b}	9.97 ^{ab}	9.84 ^{ab}	10.17 ^{ab}	0.369	0.05
White line 100 dpp	7.46	8.46	7.63	8.12	6.85	7.55	6.90	6.22	0.700	NS
White line 160 dpp	5.67 ^{ab}	6.73 ^{ab}	5.55 ^{b}	7.24 ^{a}	5.48 ^{b}	5.64 ^{ab}	6.12 ^{ab}	6.24 ^{ab}	0.387	0.01
Elastic modulus diaphragm Sole 100 dpp (N/mm^2)	18.01	14.80	14.99	15.51	14.48	21.05	13.66	18.79	2.87	NS

$^{a, b, c}$ – different letters in the same row indicate mean values that differ significantly,
NS – not significantly different

Table 4.17 ***Punch resistance, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line horn of front and hind, right and left and inner and outer claws at 100 and 160 days postpartum (continuation)***

	Front				Hind					
	Left		Right		Left		Right			
Measurement	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	sem	*P*
Elastic modulus diaphragm (N/mm^2)										
Sole 160 dpp	19.99	18.26	18.98	18.83	20.81	22.14	16.86	22.04	1.91	NS
White line 100 dpp	16.38	16.85	14.81	18.73	18.86	18.21	21.49	12.25	3.04	NS
White line 160 dpp	20.38	21.28	25.36	19.22	20.85	19.51	22.83	17.40	1.86	NS
Membrane stress (N/mm^2)										
Sole 100 dpp	2628	1619	2618	1929	2194	2596	2221	1716	621.6	NS
Sole 160 dpp	3735	3382	3540	3018	3812	3539	2944	4290	627.6	NS
White line 100 dpp	1150	1183	1126	1907	1365	1735	4163	550	693.8	NS
White line 160 dpp	1334	1699	2522	1676	2831	1865	2303	1410	412.5	NS

NS – not significantly different

At 50 days *postpartum* horn samples were not separated for the inner and outer claws of each hoof, inner and outer claws were not compared by mechanical testing and the results for the punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas of the horn are presented in Table 4.18 for front and hind, right and left claws. There were no significant differences recorded between claws in the punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas of the horn.

Table 4.18 ***Punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas of the horn of front and hind, right and left claws at 50 days postpartum***

	Front		Hind			
Measurement	Left	Right	Left	Right	sem	*P*
Punch resistance (N)						
Sole	8.66	9.04	9.02	8.63	0.334	NS
White line	7.09	7.25	5.62	6.12	0.523	NS
Work to fracture (kJ)						
Sole	10.16	10.00	10.17	10.09	0.393	NS
White line	7.45	7.24	6.66	7.42	0.561	NS
Elastic modulus (N/mm^2)						
sole	41.7	42.8	47.3	53.0	13.99	NS
White line	32.47	30.42	22.63	28.14	5.96	NS
Membrane stress (N/mm^2)						
Sole	10802.5	10093.7	18889.9	24329.9	8896.2	NS
White line	6821.9	3695.7	3251.4	6428.6	2596.4	NS

NS – not significantly different

There was a significant correlation ($P<0.001$) between punch force and work to fracture of the sole and of the white line horn (R^2adj.= 0.51). The punch force and work to fracture were significantly ($P<0.001$) greater in all periods for the sole horn when compared to the white line horn.

4.3.2.7 Elastic modulus measured though tension tests of the sole area of the horn during the postpartum period

When analysed through multiple regression analysis the factors that had significant effect on the elastic modulus of the sole area of the horn measured through tension tests were: days between sample collection and analysis, *postpartum* period and haemorrhage level of the sample. The regression equation for the elastic modulus of the sole area of the horn was:

Elastic modulus (N/mm^2) = - 12.6 + 3.24 days to analyse sample + 32.5 postpartum period - 9.55 haemorrhage, R^2adj. = 0.23, $P<0.001$

The elastic modulus measured through tension tests at 50, 100 and 160 days *postpartum* analysed through ANOVA is presented in Table 4.19. The elastic modulus measured through tension test of the sole area was significantly ($P<0.05$) lower at 100 days *postpartum* when compared to 160 days *postpartum*.

4.3.2.8 Elastic modulus measured through tension tests of the sole area of the hoof horn of front and hind, left and right claws

The elastic modulus measured through tension tests of the front and hind right and left claws analysed through ANOVA are presented in Table 4.20. The elastic modulus of the tension test of the sole area did not differ significantly between front and hind, left and right claws during days 50, 100 and 160 of the *postpartum*.

Table 4.19 ***Elastic modulus measured through tension tests of the sole area on days 50, 100 and 160 postpartum and dry matter on days 50 and 100 postpartum***

	Day				
Measurement	50	100	160	sem	*P*
Elastic modulus (N/mm^2)	96.52 [ab]	70.77 [b]	114.47 [a]	10.88	0.05
Dry matter (%)	74.11	73.92	-	0.426	NS

[a, b] – different letters in the same row indicate mean values that differ significantly,
NS – not significantly different

Table 4.20 ***Elastic modulus measured through tension tests on days 50, 100 and 160 postpartum and dry matter of the horn on days 50 and 100 postpartum (pp) of front and hind, left and right claws***

	Front		Hind			
Measurement	Left	Right	Left	Right	sem	*P*
Elastic modulus (N/mm^2)						
day 50 pp	85.49	127.21	84.20	85.32	24.14	NS
day 100 pp	58.69	79.14	82.82	66.65	11.06	NS
day 160 pp	120.40	121.40	102.00	107.20	21.12	NS
Dry matter (%)						
day 50 pp	75.62 [a]	72.36 [ab]	71.46 [b]	74.44 [ab]	0.909	0.01
day 100 pp	75.04	74.08	72.68	73.63	0.730	NS

[a, b] – different letters in the same row indicate mean values that differ significantly,
NS – not significantly different

4.3.2.9 Dry matter of the hoof horn

There was a significant difference ($P<0.01$) between the dry matter content of the horn of the front left and hind left claws at 50 days *postpartum*. The dry matter content was higher in hoof horn tissue from the front left claw compared with the hind left claw. No significant differences were measured between the dry matter content of the horn of different claws at day 100 *postpartum* (Table 4.20). There were no significant differences in dry matter content of the hoof horn between 50 and 100 days *postpartum* (Table 4.19).

4.3.2.10 Body condition score in the postpartum period

The mean body condition score increased from day 12 to day 194 *postpartum*, but this increase was not statistically significant (Figure 4.10).

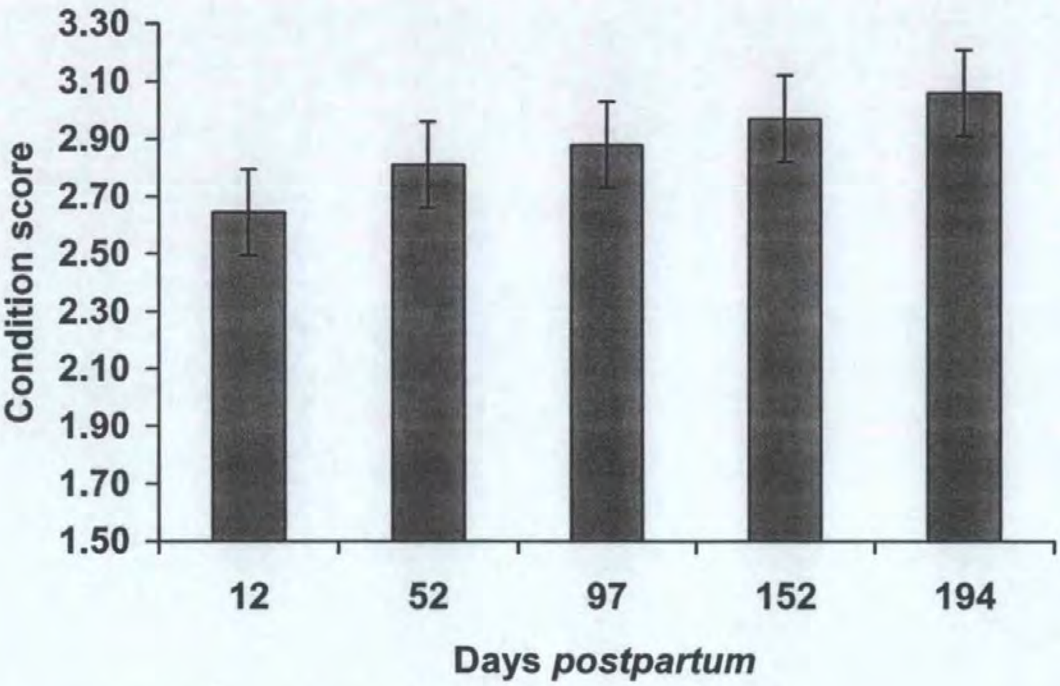


Figure 4.10 ***Body condition score in the postpartum period***

4.4 Discussion

4.4.1 Milk biotin concentration of supplemented and non-supplemented cows

The plasma and milk biotin levels have been used in the literature to test the effectiveness of oral biotin supplementation (Geyer and Schulze, 1994; Schmid, 1995; Hochstetter, 1998; Midla *et al.,* 1998; Hedges *et al.,* 2001; Higuchi *et al.,* 2004). According to Geyer and Schulze (1994) and Schmid (1995) plasma biotin levels reached high levels a short time (1 hr) after oral supplementation of biotin. After supplementation of 20 mg biotin per day Schmid (1995) described an increase of the mean biotin plasma levels from 449 ng/l before biotin supplementation, to 1643 ng/l following supplementation. Distl and Schmid (1994), Midla *et al.* (1998) and Higuchi *et al.* (2004) reported significantly higher levels (1.72 ng/ml ± 0.22) of biotin in the plasma of cows supplemented with 20 mg biotin per day compared with non-supplemented cows (0.71 ng/ml ± 0.22).

In milk Hochstetter (1998), Midla *et al.* (1998) and Hedges *et al.* (2001) found significant differences in the biotin levels of cows supplemented with 20 mg of biotin daily when compared to cows not supplemented with biotin. The levels of biotin in milk obtained in the present experiment of 17.3 μg/kg ± 7.2 for cows not supplemented with biotin and 102.6 μg/kg ± 7.7 for biotin supplemented cows, were similar to those obtained by Hochstetter (1998) and Midla *et al.* (1998). Midla *et al.* (1998) reported mean milk biotin levels of 93 ng/ml and of 35 ng/ml and Hochstetter (1998) levels of 80 - 171 μg/l and 12.6 - 15.0 μg/l for biotin supplemented and non-supplemented animals. However, according to Midla *et al.* (1998) when supplementation started after *parturition* the concentration of biotin in milk differed between groups only at the end of the lactation period (average of 293 days). These authors reported great variation in the biotin levels (93 to 162 ng/ml for cows supplemented with biotin and 35 to 78 ng/ml for cows not supplemented with biotin, sed 11 – 31). In the present experiment, milk biotin concentration differed between supplemented and not supplemented cows at day 120 *postpartum* and variation was relatively low. Similar results were reported by Hochstetter (1998) who found increased concentration of milk biotin in cows supplemented with biotin for 8 to 12 months at a daily level of 20 mg per head. In this experiment, the milk biotin concentration showed that the measured intake of compound was correct and the groups had the daily intake of biotin according to the treatment levels required. Considering a rumen biotin synthesis of 1 mg biotin /kg organic matter (OM) and a concentration of biotin in diets for lactating dairy cows ranging from 0.2 to 0.4 mg/kg of DM (Midla *et al.*, 1998), Zimmerly and Weiss (2001) estimated that the daily intake of biotin from the basal diet would be of 5 mg and the ruminal synthesis would provide 18 mg of biotin daily and if cows would be supplemented with 20 mg of biotin per head daily the supply of biotin would be 1.9 times higher for supplemented cows compared with control cows.

4.4.2 Body condition score

The role that biotin plays on the lipogenesis and gluconeogenesis (Seymour, 1998; Tomlinson *et al,* 2004) and the increased requirement for energy (Girard, 1998) and potentially biotin (Hochstetter, 1998) in the early lactation could have led to cows supplemented with biotin having a more efficient energy metabolism than cows not supplemented with biotin. An optimization of gluconeogenesis may have occurred but was not reflected in changes in the body condition score as these were not significantly different between groups. Midla *et al.* (1998) had similar results, there being no significant differences in the body condition score of biotin supplemented cows and non biotin supplemented cows. Biochemical values in the plasma of the animals are a better measure of their energy metabolism.

4.4.3 Effect of biotin supplementation on locomotion and lesion score

Biotin supplemented cows had significantly lower sole area lesion of the hind left inner claw at 160 days *postpartum* when compared with cows not supplemented with biotin. The lesion score of any other claw or measurement period and the locomotion score were not affected by the supplementation with biotin. A lower incidence of sole haemorrhages was found by Bergsten *et al.* (2002) and Bergsten *et al.* (2003). Fitzgerald *et al.* (2000) found significantly higher levels of moderate to severe damage of hind outer claws of cows that were not supplemented with biotin compared to cows supplemented with biotin. The positive effect of biotin on keratinisation would only be noticed after the newly formed horn appeared on the solear surface and this may have influenced the period biotin was first effective in reducing lesions on the solear horn area. At 160 days *postpartum* lesion scores of the sole horn of hind claws were significantly higher than in other periods and differences between treatments increased. Considering a sole thickness of approximately 7 mm in the toe and heel areas and 5 mm in the middle (Toussaint-Raven, 1985; Scott, 1987; Paulus and Nuss, 2002) and a mean growth rate of 4.3 mm per month of the coronary horn, the sole horn in the present experiment would have had a turnover of approximately 1.5 months. However, Schmid (1995) measured a slower growth rate for the sole horn compared with the coronary horn and so the turnover of the sole horn would be approximately of 2 months. Midla *et al.* (1998) stated that it took the sole horn 60 to 90 days to reach the weight-bearing surface. The growth rate of the coronary horn in the present experiment was however half of the growth rate of the coronary horn of cows in experiment 1 (6.9 to 8.4 mm/month) that was used as basis for calculating the necessary time for the horn to grow out after beginning of supplementation, thus the horn that could have suffered alterations after the supplementation with biotin took longer than expected to appear on the solear surface.

White line lesions in the present experiment increased between 50 and 100 days *postpartum* and sole lesions increased after 100 days *postpartum*. Considering the hoof horn growth the observed haemorrhages most likely originated from insults that occurred around calving for the white line area and 2 months after calving for the sole area. Lesions occurring around calving could have originated in physiological changes, diet and housing changes. The significant effect of biotin, in reducing the haemorrhage levels of the sole horn area, was obtained during the period of maximum insult (160 days *postpartum*). Bryant *et al.* (1985) reported a greater response of biotin supplementation in reducing the number of toe lesions in pigs when the prevalence of toe lesions was high. In this experiment cows not supplemented with biotin had an earlier significant increase in the lesion score of the hind right medial and hind left lateral claws at 100 days *postpartum* compared to cows supplemented with biotin which had a significant increase in the lesion score of the hind right medial and hind left lateral claws at day 160 *postpartum*. Hedges *et al.* (2001) reported a departure of the hazard function for lameness caused by white line lesions of cows supplemented and not supplemented with biotin at day 130 after the start of supplementation. Midla *et al.* (1998) and Hoblet *et al.* (2002) found a lower risk and incidence of white line separation in cows supplemented with biotin compared with cows not supplemented with biotin. Distl and Schmid (1994) observed a lower incidence of sole haemorrhages after 8 months supplementation with biotin and of dermatitis interdigitalis after 4 month supplementation. Hochstetter (1998) reported an improvement in the levels of sole haemorrhages, of dermatitis interdigitalis and of heel erosions after 8 months supplementation with biotin and no changes of these conditions were observed in the cows not supplemented with biotin. Schmid (1995) reported that cows with severe macroscopic alterations of the sole and heel area of the claw had an increase in the quality of the horn in these areas observed macroscopically and through histology that occurred after 4 months of biotin supplementation. After 5 months supplementation with biotin, the tensile strength of the sole and heel areas increased significantly and after 15 months of biotin supplementation the cows had almost no sole or heel horn alterations while the white line areas still had some tissue alterations. These results support the need for longer term biotin supplementation in dairy cattle. It is possible that long term supplementation with biotin could have been more effective and should supplementation have been continued in this experiment the differences in lesion scores of other claws than only the hind left inner claw, of cows supplemented with biotin and not supplemented with biotin could have been significantly different.

In the literature, the supplementation of the diets with biotin has been found to improve the condition of the sole, white line, heel, coronary horn areas and even the interdigital skin in several species. The supplementation of the diet with biotin has been found to significantly reduce lameness in pigs, horses and cows (Webb *et al.*, 1984; Bryant *et al.*, 1985; Distl and Schmid, 1994; Geyer and Schulze, 1994; Brooks *et al.*, 1997; Fitzgerald *et al.*, 2000). Fitzgerald *et al.* (2000) found significantly lower locomotion scores in biotin supplemented herds compared with herds not supplemented with biotin. A gradual improvement in the scoring of lameness was reported by Schmid (1995). In the present experiment cows supplemented with biotin did not present significantly different locomotion score to non supplemented cows.

Midla *et al.* (1998) did not find differences in the lesion scores of the sole horn between biotin supplemented and non-supplemented cows. Hedges *et al.* (2001) considered that biotin did not prevent the occurrence of sole ulcers because of the involvement of the suspensory apparatus in this pathology and so the improvement of the horn quality would not resolve the primary problem causing the pathology. Biotin may have a beneficial effect on keratinisation, but should some areas of the claws be under greater pressure and/or the blood circulation in the corium on these areas be compromised, it is possible that biotin would not reach the basal keratinising cell layer and no beneficial effect on horn structure would have occurred. Nonetheless, Lischer *et al.* (2001) reported that cows with poor healing parameters for sole ulcers had significantly lower serum biotin concentration compared with cows with good healing parameters.

4.4.4 Effect of biotin supplementation on mechanical testing of hoof horn

Biotin plays a role in the synthesis of long chain fatty acids, which are part of the composition of the intercellular cementing substance and in the keratin protein synthesis (Fritsche *et al.,* 1991; Mulling *et al.*, 1999; Mulling, 2000; Scaiffe, 2000). Higuchi *et al.* (2004) reported a significantly higher total lipid content of the sole horn in biotin supplemented cows when compared to control cows. Hochstetter (1998) clearly illustrated the influence of biotin on the ultra-structure and composition of the intercellular cementing substance and protein synthesis. The biotin supplementation of dairy cows resulted in an intercellular cementing substance with greater homogeneity and less lipid globules and inside the horn cells the keratin filaments and their associated proteins that were more distinguishable indicating a change in the protein composition of the cells (Hochstetter, 1998). Koster *et al.* (2002) found an increased integrity of the intercellular substance due to greater cell adhesion and absence of microcracks in biotin supplemented compared with non-supplemented dairy cows. The main function of the cementing substance is to establish cell to cell adhesion and disturbance in the synthesis of the cementing substance has been found to result in the separation of the cells and the formation of brittle horn (Baillie and Fiford, 1996; Zaun, 1997; Mulling *et al.*, 1998). In addition, the cementing substance has been found to establish a permeability barrier in the intercellular space. This barrier prevents the passage of aqueous solutions through the horn protecting the horn cells from loss of water and/or extreme hydration and thus regulates the hydration level of the hoof horn, influencing its mechanical properties (Hochstetter, 1998; Mulling and Budras, 1998; Mulling *et al.*, 1999). The intracellular content of keratin and keratin-associated proteins also has an influence over the water content of the horn cells and can be altered through biotin supplementation (Hochstetter, 1998). Higuchi *et al.* (2004) found a higher dry matter content of hoof horn in biotin supplemented cows when compared to control cows. While Lawrence *et al.* (2004) did not find differences in the fatty acid profile of the white line and fat pads between biotin supplemented and non-supplemented beef cattle.

Schmid (1995) reported an improvement in the microscopic evaluation of bovine hoof horn after biotin supplementation. The coronary horn presented fewer numbers of tubules with enlarged marrows after 10 months of biotin supplementation and lower incidence of microcracks after 15 months of biotin supplementation. The sole and heel horn improved after 4 months of biotin supplementation, presenting less microcracks and tubules with enlarged marrows and after 15 months of biotin supplementation the sole and heel horns were classified as not having microscopic alterations at the microscopic level. Horses with poor coronary horn quality that presented microcracks between the tubular cortex and intertubular horn, had this condition improved after biotin supplementation (Zenker *et al.*, 1995). Improved microscopic appearance of horses and pigs hoof horn after biotin supplementation was reported by Kempson (1990) and Kempson *et al.* (1989). Pigs supplemented with biotin had greater number of tubules and less intertubular horn (Kempson *et al.*, 1989). However, Higuchi *et al.* (2004) did not find morphologic differences in the horn tubules or intertubular horn between cows supplemented with biotin and cows not supplemented with biotin. The greater number of tubules and improvement of the microscopic appearance of the tubular and intertubular horns would likely improve the mechanical properties of the hoof horn.

In the present experiment no differences were found between the punch resistance, work to fracture, elastic modulus, membrane stress and dry matter of biotin supplemented and non-biotin supplemented cows. The authors that have measured differences in the mechanical properties of the hoof horn only reported significant changes after at least 4 month supplementation with biotin. According to Schmid (1995) the tensile strength of the sole and heel horns increased significantly after 4.5 months supplementation with biotin (from 52.73 to 64.65 N/mm^2 and 34.65 to 54.42 N/mm^2, respectively) and a significant increase of the tensile strength of the coronary horn of cows occurred after 10 months of supplementation with biotin (from 64.23 to 75.94 N/mm^2). Distl and Schmid (1994) supplemented cows for 12 months and significant changes in coronary horn hardness were measured after 4 months of supplementation. The average tensile strength and horn structure of the coronary horn in horses with histological alterations, increased after 8 and 15 months of biotin supplementation (Geyer and Schulze, 1994). Zenker *et al.* (1995) only found significant differences in the tensile strength of the coronary horn of horses supplemented with biotin when compared to non-supplemented horses after 33 months supplementation. Considering that in this experiment biotin supplementation was carried out for 5 months, this may not have been long enough to influence the mechanical properties of the sole and white line horn.

Schmid (1995) did not find changes in the moisture content of the coronary (12.43 ± 3.25 % at the start of supplementation and 11.89 ± 2.60 % after 12 months supplementation) and sole horns (22.42 ± 3.62 % at the start of supplementation and 22.21 ± 2.89 % after 12 months supplementation) after 12 months biotin supplementation. In the present experiment there was no difference in the moisture content of the hoof horn between biotin supplemented and non-biotin supplemented cows 100 days after the start of supplementation (26.03 and 26.25 %, respectively). The lack of difference in the moisture content of the horn of biotin supplemented and non-biotin supplemented cows may explain why mechanical properties did not vary significantly between cows supplemented with biotin and cows not supplemented with biotin. Hedges *et al.* (2002) measured the tensile strength of the white line on zones 2 and 3 and the hardness of the sole horn and similarly to the present experiment did not find significant differences between biotin supplemented and non-biotin supplemented cows. These authors tested the white line and sole areas of the horn samples up to day 187 after the start of the supplementation with biotin. However, samples were stored in water and frozen at -20°C. The storage of samples in water could have reduced the differences in the mechanical properties of the horn samples (Experiment 2). Considering that biotin has an effect on the water storage capacity of the horn cells and on the permeability of water between the cells (Hochstetter, 1998), the moisture content of the horn samples should be measured and standardizing the moisture content of the horn samples could have masked some of the effects of biotin on the horn composition. The freezing of samples for over 30 days also altered the mechanical properties of the hoof horn increasing its elastic modulus (Experiment 2).

The areas of the sole horn that were tested may have also influenced results. The hoof horn was sampled with a plane on the areas 5 and 2 of the sole and white line, according to the IFM (Shearer *et al.*, 2002), that were not the areas with the highest scores for lesions but enabled the collection of samples with a more uniform thickness. Areas 3 and 4 of the IFM (Shearer *et al.*, 2002) typically presented the highest scores for lesions (Leach *et al.*, 1998) and would reflect better changes in the lesion score measured between groups. Biotin supplementation in pigs increased the compressive strength and hardness (Shore A and D) of the lateral abaxial wall horn of the hoof, but not the leading edge of the wall horn (toe), sole and white line areas and decreased the hardness of the bulb (Webb *et al.*, 1984).

4.4.5 Effect of biotin supplementation on claw measurements

Biotin supplementation is known to increase hoof horn hardness in cattle (Brooks *et al.*, 1997; Distl and Schmid, 1994; Schmid, 1995 and Campbell *et al.*, 2000). Greater horn hardness could lead to changes in the growth and wear rates and subsequently to changes in the measurements of the claws. Distl and Schmid (1994) reported a greater increase in the claw angle, the height of the heel and the diagonal of the hind and height of the heel of front claws in biotin supplemented cows when compared to non-biotin supplemented cows. In their experiment, biotin supplemented cows also presented greater wall horn hardness measured through Shore D meter. Changes in claw measurements could have been related to a decrease in the wear rate in biotin supplemented animals due to the greater horn hardness. Biotin supplementation (20 mg per head daily) was carried out on in their experiment for 12 months. However, significant changes in horn hardness were measured after 4 months supplementation and of claw measurements from 8 months supplementation onwards. In the present experiment the absence of significant differences in the dorsal length (7.78 vs. 7.96 cm for front claws and 7.95 vs. 8.20 cm for hind claws), lateral height (7.84 vs. 8.13 cm for front claws and 7.51 vs. 8.01 cm for hind claws), heel height (5.91 vs. 5.73 for front claws and 4.43 vs. 4.72 cm for hind claws) and hoof angle (51.25 vs. 50.43 for front claws and 52.63 vs. 51.61 cm for hind claws) of front and hind lateral claws between biotin supplemented and non-supplemented cows, 5 months after the start of the supplementation with biotin, may be related to the absence of differences in growth (0.361

vs. 0.369 cm/month for front claws and 0.491 vs. 0.507 cm/month for hind claws) and wear rates (0.273 vs. 0.237 cm/month for front claws and 0.384 vs. 0.307 cm/month for hind claws) of front and hind claws between the two groups. Similarly, Midla *et al.* (1998) did not find significant differences in the dorsal wall length and hoof angle of biotin supplemented and non-biotin supplemented cows. However, in their experiment biotin supplemented cows had smaller height of the heel in the forelimbs when compared to non-biotin supplemented cows at mean day 100 (48.2 ± 5.6 vs. 51.5 ± 5.1 mm) and 293 postpartum (44.8 ± 4.8 vs. 48.5 ± 6.2 mm), when supplementation started after parturition. Schmid (1995) did not find significant differences in the growth rate of front and rear claws of biotin supplemented and non-biotin supplemented dairy cows. There was no alteration of the growth rate of the coronary horn in horses supplemented with biotin when compared to non-biotin supplemented horses as reported by Geyer and Schulze (1994).

4.4.6 Locomotion and lesion score in the postpartum period

In the present experiment there was an increase in the locomotion score and the incidence of white line and sole lesions during the *postpartum* period. Several authors reported similar figures (Enevoldsen *et al.,* 1991; Bergsten and Herlin, 1996; Bergsten and Frank, 1996; Leonard *et al.,* 1996; Vermunt and Greenough, 1996; Leach *et al.,* 1997; Livesey *et al.,* 1998; Whay, 1998; Chaplin *et al.,* 2000 and Offer *et al.*, 2000). Hormonal changes and the consequently softening of the collagen fibres that connect the third phalanx to the horn capsule, associated with parturition, are believed to be the major contributory factor in the development of these lesions (Webster, 1998; Holah *et al.,* 2000; Tralton and Webster, 2002). Haemorrhages in the claw horn have been found to be at their greatest levels between 100 and 120 days *postpartum* and decline in number and severity thereafter. White line lesions were found to occur earlier in the *postpartum* period than sole lesions

(Enevoldsen *et al.*, 1991; Leach *et al.*, 1997; Offer *et al.*, 1998 and 2000). Similar results were found in the present experiment, with white line lesions increasing between days 50 and 100, and sole lesions increasing after 100 days *postpartum*. Considering the recorded hoof horn growth of the wall the observed haemorrhages probably originated from insults that occurred approximately 6 to 8 weeks earlier, the greatest insults occurring around *parturition* for the white line area and two months *postpartum* for the sole area. These results are in accordance with other authors (Livesey *et al.*, 1998; Bergsten, 2001). The earlier increase in number and severity of white line lesions when compared to the sole lesions was explained through a differing response to the initial insult, differing growth and wear rates of the two areas, or a differing origin of the lesion (Leach *et al.*, 1997; Le Fevre *et al.*, 2001). In this experiment sole and white line lesions were positively correlated indicating that the formation of lesions in both areas were linked even if the period of maximum lesion scoring occurred earlier for the white line area compared to the sole area.

