Lameness of dairy cattle: Factors affecting the mechanical properties, haemorrhage levels, growth and wear rates of bovine claw horn

Louise Anne Lethbridge

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Lameness of dairy cattle: Factors affecting the mechanical properties, haemorrhage levels, growth and wear rates of bovine claw horn

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Louise Anne Lethbridge

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The present study aimed to determine with the use of mechanical testing parameters the effects of diet supplements, breed and time (prepartum /post partum) on lesion development, severity and the integrity of bovine claw horn.

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Declaration

Each chapter is set out in the style of; the Journal of Dairy Science to which the papers have been submitted. Consequently there is some repetition, particularly in the methods and references sections. The submitted papers include other authors, but for each of the chapters my input was the greatest. I, with the appropriate assistance of my co-authors / supervisors, designed and carried out the research, analysed the data and wrote the papers.

Abstract

Lameness is one of the main economic and welfare issues faced by the global dairy industry. It mainly affects the hind claws and the main causes / types are; claw horn lesions of the sole and white line (WL), along with foot rot (NZ) and digital dermatitis (UK). This thesis aims to apply and develop mechanical tests to determine the effect of dietary supplements, animal breed and number of days postpartum (dpp) on claw horn (CH) mechanical properties. Supplementation with live yeast (UK) significantly increased the puncture resistance (PR) of sole horn ($P<0.05$), level of mean sole haemorrhage percentage, total combined lesion score (TLS) and wear rates ($P<0.10$), while increasing daily mean milk yield, total milk fat and protein without significant increases in feed intake, providing an increase in feed conversion efficiency. In growing (NZ) dairy cattle PR was lower in 1, 2 (WL) compared with sole (4 and 5) while zones 4 and 5 did not differ significantly. Dairy breed (NZ) affected the PR of the CH, significantly ($P<0.001$) lower PR in CH of