The highest scores for sole lesions occurred in the outer hind claws and for the white line lesions in the hind claws. The front claws had lower scores in all measuring periods when compared to hind claws. Bergsten and Herlin (1996), Leach *et al,* (1997), Offer *et al* (2000) and Le Fevre *et al.* (2001) reported similar results. The difference in shape between front and hind and inner and outer hind claws reported by Toussaint-Raven (1985) and Scott (1987) and the increased weight bearing on the outer hind claws because of the conformation of the udder at parturition, forcing the cow to have a wider stance (Vermunt and Greenough, 1996), increase the pressure and the risk for greater number and severity of horn lesions on these claws. The cow has been found also to be more able to relieve weight from the front claws than from the hind claw (Toussaint-Raven, 1985; Frandson, 1986) and the hind feet of cattle are used more for propulsion and the front feet are used to support the front part of the body (Scott, 1987).

4.4.7 Changes in the claw measurements in the postpartum period

In the present experiment monthly hoof horn growth rates (0.499 cm/month for hind lateral claw and 0.365 cm/month for front lateral claw) were similar to the ones reported by Leach *et al.* (1997), Chaplin *et al.* (2000) and Offer *et al.* (2000) (0.417 to 0.540 cm/month) for cows kept in a cubicle system like in the present experiment. However, the net growth rate was positive for front and hind claws (0.109 and 0.171 cm/month), unlike the net growth rate of -0.25 mm per month reported by Leach *et al.* (1997). Wear rates were higher for the hooves of the hind claws (0.328 cm/month) compared with the hooves of the front claws (0.256 cm/month) and net growth rates were also higher for the hind claws. Clark and Rakes (1982) reported similar figures, the hind claws growing faster (4.17-11.08 mm/month) than the front claws (4.09-8.99 mm/month). Schmid (1995) did not find differences between the growth rate of front and hind claws. Higher wear rates for the hind claws when compared to the front claws may be related to a greater load that these claws were subjected due to the increase in udder size in the *postpartum* period (Toussaint-Raven, 1985; Scott, 1987; Singh, 1993; Vermunt and Greenough, 1996).

Changes in the shape of the claw after the start of the lactation were reported by Offer *et al.* (2000). They found that during lactation, the angle of the dorsal border became smaller while the dorsal wall became longer. These changes were considered probably to be related to changes in the growth and wear rates (Offer *et al.*, 2000). In the present experiment there was an increase in the lateral height and the angle of the dorsal border of hind hooves and in the lateral and heel height of the front hooves during the *postpartum* period. The increase in height was probably related to the effect of a positive net growth rate.

4.4.8 Changes in the mechanical properties of the hoof horn in the postpartum period

The measurements for punch force, work to fracture and elastic modulus of the diaphragm decreased between 50 to 160 days *postpartum*. An increase in the level of haemorrhages of the sole and white line horn at these measurement periods may have influenced these results. The haemorrhage level of the tested areas of the horn samples significantly (P<0.001) affected the punch force, the elastic modulus and the membrane stress of the sole and white line areas of the horn. Other factors that may have affected the measurements of punch force, work to fracture and elastic modulus of the diaphragm could have been the amount of time the animals were housed and the length of time that the hooves were exposed to slurry and concrete flooring (Wells *et al.*, 1995; Vermunt and Greenough, 1996; Berry, 2001; Offer *et al.*, 2001). Elastic modulus measured through tension testing increased at 160 days *postpartum*, unlike other tests were values decreased at this measurement period. These results were difficult to explain.

Measurements of punch force, work to fracture, elastic modulus of the diaphragm and membrane stress were consistently lower for the white line horn when compared to the sole horn. In the white line area of the solear surface of the bovine hoof, Budras *et al.* (1996) reported mean hardness values obtained with the use of the ball-impact method of 5.1 N/mm^2 for the cap-horn area and of 6.9 N/mm^2 for the terminal horn area. These values were significantly lower than the values for the sole horn (12.9 N/mm^2) and for the wall horn (27.5 N/mm^2). The white line consisted of the cap-horn, the laminar horn leaflets and the terminal horn. The cornification of the cap-horn and the laminar horn was found to occur according to hard cornification patterns and the cornification of the terminal horn according to soft cornification patterns. These morphological differences explain the high incidence of alterations in the horn formation has been found at the white line area

(Mulling *et al.*, 1994; Budras *et al.*, 1996). The outer part of the white line, where the soft horn bonds to the harder coronary horn, has been found to be especially susceptible to tearing. A high horn production rate at the abaxial termination of the white line has been found to have a negative influence on the horn quality and could be the explanation for increased number of white line lesions. A high horn production rate has been found to lead to incomplete keratinization and production of softer horn. The areas of high horn production have been found to be also more susceptible to vascular disturbances (Budras *et al.*, 1996).

At 50 days *postpartum* there were no differences between front and hind claws in punch force, work to fracture, elastic modulus of the diaphragm and membrane stress. One of the hind outer claws consistently had the lowest levels for punch force and work to fracture at 100 and 160 days *postpartum* and the highest levels were found in front claws. No explanation was found for the high levels of punch force of the hind right inner claw at 100 days *postpartum*. The lower levels of punch force and work to fracture of the sole and white line horn of hind claws in the *postpartum* period can be related to a higher increase in lesions and alterations of the sole and white line horn of these claws when compared to front claws. Mulling *et al.* (1994) found a high negative correlation between hardness and horn structure and the sites of predilection for occurrence of lesions in the ground surface of the hoof. Schmid (1995) reported significantly higher values of Shore D hardness and tensile strength for the sole, coronary and heel horn of front claws than for the hind claws. Higher values of elastic modulus measured through tension tests were also reported by Zoscher *et al.* (2000) for the front claws (405.5 N/mm) when compared to the hind claws (349.5 N/mm) and results were related to a lower dry matter content of hind claws when compared to front claws. Similarly the dry matter content of hind claws was also lower than dry matter content of front claws in the present experiment. Schmid (1995) reported higher DM content of the coronary (89.74 and 85.27%) and sole (80.20 and 74.97%) horns of front claws when compared to hind claws.

4.5 Conclusions

Biotin supplementation from 25 days *prepartum* until day 160 *postpartum* did not have a significant effect on the locomotion score, lesion score of the white line area, claw angle, claw length and heel height, body condition score, punch resistance, elastic modulus and work to fracture of the sole and white line horns of dairy cows housed in a cubicle system in the *postpartum* period. The lesion score of the sole area of the hind left inner claw at day 160 *postpartum* was significantly lower for biotin supplemented cows when compared to non-supplemented cows.

Chapter 5

Experiment 4 - Structural changes in the hoof before and during the first lactation

5.1 Introduction

Heifer genotype, rearing methods, growth rate, nutrition and the occurrence of foot lesions during the first lactation have been found to affect the occurrence of lameness in later life (Enevoldsen *et al.*, 1991; Livesey *et al.*, 1998; Thomas *et al.*, 1999; Hirst *et al.*, 2002). According to Offer *et al.* (2000) when heifers were subjected to similar environmental and nutritional challenges as multiparous cows, heifers developed more hoof lesions when compared with multiparous cows. The increase in lesion score during lactation was greater for heifers, while cows had greater scores at the beginning of the lactation and higher cumulative scores during the lactation. This confirms the importance of heifer management during first lactation in reducing the occurrence of insults to hoof tissue and thus the reduction of subsequent lameness. Heifers have been found to have similar initial lesion and locomotion scores and have been considered to be a good model for studying lameness during first lactation (Logue *et al*, 1994). However, in terms of horn structure from a group of 20 heifers that had minimal visible solear haemorrhages *prepartum*, eight of these had poor quality horn when hoof horn structure was assessed using electron microscopy (Kempson and Logue, 1993). This poor horn quality was characterized by large areas of cellular destruction, presence of red blood cells and disruption of the pattern of keratinisation and four of the heifers with poor quality horn developed severe solear haemorrhage, while two developed moderate haemorrhage during lactation (Kempson and Logue, 1993). According to these authors the hoof quality was linked to the development of haemorrhages during the first lactation.

The structure and the biomechanical properties of hoof horn have been found to be determined by internal and external factors. The internal factors include; the structure, composition and chemical bonding of keratin proteins, keratin filaments and filament-associated proteins; the structure, composition and amount of the intercellular cementing substance and the architecture of the horn, i.e. the arrangement of horn tubules and intertubular space (Pellman *et al.*, 1993; Zaun, 1997; Mulling *et al.*, 1998; Patan and Budras, 2003). The quality of keratinisation has been found to be dependent on the appropriate supply of nutrients and oxygen. The strength of the keratin proteins was found to be determined by the amount of their disulphide bonds (Mulling *et al.*, 1994). Mulling *et al.* (1994) found a high negative correlation between hardness, horn structure and the sites of predilection for occurrence of lesions in the distal surface of the hoof. Manson (1986) assessed the hardness of different areas of the hoof sole and wall, and found a significant negative correlation between the mid sole hardness and the locomotion score. The hardness of sole, heel and dorsal wall were significantly lower in the affected digits of lame cows when compared to the non-affected digit on the same leg (Tranter *et al.*, 1993). The hardness of the sole and dorsal wall and the moisture content of the sole and heel were also lower in the digits of lame cows when compared with the same digit of non-lame cows (Tranter *et al.*, 1993). Sole lesion score and heel horn erosion were significantly and negatively related to heel horn hardness (Offer *et al.*, 2001). Structurally damaged tissue of the white line had significantly ($P<0.01$) lower tensile strength (2.4 Mpa) when compared with white line tissue that was not damaged (4.5 Mpa) (Hedges *et al.*, 2002). Undamaged wall horn from horse hooves had a tensile strength of 60 N/mm^2 compared with a tensile strength of 20 to 28 N/mm^2 from wall horn with altered intertubular structure (Geyer and Schulze, 1994). This relationship between tissue damage and structural properties indicates the possible use of the measurement of structural strength of hoof tissue as an indicator of factors that increase or reduce the occurrence of hoof lesions and lameness.

The aim of this experiment was to study sole horn lesion formation and the incidence of lameness during the *pre-* and *postpartum* periods of first lactation heifers and to compare these with mechanical testing of the hoof horn and the Holstein UK Index classification for conformation to compare and potentially predict the heifers susceptibility to lameness.

5.2 Material and methods

5.2.1 Experimental design

Twenty Holstein-Friesian first lactation heifers were selected at random from the Seale-Hayne herd. The experiment had a randomised block design, with heifers (n = 20) as replicates and the blocks were the repeated measurements over time. The measurements were taken 40 days *prepartum* and repeated at 50, 100 and 150 days *postpartum*. The experiment was undertaken at the Bradmoors dairy unit of the University of Plymouth, on the Seale-Hayne campus at Newton Abbot in Devon between July 2001 and March 2002.

5.2.2 Housing and management of the experimental animals

The experiment was completed over 260 days and the majority of the heifers calved during the period of August 2002 to October 2002. The heifers were placed with the lactating multiparous cows at pasture one month prior to *parturition* in order to getting used to being handled and entered the milking parlour twice daily, this being a standard procedure on the farm. During this period the animals were kept at pasture until one week *prepartum* when the animals were transferred to a loose straw bedded yard in a separate 'heifer' group away from the multiparous lactating dairy cows throughout the whole lactation. The straw yard consisted of a straw-bedded area and a concrete area where the water troughs and feeding alley were situated. The space allocated for each heifer in the straw-bedded area was 7.5 m^2, which exceeded the area that is recommended in the Code of Recommendations for the welfare of cattle (DEFRA, 2003). The number of water facilities per cow were as recommended by BS 5052, 1990 and all housing conformed to BS 5502: part 40 (BS, 1990).

The passageway where animals consumed forage was cleaned twice a day with a scraper pulled by a tractor. The cows were milked twice daily, at 6 a.m and 3 p.m., through an Alfa-Laval 8 x 8 automatic tandem milking parlour, with automatic animal identification, cluster removal, compound feed allocation and milk recording. All animals were footbathed with formalin (5%) for the treatment and prevention of *Digital dermatitis*, between days 50 and 100 of the lactation during 2 consecutive days.

5.2.2.1 Diet

All the heifers were offered the same total mixed ration (TMR) one week *prepartum* and during the *postpartum* period. The components and composition of the TMR are presented in Table 5.1. The TMR consisted of a mixture of maize silage, brewers grain and a 38 % crude protein (CP) compound. The TMR was replaced once daily at 08.30 am. In addition, at milking, heifers were offered an in-parlour (22 % CP) compound with added bioplex minerals according to yield at 0.4 kg per litre to a maximum of 6 kg (FM/heifer/d). The composition of the components of the total diet (kg) supplemented with compound in parlour is presented in Table 5.2.

Table 5.1 ***Components and composition of the lactating heifer diet***

Components	**Quantity** (kg DM/head/day)
Maize silage	11.5
Compound (38 % CP) plus urea	1.3
Brewers grain	2.6
Minerals	0.08
In parlour dairy concentrate (22 % CP)	≤ 5.2
Composition	
Dry matter (%)	45.3
Metabolisable energy (MJ/kg DM)	22.06
Crude protein (%)	18.1
NDF (g/kg DM)	38.1
Starch + sugar (%)	26.3

Table 5.2 ***Composition of the components of the diet (kg DM) supplemented with compound in parlour***

	Maize silage	Compound (38 % CP)	Brewers grains	Dairy compound
Offered (kg DM/head/day)	11.5	1.3	2.6	<5.2
Dry matter (g/kg FM)	422	862	440	870
Ash (g/kg DM)	42.6	84	80	90
NDF (g/kg DM)	421	225	320	200
Starch (g/kg DM)	301	80	200	160
WSC (g/kg DM)	26.0	70	-	70
ME (MJ / kg DM)	12.0	12.3	11.6	12.9
Oil (g/kg DM)	4	4.5	40	5
Crude protein (g/kg DM)	89.8	436	200	252

5.2.3 Measurements and Records

5.2.3.1 Locomotion score

The heifers were scored weekly for locomotion, using a scoring system with 5 points according to Tranter and Morris (1991) and description of the locomotion scoring was detailed in Experiment 1 (2.2.4.6). Any causes of lameness were investigated and recorded.

5.2.3.2 Hoof lesion score

The heifers had all claws assessed for sole ulcer, sole haemorrhage and heel erosion at 40 days *prepartum* and 50, 100 and 150 days *postpartum*. The lesions on each claw were scored according to Leach *et al.* (1998) as described in Experiment 1 (2.2.4.4).

5.2.3.3 Growth rate and measurements of the hooves

The hoof growth and wear rates were measured on the right front and rear claws. A mark was made on the hoof wall 1 cm below the coronary border and the distance from the coronary border to the mark and from the mark to the distal end of the wall was measured. A new mark was made 1 cm below the coronary border, every time the hooves were measured at 50, 100 and 150 days *postpartum*. A monthly growth and wear rate was estimated from these measurements. The hoof angle and length of the front hoof wall and height of the heel were also measured at the same time using the method described in Experiment 1 (2.2.4.5).

5.2.3.4 Collection of hoof horn samples

The samples of hoof sole tissue were collected from all the claws of all experimental heifers at 40 days *prepartum* and at 50, 100 and 150 days *postpartum*. The samples were taken using a hand held woodplane to reduce the variation in sample thickness. The first outer layer of horn (1 mm) was discarded and a sliver of 0.05 to 0.25mm thickness was placed in a sealed plastic bag and stored in a refrigerator at a temperature of 2 °C until punch resistance tests and tension tests were completed using a texture analyser (Hinterhofer *et al,* 1998). The samples were taken from zones 2, 4 and 5 of the hoof, according to the International Foot Map (Shearer *et al.*, 2002).

The method for punch resistance and tension testing were described in Experiment 1 and 2, respectively. The equations of the large deflection of circular plates used for the calculation of the elastic modulus of the diaphragm and membrane stress in the punch resistance test were described in Experiment 2.

5.2.3.5 Dry matter

Hoof tissue samples were weighed and placed in the oven (100 °C for 72 hrs) for the determination of the dry matter content.

5.2.3.6 Scanning electron microscopy

The samples were kept in sealed plastic bags, frozen and processed within 7 days. The scanning electron microscopy (SEM) was completed on samples of hoof horn, following punch resistance and tension tests, in order to visualise the fracture surface of the tests to identify how the structures of the horn were involved in the fracture process. Two to three samples of the sole and of the white line areas of 5 to 6 different animals were analysed and representative pictures of each area are presented in Figures 5.6 to 5.13. These samples were coated in gold and observed under high vacuum with a JSM-6100 Scanning electron microscope (JOL, Ltd. Akishima, Japan).

5.2.3.7 Live weight and condition score

During the *postpartum* period, live weight and condition score were measured weekly. The same person completed the scoring of condition, using a 5 point scale with half points (Lowman *et al.*, 1976) as described in Experiment 1.

5.2.3.8 Milk yield

Milk yield was recorded automatically twice a day and the mean weekly yield was calculated and used for comparison with locomotion score, HUKI, condition score and live weight data.

5.2.3.9 Holstein UK Index (HUKI)

The heifers were scored once during the first lactation by an independent assessor from the Holstein UK index for the linear type traits stature, chest width, body depth, angularity, rump angle, rump width, rear legs, foot angle, locomotion, fore udder attachment, rear udder height, central ligament, udder depth, teat placement rear view, teat position side view and teat length. The linear type traits were scored using a scale of 1 to 9. Using the linear type traits the composite traits were calculated on a scale of 40 to 100 points and included total body score, total legs and feet score, total dairy score, total mammary score and final score. The final score was calculated through the addition of composite traits, with a different weighting being given for every composite trait as follows: 15% for total body score, 30 % for total legs and feet score, 15 % for total dairy score and 40 % for total mammary score. The scores for the linear type traits are presented in Table 5.3 and the composite traits are presented in Table 5.4.

Table 5.3 ***Scores for the linear type traits used by the Holstein UK breed***

Linear type traits	**Lower scores (1)**	**Higher scores (9)**
Stature	Small (130 cm)	Tall (154 cm)
Chest width	Narrow	Wide
Body depth	Shallow	Deep
Angularity	Coarse	Sharp
Rump angle	High pins	Low pins
Rump width	Narrow pins	Wide pins
Rear legs side view	Straight	Sickled
Foot angle	Low	Steep
Locomotion	Poor	Excellent
Fore udder attachment	Loose	Right
Rear udder height	Very low	Very high
Central udder ligament	Broken	Strong
Udder depth	Below hocks	200 cm above hocks
Teat position, rear view	Outside	Close
Teat position, side view	Close	Wide
Teat length	Short	Long

Table 5.4 ***Scores for composite traits used by the Holstein UK breed***

Score	Description
40 – 64	Poor
65 – 74	Fair
75 – 79	Good
80 – 84	Good plus
85 – 89	Very good
90 - 100	Excellent

5.2.4 Statistical analysis

The experiment was a randomised block design, using individual heifers as replicates. The milk yield, live weight, condition score, locomotion score, hoof measurements, dry matter of the hoof horn, elastic modulus of the tension test, punch force and work to fracture data were all normally distributed. The lesion score, elastic modulus of the diaphragm and membrane stress data were normalised using log 10 transformation before analysis. All the parameters were compared by observation period, using analysis of variance (ANOVA), general linear model command (Minitab 12.0). A series of ANOVAs were used to compare differences of lesion score, punch force, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus between claws. The thickness of each sample was used as a covariate when punch force and work to fracture data were analysed (ANCOVA). The comparison of means was completed using the Tukeys test. The difference between the sole and white line areas of the horn was compared using a paired-t test. Regression analysis was used to test the effect of days *postpartum* on the locomotion score, total lesion score of the sole and white line area of the horn and the effect of sample thickness on the punch force of the sole and white line areas of the horn. Correlation analysis was used to assess relationships between variables using Pearsons correlation. Multivariate stepwise regression was used to estimate which parameters influenced the variation in lesion score, punch force, elastic modulus and membrane stress data.

5.3 Results

5.3.1 Locomotion score

5.3.1.1 Locomotion score in the postpartum period

The locomotion score was found to increase significantly ($P<0.001$) from day 7 up until day 120 of the *postpartum* period and subsequently decreased after day 150 *postpartum*. The quadratic regression equation of the mean locomotion score and days in the *peripartum* period is presented in Figure 5.1.

Locomotion score = -8×10^{-5} days^2 + 0.0212 days + 0.9759, R^2adj. = 0.80, $P<0.001$

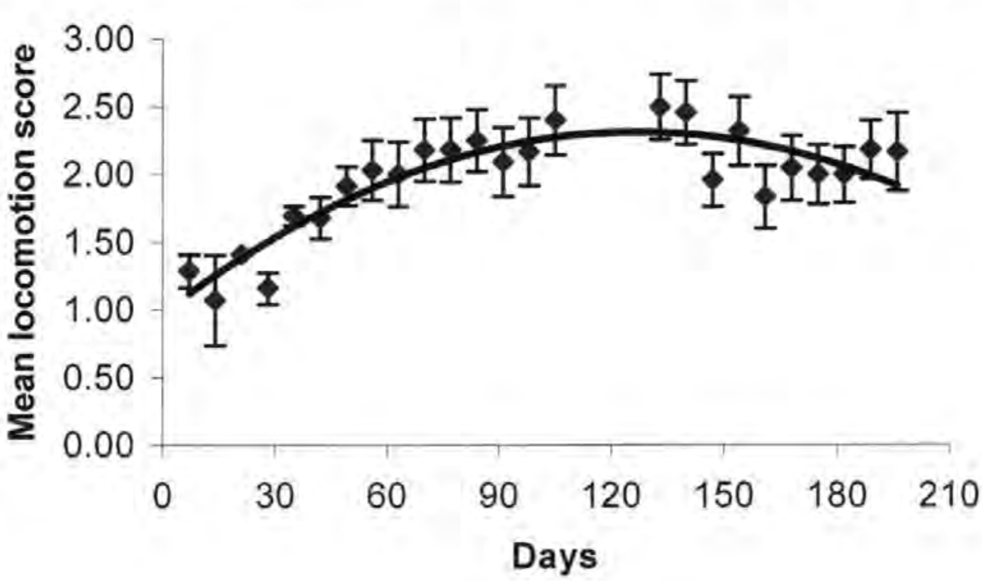


Figure 5.1 ***Mean locomotion score of primiparous dairy cattle in the postpartum period***

5.3.1.2 Lameness incidence and factors that predispose heifers to lameness

The lameness incidence and the incidence of the factors that were associated with lameness during the *peripartum* period are presented in Table 5.5. Lame animals were considered animals that had a locomotion score greater than 3.0.

Table 5.5 ***Lameness incidence and incidence of the factors that were associated with lameness during the peripartum period***

	Days peripartum					
	-40	50	100	150	sem	*P*
Lameness > 3 incidence (%)	0.05^{b}	0.35^{ab}	0.45^{a}	0.45^{a}	0.097	0.05
Lameness > 4 incidence (%)	0.00	0.15	0.25	0.10	0.070	NS
Claw horn haemorrhage sole (%)	0.14	0.48	0.48	0.29	0.102	NS
Severe claw horn haemorrhage sole (%)	0.00	0.05	0.14	0.14	0.060	NS
Claw horn haemorrhage white line (%)	0.29	0.33	0.38	0.19	0.101	NS
Severe claw horn haemorrhage white line (%)	0.00	0.05	0.00	0.05	0.034	NS
Digital dermatitis (%)	0.00^{b}	0.10^{ab}	0.25^{a}	0.05^{ab}	0.063	0.05
Heel erosion (%)	0.04	0.08	0.09	0.03	0.033	NS
White line separation (%)	0.06^{a}	0.00^{b}	0.01^{ab}	0.03^{ab}	0.123	0.01
White line crack, perpendicular (%)	0.00^{b}	0.04^{b}	0.01^{b}	0.33^{a}	0.021	0.001
Stone puncture (%)	0.03^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.007	0.01
Swollen hock (%)	0.00	0.01	0.00	0.00	0.004	NS

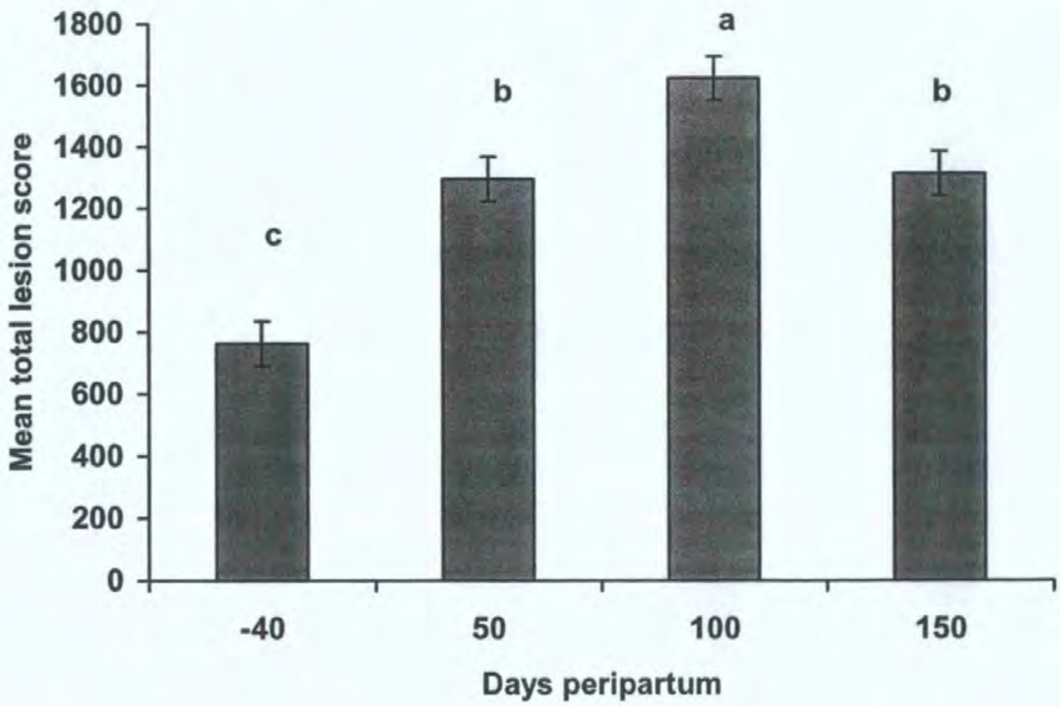
$^{a, b}$ – different letters in the same row indicate values that differ significantly, NS – not significantly different

One animal in the group was severely affected by *Digital dermatitis* and became severely lame (locomotion score 4) while all the other cases of lameness were mild to moderate (locomotion score 2 and 3).

5.3.2 Hoof horn lesion score

5.3.2.1 Hoof horn lesion score in the peripartum period

Hoof lesion score was calculated separately for the sole and white line areas of each claw of each hoof. A total score was determined for each animal. The mean total lesion score of the sole and white line areas was found to increase significantly ($P<0.001$) from the *prepartum* to the *postpartum* period and during the *postpartum* period. This increase occurred up to day 100 of the *postpartum* period for the white line area and the sole area. The mean total lesion score of the sole area and the white line area compared between measurement periods through ANOVA are presented in Figure 5.3 and 5.5 respectively. When analysed through regression analysis the lesion score of the sole and white line areas increased significantly and quadratically in the *peripartum* period (Figure 5.4 and 5.6, respectively).



a, b,c – different letters indicate values that differ significantly, P<0.001

Figure 5.3 ***Mean total lesion score of the sole area in the peripartum period***

Total lesion score of the sole horn = 957.144 + 8.64691 day - 0.0317169 day^2, R^2adj. = 0.52, $P< 0.001$

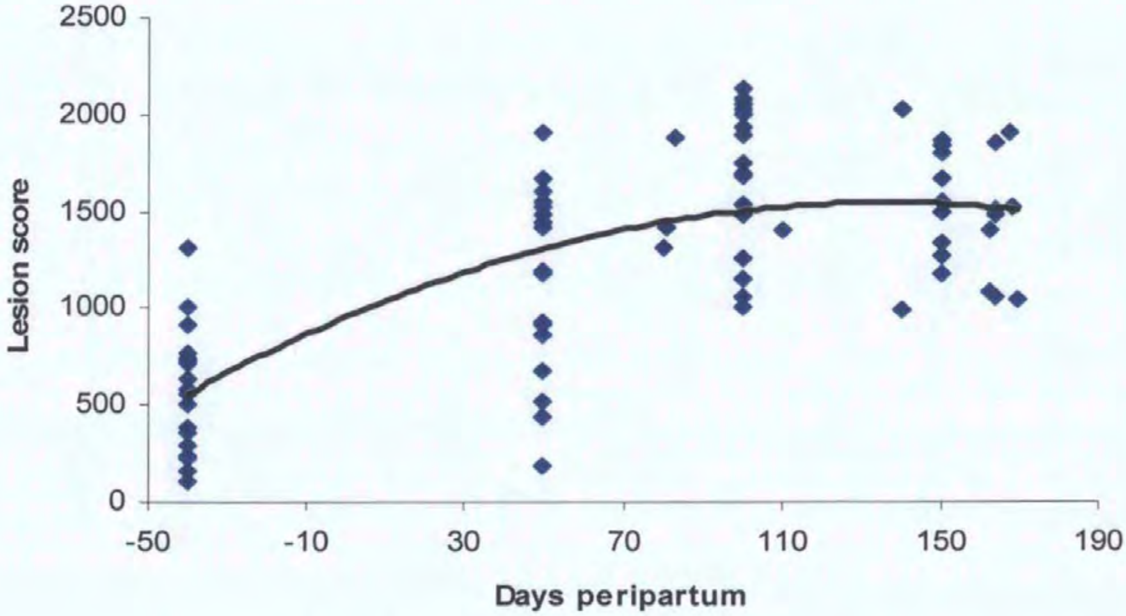
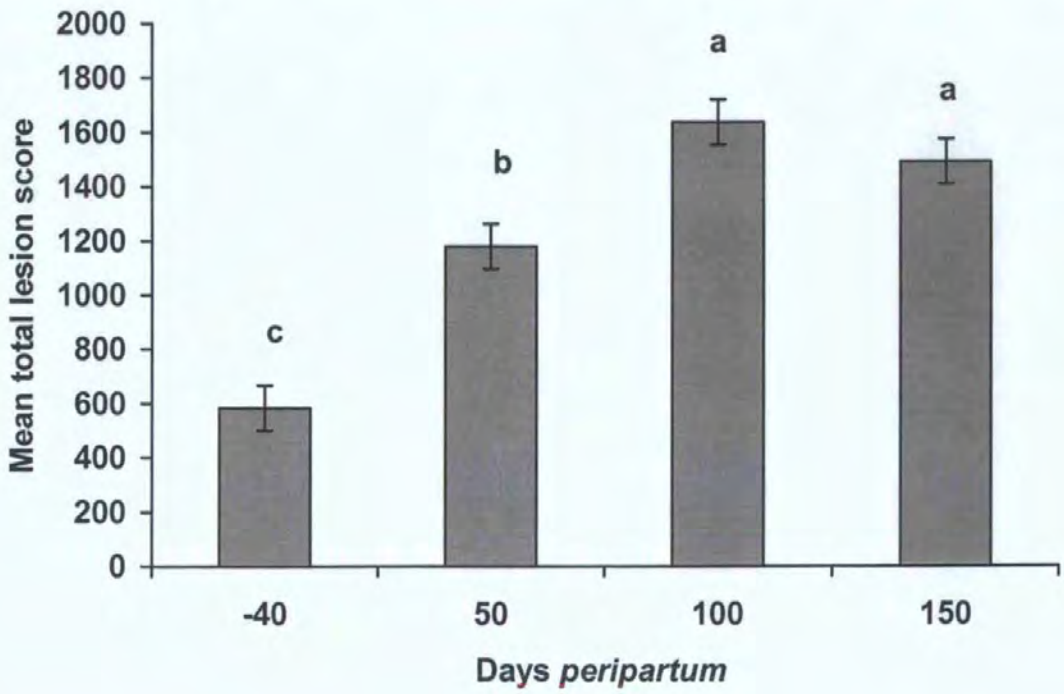


Figure 5.4 ***Total lesion score of the sole area in the peripartum period***



a, b,c – different letters indicate values that differ significantly, $P<0.001$

Figure 5.5 ***Mean total lesion score of the white line area during the peripartum period***

Total lesion score of the white line horn = 1156.34 + 8.25110 day - 0.0453115 day^2, $R^2adj.$ = 0.52, $P< 0.001$

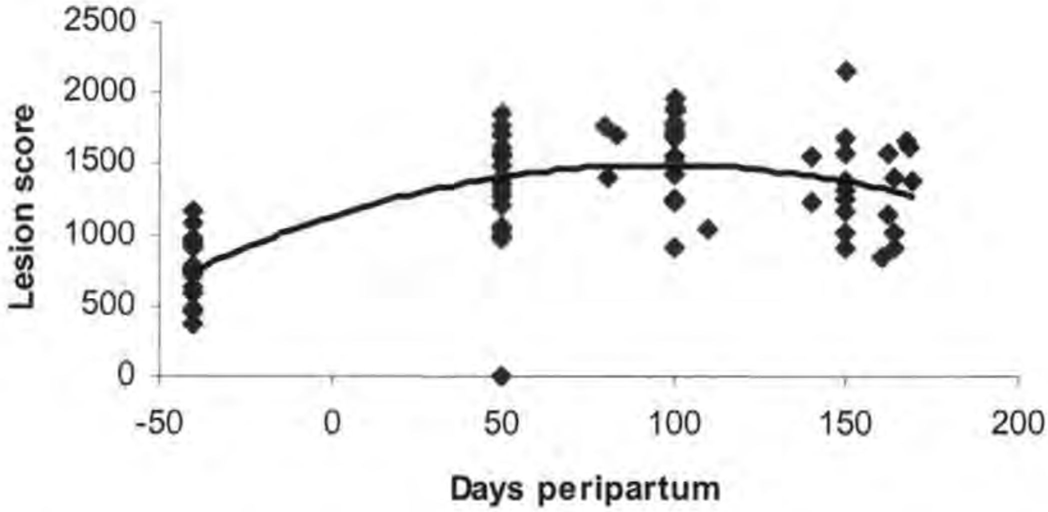


Figure 5.6 ***Total lesion score of the white line area during the peripartum period***

Mean total sole and white line lesion scores were significantly (P<0.001) and positively correlated at days -40, 50, 100 and 150 of the *peripartum*, the highest correlation factors were found at days 100 and 150 *postpartum* (R = 0.57).

5.3.2.2 Hoof horn lesion score of different claws

The mean lesion score of the sole and the white line areas for each claw, in the *peripartum* period analysed through ANOVA are presented in Tables 5.6 and 5.7, respectively. At days -40, 50, 100 and 150 *peripartum* for the sole area and days 50, 100 and 150 *peripartum* for the white line area significant differences were found between the mean lesion score of different claws. The mean lesion scores of front and hind claws were significantly different ($P<0.001$), with hind claws consistently having the highest scores and front claws the lowest scores. During the *prepartum* period no significant differences were measured between the lesion scores of the white line area of the different claws and the sole area of the hind outer claw presented significantly higher ($P<0.05$) lesion scores compared with the hind right inner and front right inner claws. During the postpartum period the hind left inner claw had the significantly ($P<0.001$) highest lesion scores of the sole area of the horn and the lowest scores were of the sole area were found on the front claws and hind right inner claws. The increase of lesion scores of the sole area between the *prepartum* period and day 150 *postpartum* was significantly ($P<0.001$) greater for the hind left claws when compared with the front left inner and front right outer claw. The lesion scores of the white line area were significantly ($P<0.001$) greater for the hind left outer claw at days 50 and 100 *postpartum* when compared to the front left outer and front right claws at day 50 *postpartum* and to the front right and hind right inner claws at day 100 *postpartum*. At day 150 postpartum the hind claws had significantly ($P<0.001$) greater lesion scores of the white line area when compared with the front right claws. The increase in lesion score of the white line area between the *prepartum* period and day 100 *postpartum* was significantly ($P<0.05$) greater for the hind left outer claw when compared with the front right outer claw. The increase in lesion score of the white line area between the *prepartum* period and day 150 *postpartum* was significantly ($P<0.001$) greater for the hind left outer claw when compared with the front claws.

Table 5.6 ***Mean lesion score of the sole area of hind right and left and front right and left inner and outer claws at days –40, 50, 100 and 150 peripartum and change in lesion scores between days 150 and –40 peripartum***

	Hind				Front					
	Right		Left		Left		Right			
Days *peripartum*	inner	outer	inner	outer	inner	outer	inner	outer	sem	*P*
- 40 days	49.2 [b]	99.8 [a]	92.1 [ab]	59.0 [ab]	89.9 [ab]	72.4 [ab]	43.8 [b]	78.2 [ab]	11.8	0.05
50 days	132.4 [bc]	202.9 [ab]	216.0 [a]	150.0 [abc]	133.5 [bc]	122.4 [bc]	125.5 [bc]	97.6 [c]	18.3	0.001
100 days	202.0 [bc]	245.5 [ab]	285.5 [a]	236.5 [ab]	179.1 [bc]	171.0 [c]	157.3 [c]	160.0 [c]	15.8	0.001
150 days	181.5 [abc]	235.8 [ab]	243.7 [a]	234.0 [ab]	146.5 [c]	173.4 [bc]	158.0 [c]	119.4 [c]	14.4	0.001
Change -40 to 150	132.3 [ab]	135.9 [ab]	151.6 [a]	175.0 [a]	56.6 [bc]	101.0 [abc]	114.2 [abc]	41.2 [c]	19.3	0.001

[a, b, c] – different letters in the same row indicate values that differ significantly

Table 5.7 ***Mean lesion score of the white line area of hind right and left, front right and left inner and outer claws at days –40, 50, 100 and 150 peripartum and change in lesion scores between days 150 and –40 and days – 40 and 100 peripartum***

	Hind				Front					
	Right		Left		Left		Right			
Days *peripartum*	inner	outer	inner	outer	inner	outer	inner	outer	sem	*P*
-40 days	87.0	95.7	94.1	90.2	103.6	107.4	85.6	100.6	9.6	NS
50 days	186.4 [abc]	192.9 [abc]	200.2 [ab]	213.3 [a]	154.3 [abc]	130.8 [c]	145.9 [bc]	139.5 [bc]	15.4	0.001
100 days	181.0 [b]	223.1 [ab]	204.2 [ab]	242.9 [a]	207.2 [ab]	202.2 [ab]	184.4 [b]	178.2 [b]	13.0	0.01
150 days	180.3 [a]	187.1 [a]	197.5 [a]	222.9 [a]	148.7 [ab]	140.8 [ab]	119.7 [b]	116.3 [b]	14.0	0.001
Change -40 to 100	94.1 [ab]	127.4 [ab]	114.0 [ab]	148.8 [a]	99.9 [ab]	98.7 [ab]	98.9 [ab]	77.6 [b]	15.3	0.05
Change -40 to 150	93.3 [abc]	91.4 [abc]	107.3 [ab]	128.8 [a]	41.3 [bcd]	37.2 [bcd]	34.1 [cd]	16.3 [d]	16.2	0.001

[a, b, c, d] – different letters in the same row indicate values that differ significantly,
NS – not significantly different

When testing through multiple regression analysis for the predictors that had a significant influence over the lesion score of the sole and white line areas of the horn non of the variables; milk yield corrected for 305 days, cow, condition score day 100 *postpartum,* change in weight from day 21 to 100 *postpartum,* HUKI final score and legs and feet score, heel height, claw length and claw angle at day 50 *postpartum*, were found to be significant.

5.3.2.3 Relationship between the locomotion score and lesion score on different days of the peripartum period

In Tables 5.8 and 5.9 the locomotion score of the animals at days –21, 14, 49, 91, 154 and 189 *postpartum* are compared with the mean lesion scores of the white line (Table 5.8) and sole areas (Table 5.9) of each claw on days –40, 50, 100 and 150 *postpartum*.

Table 5.8 ***Significant correlations (r) between the mean locomotion score at days –21, 14, 49, 91, 154 and 189 and the mean lesion score of the white line area of hind right and left and front right and left inner and outer claws at days –40, 50, 100 and 150 peripartum***

Locomotion at Day peripartum	Lesion score Claw	 day	*P*	r
91	Front left inner	50	0.05	0.45
91	Hind left outer	100	0.05	0.44
154	Total score	50	0.001	0.70
154	Front left inner	50	0.05	0.49
154	Front right inner	50	0.05	0.52
154	Hind left inner	50	0.05	0.50
189	Total score	50	0.05	0.46
189	Front right inner	50	0.05	0.48

Note: all *prepartum* correlations were NS

The locomotion scores measured at days 91, 154 and 189 *postpartum* increased significantly (P<0.05) when the lesion score of the of the white line area of the front inner claws measured at day 50 *postpartum* increased. The locomotion scores measured at days 154 and 189 increased significantly (P<0.05) when the total lesion score of the animal of the white line area measured at day 50 *postpartum* increased. The locomotion scores measured at day 91 increased significantly (P<0.05) when the lesion score of the hind left outer claw increased during the same period and the locomotion scores measured at day 154 increased significantly (P<0.05) when the lesion score of the hind left inner claw increased at day 50 *postpartum.*

Table 5.9 ***Significant correlations (r) between the mean locomotion score at days –21, 14, 49, 91, 154 and 189 and the mean lesion score of the sole area of hind right and left and front right and left inner and outer claws at days –40, 50, 100 and 150 peripartum***

Locomotion Day peripartum	Lesion score Claw	 day	*P*	r
-21	Front inner	50	0.05	0.48
14	Front inner	-21	0.05	0.53
49	Total score	50	0.05	0.46
49	Front left	50	0.05	0.51
49	Front left inner	50	0.05	0.53
49	Hind left inner	50	0.05	0.55
49	Hind left	50	0.05	0.53
49	Hind left	150	0.05	0.45
49	Hind left outer	150	0.05	0.50
91	Hind left	100	0.05	0.55
91	Front left	100	0.05	0.51
91	Total score	100	0.05	0.51
91	Hind left outer	100	0.05	0.54
91	Front left inner	100	0.05	0.50
189	Hind left outer	50	0.05	0.45
189	Front right inner	100	0.05	0.45

The lesion score of the sole area of the front inner claws in the *prepartum* period and at day 50 *postpartum* were significantly correlated to the locomotion scores measured in the *prepartum* period and early *postpartum* period. The locomotion score measured at day 49 *postpartum* increased significantly ($P<0.05$) when the total lesion score of the animal, the lesion score for the front left and hind left claws for of the sole area measured in the same period increased and when the lesion score of the hind left claws measured at day 150 *postpartum* increased. The locomotion score measured at day 91 *postpartum* increased significantly ($P<0.05$) when the total lesion score of the sole area of the animal and the lesion score of the sole area of the hind left and front left claws measured in the same period increased. Locomotion score measured at day 189 *postpartum* increased significantly ($P<0.05$) when the lesion score of the sole area of the hind left outer claw measured at day 50 *postpartum* and the lesion score for the sole area of the front right inner claw measured at day 100 *postpartum* increased.

5.3.3 Measurement of hoof growth and wear rates, angle and length of the front hoof wall and height of the heel

5.3.3.1 Measurements of the hooves during the postpartum period

The mean measurements of hoof angle, length of the front wall, height of the side wall and height of the heel of the front and hind outer claws at 50, 100 and 150 days *postpartum* when analysed through ANOVA are presented in Table 5.10. The height of the heel of front and hind claws and the angle of the hind claw increased significantly ($P<0.05$ and 0.001) between day 50 and 150 *postpartum*. No significant differences were found in the length of the front wall and height of the side wall for front and hind claws.

Table 5.10 ***Hoof angle, length of the front wall, height of the side wall and height of the heel of the front and hind outer claws at 50, 100 and 150 days postpartum***

Days *postpartum*	50	100	150	sem	*P*
Length front wall, front claw (cm)	8.05	7.83	8.04	0.125	NS
Length front wall, hind claw (cm)	7.89	7.87	7.89	0.142	NS
Height side wall, front claw (cm)	7.82	8.14	8.29	0.154	NS
Height side wall, hind claw (cm)	7.71	7.90	7.78	0.147	NS
Height heel, front claw (cm)	5.28 [b]	5.90 [a]	6.35 [a]	0.170	0.001
Height heel, hind claw (cm)	4.47 [b]	4.80 [ab]	4.82 [a]	0.111	0.05
Hoof angle, front claw (degrees)	48.84	50.94	51.82	0.885	NS
Hoof angle, hind claw (degrees)	46.63 [b]	48.05 [ab]	50.30 [a]	0.971	0.05

[a, b] – different letters in the same row indicate values that differ significantly,
NS – not significantly different

Monthly hoof growth and wear of front and hind claws between 50 to 100 and 100 to 150 days *postpartum* are presented in Table 5.11.

5.3.3.2 Differences in the measurements between front and hind claws

The front claws had significantly (P<0.001) higher height of the heel at 50 (front – 5.28, hind – 4.47), 100 (front – 5.90, hind – 4.80 mm) and 150 (front – 6.355, hind – 4.82 mm) days *postpartum*, significantly higher (P<0.01) hoof angle at 50 day *postpartum* (front – 49.85, hind – 46.63 mm) and significantly (P<0.05) higher height of the side wall at 150 day *postpartum* (front – 8.29, hind – 7.78 mm) compared with hind claws. There was no difference in the monthly growth rate of front and hind claws, however, wear rates were significantly (P<0.05) higher for the hind claws between 50 to 100 (front - 0.44, hind –

0.58 cm/month) and 100 to 150 (front - 0.56, hind – 0.74 cm/month) days *postpartum* and consequently the monthly net growth rate was lower between 50 to 100 (front - 0.14, hind – 0.02 cm/month) and 100 to 150 (front - 0.11, hind – -0.08 cm/month) days *postpartum* for the hind claws when compared with front claws.

Table 5.11 ***Monthly hoof growth and wear of front and hind claws between days 50 and 100 and 100 and 150 postpartum***

Period	50 to 100 days	100 to 150 days	sem	*P*
Growth front (cm/month)	0.58	0.67	0.049	NS
Wear front (cm/month)	0.44	0.56	0.077	NS
Net growth front (cm/month)	0.14	0.11	0.064	NS
Growth hind (cm/month)	0.60	0.66	0.038	NS
Wear hind (cm/month)	0.58	0.74	0.075	NS
Net growth hind (cm/month)	0.02	- 0.08	0.058	NS

NS – not significantly different

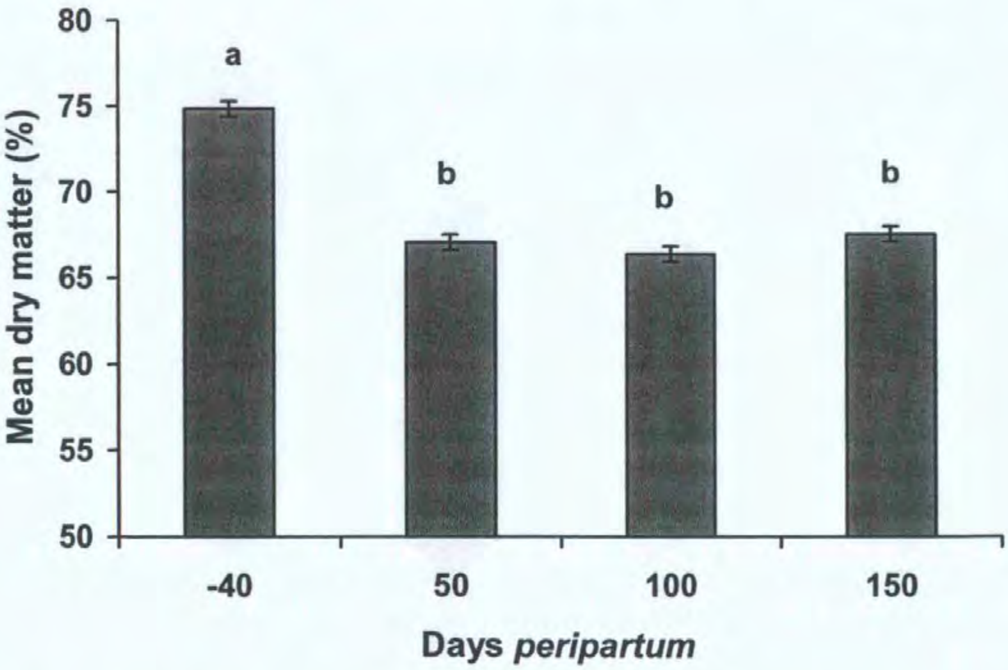
5.3.3.3 Correlations between hoof measurements, locomotion score and lesion score

The length of the hind claws at 150 days *postpartum* was significantly ($P<0.01$) and positively correlated to the lesion score of the white line area in the same period (r = 0.60). The height of the side wall of the front claws at 100 days *postpartum* was significantly ($P<0.05$) and positively correlated to the locomotion score in the same period (r = 0.48) and to the lesion score of the white line at 150 days *postpartum* (r = 0.51). The height of the side wall of the hind claws at 150 days *postpartum* was significantly ($P<0.01$) and positively correlated to the lesion score of the white line at the same period (r = 0.56) and to the locomotion score at 189 days *postpartum* (r = 0.52). The height of the heel of the front claws at 150 days *postpartum* was significantly ($P<0.05$) and positively correlated to the locomotion score in the same period (r = 0.45). The foot angles of front and hind claws were significantly ($P<0.05$) and positively correlated to the locomotion score at 14 days *postpartum* (r = 0.45 and 0.60, respectively).

5.3.4 Dry matter of the hoof horn

5.3.4.1 Dry matter of the hoof horn in the peripartum period

The mean dry matter of the hooves of each animal decreased significantly ($P< 0.001$) from the *prepartum* (-40 days) period until 50 days *postpartum*. There were no significant differences in the dry matter content of the hoof tissue between 50, 100 and 150 days *postpartum*. The mean dry matter content of the hoof tissue at days – 40, 50, 100 and 150 *peripartum* are presented in Figure 5.7.



a,b – different letters indicate values that differ significantly, $P < 0.001$

Figure 5.7 ***Mean dry matter content (%) of the hoof horn of heifers on days –40, 50, 100 and 150 of the peripartum period***

5.3.5 Punch force, work to fracture, elastic modulus of the diaphragm, membrane stress of the punch test and elastic modulus of the tension test

Using multivariate stepwise regression analysis to test for the factors that had a significant effect on the punch force of the sole horn, sample thickness, dry matter of the sample, and days *peripartum* had a significant (P<0.001 and <0.01) effect, while cow and haemorrhage level of the sample did not have a significant effect. The regression equation for punch force of the sole area of the horn was:

Punch force of the sole area (N) = - 10.5 + 0.225 DM (%) + 0.0180 days + 23.8 thickness (mm) , R^2adj. = 0.60, P <0.001.

The regression equation between punch force of the sole horn and sample thickness is presented in Figure 5.8. Sample thickness was used as a covariate when punch force data were analysed using ANCOVA.

Punch force of the sole horn (N) = 1.70539 + 97.1141 thickness (mm) - 261.762 thickness^2 (mm), R^2adj. = 0.40, P <0.001.

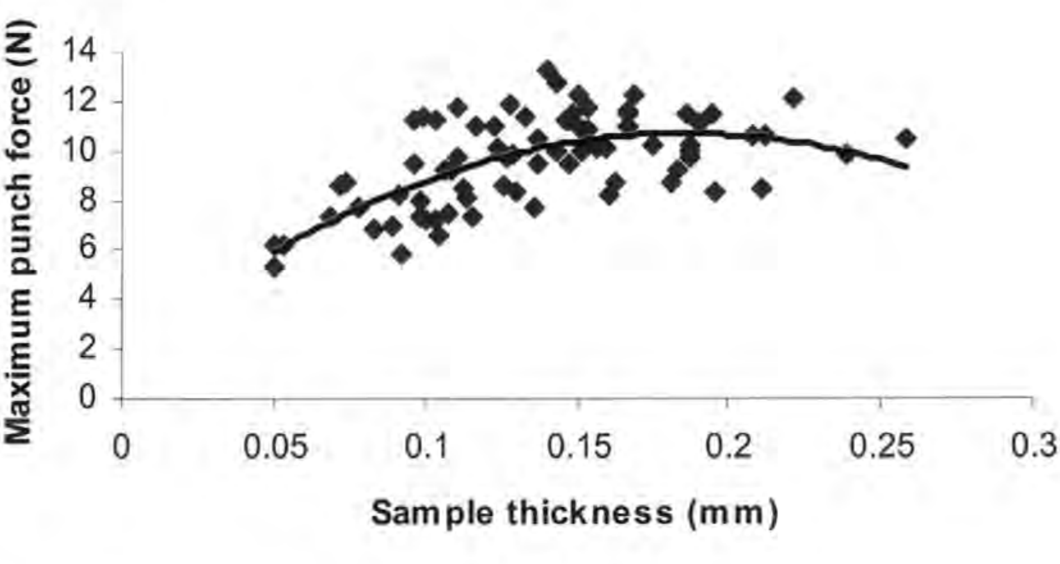


Figure 5.8 ***Effect of sample thickness on punch force of the sole horn***

The factors that had a significant ($P<0.001$) effect on the elastic modulus of the diaphragm of the sole horn when using multiple regression analysis were, the dry matter of the sample, and days *peripartum,* while thickness, cow and haemorrhage level of the sample did not have a significant effect. The regression equation for elastic modulus of the diaphragm of the sole horn was:

Elastic modulus of the diaphragm of the sole horn (N/mm^2) = - 69.4 + 1.16 Dm (%) + 0.0900 days , R^2adj. = 0.47, $P<0.001$.

The factors that had a significant effect on the membrane stress of the sole horn (using multiple regression analysis), thickness, dry matter of the sample, and days *peripartum* did have a significant ($P<0.001$) effect, while cow and haemorrhage level of the sample did not have a significant effect. The regression equation for membrane stress of the sole horn was:

Membrane stress of the sole horn (N/mm^2) = - 12600 + 243 DM (%) + 16.2 days - 22350 thickness (mm), R^2adj. = 0.49, $P<0.001$.

Using multiple regression analysis to test the factors that had a significant effect on the punch force of the white line area of the horn, thickness, dry matter of the sample, days *peripartum* and haemorrhage level of the sample had a significant ($P<0.001$ and <0.01) effect while cow did not have a significant effect. The regression equation for punch force of the white line area was:

Punch force of the white line area (N) = - 11.6 + 0.217 Dm (%) + 0.00543 days + 18.0 thickness (mm) - 0.604 haemorrhage level (1 to 5), R^2adj. = 0.32, $P<0.001$.

The factors that had a significant effect (P<0.001) on the elastic modulus of the diaphragm of the white line horn when using multiple regression analysis were: thickness, dry matter of the sample, haemorrhage level of the sample and days *peripartum* , while cow did not have a significant effect. The regression equation for elastic modulus of the diaphragm of the white line area was:

Elastic modulus of the diaphragm of the white line area (N/mm^2) = - 61.6 + 1.21 DM (%) - 46.5 thickness (mm) - 2.42 haemorrhage level (1 to 5) + 0.0494 days, R^2adj. = 0.43, P <0.001.

When testing through multiple regression analysis for the factors that had a significant effect on the membrane stress of the white line horn, sample thickness was the only factor found to be significant. The regression equation for membrane stress of the white line area was:

Membrane stress (N/mm^2) = 3383 - 15007 thickness (mm), R^2adj. = 0.35, P <0.001.

5.3.5.1 Mechanical tests in the peripartum period

The mean punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the punch test and mean elastic modulus of the tension test of the sole and white line areas of the horn of the heifers at -40, 50, 100 and 150 days *peripartum* when analysed through ANCOVA are presented in Table 5.12. The mean punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas and mean elastic modulus of the tension test of the sole area changed significantly during the *peripartum* period. The days of measurement in the *peripartum* period had no significant effect on mean elastic modulus of the tension test of the white line area. The punch force, elastic modulus of the diaphragm and of the tension test of the sole area increased significantly at 100 and 150 days *postpartum*. The work to fracture and membrane stress of the sole area of the horn and punch force and the work to fracture, elastic modulus of the diaphragm and membrane stress of the white line area decreased significantly between 40 days *prepartum* and 50 and 100 days *postpartum*, but was not significantly different at 150 days *postpartum* when compared to the *prepartum* period.

The punch force and work to fracture were significantly ($P<0.001$) lower for the white line area of the horn during all measurement periods when compared with the sole area. The elastic modulus of the diaphragm and membrane stress of the white line area of the horn were significantly ($P<0.001$) lower at 150 days *postpartum* compared with the sole area.

5.3.5.2 Differences of the mechanical tests between claws

There were no significant differences for the punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the sole and white line areas of the horn and mean elastic modulus of the tension test between front inner and outer and hind inner and outer claws at any of the test periods. As a consequence, only differences between front and hind claws were presented.

The mean punch force, work to fracture, elastic modulus of the diaphragm and membrane stress of the punch test and mean elastic modulus of the tension test of the sole and white line areas of the horn of the hind and front inner and outer claws of the heifers at -40, 50, 100 and 150 days *peripartum* analysed through ANCOVA are presented in Table 5.13, 5.14, 5.15 and 5.16, respectively. There were no significant differences between claws for any of the measured parameters at 40 days *prepartum*.

Table 5.12 ***Punch force, work to fracture, elastic modulus of diaphragm, membrane stress, elastic modulus of tension test of the sole and white line areas of the horn and dry matter at days –40, 50, 100 and 150 of the peripartum period***

	Days *peripartum*					
	- 40	50	100	150	sem	*P*
Sole horn						
Punch force (N)	8.68 ^{b}	8.60 ^{b}	10.57 ^{a}	10.74 ^{a}	0.29	0.001
Work to fracture (kJ)	12.72 ^{a}	10.47 ^{b}	12.69 ^{a}	12.00 ^{a}	0.32	0.001
Elastic modulus of the diaphragm (N/mm^2)	13.43 ^{b}	13.86 ^{b}	16.33 ^{b}	22.61 ^{a}	1.17	0.001
Membrane stress (N/mm^2)	3011.7 ^{a}	751.3 ^{b}	1397.3 ^{b}	3101.8 ^{a}	296.0	0.001
Elastic modulus of the tension test (N/mm^2)	88.76 ^{b}	71.49 ^{b}	97.96 ^{b}	151.60 ^{a}	9.80	0.001
White line horn						
Punch force (N)	6.92 ^{a}	5.24 ^{c}	5.88 ^{bc}	6.24 ^{ab}	0.23	0.001
Work to fracture (kJ)	8.29 ^{a}	6.17 ^{b}	6.70 ^{b}	6.88 ^{b}	0.31	0.001
Elastic modulus of the diaphragm (N/mm^2)	19.86 ^{a}	9.62 ^{b}	12.18 ^{b}	17.76 ^{a}	1.38	0.001
Membrane stress (N/mm^2)	1806.6 ^{a}	232.0 ^{b}	453.2 ^{b}	1300.5 ^{a}	257.4	0.001
Elastic modulus of the tension test (N/mm^2)	91.32	77.78	78.62	104.12	10.25	NS
Dry matter content of the horn (%)	74.81 ^{a}	67.07 ^{b}	66.37 ^{b}	67.55 ^{b}	0.47	0.001

$^{a, b, c}$ – different letters in the same row indicate values that differ significantly, NS – not significantly different

At 50 days *postpartum* the front claws had significantly ($P<0.001$) higher levels of punch force and work to fracture of the white line area and dry matter content when compared with the hind claws. The punch force of the sole horn of the front right claw was significantly ($P<0.05$) higher when compared with the hind right claw. No significant differences were measured between the work to fracture, membrane stress and elastic modulus measured through the tension test of the different claws. At 100 days *postpartum* the punch force of the white line area of the horn of the front right claw was significantly higher when compared with the hind left claw. There were no significant differences of the punch force, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus of the tension test of the sole horn and work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus of the tension test of the white line area between front and hind claws. At 150 days *postpartum* the punch force of the white line area was significantly ($P<0.05$) higher on the front left claws when compared with the hind claws. The work to fracture of the white line area of the horn was significantly ($P<0.05$) higher on the hind right claws compared with the hind left claws. The elastic modulus of the diaphragm of the white line area of the horn was significantly ($P<0.05$) higher on the front left claws when compared with the hind right claws. There were no significant differences between front and hind claws when the punch force, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus of the tension test of the sole area and membrane stress of the white line area were tested.

Table 5.13 ***Punch force, work to fracture, elastic modulus of diaphragm, membrane stress, elastic modulus of tension test of the sole and white line horn and dry matter of the hind right, hind left, front right and front left claws at 40 days prepartum***

	Hind		Front			
	Right	Left	Right	Left	sem	Sig.
Sole area of the horn						
Punch force (N)	7.36	8.04	7.99	8.32	0.38	NS
Work to fracture (kJ)	11.47	12.51	12.07	12.83	0.58	NS
Elastic modulus of the diaphragm (N/mm^2)	11.89	14.42	14.33	13.62	1.95	NS
Membrane stress (N/mm^2)	2431.8	3519.9	3026.7	2835.9	751.2	NS
Elastic modulus of the tension (N/mm^2)	86.80	-	92.78	-	17.0	NS
White line area of the horn						
Punch force (N)	6.38	5.51	6.72	5.78	0.48	NS
Work to fracture (kJ)	7.52	6.22	8.48	6.92	0.61	NS
Elastic modulus of the diaphragm (N/mm^2)	20.32	25.44	17.88	20.15	3.25	NS
Membrane stress (N/mm^2)	2381.8	5204.2	1529.9	2196.9	1250.0	NS
Dry matter content of the horn (%)	75.06	-	74.46	-	0.44	NS

NS – not significantly different

Table 5.14 ***Punch force, work to fracture, elastic modulus of diaphragm, membrane stress, elastic modulus of tension test of the sole and white line areas of the horn and dry matter of the hind right, hind left, front right and front left claws at 50 days postpartum***

	Hind		Front			
	Right	Left	Left	Right	sem	P
Sole area of the horn						
Punch force (N)	8.61 [b]	9.26 [ab]	9.29 [ab]	9.62 [a]	0.24	0.05
Work to fracture (kJ)	10.68	10.50	10.88	11.55	0.29	NS
Elastic modulus of the diaphragm (N/mm^2)	12.97 [b]	18.90 [a]	17.38 [ab]	17.08 [ab]	1.64	0.05
Membrane stress (N/mm^2)	1339	2530	2584	1474	566.4	NS
Elastic modulus of the tension (N/mm^2)	74.57	63.18	78.79	70.72	11.59	NS
White line area of the horn						
Punch force (N)	5.31 [b]	5.08 [b]	6.07 [a]	6.13 [a]	0.20	0.001
Work to fracture (kJ)	6.16 [b]	5.75 [b]	7.24 [a]	7.17 [a]	0.30	0.001
Elastic modulus of the diaphragm (N/mm^2)	9.94	10.00	12.05	11.91	0.95	NS
Membrane stress (N/mm^2)	386	601	437	528	153.1	NS
Dry matter content of the horn (%)	66.27 [b]	-	67.86 [a]	-	0.43	0.05

[a, b] – different letters in the same row indicate values that differ significantly, NS – not significantly different

Table 5.15 ***Punch force, work to fracture, elastic modulus of diaphragm, membrane stress, elastic modulus of tension test of the sole and white line areas of the horn and dry matter of the hind right, hind left, front right and front left claws at 100 days postpartum***

	Hind		Front			
	Right	Left	Left	Right	sem	*P*
Sole area of the horn						
Punch force (N)	10.38	10.13	11.09	11.04	0.34	NS
Work to fracture (kJ)	12.71	11.80	13.37	13.31	0.46	NS
Elastic modulus of the diaphragm (N/mm^2)	17.36	19.51	17.50	20.62	1.66	NS
Membrane stress (N/mm^2)	2884	3834	1900	3603	805.4	NS
Elastic modulus of the tension (N/mm^2)	87.34	115.17	101.09	91.25	12.73	NS
White line area of the horn						
Punch force (N)	5.91 [ab]	5.46 [b]	6.28 [ab]	6.57 [a]	0.24	0.01
Work to fracture (kJ)	6.56	6.20	7.10	7.34	0.34	NS
Elastic modulus of the diaphragm (N/mm^2)	13.78	12.49	14.80	16.33	1.21	NS
Membrane stress (N/mm^2)	692	674	1123	1032	271.8	NS
Dry matter content of the horn (%)	66.17	-	66.56	-	0.44	NS

[a, b] – different letters in the same row indicate values that differ significantly, NS – not significantly different

Table 5.16 ***Punch force, work to fracture, elastic modulus of diaphragm, membrane stress, elastic modulus of tension test of the sole and white line areas of the horn and dry matter of the hind right, hind left, front right and front left claws at 150 days postpartum***

	Hind		Front			
	Right	Left	Left	Right	sem	*p*
Sole area of the horn						
Punch force (N)	10.54	10.75	11.14	11.08	0.24	NS
Work to fracture (kJ)	12.25	11.65	12.38	12.38	0.26	NS
Elastic modulus of the diaphragm (N/mm^2)	20.26	21.51	24.26	24.56	1.84	NS
Membrane stress (N/mm^2)	2103	2318	4762	4710	920.8	NS
Elastic modulus of the tension test (N/mm^2)	131.3	131.4	158.8	176.3	153	NS
White line area of the horn						
Punch force (N)	6.14 ^{b}	5.49 ^{b}	6.61 ^{a}	6.44 ^{ab}	0.29	0.05
Work to fracture (kJ)	7.21 ^{a}	5.83 ^{b}	7.15 ^{ab}	7.01 ^{ab}	0.37	0.05
Elastic modulus of the diaphragm (N/mm^2)	15.37 ^{b}	16.18 ^{ab}	20.00 ^{a}	19.46 ^{ab}	1.65	0.05
Membrane stress (N/mm^2)	1382	1081	1133	1457	356.5	NS
Dry matter content of the horn (%)	66.85	-	68.26	-	0.54	NS

$^{a, b}$ – different letters in the same row indicate values that differ significantly, NS – not significantly different

5.3.5.3 Correlations between the mechanical tests

There was a highly significant positive correlation between punch force and work to fracture of the sole and white line areas of the horn ($P<0.01$ and 0.001) (R = 0.68 to 0.96). The elastic modulus of the diaphragm and membrane stress of the sole area were highly significantly ($P<0.01$ and 0.001) and positively correlated (R = 0.68 to 0.93) when tested for all claws and periods.

The punch force was significantly ($P<0.05$) moderately positively correlated to elastic modulus of the diaphragm (r = 0.50) when the sole horn of hind left and front outer claws were tested in the *prepartum* period and the sole horn of the front right and hind right outer claws and the white line horn of the front left inner claw were tested at 50 days *postpartum* (r = 0.50 to 0.67, $P<$ 0.01 to 0.05). However, when the white line horn of the front left inner claw was tested at 100 days *postpartum* and the front right inner and front left outer claws were tested at 150 days *postpartum* punch force was significantly ($P<0.01$), negatively correlated with the elastic modulus of the diaphragm (r = - 0.50 to - 0.69). Punch force was significantly ($P<0.05$) negatively correlated (r = -0.50 to 0.57) with membrane stress when the sole and white line horn of the hind outer claws was tested at 50 and 100 days *postpartum* and of the front inner claws at 50 days *postpartum*. Work to fracture was significantly ($P<0.05$) negatively correlated (r = -0.53 to –0.66) to membrane stress and elastic modulus of the diaphragm when the sole horn of the hind outer claws was tested at -40, 50 and 100 days *postpartum* and the white line horn of the front right, front left outer and hind left inner and right outer claws were tested at 50 days *postpartum* (r = - 0.50 to - 0.54), front left inner and hind left outer were tested at 100 days *postpartum* (r = - 0.50 to - 0.56) and front right inner and front left outer claws were tested at 150 days *postpartum* (r = -0.55 to -0.73). The correlation factors were low.

Elastic modulus of the tension test was significantly (*P*<0.001 to 0.05) positively correlated (r = 0.55 to 0.91) to the elastic modulus of the diaphragm and membrane stress when the sole area of the hind right inner claw was tested at 50, 100 and 150 days *postpartum* and the sole area of the front inner claws were tested at 100 and 150 days *postpartum*. Elastic modulus of the tension test was significantly and positively correlated to work to fracture (r = 0.62 to 0.81, *P*<0.01) and to punch force (r = 0.50, *P*<0.05) when the sole horn was tested in the front left inner claw at 100 days *postpartum* and the hind left inner claw at 150 days *postpartum*.

5.3.5.4 Correlations between the mechanical tests and the lesion score

The lesion score of the sole and white line areas of the respective claws were significantly (*P*<0.05 to 0.01) negatively correlated to work to fracture and punch force when the sole horn of the front outer claws and the white line horn of the front right claws were tested at 40 days *prepartum* (r = -0.52 to -0.80), when the sole horn of the hind right inner claw was tested at 50 days *postpartum* (r = -0.51 to -0.64) and the white line horn of the hind right claws were tested at 150 days *postpartum* (r = -0.52 to - 0.54). However, the lesion score of the white line area was significantly (*P*< 0.05 to 0.01) positively correlated to work to fracture and punch force when the white line horn of the hind left, hind right inner and front outer claws were tested at 100 days *postpartum* (r = 0.55 to 0.60). At 50 days *postpartum* the lesion score of the white line area was significantly (*P*<0.05 to 0.01) positively correlated to the elastic modulus of the diaphragm and membrane stress when the horn of the white line area of all front claws (r = 0.45 to 0.77) and of the hind inner and left outer claws (r = 0.52 to 0.60) were tested. Lesion score of the front left inner claw at 40 days *prepartum* (r = - 0.52), of the front right outer claw at 50 (r = - 0.68) and 150 (r = -

0.50) days *postpartum* was significantly (P<0.05) negatively correlated to the dry matter content of the horn.

5.3.5.5 Correlation between mechanical tests and dry matter content

The dry matter content was significantly (P<0.05) positively correlated to punch force and work to fracture when the horn of the white line area of the front outer claws was tested at 40 days *prepartum* (r = 0.55). The dry matter content was significantly (P<0.05) positively correlated to the elastic modulus of the tension test when the sole horn of the front right claws were tested at 150 days (r = 0.52) and to the elastic modulus of the diaphragm and membrane stress when the sole area of the hind left outer claw (r = 0.57) and the white line area of the horn of the front left outer and front right inner claws were tested at 150 days *postpartum* (r = 0.50 to 0.61) and the front outer claw was tested at 50 days *postpartum* (r = 0.69).

5.3.5.6 Correlation between mechanical tests, locomotion score and lameness

The locomotion score at days 49, 154 and 189 *postpartum* were significantly (P<0.05) positively correlated to the membrane stress of the sole horn at 50 days *postpartum* (r = 0.50 to 0.62) and locomotion score at 154 days *postpartum* was significantly (P<0.05) positively correlated to membrane stress of the white line horn at days 50 to and 150 *postpartum* (r = 0.50 to 0.54). The locomotion score at days 49, 154 and 189 *postpartum* were significantly (P<0.01) and positively correlated and elastic modulus of the tension test of the sole horn at 50 days *postpartum* (r = 0.57 to 0.68). The elastic modulus of the tension test of the sole at day 100 *postpartum* was significantly (P<0.01) negatively correlated to locomotion score at day 154 *postpartum* (r = - 0.61). At 50 days *postpartum* the elastic modulus of the tension test of the white line was significantly ($P<0.05$) positively correlated to locomotion score at day 154 *postpartum*.

The number of days that the heifers were lame throughout the lactation, corresponded to heifers with a locomotion score > 3, and the number of days animals were severely lame, corresponded to heifers with a locomotion score > 4, throughout the lactation, were significantly ($P<0.50$ to 0.001) positively correlated to the elastic modulus of the diaphragm of the sole and white line areas of the horn at 50 days *postpartum* (r = 0.61 to 0.78), the membrane stress of the white line area at 150 days *postpartum* (r = 0.50 to 0.54), the elastic modulus of the tension test of the sole area of the horn at 50 days *postpartum* (r = 0.53 to 0.60) and negatively correlated to the punch force of the sole area at 100 days *postpartum* (r = -0.50).

5.3.5.7 Correlation between mechanical tests and the length, the height, the height of the heel, the angle and the growth of front and hind claws

The correlation between the mechanical tests of the sole area and the claw length, claw height, heel height, hoof angle and the growth rate of the front claw are presented in Table 5.17 and of the hind claw are presented in Table 5.18.

The correlation between the mechanical tests of the white line area and the claw length, claw height, heel height, the angle and the growth rate of the front claws are presented in Table 5.19 and of the hind claw are presented in Table 5.20.

The length of the front and hind claws was negatively correlated to the punch force and work to fracture of the sole and white line areas of the horn. However, in the early *postpartum* period the punch force of the sole area of the front claws was positively correlated to the length of the claw. The elastic modulus of the diaphragm and membrane stress were generally positively correlated to the length of the claws.

The lateral height of the claws was positively correlated to the elastic modulus, elastic modulus of the diaphragm and membrane stress and negatively correlated to the punch force and work to fracture of the sole and white line areas of the claw horn. The heel height was positively correlated to the punch force, work to fracture and elastic modulus of the diaphragm.

The angle of the claws was negatively correlated to the punch force and positively correlated to the elastic modulus. The results of the elastic modulus of the diaphragm differed between the sole and the white line areas, being positive correlated to the angle of the claws when testing the white line area and negatively correlated to the angle of the claws when testing the sole area. Growth rate of the claws was negatively correlated to the punch force and positively correlated to the elastic modulus.

Table 5.17 ***Pearsons correlation between the length, the height, the height of the heel, the angle and the growth of the front claws at days 50, 100 and 150 of the postpartum period and the elastic modulus (EM), punch force (PR), work to fracture (w), elastic modulus of the diaphragm (EMd) and membrane stress (ms) of the sole area of front claws***

Claw measurements	EM	PR	w	EMd	ms
Day 50 postpartum					
Length	NS	0.62**	0.65**	0.60**	0.60**
Height, lateral	NS	NS	NS	0.50*	NS
Heel height	NS	NS	NS	NS	- 0.53*
Angle	- 0.69**	NS	NS	0.46*	NS
Growth	NS	NS	NS	NS	NS
Day 100 postpartum					
Length	NS	0.50*	NS	- 0.50**	0.67**
Height, lateral	0.63**	NS	NS	0.52*	NS
Heel height	NS	0.55*	NS	NS	- 0.54*
Angle	- 0.63*	NS	NS	NS	- 0.54*
Growth	- 0.57*	NS	NS	- 0.80***	- 0.80***
Day 150 postpartum					
Length	NS	NS	NS	0.55**	0.55**
Height, lateral	NS	NS	NS	0.50*	NS
Heel height	0.52*	NS	- 0.50*	NS	NS
Angle	NS	0.59*	0.61*	NS	NS
Growth	- 0.67**	NS	NS	NS	NS

* - significant at $P<0.05$, ** - significant at $P< 0.01$, *** - significant at $P< 0.001$

Table 5.18 ***Pearsons correlation (R) between the length, the height, the height of the heel, the angle and the growth of the hind claws at days 50, 100 and 150 of the postpartum period and the elastic modulus (EM), punch force (PR), work to fracture (w), elastic modulus of the diaphragm (EMd) and membrane stress (ms) of the sole area of the hind claws***

Claw measurement	**EM**	**PR**	**w**	**EMd**	**ms**
Day 50 postpartum					
Length	0.50*	NS	NS	NS	0.55*
Height, lateral	NS	- 0.50*	- 0.55*	0.50*	NS
Heel height	NS	NS	- 0.50*	0.50*	- 0.50*
Angle	NS	NS	NS	NS	NS
Growth	NS	NS	NS	NS	NS
Day 100 postpartum					
Length	NS	- 0.50*	NS	NS	- 0.50*
Height, lateral	NS	- 0.53*	NS	NS	- 0.61**
Heel height	NS	NS	NS	NS	NS
Angle	NS	NS	NS	- 0.50**	0.62**
Growth	- 0.63*	- 0.63**	NS	- 0.74***	- 0.74**
Day 150 postpartum					
Length	NS	- 0.50*	- 0.50*	NS	0.53*
Height, lateral	0.62**	- 0.60**	- 0.60**	NS	0.60*
Heel height	NS	0.50*	0.50*	NS	NS
Angle	NS	NS	0.50*	NS	NS
Growth	NS	NS	0.52*	0.61**	0.72**

* - significant at $P<0.05$, ** - significant at $P<0.01$, *** - significant at $P<0.001$

Table 5.19 ***Pearsons correlation between the length, the height, the height of the heel, the angle and the growth of the front claws at days 50, 100 and 150 of the postpartum period and the elastic modulus (EM), punch force (PR), work to fracture (w), elastic modulus of the diaphragm (EMd) and membrane stress (ms) of the white line area of front claws***

Claw measurement	EM	PR	w	EMd	ms
Day 50 postpartum					
Length	NS	0.60**	0.60**	0.50*	0.55*
Height, lateral	NS	0.50*	NS	0.50*	NS
Heel height	NS	NS	NS	0.50*	NS
Angle	NS	NS	0.51*	NS	NS
Growth	NS	NS	NS	NS	NS
Day 100 postpartum					
Length	NS	- 0.50*	NS	NS	- 0.50*
Height, lateral	NS	- 0.53*	NS	NS	- 0.61**
Heel height	NS	0.71**	0.60**	0.50*	NS
Angle	NS	NS	- 0.53*	NS	NS
Growth	NS	NS	NS	NS	NS
Day 150 postpartum					
Length	NS	- 0.62**	- 0.55**	NS	NS
Height, lateral	NS	NS	NS	0.50*	NS
Heel height	NS	NS	NS	0.79***	0.70***
Angle	NS	0.65**	0.72**	NS	NS
Growth	NS	NS	0.52*	NS	0.51*

* - significant at $P<0.05$, ** - significant at $P< 0.01$, *** - significant at $P< 0.001$

Table 5.20 ***Pearsons correlation between the length, the height, the height of the heel, the angle and the growth of the hind claws at days 50, 100 and 150 of the postpartum period and the elastic modulus (EM), punch force (PR), work to fracture (w), elastic modulus of the diaphragm (EMd) and membrane stress (ms) of the white line area of hind claws***

Claw measurement	**EM**	**PR**	**w**	**EMd**	**ms**
Day 50 postpartum					
Length	NS	NS	0.62*	- 0.75**	- 0.61**
Height, lateral	NS	- 0.79**	- 0.64**	NS	NS
Heel height	NS	NS	- 0.58*	NS	NS
Angle	NS	NS	0.50*	0.64*	NS
Growth	NS	NS	NS	NS	NS
Day 100 postpartum					
Length	NS	NS	0.57*	NS	NS
Height, lateral	NS	NS	NS	NS	- 0.69**
Heel height	NS	NS	NS	NS	NS
Angle	NS	0.68**	0.68**	0.60*	0.56*
Growth	NS	- 0.64**	- 0.57**	NS	- 0.69**
Day 150 postpartum					
Length	NS	NS	0.61*	NS	0.52*
Height, lateral	NS	NS	0.74***	0.62*	0.52*
Heel height	NS	0.52*	NS	NS	NS
Angle	NS	NS	NS	0.54*	- 0.50*
Growth	NS	0.58**	0.53**	0.55*	0.50*

* - significant at $P<0.05$, ** - significant at $P< 0.01$, *** - significant at $P< 0.001$

5.3.6 Scanning electron microscopy of the crack surface and fracture path of tension and punch tests

5.3.6.1 Scanning electron microscopy images of the fracture surface of tension tests

The fracture surfaces of tension tests of the sole area of horn with the greatest elasticity, which were measured in a perpendicular direction to the tubules, are presented in Figures 5.9 and 5.10. In Figure 5.9 the pictures are of high resolution (x 200 and x 300) and the structures are shown in great details. The fracture surface of these tests was irregular with a higher level of fibre pull out, indicating resistance to deformation and inelastic deformation.

The fracture surfaces of tension tests of the sole area of horn that had a low elasticity and were taken in a perpendicular direction to the tubules are presented in Figures 5.11. The fracture surface of these tests was more uniform and the level of fibre pull out was low. Figure 5.12 demonstrates again the fracture surface of tension tests of the sole area measured in a perpendicular direction to the tubules. In pictures A and B the deviation of the fracture path from the tubules are presented and in pictures C and D the wave like appearance of the intertubular horn is shown.

The fracture surface of tension tests of horn of the white line area parallel to the laminae of the laminar horn is presented in Figure 5.13 and 5.14. The fracture surfaces in Figure 5.13 appear smooth, with low level of fibre pull out, demonstrating the low elasticity of the horn of the white line area when compared with the horn of the sole area. In Figure 5.14 the non-uniform fracture surface of the area characteristic of the white line area is presented and in picture A and B a pull out of laminae is presented.

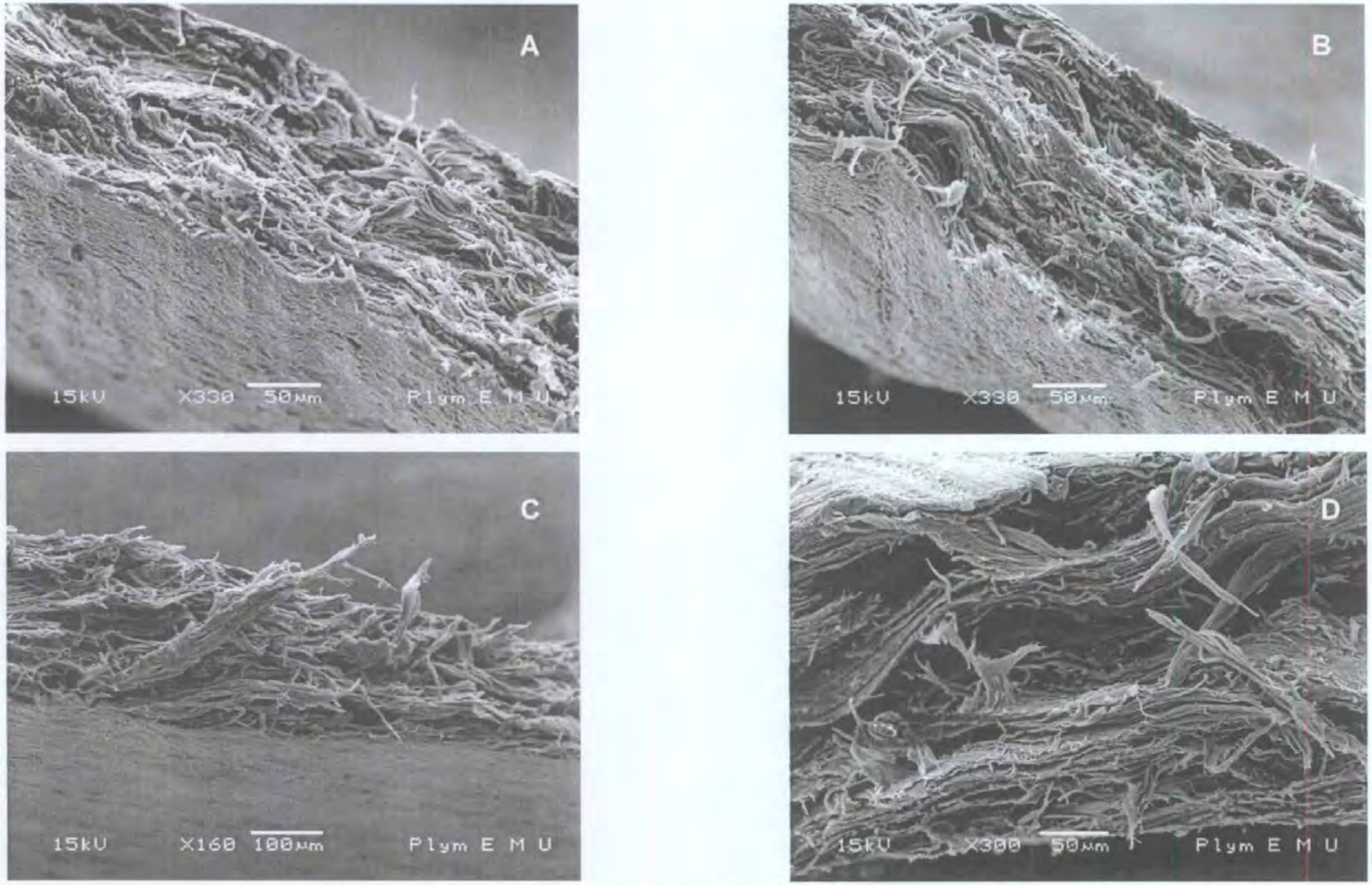


Figure 5.9 SEM image of fracture surface of tension tests of hoof horn of the sole area perpendicular to the direction of the tubules, horn with great elasticity showing fibre pullout of intertubular horn (Picture A and B x 330, scale line = 50µm; picture C x 160, scale line = 100µm and picture D x 300, scale line = 50µm)

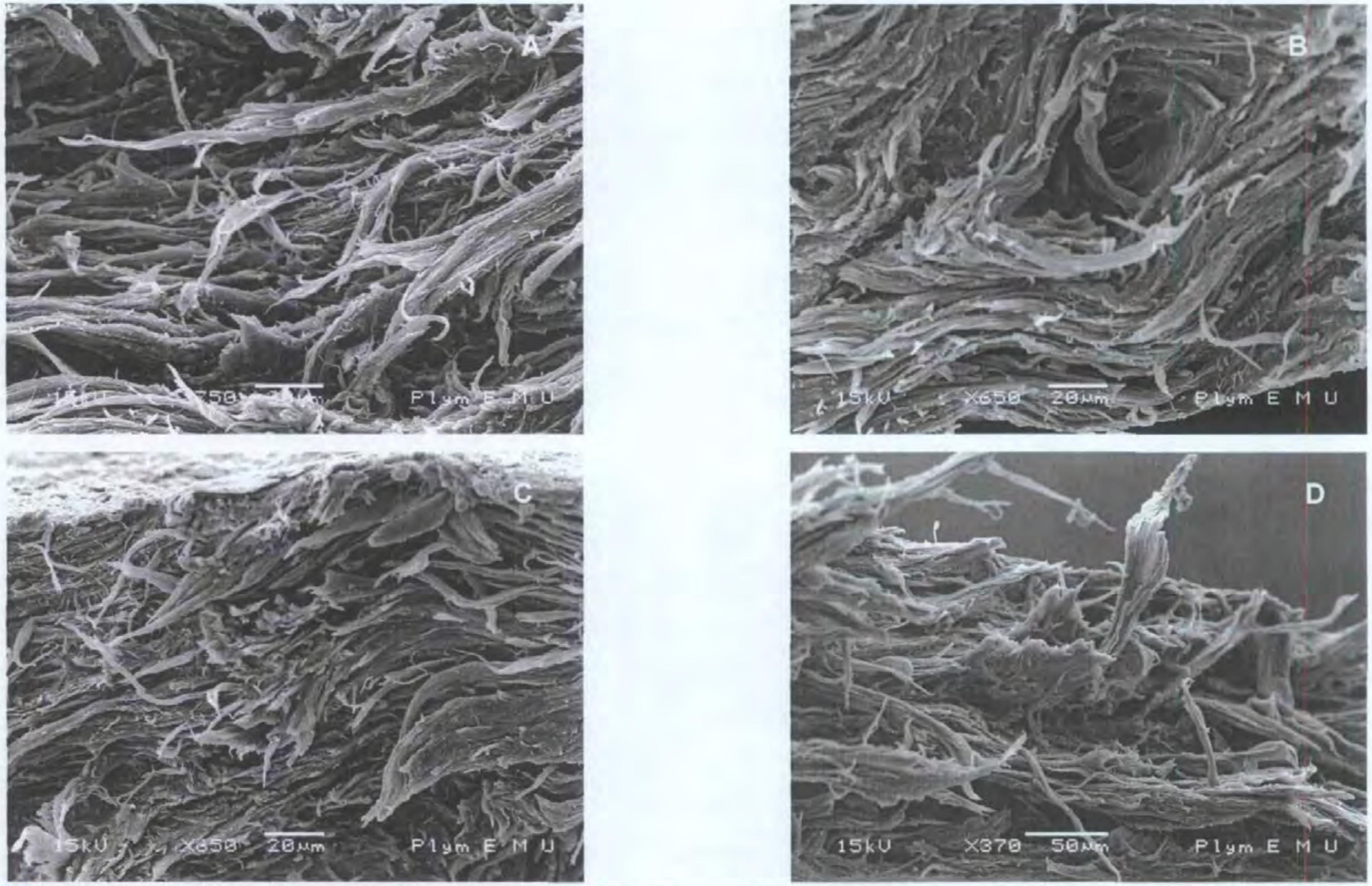


Figure 5.10 SEM image of fracture surface of tension tests of hoof horn of the sole area perpendicular to the direction of the tubules, horn with great elasticity showing fibre pullout of intertubular horn (Picture A x 750, scale line = 20µm; picture B and C x 650, scale line = 20µm and picture D x 370, scale line = 50µm).

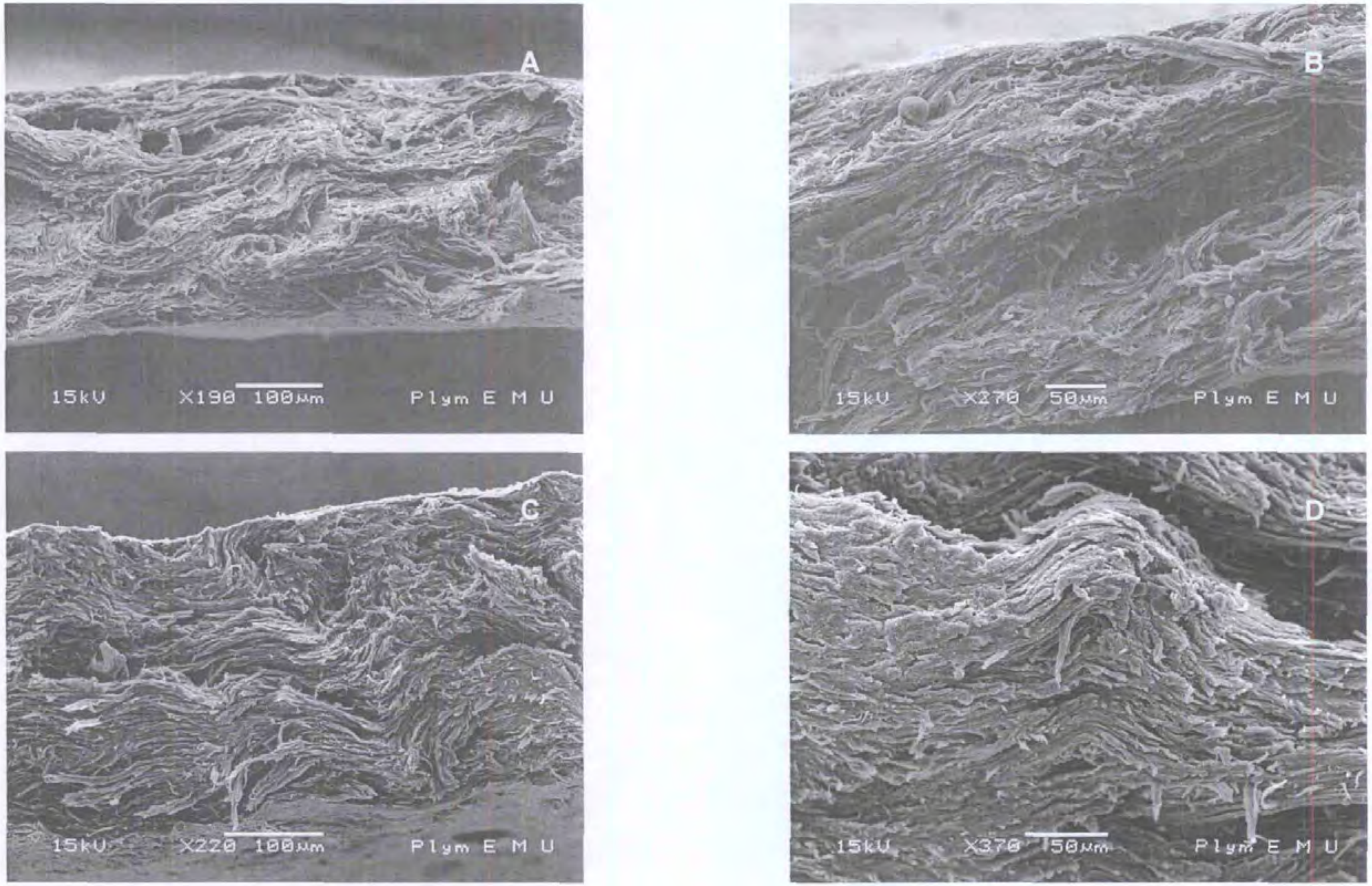


Figure 5.11 SEM image of fracture surface of tension tests of hoof horn of the sole area perpendicular to the direction of the tubules, horn with low elasticity showing smooth fracture surface with low level of fibre pullout of intertubular horn (Picture A x 270, scale line = 50µm; picture B x 190, scale line = 100 µm; C x 220, scale line = 100µm and picture D x 370, scale line = 50µm).

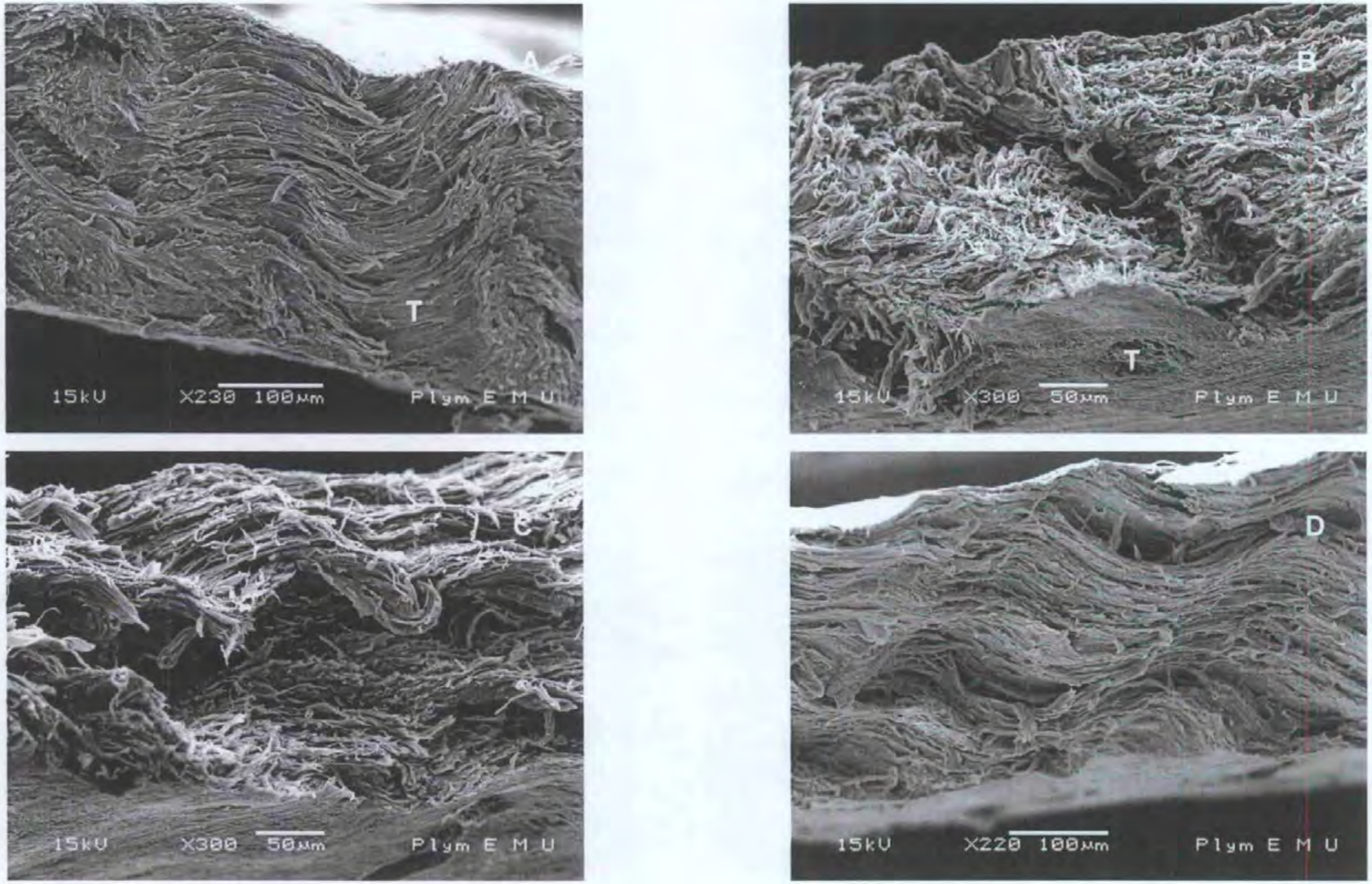


Figure 5.12 SEM image of fracture surface of tension tests of hoof horn of the sole area perpendicular to the direction of the tubules, fracture surface deviating from tubule (T) (A and B), wave like appearance of intertubular horn (C and D) (Picture A x 200, scale line = 100μm; picture B and C x 300, scale line = 50 μm and picture D x 220, scale line = 100μm).

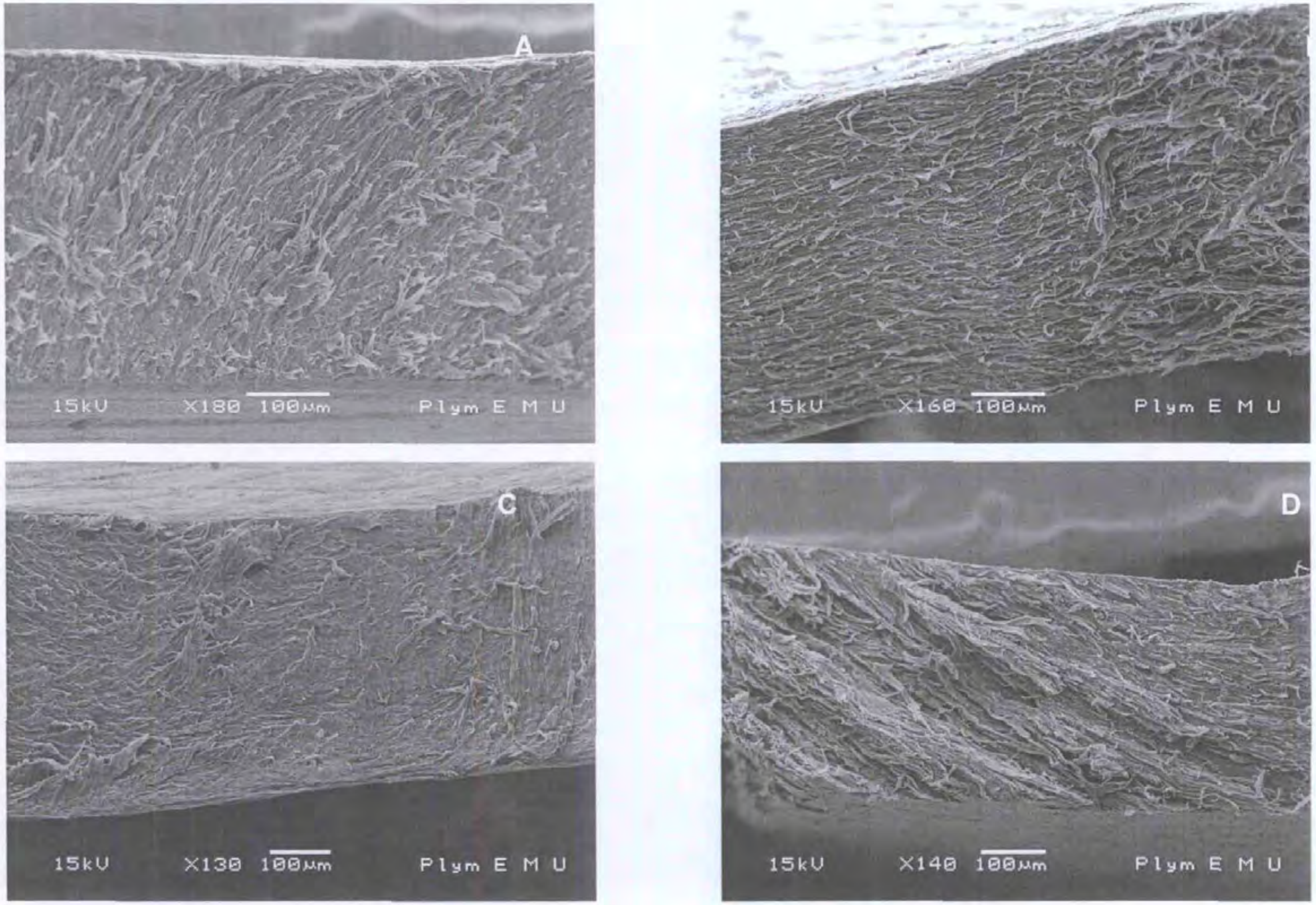


Figure 5.13 SEM image of fracture surface of tension tests of hoof horn of the white line area parallel to laminaes of laminar horn, horn with low elasticity showing smooth fracture surface with low level of fibre pullout (Picture A x 100, scale line = 100µm; picture B x 160, scale line = 100µm; C x 130, scale line = 100 µm and picture D x 140, scale line = 100µm).

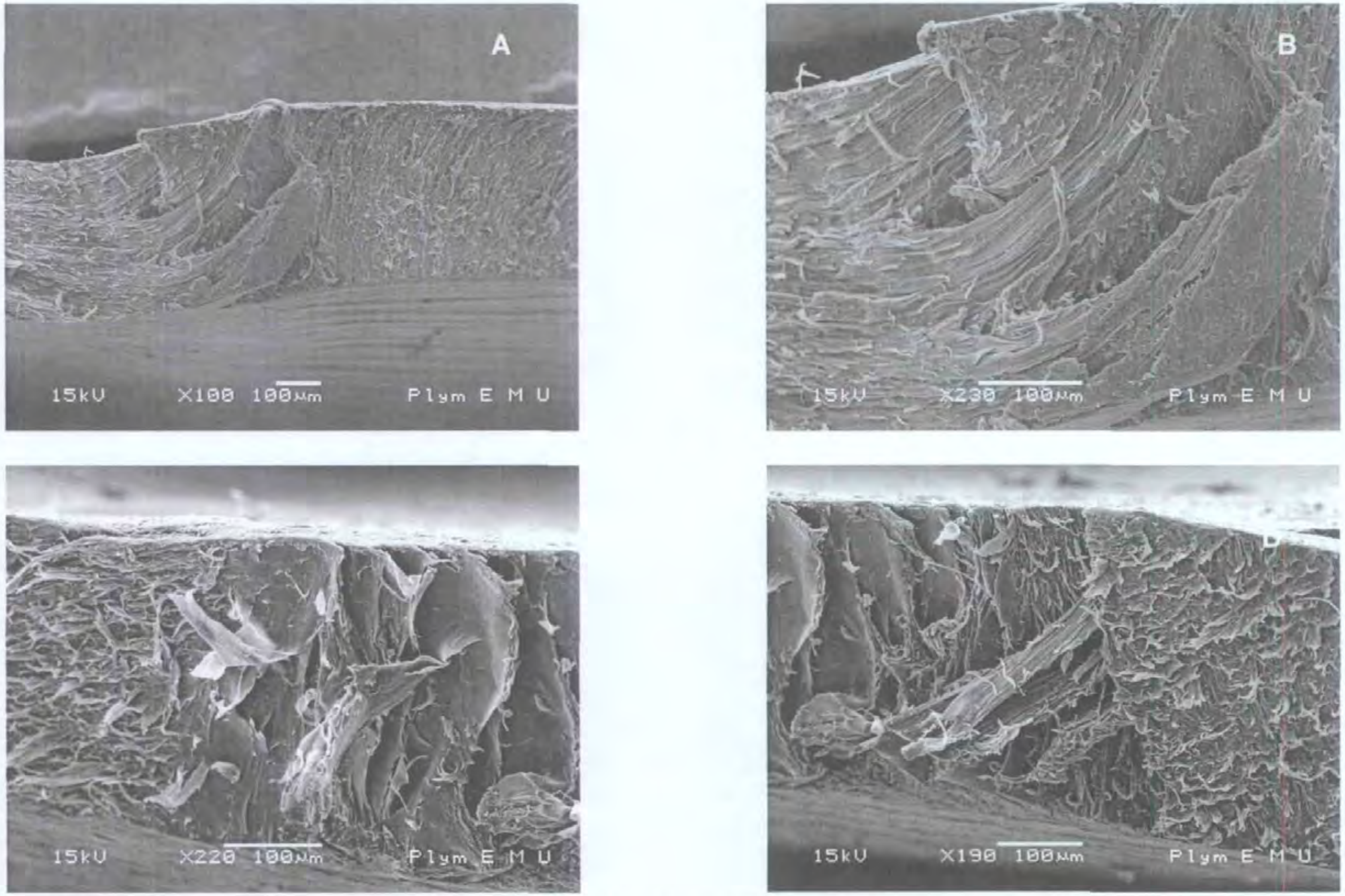


Figure 5.14 SEM image of fracture surface of tension tests of hoof horn of the white line area parallel to laminaes of laminar horn, fracture surface showing pull-out of laminaes (A and B) and non-uniformity of this area (C and D), picture B is close up of picture A (Picture A x 100, scale line = 100µm; picture B x 230, scale line = 100µm; C x 220, scale line = 100 µm and picture D x 190, scale line = 100µm).

5.3.6.2 Scanning electron microscopy images of the fracture path of punch tests

The fracture path of punch tests of horn of the sole area parallel to the tubules is presented in Figure 5.15 and the pictures show punch zone and crack propagation. The crack propagation area is much larger than the punch area, indicating the high pressure to which the samples were subjected. The crack path avoided the areas where tubules were situated.

The fracture path of punch tests of horn of the white line area parallel to the tubules is presented in Figure 5.16. The crack area of the tests completed on tissue from the white line area was smaller than the crack area of the tests completed on the sole area, with the crack following existing structures such as laminae.

5.3.7 Milk yield

The mean milk yield of the animals at the end of the first lactation, corrected for 305 days, was 7,909 litres (4234 – 9656 l) (sem = 289). Milk yield was significantly ($P<0.05$) and positively correlated with locomotion score at day 91 *postpartum* (r = 0.48) and negatively correlated with the difference in weight gain between days 21 and 100 of the *postpartum* period and condition score at day 180 *postpartum* (r = - 0.46 and – 0.51).

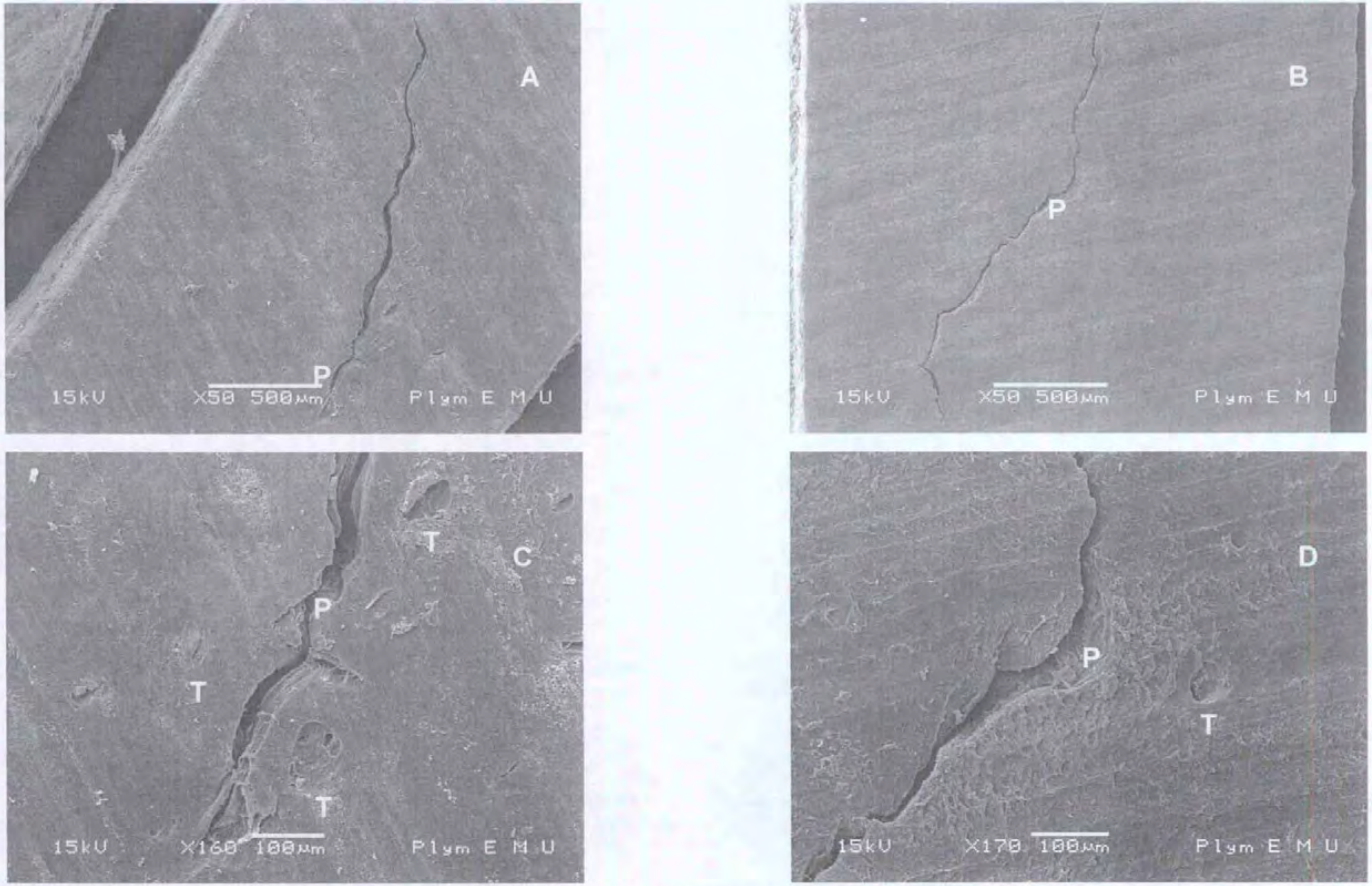


Figure 5.15 SEM image of the fracture path of the punch test of hoof horn of the sole area performed parallel to the tubules, P = punch zone, T = tubule, Picture C is an enlargement of picture A and picture D is an enlargement of picture B (Picture A and B x 50, scale line = 500µm; C x 160, scale line = 100 µm and picture D x 197, scale line = 100µm).

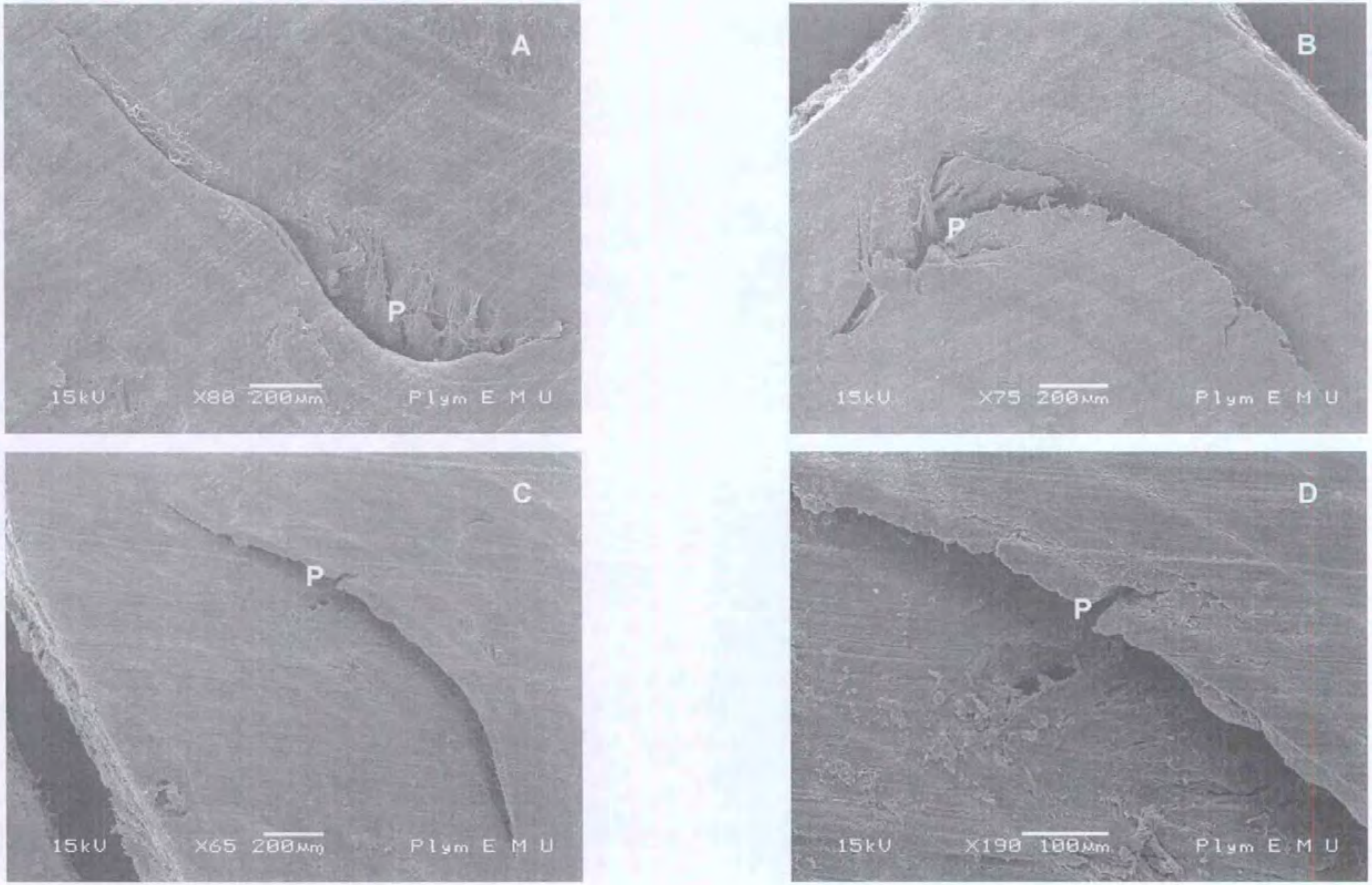
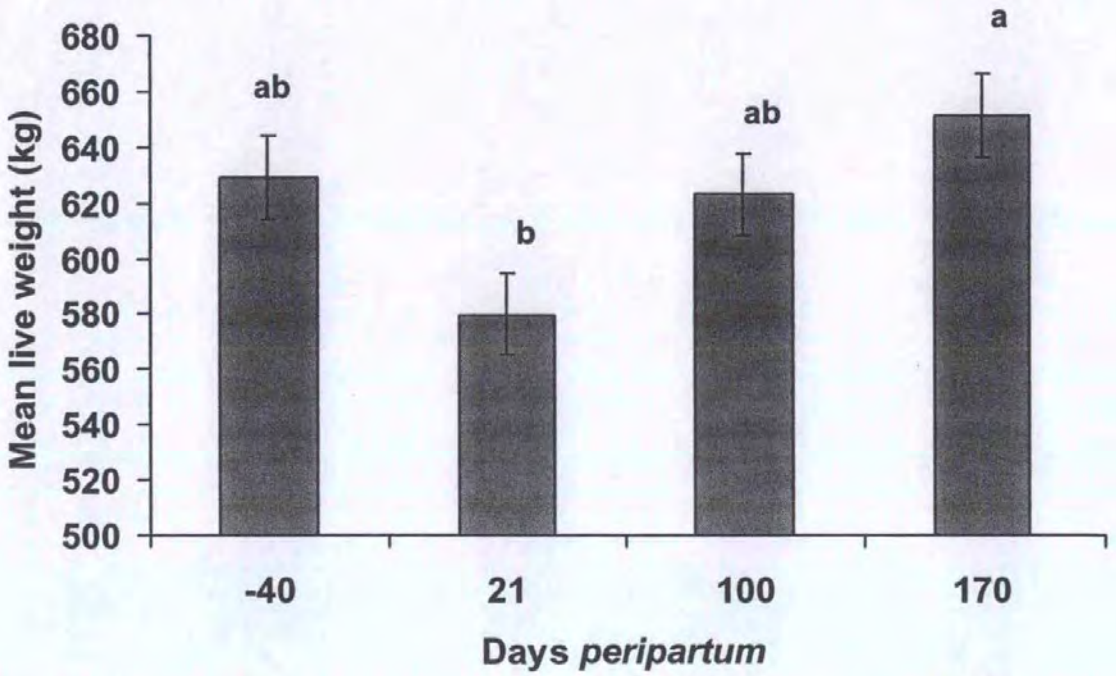


Figure 5.16 SEM image of the fracture path of the punch test of hoof horn of the white line area performed perpendicular to the laminaes of the laminar horn, P = punch zone, Picture D is an enlargement of picture C (Picture A x 00, scale line = 200µm; B x 75, scale line = 200µm; C x 65, scale line = 200 µm and picture D x 190, scale line = 100µm).

5.3.8 Condition score and weight gain

5.3.8.1 Live weight of the animals in the peripartum period

Mean live weight was significantly (*P*<0.01) lower in the early *postpartum* period and increased until day 170 *postpartum* (Figure 5.17).



a, b – different letters indicate values that differ significantly, p<0.01

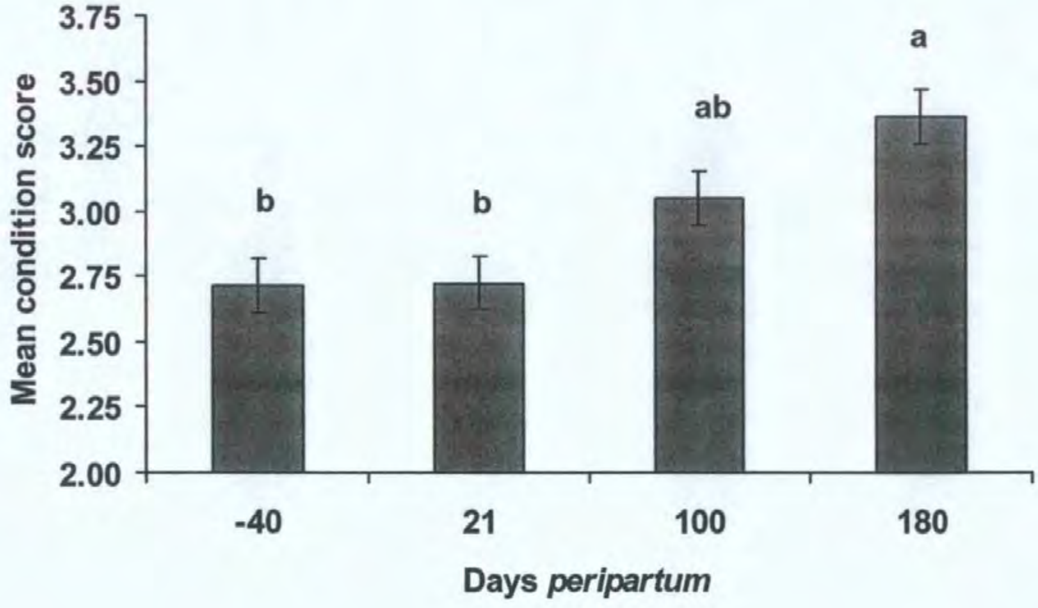
Figure 5.17 ***Mean live weight in the peripartum period***

5.3.8.2 Pearsons correlations between live weight, locomotion score and lameness

Live weight *prepartum* was significantly (*P*<0.05) and positively correlated to number of days lame throughout the lactation, corresponding to heifers with locomotion score > 3, and locomotion score at 91 days *postpartum* (r = 0.46). Live weight at 21 days *postpartum* was significantly (*P*<0.05 to 0.01) and positively correlated with number of days lame throughout the lactation, corresponding to heifers with locomotion score > 3, and locomotion score at day 49, 154 and 189 *postpartum* (r = 0.50 to 0.60). Live weight at 100 days *postpartum* was significantly ($P<0.05$ to 0.01) and positively correlated with locomotion score at day 154 *postpartum* (r = 0.45). The live-weight gain between day 21 and 100 of the *postpartum* period was significantly ($P<0.05$) and negatively correlated to number of days animals were severely lame, corresponding to heifers with a locomotion score > 4, throughout the lactation and lesion score of the white line area at day 150 *postpartum* (r = - 0.65 and 0.51).

5.3.8.3 Condition score in the peripartum period

The mean condition score increased significantly ($P<0.001$) from day 21 to day 180 *postpartum* (Figure 5.18).



a,b – different letters indicate values that differ significantly, $P < 0.001$

Figure 5.18 ***Mean condition score in the peripartum period***

5.3.8.4 Pearsons correlations between condition score, lesion score, locomotion score and lameness

The condition score at 21 days *postpartum* was significantly ($P<0.05$) positively correlated with locomotion score at 154 days *postpartum*, number of days the animals were severely lame throughout the lactation, corresponding to heifers with a locomotion score > 4, and lesion score of the white line area at 50 days *postpartum* (r = 0.50 to 0.59). The condition score at 100 days *postpartum* was significantly ($P<0.05$) and positively correlated with locomotion score at 154 days *postpartum* (r = 0.45). The change in condition score between 21 days *postpartum* and 40 days *prepartum* was significantly ($P<0.05$) and positively correlated with locomotion at 49 and 154 days *postpartum*, number of days the animals were lame throughout the lactation, corresponding to heifers with a locomotion score > 3, and lesion score of the white line area at 50 days *postpartum* (r = 0.50 to 0.62). The difference in condition score between 21 and 100 days *postpartum* was significantly ($P<0.05$ to 0.01) and negatively correlated with number of days the animals were severely lame throughout the lactation, corresponding to heifers with a locomotion score > 4, and lesion score of the white line area at 50 days *postpartum* (r = - 0.54 to – 0.61).

5.3.9 Holstein UK Index (HUKI)

The mean values for the HUKI score for rear legs, feet, locomotion, body score, dairy score, total legs and feet score, mammary score and final score are presented in Table 5.21.

Table 5.21 ***Holstein UK Index score for rear legs, feet, locomotion, total legs and feet score, body score, dairy score, mammary score and final score***

Traits	Mean	sem	Range
Rear legs	5	0.23	3 – 6
Feet	5	0.26	2 – 7
Locomotion	5	0.29	2 – 7
Total legs/feet	76	1.76	50 – 83
Body score	76	1.10	67 – 86
Dairy score	76	1.94	60 – 87
Mammary score	75	1.32	65 – 85
Final score	76	1.06	67 - 83

5.3.9.1 Pearsons correlation between the HUKI linear traits for legs and feet and composite trait for legs and feet and mechanical tests, locomotion score, condition score and live weight

The HUKI score for rear legs was significantly ($P<0.05$) and positively correlated with the punch force of the sole and white line horn at 40 days *prepartum* (r = 0.55 and 0.50), the membrane stress of the punch test of the sole horn at 40 days *prepartum* and 50 days *postpartum* (r = 0.56 and 0.54), the elastic modulus of the diaphragm and elastic modulus of the tension test at 50 days *postpartum* (r = 0.50 and 0.53), the locomotion score at 154 and 180 days *postpartum* (r = 0.51 and 0.52) and the live weight and condition score at 21 days *postpartum* (r = 0.63 and 0.64).

The HUKI score for feet was significantly ($P<0.05$ to 0.001) and positively correlated with the punch force, elastic modulus of the diaphragm and membrane stress of the white line horn at 100 days *postpartum* (r = 0.56, 0.60 and 0.47) and negatively correlated with lesion score of the white line area at 40 days *prepartum* (r = - 0.50) and with the number of days the animals were severely lame throughout the lactation, corresponding with a locomotion score > 4 (r = - 0.50). The HUKI locomotion score was significantly ($P<0.05$) and positively correlated with the punch force of the white line horn at 100 days *postpartum* (r = 0.50).

The HUKI total score for legs and feet was significantly ($P<0.05$ to 0.01) negatively correlated with the punch force of the white line horn at 150 days *postpartum* (r = - 0.50), with the elastic modulus of the diaphragm and membrane stress of the white line horn at -40 days *prepartum,* 50 and 150 days *postpartum* (r = - 0.60 to - 0.68), with the membrane stress of the sole horn at 50 days *postpartum* (r = - 0.50) and the number of days the animals were severely lame throughout the lactation, corresponding with a locomotion score > 4 (r = - 0.48).

5.3.9.2 Pearsons correlation between the HUKI linear and composite traits for body and dairy scores and locomotion score, lameness, lesion score, condition score and live weight

The HUKI score for stature was significantly ($P < 0.05$ to 0.001) and positively correlated with live body weight at -40, 21 and 100 days *postpartum* (r = 0.55 to 0.74), with locomotion score at 50 days *postpartum* (r = 0.55) and with number of days the animals were lame throughout the lactation, corresponding with a locomotion score > 3 (r = 0.46). The HUKI score for chest width was significantly ($P < 0.05$ to 0.01) and positively correlated with live body weight at 40 days *prepartum* and 21, 100 and 170 days *postpartum* (r = 0.47 to 0.67), with condition score at 100 and 180 days *postpartum* (r = 0.53 to 0.67) and with lesion score of the white line at 150 days *postpartum* (r = 0.46).

The HUKI score for body depth was significantly ($P < 0.05$) and positively correlated with the number of days the animals were lame throughout the lactation corresponding with a locomotion score > 3 (r = 0.50). The HUKI score for angularity was significantly ($P<0.05$ to 0.01) negatively correlated with the condition score at 21, 100 and 180 days *postpartum* (r = -0.50 to -0.64) and with lesion score of the sole area at 40 days *prepartum* (r = -0.47).

The HUKI score for rump angle was significantly ($P<0.05$) negatively correlated with the condition score at 21 days *postpartum* (r = - 0.52). The HUKI score for rump width was significantly ($P < 0.05$) positively correlated with the live weight at 21 and 100 days *postpartum* (r = 0.53 and 0.57) and with locomotion score and lesion score of the sole horn at 50 days *postpartum* (r = 0.46 and 0.49). The HUKI total body score was significantly ($P < 0.05$) positively correlated with the number of days the animals were lame throughout the lactation corresponding with a locomotion score > 3 (r = 0.55).

The HUKI score for fore udder attachment was significantly ($P< 0.05$ to 0.01) negatively correlated with the lesion score of the white line area at 100 days *postpartum* (r = - 0.64) and with the lesion score of the sole area at 50 days *postpartum* (r = - 0.56).

The HUKI total dairy score was significantly ($P<0.05$) negatively correlated with the punch force of the white line horn at 50 days *postpartum* (r = - 0.50), with the punch force of the sole horn at 100 and 150 days *postpartum* (r = - 0.50), with the elastic modulus of the diaphragm of the sole horn at 50 days *postpartum* (r = - 0.50) and of the sole and white line horn at 100 days *postpartum* (r = - 0.53 and – 0.59), with the condition score at 180 days *postpartum* (r = - 0.54) and with the lesion score of the sole area at 40 days *prepartum* (r = - 0.51).

The HUKI total mammary score was significantly ($P<0.05$ to 0.001) negatively correlated with the punch force, elastic modulus of the diaphragm and membrane stress of the white line horn at 50 and 100 days *postpartum* (r = - 0.50 and - 0.69), with the punch force of the sole horn at 150 days *postpartum* (r = - 0.60), with condition score at 100 and 180 days *postpartum* (r = - 0.48 and -.0.60) and with lesion score of the white line area at 100 days *postpartum* (r = - 0.47).

5.3.9.3 Pearsons correlation between the HUKI Total final score and mechanical tests and lesion score

The HUKI total final score was significantly ($P<0.05$ to 0.01) negatively correlated with the punch force of the white line horn at 50 and 150 days *postpartum* (r = - 0.50), with the punch force of the sole horn at 150 days *postpartum* (r = - 0.60), with the elastic modulus of the diaphragm of the sole horn at 50 days *postpartum* (r = - 0.51) and of the white line horn at 50 and 100 days *postpartum* (r = - 0.61 and – 0.57), with the membrane stress of the white line horn at 100 days *postpartum* (r = - 0.54) and with the lesion score of the sole area at 40 days *prepartum* (r = - 0.51).

5.4 Discussion

5.4.1 The development of the locomotion and lesion scores in the peripartum period

Claw horn haemorrhages of the sole and white line areas and *Digital dermatitis* were the most common pathological changes observed in the claws during the *postpartum* period. However, only 0.14 of animals had severe haemorrhages of the sole area and 0.05 severe haemorrhages of the white line area, which was consistent with the study by Offer *et al.* (2000) who reported that very few cases of clinical lameness were related to claw horn lesions in the first lactation. Heifers in the present experiment were housed in a straw yard and this housing system could have helped to keep low the severity level of claw horn haemorrhages. According to Chaplin *et al.* (2000) and Webster (2001) housing had a significant effect on the development of sole and white line lesions and fewer lesions were found in heifers housed on loose bedded systems compared with those housed in cubicle housing systems (Webster, 2001).

At 40 days *prepartum* the incidence of stone puncture (3%) and white line separation (6%) were at their highest, but this levels were still relatively low when compared with the incidence of haemorrhages of the sole and white line areas of the horn (14 % and 29 %, respectively). Alterations of the white line area and stone punctures were probably related to management while at pasture and the greater walking distances the animals were subjected to during the grazing period. White line cracking and separation, heel horn erosion and stone puncture were found not to cause extensive damage to the claw horn, but these indicated an increased pressure on specific areas of the claw leading to greater alterations over time and could predispose to the entrance of pathogens and occurrence of infections in the dermis and/or epidermis of the claw.

However, in the present experiment the locomotion score increased significantly ($P<0.001$) until 120 days *postpartum* and decreased after 150 days *postpartum.* This increase could be linked to the length of time the animals were housed and to *parturition.* The scores for white line and sole lesions increased significantly ($P<0.001$) during the *postpartum* period and were significantly correlated (r =0.45 to 0.70) to the increase in locomotion score. Locomotion score reached a peak at approximately 120 days *postpartum* which was at a similar time to the peak in sole and white line lesion scores (approximately 100 days). Physiological changes occurring during early lactation were found to have a greater effect on the development of sole and white line lesions. Hormonal changes and the subsequently softening of the collagen fibres of the suspensory weight bearing mechanism that connect the third phalanx to the horn capsule, are believed to be the major contributory factor in the development of these lesions (Webster, 1998; Holah *et al.*, 2000; Tarlton and Webster, 2002). Several authors have reported an increase in the incidence of sole and white line lesions and the locomotion score in the *postpartum* period (Enevoldsen *et al.*, 1991; Bergsten and Herlin, 1996; Bergsten and Frank, 1996; Leonard *et al.*, 1996; Vermunt and Greenough, 1996; Leach *et al.*, 1997; Boelling and Pollot, 1998b; Livesey *et al.*, 1998; Whay, 1998; Chaplin *et al.*, 2000 and Offer *et al.*, 2000).

Haemorrhages in the claw horn have been found to be at their greatest levels between 100 and 120 days *postpartum* and to decline in number and severity thereafter (Enevoldsen *et al.*, 1991; Leach *et al.*, 1997; Offer *et al.*, 1998 and 2000). Leach *et al.* (1997) reported in *primiparous* heifers that the highest levels of white line and sole lesions occurred at 63 and 108 days *postpartum*, respectively. White line lesions occurred earlier in the *postpartum* period than sole lesions. The earlier increase in number and severity of white line lesions when compared to the sole lesions was thought to be due to a differing response to the initial insult, differing growth and wear rates of the two areas and/or a differing origin of the lesion (Leach *et al.*, 1997; Le Fevre *et al.*, 2001). In the present experiment the greatest levels of sole and white line haemorrhages occurred both around 100 days *postpartum*. Webster (2001) found that the increase in lesion score of sole and white line occurred earlier (28 days) in animals housed in loose housed straw bedded yards, but that the lesion scores reached a peak at the same time for both areas. Webster (2001) also described that when animals were housed in cubicles the peak lesion score was later at 56 days *postpartum* when compared with heifers housed in the straw yards (28 days). These results suggested that there is no specific set time lag between the appearance of white line and sole haemorrhages and indicate that different housing conditions affect the development and occurrence of sole and white line lesions. There was a higher occurrence of severe haemorrhages of the sole area when compared with haemorrhages of the white line area (Table 5.5). Similar results were described by Offer *et al.* (2000) and Webster (2001) for first calving heifers. The mean sole and white line lesion scores were significantly (P<0.001) and positively correlated (r =0.57) and Manske *et al.* (2002) reported similar significant correlations.

Taking into consideration the hoof horn wall growth rate (0.58 to 0.66 cm/month) measured in this experiment, the observed haemorrhages of the sole horn probably originated from insults that occurred approximately 30 to 35 days earlier. This would indicate that the greatest insults occurred around 60 days following *parturition* for the sole and white line areas. A greater incidence of heel erosion has been found to occur in animals housed in straw yards (Livesey *et al.*, 1998; Webster, 2001) and the severity was found to increase over the *postpartum* period similar to claw horn lesions (Enevoldsen *et al.*, 1991; Vermunt and Greenough, 1991; Livesey *et al.*, 1998; Offer *et al.*, 2001). In this study, the incidence of heel horn erosion increased between days 50 and 100 *postpartum*, however, only 0.10 of the animals were affected.

There were no differences found in the lesion score between the outer and inner claws of front and hind hooves. However, several authors have reported that the majority of lesions occurred on the outer hind claws (0.75) and inner front claws (0.25) (Bergsten and Herlin, 1996; Leach *et al.,* 1997; Offer *et al.,* 2000; Le Fevre *et al.,* 2001). This increased predisposition of hind outer claws to increased number and severity of horn lesions may be due to the difference in shape between inner and outer hind claws reported by Toussaint-Raven (1985) and Scott (1987). While others thought that the increased weight bearing on the outer hind claws was due to the conformation of the udder around *parturition* that forced the cow to have a wider stance (Vermunt and Greenough, 1996). Similar results have been found even in relatively young dairy replacements; Singh *et al.* (1994) found that calves between 9 and 10 months of age and heifers between 15 and 18 months of age had greater levels of sole lesions on the outer claw of the hind feet when compared with other claws. However, in these experiments animals were housed in cubicles possibly leading to a different pressure distribution between inner and outer claws when compared with animals housed in a straw yard such as those in the present experiment. It has been found that a yielding surface distributed the load more evenly over the weight-bearing surface of the individual claw and between the lateral and medial claw of each limb (Vermunt and Greenough, 1994; Wandel *et al.*, 2002). As a consequence, in the present experiment the concentration of sole and white line lesions would have been spread between claws due to the yielding nature of loose straw bedded housing systems. Le Fevre *et al.* (2001) reported that sole lesions were significantly more concentrated in some claws and white line lesions were spread more evenly between claws in cows housed in cubicles.

Vermunt and Greenough (1996), Livesey *et al.* (1998) and Webster (2001) found higher lesion scores for the sole and white line areas for heifers housed in cubicles when compared to heifers housed in dry lots and straw yards. According to Colam-Ainsworth *et al.* (1989) heifers housed in cubicles spend less time lying down when compared to heifers housed in straw yards and this would impose greater stress on the hooves. Housing cattle on concrete floors has been associated to a greater wear rate that results in the loss of the concavity of the sole and increased pressure on the sole area (Scott, 1987; Tranter and Morris, 1992).

Outer and inner hind claws exhibited the highest lesion scores for sole and white line areas in the *postpartum* period when increase in lesion scores occurred. Increased lesion scores on hind claws may be related to the biomechanical differences of front and hind claws. The cow has been found to be better able to relieve weight from the front claws than from the hind claw (Toussaint-Raven, 1985; Frandson, 1986) and greater forces are applied by the hind legs of cattle than by the front legs (Scott, 1987; Vermunt and Greenough, 1996). There was a greater increase in the lesion score of the white line of hind claws at 50 days *postpartum* when compared with front claws and at 150 days *postpartum* the decrease in lesion score was also greater for the front claws. Le Fevre *et al.* (2001) also reported a quicker recovery rate of lesions of the front claws when compared with the hind claws. Tarlton and Webster (2002) found an increased loosening of the connective tissue of the dermis which occurred for a longer period of time in the hind claws (Tarlton and Webster, 2002) compared with the front claws.

In this experiment locomotion scores were significantly ($P<0.05$) correlated to the lesion scores of the sole and white line areas of front and hind and inner and outer claws and total scores of the animal at different periods of the *postpartum*, however most significant correlations were found at days 50 and 100 *postpartum* (R= 0.45 to 0.55) when lesion and locomotion scores were higher. The locomotion score was significantly ($P<0.05$) correlated mainly to the lesion scores of the sole area of the hind left and front left claws and the hind left claw had also consistently the highest scores for lesion. Webster (2001) found a significant ($P<0.001$) relationship (measured through regression analysis) between locomotion score and lesion scores, however, the R^2 of this relationship value was low (0.238). The author stated that it was difficult to predict the association of a moderate degree of claw horn lesions with the locomotion score, while claw horn lesions with high scores were more frequently associated with the locomotion score.

5.4.2 The hoof horn growth and wear rates and development of claw angle, claw and heel height and the length of the dorsal border in the postpartum period and their relationship with locomotion score, lesion scores and mechanical tests

The monthly hoof horn growth (0.58 to 0.67 cm/month) and wear rates (0.44 to 0.74) were similar to those reported by Webster (2001) for first lactation heifers (growth – 0.48 to 0.62 cm/month, wear – 0.46 to 0.53 cm/month). The hooves of the hind and front claws grew at similar rates (front - 0.58 to 0.67 cm/month, hind – 0.60 to 0.66 cm/month) and this was in agreement with Clark and Rakes (1982) and Whay (1998), however the hind claws had higher wear rates (0.58 to 0.74 cm/month) than front claws (0.44 to 0.56 cm/month) resulting in the hind claws having a negative net growth rate (-0.08 cm/month) between 100 to 150 days *postpartum*, similar to the findings reported by Leach *et al.* (1997) during the housing period (-0.25 mm/month). The higher wear rates for hind claws compared with the front claws may be related to a greater load that these claws are subjected to due to the increase in udder size in the *postpartum* period (Vermunt and

Greenough, 1996) and to the propulsion movement performed by the hind claws (Toussaint-Raven, 1985, Scott, 1987). In this experiment, six of the twenty heifers had udder oedema to varying degrees during the *peripartum* period and had good udder 'type' traits, causing them to have a wider stance which would have increased the pressure on the hind outer claws.

In previous studies, it was found that it took 8 to 10 months for the wall horn produced at the coronary border to reach the ground wearing surface (Kempson and Logue, 1993) and that it took approximately 6 to 8 weeks for the newly formed sole and heel horn to reach the weight bearing surface on the ground (Livesey *et al.*, 1998; Hoblet, 2001). With a mean height of the side wall of 79.8 mm and a mean growth rate of 6.3 mm the coronary horn would take 12.7 months to reach the ground surface in this experiment. Assuming a sole thickness of approximately of 7 mm in the toe and heel areas and 5 mm in the middle (Toussaint-Raven, 1985; Scott, 1987; Paulus and Nuss, 2002) the sole horn in the present experiment would have a turnover rate of approximately 1 month. However, Schmid (1995) measured a slower growth rate for the sole horn when compared with the coronary horn and as a consequence the turnover rate of the sole horn was estimated to have been between 1 and 1.5 months, which are similar the figures reported by Livesey *et al.* (1998).

In this experiment wear rates did not differ between the periods of 50 to 100 days *postpartum* (front - 0.44, hind – 0.58 cm/month) and 100 to 150 days *postpartum* (front - 0.56, hind – 0.74 cm/month). Leach *et al.* (1997), Livesey *et al.* (1998) and Offer *et al.* (2001) reported a significant increase in wear rates during the *postpartum* period. However, the difference in the results from this experiment and those found by Offer *et al.* (2001) may be explained by the different housing conditions. Offer *et al.* (2001) reported higher wear rates between 28 to 63 days *postpartum*. The variation in wear rates (0.44 to 0.74 cm/month) that occurred in the present experiment was similar to the variation (0.42 to 0.74 cm/month) reported by Offer *et al.* (2001). The animals were housed in cubicles in the experiments from Leach *et al.* (1997), Livesey *et al.* (1998) and Offer *et al.* (2001) whilst in the present experiment they were housed in a straw yard. The wear rates tend to be higher in cubicle housing systems (Leach *et al.* 1997, Livesey *et al.*, 1998; Whay, 1998; Offer et al, 2000; Vokey *et al.*, 2001). Conversely, Webster (2001) did not find differences in the wear and growth rates of hooves of *primiparous* animals housed in a cubicle system with concrete flooring (wear – 0.48 to 0.52 cm/month, growth – 0.51 to 0.56 cm/month) when compared with those housed in a straw yard (wear – 0.47 to 0.52 cm/month, growth – 0.48 to 0.62 cm/month). However, it is worth noting that concrete finishes can vary in the abrasion level. The hoof growth rates were found not to vary throughout the lactation in this experiment and this is in agreement with the findings of Livesey *et al.* (1998) and Offer *et al.* (2001).

In this experiment the growth rate between 50 and 100 days *postpartum* was significantly (P<0.05 to 0.001) negatively correlated to the elastic modulus, elastic modulus of the diaphragm, membrane stress of the sole horn and to the punch resistance of the horn of the sole and white line areas at 100 and 150 days *postpartum*. These results indicated that higher growth rates are associated with a decrease in the elastic modulus. McCallum (1999) found that the pressure on the horn stimulated the hoof horn growth rate to increase. The epidermal cells receive a supply of nutrients and oxygen via diffusion from the blood vessels in the corium. The living epidermal cells are very susceptible to any disturbance in the circulation within the vessels of the corium (Mulling *et al.*, 1998). Increased pressure could lead to disturbances in the circulation within the vessels of the corium and the formation of horn with inferior quality. Mulling *et al.*, (1994) found a high negative correlation between hardness and horn structure and the sites of predilection for occurrence of lesions in the ground surface of the hoof. Moreover, in this experiment horn with a greater punch resistance at the start of lactation was associated with a lower horn growth rate of the claws. However, when the lactation period progressed (day 150 *postpartum*), increased growth rates were not associated with decreases in the punch resistance or elastic modulus of the horn, indicating that in the early *postpartum* period the hooves were more susceptible to the influences of external pressures.

Differences have been found in the shape of the front and hind claws (Toussaint-Raven, 1985), with the front claws of cows typically having a higher heel and a more horizontal "coronary line" than hind claws. Similar results were found in this experiment and the front claws had a higher heel (5.28 – 6.35 cm) and greater angle of the dorsal wall (48.84 – 51.82 °) compared with hind claws (4.47 – 4.82 cm and 46.63 – 50.30 °).

Changes in the shape of the claw after the start of the lactation were reported by Offer *et al.* (2000), who found that during lactation, the angle of the dorsal border became significantly smaller while the dorsal wall became significantly longer. These changes were considered to be related to changes in the growth and wear rates (Offer *et al.*, 2000). Housing system has been found to affect heel height and Phillips and Schofield (1994) reported an increase in the depth of the heel of animals kept in a straw yard, while heel depth decreased in animals housing in a cubicle system. Moreover, Boelling and Pollot (1998) reported lengthening of the dorsal border and decrease in the foot angle when cows were at pasture and decreased dorsal border and steeper foot angle when cows were housed on concrete. Similar results were found in this experiment, where animals were kept in a straw yard and there was an increase in the angle of the dorsal border and in the heel height in the *postpartum* period. These results indicate that there are greater similarities between housing at pasture and housing in loose straw bedded yards and that the associated pressures on the hoof affects the shape and wear and growth rates of the hoof. This potentially indicates that housing cows on more solid concrete floors with little or no bedding to 'cushion' the impact on the hoof when animals walk seems more likely to affect the growth, wear rate, heel height, length and angle of the claw and that this may lead to thinner soles, greater impact of pressure within the hoof which could affect the tissue differentiation, oxygen and nutrient supply to the lamellae which may result or increase the opportunity for damage to hoof tissue and the formation of lesions.

A higher incidence of lameness has been related to a smaller angle of the dorsal border and a longer dorsal border of the claws (Boelling and Pollot, 1998; Manson and Leaver, 1989). A steep foot angle has been related to a good walking ability (Boettcher *et al.*, 1997; Boelling and Pollot, 1998b), while hoof length was negatively correlated and hoof angle was positively correlated with animal survival rates (Choi and MacDaniel, 1993; McDaniel, 1995). In this experiment, a longer dorsal border of the hind claws at 150 days *postpartum* was related to a higher lesion score of the white line, which confirmed the results from Boettcher *et al.* (1997), Boelling and Pollot (1998b), Choi and MacDaniel (1993) and McDaniel (1995). The lesion score of the white line and the locomotion score were also positively correlated with the height of the heel and of the side wall at days 100 and 150 *postpartum* (r =0.48 to 0.51). However, the angle of the dorsal border of the front and hind claws were positively correlated to locomotion score at the beginning of the lactation period (r =0.45 to 0.60) contradicting the results obtained by Boelling and Pollot

(1998) and Manson and Leaver (1989). However, there were no animals that were severely lame at this period.

5.4.3 Factors that had an influence over the mechanical parameters of the hoof horn

In this experiment the decrease in the dry matter of the horn from the *prepartum* to the *postpartum* period was most likely related to the change in housing from pasture to the straw yard. The work required to fracture and membrane stress of the sole horn and work to fracture, elastic modulus of the diaphragm and membrane stress of the white line significantly decreased between 40 days *prepartum* and 50 days *postpartum*, indicating that this difference in dry matter was probably one of the factors influencing the results from the mechanical tests. Measurements of hoof hardness and elastic modulus have been found to be affected by the moisture content of the hoof horn, decreasing with increasing moisture content (Collins *et al.*, 1998; Hinterhofer *et al.*, 1998). This may be one reason why altering the 'natural' moisture content of the hoof horn by hydration of hoof samples as part of the method of analysis may not be desirable when trying to determine the factors *in vivo* that have an influence over the mechanical properties of the hoof horn. However, hydration of samples to a standard moisture content would clearly reduce some of the variability in the results.

In this experiment during the *postpartum* period the increase in the level of haemorrhages of the sole and white line horn and the higher incidence of claw horn alterations such as heel horn erosion and crack of the white line may have also influenced the measurements of elastic modulus, puncture resistance, work to fracture and stress of the hoof horn. At 150 days *postpartum* there was an increase in the values of the majority of the parameters measured in the mechanical tests. At 150 days *postpartum* haemorrhages of the white line horn were declining and remained the same as at 100 days *postpartum* for the sole horn. These changes in the lesion score would have influenced the results of the mechanical tests. The punch resistance, work to fracture and elastic modulus were negatively correlated with the lesion score of the same claws and to locomotion score of the animals. Hedges *et al.* (2002) reported that white line with structural damage had a significant lower tensile strength (2.4 Mpa) than white line with no structural damage (4.5 Mpa). Working with horses, Geyer and Schulze (1994) reported reduction in horn strength, with unaltered wall having a tensile strength of 60 N/mm^2 compared with horn presenting alterations 20 to 28 N/mm^2. These results clearly indicate that horn structural strength and elasticity were reduced by structural alterations to the hoof horn.

In terms of the comparison of the structural strength of the sole and white line areas of the horn the results in this experiment were similar to those found by other authors. The punch force, elastic modulus, work to fracture were all found to be lower in the white line, thus indicating the white line to be weaker and less elastic compared with sole tissue. Working on the white line area of the solear surface of the bovine hoof, Budras *et al.* (1996) reported mean hardness values obtained with the use of the ball-impact method of 5.1 N/mm^2 for the cap-horn area and of 6.9 N/mm^2 for the terminal horn area. These values were significantly lower than the values for the sole horn (12.9 N/mm^2) and for the wall horn (27.5 N/mm^2). A high incidence of alterations in the horn formation has been found at the white line area (Mulling *et al.*, 1994) and the outer part of the white line, where the soft horn bonds to the harder coronary horn, has been found to be especially susceptible to tearing (Budras *et al.*, 1996). Differences between the sole and white line horn are demonstrated also in the SEM pictures. The crack propagation area during the punch resistance test was greater for the sole area when compared to the white line area. The white line horn presented constantly a more uniform fracture surface with low level of fibre pull out after the tension tests were completed, further indicating in addition to the mechanical tests the lower elasticity of the horn of the white line area when compared to the horn of the sole area. In the fracture surface of tension tests of the sole area the fracture path deviates from the tubules. The deviation probably occurs due to the different alignment of the cells around the tubules when compared with the intertubular horn and because the cells of the intertubular horn diverge around the tubules (Baillie and Fiford, 1996). The wave like appearance of the intertubular horn that is related to the way the keratinised cells develop and the deposition of overlapping flakes (Baillie and Fiford, 1996).

At 40 days *prepartum* there were no differences between front and hind claws in any of the parameters measured. In the *postpartum* period values for punch resistance and work to fracture of the sole and white line horn were always higher for the front claws when compared to hind claws, being significantly different in all periods for the white line horn and only at 50 days *postpartum* for the sole horn. Lower values of punch resistance and work to fracture of the sole and white line horn of hind claws in the *postpartum* period were probably related to a higher increase in lesions and alterations of the sole and white line horn of these claws when compared with front claws. Also different claw measurements, such as higher heel and dorsal angle, and lower wear rates of the front claws may have subjected the white line and sole horn to different pressures affecting the structure and composition of the horn. Mulling *et al.* (1994) found a high negative correlation between hardness and horn structure and the sites of predilection for occurrence of lesions in the distal surface of the hoof. Schmid (1995) reported significantly higher values of Shore D hardness and tensile strength for the sole horn, incubated at 65 %

relative humidity, of front claws (52.51 ± 8.43 and 64.25 ± 6.16 N/mm^2) than for the sole horn of the hind claws (44.19 ± 8.11 and 59.12 ± 6.32 N/mm^2). The differences in Shore D hardness were related to a lower moisture content of the sole horn of the front hooves when compared with the hind hooves (19.80 and 25.03 %, respectively). Van Amstel *et al.* (2004) reported higher moister content for the hind claws (33.1%) when compared to the front claws (31.1 %). Higher values of elastic modulus measured through tension tests were also reported by Zoscher *et al.* (2000) for the front claws (405.5 N/mm) when compared to the hind claws (349.5 N/mm). These results were related to the dry matter of these claws. Dry matter was significantly higher in claws that had higher values of elastic modulus and the dry matter of the claw horn in the present experiment was positively correlated to the elastic modulus of the horn of the same claws. These results indicated that the mechanical measurements used in this experiment were successful in evaluating the differences in hoof tissue strength between front and hind claws.

The elastic modulus measured through the tension test decreased from the *prepartum* period to 150 days *postpartum*, however no differences were found between claws. These results are probably related to a smaller number of tests that were completed due to the shape of the test sample and to a lower repeatability of results. The calculation of membrane stress and elastic modulus of the diaphragm involved also the use of measurements that were more subjected to intra-test variation, resulting in greater variability of results and lower significance. There were no differences found between inner and outer claws of front and hind hooves, these results being similar to the lesion scoring that found no difference between these claws. Hedges *et al.* (2002) reported that the white line of the inner claw had a greater tensile strength than the white line of the outer claw.

5.4.4 The changes in the live weight and condition score in the peripartum period

Changes in the live weight gain and condition score observed in the *peripartum* period were typical to those of lactating animals. Live weight and condition score were positively correlated to the number of days lame and locomotion score in different periods of the *peripartum*, indicating that heavier animals had higher locomotion scores. No correlation was found between live weight gain, condition score and the total lesion score of the sole and white line areas of the horn. Bowell *et al.* (2003) reported a similar significant positive relationship between body condition score and locomotion score. However, Van Dorp *et al.* (2004) found a significant but low negative phenotypic correlation of locomotion with body condition score, indicating that cows with poorer locomotion had lower body condition. These authors analysed data from 26 Canadian Holstein herds, the type of animals being different from Holstein-Friesians, and they looked not only at heifers but also at multiparous cows. The management of the body condition score during late lactation and the dry period has been found to be an important factor in determining the metabolic load of the cow during the *postpartum* period (Olsson *et al.*, 1998; Ingvartsen *et al.*, 1999; Thomas *et al.*, 1999). Lame animals were found to alter their feeding behaviour (Margerison *et al.*, 2002) and when animals are severely lame feed intake could be compromised leading to a loss of body condition.

5.4.5 Factors that correlated with the milk yield and the Holstein UK Index

Negative correlations of milk yield with condition score were considered typical to lactating animals around the peak of lactation, higher milk yield being related to greater negative energy balance and greater use of body reserves. Milk yield was also positively correlated with locomotion score at day 91 *postpartum*, indicating that cows producing more milk were significantly more lame at this stage of the lactation. However, no relationship was found between total lesion score of the sole and white line horns and milk yield corrected for 305 days. According to Deluyker *et al.* (1991), higher milk yield in the first 5 days of lactation and at day 21 of lactation was associated with significant increase in clinical lameness at the beginning of lactation and the majority of the lameness was associated with sole and white line lesions. Similarly, Enevoldsen *et al.* (1991) found that milk yield in early lactation and high milk yield combined with high body weight in the first lactation, were positively correlated with an increase in the levels of sole ulcers. A negative relationship has been found between milk yield and dairy cow health which included, higher incidence of mastitis, ketosis and milk fever, reproductive and locomotive problems (Emanuelson, 1988; Lyons *et al.,* 1991; Pryce *et al.*, 1998; Wassmuth *et al.*, 1999).

HUKI total dairy and mammary score and final score were negatively correlated with punch resistance and elastic modulus. Boettcher *et al.* (1997) found moderately high genetic correlations between dairy form and clinical lameness. According to Distl (1999) the selection of cows for higher milk yield may compromise the soundness of feet and legs. In addition, the selection of cows for higher milk yield had increased risk of metabolic stress (Emanuelson, 1988; Pryce and Lovendahl, 1999). Higher genetic merit cows have been found to partition a greater level of energy into milk production and significantly less energy to body reserves (Knight *et al.*, 1999; Thomas *et al.*, 1999; Veerkamp and Koenen, 1999). While high genetic merit cows were taller, they had lower levels of body condition than low genetic merit cows (Veerkamp and Koenen, 1999). Fregonesi and Leaver (2001) found that cows with low milk yield (≅ 19.1 kg/day) had greater live weight and body condition score than high yielding cows (≅ 32.1 kg/day) and a lower dry matter intake. This was supported by Dillon *et al.* (1999) who reported similar results, with high genetic merit cows having a greater live-weight loss up to week 10 of lactation, a lower live-weight gain between the tenth week and the end of the lactation and higher live weight gains during the dry period compared to low genetic merit cows.

The HUKI score for legs was positively correlated with locomotion score at 150 days *postpartum*, indicating that animals with straighter legs had better scores for locomotion. Boelling and Boelling and Pollot (1998), Pollott (1998b) and Van Dorp *et al.* (2004) found an association of sickle shaped legs with higher scores for locomotion. However, Boettcher *et al.* (1997) did not find any correlation between rear leg side view and clinical lameness. Animals with lower HUKI score for feet had lower foot angle, had higher lesion scores of the white line area and spent more days lame during the lactation. Similarly, a steep foot angle (Boettcher *et al.*, 1997) and straight rear legs have been related to a good walking ability (Boelling and Pollot, 1998b; Van Dorp *et al.*, 2004) and a lower dorsal angle and longer dorsal border of the claw has been found to be negatively correlated to the incidence of lameness (Boelling and Pollot, 1998; Manson and Leaver, 1989). While, the length of the hoof was negatively correlated and hoof angle was positively correlated with survival (Choi and MacDaniel, 1993; McDaniel, 1995). In this experiment animals with higher HUKI total score for legs and feet, indicating animals with better conformation of feet and legs, were lame for less days throughout the lactation. Boettcher *et al.* (1997) reported moderately high genetic correlations of the feet and legs score with clinical lameness. Van Dorp *et al.* (2004) found a weak phenotypic and a high genetic correlation of feet and legs score with locomotion, animals with better feet and legs score having a better locomotion score. However, no relationship was found between the total lesion score for the sole and white line horns and the HUKI score for legs and feet. Laven *et al.* (2004) reported similar results not finding a significant correlation between the sire leg and feet score and the occurrence of claw lesions in cows.

Animals with steeper foot angle also had higher values for mechanical tests, indicating stronger hoof horn. The animals that had a better score for the HUKI locomotion score had greater punch resistance for the white line area. However, in this experiment there were some animals with straighter legs that had better scores for locomotion and animals with higher total scores for legs and feet, indicating better conformation, had lower values for the mechanical tests. The significant correlation of the mechanical tests with the HUKI scores for rear leg occurred in the *prepartum* period and early in the *postpartum* period and the horn tested at this period was probably formed in late gestation. During this period the pressures that the locomotory system was subjected to may differ from pressures occurring in the *postpartum* period, changing the influence that the conformation had on the pressures on the hooves. Similarly, the change in housing that occurred during this time may have also altered the pressures exerted over the hoof horn. The range of scores given for the HUKI rear legs score (3 to 6) was not as wide as the HUKI scores for feet and for locomotion. No cow was scored for having very sickled legs and so the comparison was made between cows that had straighter and not so straight legs. There was a negative correlation between the HUKI total score for legs and feet with the punch force, elastic modulus of the diaphragm and membrane stress of the horn of the white line area. Higher values of the HUKI total score for legs and feet indicate animals with a better conformation of the locomotory system and animals with better conformation were expected to have higher values for the mechanical tests of the hoof horn. However, the HUKI total score for legs and feet was also not correlated to the lesion scores of the sole and white line areas.

Higher HUKI scores for body depth, chest width and rump width were positively correlated with the number of days spent lame, locomotion score and lesion score of the sole and white line areas, indicating that heavier animals had more locomotive problems. Boettcher *et al.* (1997) reported also a positive correlation between body depth and lameness and wider rumps and lameness, indicating that sires that produced larger daughters had daughters that were more predisposed to lameness. Van Dorp *et al.* (2004) found that cows with tall and deep bodies had a predisposition to poorer locomotion. However, Collard *et al.* (2000) reported that a greater chest width was associated with lower levels of locomotive problems.

Boelling *et al.* (2001) obtained significant correlations between measurements such as, size of the claw and length, width and circumference of the cannon bone, made on bulls at the end of their performance test and the traits hock quality and bone structure of their daughters. Boettcher *et al.* (1997) and Distl (1999) reported that the daughters of bulls that transmitted improved conformation of feet and legs were less clinically lame. Van Dorp *et al.* (2004) found a high genetic correlation of foot angle, rear leg set and feet and leg score with locomotion and concluded that the selection for a low locomotion score might improve the walking ability of cows, when included into an index for feet and legs. Boelling and Pollot (1998b) analysed records of cows from the Holstein-Friesian Society of Great Britain from 1978 to 1987 and found that the traits rear leg side view and foot angle improved in the preferred direction during these 10 years. Those traits have been considered important breeding objectives and sires have been selected to improve the traits, the foot angle was aimed to become straighter and the rear leg steeper. The correlation of those traits was very high and the selection for a straighter leg favours the selection for a steep foot angle. However, the authors considered it difficult to determine which trait had a greater influence over the other trait (Boelling and Pollot, 1998b; Distl, 1999). Veerkamp *et al.* (1995) incorporated 4 linear traits (udder depth, angularity, teat length and foot angle) and 3 milk yield traits in a selection index (ITEM) to increase the longevity, or longer herd life, of cows. Choi and McDaniel (1993) and Distl (1999) considered also the inclusion of the foot angle in a selection index because of its favourable relationship with survival. The genetic correlation between survival and foot angle (Brotherstone and Hill, 1991; Choi and McDaniel, 1993) and heel depth (Choi and McDaniel, 1993) was high. Also foot angle on the first lactation was positively related to survival rates to various ages (Choi and McDaniel, 1993; McDaniel, 1995). According to Boelling and Pollot (1998b) and Van Dorp *et al.* (2004) heritability for locomotion was low, however, conformation traits such as feet and leg score, bone quality and rear leg set had a moderate to moderately high heritability. Choi and McDaniel (1993) and Distl (1999) reported moderate heritability for foot angle and hoof length. Boelling and Pollot (1998b) considered that the selection response for locomotion would be negligible however; sires with very bad breeding values for locomotion should be identified and removed. Lyons *et al.* (1991), Boettcher *et al.* (1997) and Distl (1999) found that heritabilities for locomotive traits were high enough for them to be included in a selection program.

5.5 Conclusions

The mechanical tests used in this experiment reflected the changes in housing and in haemorrhage levels that occurred between the *prepartum* and *postpartum* period. The correlation between the HUKI conformation traits and punch resistance and elastic modulus of the horn indicate that certain types of conformation may predispose the heifers to have a weaker hoof horn structure. Sole horn conformation is related to the pressure exerted by the weight distribution in the claws that is related to the conformation of feet and legs. Lower punch resistance and elastic modulus was found in heifers with less straighter rear legs, lower foot angle, poor HUKI locomotion score, lower scores for the composite trait legs and feet and a higher final total score. Using a combination of scoring for conformation traits, mechanical tests and lesion scoring of the horn there is the potential for a selection of animals with increased hoof horn quality at the beginning of the first lactation.

Chapter 6 – Final Discussion

The present study aimed to develop mechanical methods of assessment to measure the changes in the mechanical properties of the sole and white line areas of the hoof horn and to compare these with standard methods of assessment of lameness i.e. locomotion scoring (Tranter and Morris, 1991) and scoring of sole bruising (Leach *et al.*, 1998). The mechanical tests were used to assess the changes in the structural strength of hoof horn due to; lactation period (in heifers and cows), the presence and level of horn haemorrhage and dietary supplementation with biotin (22 mg/h/d ± 2mg from 25 (± 5 days) *prepartum* until 160 d postpartum). Throughout this thesis individual claws and front and hind feet were compared, horn growth and wear rates, claw measurements and dry matter content of the horn were assessed. In the experiment using heifers the assessment of animal conformation was included and this was compared with the results of mechanical tests, locomotion score and sole bruising assessments.

6.1 Factors affecting punch force and tension testing of hoof horn

6.1.1 Test settings and repeatability of results

In Experiment 1 the punch test was completed on hoof horn samples looking at maximum punch force. The punch force measures the strength at which the tested material fractures and the work to fracture and was selected because it was suited to test specific small areas and it has been found to be a repeatable method (Aranwela *et al.*, 1999). These punch tests have been frequently used in other experimental work to determine mechanical properties of small or miniature specimens of a number of different materials (Husain *et al.*, 2002; Lewis, 2002). In this thesis the force-displacement curves of punch tests exhibited similar distinctive features, which were highly reproducible. Initially, the curve displayed a nonlinear toe-in region followed by a linear stiffness and a peak load. In this test throughout this thesis, the high radial clearance (5mm) and low sample thickness (< 0.4mm) would have caused bending effects to occur and the subsequent stresses that occurred were probably mainly in tension and not in shear.

In the subsequent experiments (2, 3 and 4) a standard tension test was added to the punch test for comparative purposes and with the aim of obtaining results that could be compared more widely with results described in the literature (Bertram and Gosline, 1987; Geyer and Schulze, 1994; Schmid, 1995; Zenker *et al.*, 1995; Douglas *et al.*, 1996; Kasapi and Gosline, 1996 and 1997; Hinterhofer *et al.*, 1998; Baillie *et al.*, 2000; Zoscher *et al.*, 2000; Hedges *et al.*, 2002). The variation in results of the tension tests in the experiments presented in this thesis was greater when compared with the punch test, which was found to be very repeatable (Tables 3.1, 3.2, 4.11, 4.20). A smaller number of tension tests were completed when compared with punch tests, because of the greater size of the test material required by this test (2x20x0.05 mm) and only a small number of test pieces (2 to 3) were obtained from the sole and white line area of each claw. This would explain the greater variability of tension test results. However Aranwela *et al.* (1999) also reported greater variation of tension test results when measuring the tensile strength of leaves.

In all the experimental work in this thesis there was variation in sample thickness of the sole area and white line areas tested. This was unavoidable and was mainly related to the concave shape of the claw and the method of sample collection. The variation in sample thickness was greatest in experiment 1 for sole 0.02 to 1.8 mm and white line areas 0.03 to 1.5 mm, when samples were collected with a standard hoof trimming knife. This variation was subsequently reduced in experiments 2, 3 and 4; to 0.04 to 0.3 mm for the sole area and 0.03 to 0.35 for the white line area with the use of a standard hand held wood plane. The effect of sample thickness was assessed and in experiment 1 sample thickness accounted for 0.30 to 0.35 (proportion) of the variation of the punch force and work to fracture results of the sole and white line area. In experiment 2 when different thicknesses of the same hooves were tested, sample thickness accounted for a greater proportion (0.75 to 0.80) of the variation of the punch force and work to fracture and in experiments 3 and 4 the sample thickness accounted for 0.30 to 0.40 of the variation of the punch force and work to fracture. The sample thickness was the variable that had the greatest influence over the punch force and work to fracture in all the experiments. As a consequence, when analysing the data of the punch force of hoof horn tissue from the sole and white line areas the thickness of the tested area was measured and included as a covariant in the statistical analysis of the punch force data.

In punch tests the calculation of the bulk material properties, such as elastic modulus and ultimate tensile strength, is complicated due to the contact mechanics involved (Aranwela *et al.*, 1999; Lewis, 2002). The calculation of the elastic modulus of the diaphragm and membrane stress from the equation for maximum lateral deflections of circular plates (Alexander Blake, 1982) was an attempt to better interpret the punch test results and to compare these with the tension tests. Liu and Piggot (1998) and Lewis (2002) obtained a good correlation between results of the small punch test and results of standard ASTM tension tests, with results varying within 0.025 to 0.26 (proportion) of each other. The results for the punch force, work to fracture, elastic modulus of the diaphragm and membrane stress were correlated and the correlation factor varied ($r = 0.50$ to 0.95). The punch force was sometimes found to be negatively correlated to elastic modulus of the diaphragm and membrane stress possibly due to an effect of sample thickness, thinner samples requiring less force to be punctured however the membrane stress and elasticity being high. The correlations between the punch force and work to fracture, elastic modulus of the diaphragm and membrane stress and elastic modulus of the diaphragm and elastic modulus measured through tension tests were found to be consistently highly significantly ($P<0.001$) correlated (r = 0.68 to 0.95). The punch force measures the strength at which the tested material fractures (Aranwela *et al.*, 1999) and as the work to fracture is the measurement of the area under the force-displacement curve, an increase in the punch force leads to an increase in the work to fracture. It was anticipated that the elastic modulus of the diaphragm would be related to the elastic modulus measured through tension as in the punch test the stress that occurred was probably mainly in tension. In the future, the use of finite element analysis may be used to elucidate the differing stages of the punch test. The finite element analysis has been used to compare elastic modulus results obtained from standard tests and the initial slope of the force-displacement curve of punch tests. The initial slope of the force-displacement curve was found to be positively and linearly related to the elastic modulus (elastic modulus = 0.283 stiffness of load–displacement curve) (Kurtz *et al.*, 1999; Kurtz *et al.*, 2002).

6.1.2 Effect of moisture content of the hoof horn samples on mechanical properties of the sole and white line areas

The maximum punch force, work to fracture, elastic modulus of the diaphragm, membrane stress and elastic modulus measured through tension of the sole and white line areas of the horn were significantly ($P<0.001$) and strongly (R^2adj. = 0.55 to 0.90) affected by the moisture content of the hoof horn. Kitchener and Vincent (1987) stated that the mechanical properties of keratinous materials are strongly affected by the state of hydration and

Bertram and Gosline (1987), Douglas *et al.* (1996), Collins *et al.* (1998), Hinterhofer *et al.* (1998), Baillie *et al.* (2000) and Dyer *et al.* (2004) demonstrated that the moisture content has a significant effect on the measurement of hardness, elastic modulus, bending stiffness and fracture toughness of the hoof horn. Collins *et al.* (1998) measured the elastic modulus of donkey hoof wall through bending tests and the samples were tested under a similar range of moisture contents (2, 18, 33 and 34 %) as in experiment 2, and found that the mean elastic modulus for samples with 34, 18 and 2 % moisture content was 145.3, 853.0 and 2167.6 N/mm^2, respectively. In experiment 2 hoof horn samples of the sole and white line areas of cattle were placed in environments containing 11, 33, 58, 75 and 97 % RH and the moisture content of the tested samples varied between 9 and 36 %, after tests of samples placed in the environment of 11 % RH were rejected because the samples were brittle and fractured when they were placed in the roller grips or between the metal plates. This allowed a regression equation to be fitted and punch force of the sole and white line areas, work to fracture of the white line area and elastic modulus measured through tension tests had a highly significantly ($P<0.001$) positive relationship to the dry matter content of the horn (R^2adj. = 0.65 to 0.89). The work to fracture of the sole area, elastic modulus of the diaphragm and membrane stress had a significantly ($P<0.01$) positive relationship to the dry matter content of the horn but R^2adj. values were lower (R^2adj. = 0.37 to 0.55). A wide range of moisture levels of the hoof horn was tested and a clear and strong relationship between the mechanical properties of the bovine horn and moisture content of the sample was demonstrated.

Kitchener and Vincent (1987) reported a mean bending stiffness of oryx horn with 0, 20 and 40 % moisture content of 6100 (s.e. 160), 4300 (s.e. 90) and 1800 N/mm^2 (s.e. 90), respectively. Bertram and Gosline (1987) tested the elastic modulus of the equine hoof wall horn in fully hydrated and dry samples, the elastic modulus being 410 N/mm^2 and 14,600 N/mm^2, respectively. In experiment 2 the mean elastic modulus of the sole horn varied from 85.5 to 751.88 N/mm^2 when the moisture content of the horn was 29.2 and 9.7 %, respectively. Collins *et al.* (1998), Baillie *et al.* (2000) and Bertram and Gosline (1987) tested horn with 0% moisture content and therefore found higher values of elastic modulus when compared to the results in experiment 2 were the lowest mean moisture content was 9.7 %. Collins *et al.* (1998) and Bertram and Gosline (1987) found higher values of elastic modulus for fully hydrated samples (145.3 and 410 N/mm^2) when compared to the elastic modulus of hydrated samples in experiment 2 (85.5 N/mm^2) and the differences may be related to the area tested and different species. Collins *et al.* (1998) and Bertram and Gosline (1987) tested the wall horn and it had a greater elastic modulus compared with the horn of the sole area that was tested in the experiment 2 (Hinterhofer *et al.*, 1998). The elastic modulus of the bovine sole and white line areas of the hoof horn has not presviously been tested using horn samples with the range of moisture levels such at that presented in experiment 2. Moreover, the punch force has not been tested before on bovine hoof horn samples and it was important to establish the effected of moisture content of the horn on the punch force.

The dry matter content of the bovine toe, lateral wall, sole horn area and front and hind claws have been found to be 74.6, 73.4, 68.8, 73.1 and 71.3 %, respectively (Zoscher *et al.*, 2000). These differences in dry matter content were found to be significant and the areas that had a significantly higher dry matter content had higher levels of modulus of elasticity; 613.5 (sd. 203.6), 375.3 (sd. 133.8), 134.9 (sd. 104.1), 405.5 (sd. 251.0) and 349.5 N/mm^2 (sd. 242.5) for toe, lateral wall, sole horn area and front and hind claws. The mean DM content of bovine sole horn presented by Van Amstel *et al.* (2004) and Zoscher *et al.*

(2000) of 67.9 and 68.8 %, respectively, is very similar to the mean dry matter content for the sole horn of 70.9 (± 0.7) % found in experiment 2, 64.8 % found in experiment 1, 74.01 % found in experiment 3 and 66.37 to 74.81 % found in experiment 4. The mean elastic modulus of the sole area of the hoof horn with physiological moisture content was 116.9 N/mm^2 (sem. 39.5) in experiment 2, 70.77 to 114.47 N/mm^2 (sem. 10.88) in experiment 3 and 77.78 to 104.12 N/mm^2 (sem. 10.25) in experiment 4 and is similar to the elastic modulus of the sole horn area with physiological moisture content presented by Zoscher *et al.* (2000) of 134.9 N/mm^2 (sd. 104.1). In both experiments the elastic modulus was measured through tension tests. The range in the elastic modulus values was very similar in experiment 3 and 4, indicating repeatability of results.

The results from experiment 2 demonstrate that the control of the loss of moisture from hoof horn samples is clearly of vital importance when testing hoof horn tissue at physiological moisture content. Therefore, to achieve this hoof horn tissue samples should be stored in sealed plastic bags or wrapped in 3 layers of Parafilm (Collins *et al.*, 1998) and kept in the refrigerator. In Experiment 2 it was found that hoof horn samples could be kept in these conditions for up to 8 days without significant changes in dry matter content and mechanical properties. This period of time (8 days) provides a greater flexibility of time between sample collection and analysis, allowing samples to be send in for example by post.

6.1.3 Effect of level of haemorrhage on horn samples on the mechanical properties of the hoof horn of the sole area

In experiment 1 the punch force of the sole area of the claw horn was found to decrease significantly ($P<0.001$) and gradually (8.72, 8.53, 8.06, 7.75, 6.08, 4.99 N, sem 0.078 to

0.460) when haemorrhage levels of the tested area increased (from 0, 1, 2, 3, 4 to 5 respectively). This indicates a direct relationship between horn structural strength and haemorrhage level which has not been previously demonstrated. Moreover, when testing through multiple regression analysis for the factors that had a significant effect of haemorrhage level on the punch force, elastic modulus of the diaphragm and membrane stress, it was found that the haemorrhage level of the hoof sample consistently had a significant ($P<0.001$) negative effect on the mechanical properties of hoof horn collected from multiparous cows and also had a significant ($P<0.001$) negative effect on the mechanical properties of hoof samples collected from the white line area of heifers. Borderas *et al.* (2004) found significant ($P<0.05$ to 0.01) negative correlations (-0.36 to -0.56) between claw hardness measured with a durometer and haemorrhages of the sole and white line areas, heel erosion and sole ulcers. Clearly, hoof horn with haemorrhages was inferior in terms of mechanical properties, i.e. strength and elasticity and would provide less cushioning impact then horn without haemorrhages, potentially increasing the trauma of the corium and basal cell layers of the dermis. A horn with less elasticity and hardness values may wear faster thus decreasing sole thickness and again increasing the risk for trauma of the corium.

6.1.4 Difference in the mechanical properties of the sole and white line areas

The differences in the punch force measurements of sole and white line horn areas in Experiments 1, 3 and 4 were consistent with the results of Budras *et al.* (1996) that reported lower hardness levels of the horn tissue of the white line area when compared with the sole area using a durometer. In experiment 4 the scanning electron micrographs of the fracture surfaces of horn samples fractured in tension tests were further investigated to compare the sole and white line areas and horn tissue that had higher and lower values of elastic modulus. The scanning electron micrographs of the fracture surfaces of horn samples fractured in tension tests showed the low elasticity of the horn of the white line area when compared with the horn of the sole area. In the micrographs the fracture surface of the hoof horn of the sole area had a higher level of fibre pull-out, indicating resistance to deformation and an inelastic type of deformation. Moreover, in the micrographs, the fracture surface of the white line area was shown to be non-uniform thus demonstrating the heterogeneous nature of the white line area of the hoof horn. This gives some insight into how the morphological differences of the sole and white line areas have an influence over the fracture process and helps to explain the susceptibility of the white line to suffer damage (Mulling *et al.*, 1994).

6.2 Factors that affected the development of the locomotion and lesion scores

Levels of locomotion score in multiparous cows (Experiment 1 and 3) were high and the incidence of claw horn problems (0.50 to 0.60), such as sole ulcers, white line separation and severe haemorrhage of the sole and white line areas of the hoof horn, and digital dermatitis (0.25 to 0.33) were similar to the occurrence reported in other herds (Kossaibati *et al.*, 1999). The Seale-Hayne herd had a high incidence of white line disruption (0.30) with incidence of stone punctures and white line separation being higher when animals were at pasture (0.06) compared to when the animals were housed (0.00). This indicates that at pasture cows were exposed to potential sole and white line puncture to a greater extent than when housed. In practice the farm did use some general purpose roads to allow the dairy herd access fields for grazing, exposing cows to small stones.

6.2.1 Effect of days in the peripartum period on the locomotion sore of cows and the lesion score of the claw horn of the sole and white line areas

This research showed that locomotion score and lesion scores of the claw horn in multiparous cows (Experiment 1 and 3) increased during the *postpartum* period. Several authors have reported similar findings (Enevoldsen *et al.*, 1991; Bergsten and Herlin, 1996; Bergsten and Frank, 1996; Leonard *et al.*, 1996; Vermunt and Greenough, 1996; Leach *et al.*, 1997; Livesey *et al.*, 1998; Whay, 1998; Chaplin *et al.*, 2000 and Offer *et al.*, 2000). The hormonal changes and the consequent softening of the collagen fibres that connect the third phalanx to the horn capsule, associated with parturition, are believed to be the major contributory factor in the development of hoof lesions (Webster, 1998; Holah *et al.*, 2000; Tralton and Webster, 2002).

The locomotion score in experiment 1 was highest between days 90 and 120 *postpartum* and in experiment 3 increased linearly until day 200 *postpartum*. The lesion scores of the sole area of the claw horn increased earlier in experiment 1 (between days 30 and 60 *postpartum*) when compared with experiment 3 (between days 100 and 160 *postpartum*). However, in experiment 1 the scores for lesion of the sole area of the claw horn remained high until day 160 *postpartum*. While it is not possible to make direct comparisons between these experiments, the cows in experiment 1 were kept in a cubicle system three weeks before calving and cows in experiment 3 were only introduced into the cubicle housing following parturition. This difference in housing management around *parturition* could have resulted in greater pressure on the sole horn in the critical *peripartum* period leading to an earlier increase in the scores for locomotion and lesion of the sole area of the claw horn. Housing cows in cubicles resulted in a significantly higher incidence of lameness and claw horn haemorrhages when compared with housing animals in straw yards and tie-stalls (Rowlands *et al.*, 1983; Singh *et al.*, 1993; Bergsten and Herlin, 1996; Livesey *et al.*, 1998; Weaver, 1998; Meyer and Galbraith, 1998; Webster, 2001; Somers *et al.*, 2002; Bowell *et al.*, 2003; Somers *et al.*, 2003; Laven and Livesey, 2004; Whitaker *et al.*, 2004).

In Experiment 1 the lesion scores for the white line area of the claw horn were constantly high, but no significant increase was observed in the *postpartum* period. These results differed from results obtained in Experiment 3 were the lesion score of the white line area of the claw horn increased significantly between day 50 and 100 *postpartum* and which may be related to the different management conditions during the *peripartum* period. In Experiment 3 the lesion scores of the white line area increased earlier in the *postpartum* period (days 50 and 100 *postpartum*) when compared with the lesion scores for the sole area (days 100 and 160 *postpartum*). Similar results were obtained by Enevoldsen *et al.* (1991), Leach *et al.* (1997) and Offer *et al.* (1998 and 2000).

In Experiment 1 lesion scores of the sole area decreased again at the end of the lactation period (270 days *postpartum*) when cows were turned out to pasture. Singh *et al.* (1993) and Kerr (1998) also found lower incidences of lameness when cattle were turned out to pasture during the spring and summer. This decrease in lesion scores would probably related to several factors, rather than any one single effect, which include change in environment, lower metabolic stress that cows are subjected to later in lactation and the longer day light and the more favourable conditions for hoof horn growth and keratinisation (McCallum *et al.*, 2002).

In heifers (experiment 4) the lesion scores for the sole and white line areas increased at the same time (100 days *postpartum*) and the highest scores for the sole area were found earlier (100 days *postpartum*) during the *postpartum* period compared with the scores for the multiparous cows (160 days *postpartum*) in Experiment 1 and 3. The lesion scores for the sole area in heifers declined at 150 days *postpartum* when the sole lesion scores were highest in the multiparous cows. In heifers, Offer *et al.* (2000) found that white line and sole lesions appeared at their greatest levels 7 days prior to cows in first lactation heifers, however, this difference increased with increasing numbers of parities and that the period of time to maximum lesion score of the white line and sole became progressively longer during subsequent lactations. The difference in the pattern of lesion formation of the sole and white line between the first and subsequent lactation animals may be related to repeated lesion formation, cumulative damage and to animals calving some time after housing in later lactations, as opposed to simultaneously in the first lactation (Offer *et al.*, 2000). It has been found that in *primiparous* heifers sole haemorrhages subsided more quickly when compared to multiparous cows (Greenough and Vermunt, 1991; Huang *et al.*, 1995; Bergsten and Herlin, 1996).

6.2.2 Effect of different claws on the lesion score of the claw horn of the sole and white line areas

During the *postpartum* period the hind outer claws of multiparous cows (experiments 1 and 3) had a significantly higher lesion score for the sole area when compared with front and inner hind claws. Similar results were reported by Bergsten and Herlin (1996), Leach *et al.* (1997), Offer *et al.* (2000) and Le Fevre *et al.* (2001). Due to the conformation of the coxo femural joint the cow has been found to be more able to relieve weight from the front claws than from the hind claw (Toussaint-Raven, 1985; Frandson, 1986) and the alteration of forces, i.e. the difference of the minimum to the maximum load, rather than the absolute load, was considered to damage to a greater extent the hind outer claws (Toussaint-Raven, 1985; Scott, 1987; Singh *et al.*, 1993). Moreover, at *parturition* the conformation of the udder has been found to force the cow to have a wider stance, causing an increased weight bearing on the outer hind claws (Vermunt and Greenough, 1996). Cows in experiment 1 had significantly higher lesion scores of the white line area of the hind outer claws at day 160 *postpartum* and in Experiment 3 lesion scores for the white line area were significantly higher for hind claws when compared to front claws. Conversely, Le Fevre *et al.* (2001) found white line lesions to be more evenly distributed between claws when compared with sole lesions and white line lesions were also less concentrated in a single claw. The difference in scores between the claws was not so pronounced in heifers (experiment 4) as in the multiparous cows (experiments 1 and 3). However, similarly in heifers, the scores were significantly higher for hind claws when compared with front claws and were higher in the sole area for the hind left claw. This difference between hind left and hind right claws may potentially be related to the left hand turn that cows have to make when leaving the parlour. The initial assessment of the housing system indicated that there was the potential for animals to have to turn sharply left through almost 360° as they left the milking parlour in the experiments completed in this thesis. However, the higher incidence of lesions in the hind left outer claw has been observed in experiments completed in other housing systems (Personal communication Van Helan, 2004).

6.3 Claw measurements in the *postpartum* period

At housing the growth and wear rates for front and hind claws were greater for cows in experiment 1 (growth rates: 0.69 and 0.84 cm/month, wear - 0.90 and 0.66 cm/month) when compared with cows in experiment 3 (growth rates: 0.37 and 0.50 cm/month, wear rates: 0.26 and 0.33 cm/month), while it is not possible to compare experiments from differing years with potential for variation in a number of factors, such as forage quality. The greater growth and wear rates of cows in experiment 1 could be related to cows in experiment 1 being housed in a cubicle system 3 weeks before calving and possible greater pressure the hooves were subjected to in the *peripartum* period. However, it is important to note that there seemed to be relatively little difference in diet, forage quality or dry matter content and despite this, considerable differences were found in the growth rates from one year to the next. This was particularly important in terms of experimental planning and application, having used estimates from Leach *et al.* (1997), Chaplin *et al.* (2000) and Offer *et al.* (2000) and growth rates from experiment 1 to plan the feeding period for experiment 3 and this was an important factor that may have affected the results from experiment 3. The growth rates of front and hind claws were greater again in the cows in experiment 1 when cows were turned out to pasture (0.97 and 0.78 cm/month) and wear rates increased for the hind claws (0.72 cm/month) but not for front claws (0.67 cm/month). The increase in wear rates of hind claws when animals were at pasture may be related to increased walking distances and rough walking surfaces. Increase in growth rate when at pasture could be related to a combination of several factors including; photoperiod, a reaction to the increase in wear rate and changes in the underfoot environment from the concrete flooring to pasture (Tranter and Morris, 1992; MacCallum *et al.*, 1998 and 2002).

The growth and wear rates were higher for the hooves of the hind claws compared with the hooves of the front claws (growth rates: 0.71 and 0.58 cm/month, wear rates: 0.33 and 0.26 cm/month). These higher wear rates for the hind claws when compared with the front claws could be related to a greater load that these claws were subjected due to the increase in udder size in the *postpartum* period (Toussaint-Raven, 1985, Scott, 1987, Singh, 1993, Vermunt and Greenough, 1996). The net growth rates were higher for the hind claws when animals were housed and positive for front and hind claws in Experiment 3 (0.11 and 0.17 cm/month) and for hind claws in Experiment 1 (0.17 cm/month). Similar levels were reported by Clark and Rakes (1982). The front claws in Experiment 1 had negative net growth rate (- 0.18 cm/month). Leach *et al.* (1997) also reported negative net growth rates (-0.25 cm/month) when animals were housed on concrete floors. This would indicate the potential for sole thinning in some housing conditions and the potential for a reduction in the impact absorption and protection of the corium which could increase sole bruising. This needs further investigation and may have the potential to be a method by which the effect of floor conditions on lameness is studied.

The heifers in experiment 4 were housed in a loose bedded yard and had a monthly hoof horn growth of 0.58 to 0.66 (cm/month) and wear rates of 0.44 to 0.74 (cm/month). These figures were similar to the levels reported by Webster (2001) for first lactation heifers housed in either cubicles or loose bedded yards (growth – 0.48 to 0.62 cm/month, wear – 0.47 to 0.52 cm/month). In the heifers, the hooves of the hind claws did not grow faster than the hooves of the front claws as found for the housed cows in Experiment 1 and 3 and reported by Clark and Rakes (1982) and Whay (1998). However, the hind claws of the heifers (experiment 4) did have a higher wear rate (0.44 to 0.56) when compared with (0.58 to 0.74 cm/month) the front claws. As a result, the hind claws had a negative net growth rate between 100 to 150 days *postpartum* (-0.08 cm/month). This is similar to the finding reported by Leach *et al.* (1997) for the housing period (- 0.25 mm/month). The higher wear rates for the hind claws when compared with the front claws may be related to a greater load that these claws were subjected due to the increase in udder size during the *postpartum* period and to the propulsion movement performed by the hind claws (Toussaint-Raven, 1985, Scott, 1987, Singh, 1993, Vermunt and Greenough, 1996). Six out of twenty heifers in experiment 4 had oedema of the udder in different degrees during the *peripartum* period, causing them to have a wider stance and increasing the pressure on the hind outer claws.

Changes in the shape of the claw after the start of the lactation were reported by Offer *et al.* (2000). They found that during lactation, the angle of the dorsal border became smaller while the dorsal wall became longer. These changes were considered probably to be related to changes in the growth and wear rates (Offer *et al.*, 2000). In Experiment 1 and 3 there was an increase in the lateral height and the angle of the dorsal border of hind hooves and in the lateral and heel height of the front hooves in the *postpartum* period. This increase in the lateral and heel height of the front hooves was most likely related to the effect of a positive net growth rate. In the cows that were at pasture at 270 days *postpartum* (experiment 1) the claws were found to be longer and the height of the heel shorter which was most likely equally due to the higher growth rate while at pasture compared with winter housing. Offer *et al.* (2000) reported smaller claw angles and longer claws when animals were out at pasture when compared with the winter housing period. The shorter height of the heel was probably related to a change in the foot angle due to the longer claw.

In Experiment 4, where the heifers were kept in a straw yard, there was an increase in the angle of the dorsal border and in the heel height in the *postpartum* period. The housing system has been found to affect heel height and Phillips and Schofield (1994) reported an increase in the depth of the heel of animals kept in a straw yard, compared with a heel depth that decreased in animals housed in a cubicle system. In heifers (experiment 4) the front claws had a higher heel (5.28 – 6.35 cm) and greater angle of the dorsal wall (48.84 – 51.82 °) compared with hind claws (4.47 – 4.82 cm and 46.63 – 50.30 °). Similar differences have been found in the shape of the front and hind claws (Toussaint-Raven, 1985), with the front claws of cows typically having a higher heel and a more horizontal "coronary line" than hind claws.

6.4 The effect of days *postpartum* and the lesion score on the results of mechanical tests

The punch force of the sole and white line areas of the claw horn decreased during the *postpartum* period in multiparous cows (Experiments 1 and 3), reaching the lowest level at day 160 *postpartum* when lesion scores of the sole area was at the highest. The punch force increased as lesions score decreased and in experiment 1 at the end of lactation this coincided with the period when cows were turned out to pasture. The housing environment change, from a cubicle system with concrete floor to pasture, and the late lactation period could both have had a positive effect on the punch force of the horn.

The punch force of the sole and white line areas was consistently significantly higher for the front claws in experiments 1, 3 and 4 when compared to the hind claws and in experiment 3 it was lowest for the hind outer claws for the sole area and lowest for the hind left claws for the white line area. In multiparous cows that had higher scores for lesions of the claw horn when compared to the heifers, the punch force decreased in periods when the cows had greater lesion scores and was lower in claws that had higher lesion scores. The lower punch force of the hind left claws of cows may have been related to the left turn the cows have to make when leaving the parlour. In heifers the punch force decreased for the white line area at 50 days *postpartum*, but increased for the sole area at 100 days *postpartum*. A greater number of animals suffered from white line haemorrhages than sole haemorrhages in the *prepartum* period and this increasing level of haemorrhage may have affected negatively the punch force test for the white line area. White line separation and stone puncture were also higher in the *prepartum* period. The punch force of the white line area increased again at 150 days *postpartum*. The increase in the punch force of the horn of the sole area during the *postpartum* period was probably affected by the change in environment that was reflected also in the decrease of the dry matter content of the horn. Heifers had lower overall lesion scores of the sole and white line areas when compared with the multiparous cows and the mechanical tests were not affected as greatly by the haemorrhage level of the horn.

In heifers, the lower punch force measured in hind claws when compared to front claws in the *postpartum* period was not observed during the *prepartum* period, indicating probably a low level of bruising of all claws. In the *postpartum* period the higher increase in lesion score of hind claws when compared with front claws increased the differences in the punch force of the horn. At 50 days *postpartum* front claws had a higher dry matter content than hind claws (67.86 vs. 66.27 %) and this difference had an effect on the results of the punch force test, the punch force of the front claws being higher than the punch force of the hind claws. However, other authors reported greater lesion score of hind claws in 9 to 10 month old calves and 15 to 18 month old heifers (Bradley *et al.*, 1989; Singh *et al.*, 1994). The rearing of heifers, their growth rate and feeding were found to influence the overall health of the feet of animals throughout their life (Thomas *et al.*, 1999) and could explain why scores for lesions were not high in heifers in the *prepartum* period in the present experiment.

6.5 The effect of biotin supplementation on the scoring of lesions and the mechanical test of the claw horn

Biotin supplementation from 25 days *prepartum* until day 160 *postpartum* did not have an influence over the locomotion score, lesion score of the white line area of the sole, measurement of claw angle, claw length and heel height, condition score, punch force, elastic modulus and work to fracture of the sole and white line horns of dairy cows housed in a cubicle system in the *postpartum* period. The lesion score of the sole area of the hind left inner claw at day 160 *postpartum* was significantly (P<0.05) lower for biotin supplemented cows when compared to non-supplemented cows. Distl and Schmid (1994) and Hochstetter (1998) observed a positive effect of biotin on the incidence of sole haemorrhages in dairy cows after 8 month supplementation, so maybe greater differences would have been measured with a longer supplementation period. However, considering the hoof growth rate of experiment 1 the supplementation time was believed to be appropriate at the start of the experiment.

6.6 The effect of claw measurements and heifer conformation on the lesion score of the claw horn and on the results of mechanical tests

The growth rate between 50 and 100 days *postpartum* was negatively correlated to the elastic modulus, membrane stress of the sole horn and to the punch force of the horn of the sole and white line areas at 50 days *postpartum*. These results indicated that higher growth rates were associated with a decrease in the elastic modulus. McCallum (1999) found that the pressure on the horn stimulated the hoof horn growth rate to increase. Epidermal cells receive a supply of nutrients and oxygen via diffusion from the blood vessels in the corium. The living epidermal cells are very susceptible to any disturbance in the circulation within the vessels of the corium (Mulling *et al.*, 1998). Increased pressure could lead to disturbances in the circulation within the vessels of the corium and the formation of horn with inferior quality. Also, horn with a greater punch force in the beginning of the lactation was associated with a lower horn growth rate of the claws (r = -0.63).

A longer dorsal border of the hind claws at 150 days *postpartum* was correlated to a higher lesion score of the white line (r = 0.60). A higher incidence of lameness has been related to a smaller angle of the dorsal border and a longer dorsal border of the claws (Boelling and Pollot, 1998; Manson and Leaver, 1989). The lesion score of the white line and the locomotion score were also positively correlated with the height of the heel (r =0.45) and of the side wall(r =0.48) at days 100 and 150 *postpartum*.

In experiment 4 the HUKI score for legs was positively correlated with locomotion score at 150 days *postpartum*, indicating that animals with straighter legs had better scores for locomotion. Boelling and Pollot (1998), Boelling and Pollott (1998b) and Van Dorp *et al.* (2004) found an association between sickle shaped legs and higher scores for locomotion. Animals with lower HUKI score for feet had lower foot angle, had higher lesion scores of the white line area and spent more days lame during the lactation, indicating that this was an effective method of animal selection for reduced lameness. Similarly, a steep foot angle (Boettcher *et al.*, 1997) and straight rear legs have been related to a good walking ability (Boelling and Pollot, 1998b; Van Dorp *et al.,* 2004) and a lower dorsal angle and longer dorsal border of the claw has been found to be negatively correlated to the incidence of lameness (Boelling and Pollot, 1998; Manson and Leaver, 1989). Animals with higher HUKI total score for legs and feet, indicating animals with better conformation of feet and legs, were lame for fewer days throughout the lactation. Boettcher *et al.* (1997) reported moderately high genetic correlations of the feet and legs score with clinical lameness. Van

Dorp *et al.* (2004) found a weak phenotypic and a high genetic correlation of feet and legs score with locomotion, animals with better feet and legs score having a better locomotion score. However, in experiment 4 no relationship was found between the total lesion score for the sole and white line horns and the HUKI score for legs and feet, indicating that this composite trait was not an effective method to select for animals with lower levels of haemorrhage of the sole and white line areas. Laven *et al.* (2004) reported similar results not finding a significant correlation between the sire leg and feet score and the occurrence of claw lesions in cows.

Animals with steeper foot angle also had higher values for mechanical tests, indicating stronger hoof horn. The animals that had a better score for the HUKI locomotion score had greater punch force for the white line area, indicating that potentially mechanical tests could be used to select animals for reduced susceptibility to lameness. However, in experiment 4 there were some animals with straighter legs that had better scores for locomotion and animals with higher total scores for legs and feet, had lower values for the mechanical tests. The significant correlation of the mechanical tests with the HUKI scores for rear leg occurred in the *prepartum* period and early in the *postpartum* period and the horn tested at this period was probably formed in late gestation. During this period the pressures that the locomotory system was subjected to may differ from pressures occurring in the *postpartum* period, changing the influence that the conformation had on the pressures on the hooves. Similarly, the change in housing that occurred during this time may have also altered the pressures exerted over the hoof horn. The range of scores given for the HUKI rear legs score (3 to 6) was not as wide as the HUKI scores for feet and for locomotion. There were no heifers scored for having very sickled legs and so the comparison was made between cows that had straighter and not so straight legs. Further tests should be carried out on a wider range of animals to model those relationships. However, there was a negative correlation between the HUKI total score for legs and feet with the punch force, elastic modulus of the diaphragm and membrane stress of the horn of the white line area and this could be related to the absence of relationship between the total lesion score for the sole and white line horns and the HUKI score for legs and feet.

HUKI total dairy and mammary score and final score were negatively correlated with punch force and elastic modulus. Boettcher *et al.* (1997) found moderately high genetic correlations between dairy form and clinical lameness. According to Distl (1999) the selection of cows for higher milk yield may compromise the soundness of feet and legs and subsequent the hoof horn quality.

Van Dorp *et al.* (2004) found a high genetic correlation of foot angle, rear leg set and feet and leg score with locomotion and concluded that the selection for a low locomotion score might improve the walking ability of cows, when included into an index for feet and legs. Boelling and Pollot (1998b) analysed records of cows from the Holstein-Friesian Society of Great Britain from 1978 to 1987 and found that the traits rear leg side view and foot angle improved in the preferred direction during these 10 years. Those traits have been considered important breeding objectives and sires have been selected to improve the traits, the foot angle was aimed to become straighter and the rear leg steeper. The correlation of those traits was very high and the selection for a straighter leg favours the selection for a steep foot angle. However, the authors considered it difficult to determine which of the traits had a greater influence over the other trait (Boelling and Pollot, 1998b; Distl, 1999). Choi and McDaniel (1993) and Distl (1999) considered also the inclusion of the foot angle in a selection index because of its favourable relationship with survival. The genetic correlation between survival and foot angle (Brotherstone and Hill, 1991; Choi and McDaniel, 1993) and heel depth (Choi and McDaniel, 1993) was high. Also foot angle on the first lactation was positively related to survival rates to various ages (Choi and McDaniel, 1993; McDaniel, 1995). According to Boelling and Pollot (1998b) and Van Dorp *et al.* (2004) heritability for locomotion was low, however, conformation traits such as feet and leg score, bone quality and rear leg set had a moderate to moderately high heritability. Boelling and Pollot (1998b) considered that the selection response for locomotion would be negligible however; sires with very poor breeding values for locomotion should be identified and removed from sire lists. Lyons *et al.* (1991), Boettcher *et al.* (1997) and Distl (1999) found that heritabilities for locomotive traits were high enough for them to be included in a selection program.

The different measurements of claw in all experiments and leg and the cow conformation in experiment 4 were consistently correlated to the lesion score, the punch force and the tensile strength of the horn indicating that certain types of conformation may predispose the dairy cows to have a weaker hoof horn structure. Lower punch force and elastic modulus was found in heifers with less straight rear legs, lower foot angle, poor HUKI locomotion score, lower scores for the composite trait legs and feet and a higher final total score. Mechanical testing could be used in a selection index in complement to the HUKI conformation scoring and the lesion scoring to select for dairy cattle with stronger hoof horn and lower incidence of haemorrhages in the claw horn.

6.7 Conclusions

The punch and tension tests proved to be adequate methods for the measurement of changes in the mechanical properties of the sole and white line areas of the hoof horn of dairy cattle. Punch force, work to fracture and elastic modulus differed between the sole and white line areas of the horn and clearly decreased when the haemorrhage level of the horn increased. Therefore the increase in the scoring for lesions of the claw horn and the locomotion score of cows that occurred in the *postpartum* period were related to a decrease in those parameters. The moisture content of the horn had a strong influence over results of punch and tension tests underlining the importance of the control of the loss of moisture of the samples. In occasions were the change in housing was followed by a change in the dry matter content of the horn the mechanical properties changed also accordingly. The supplementation of biotin did not affect the punch force and elastic modulus of the sole and white line areas of the horn. The correlation between the HUKI conformation traits and punch resistance and elastic modulus of the horn indicate that certain types of conformation may predispose the heifers to have a weaker hoof horn structureLower punch resistance and elastic modulus was found in heifers with less straighter rear legs, lower foot angle, poor HUKI locomotion score, lower scores for the composite trait legs and feet and a higher final total score. Using a combination of scoring for conformation traits, mechanical tests and lesion scoring of the horn there is the potential for a selection of animals with increased hoof horn quality at the beginning of the first lactation.

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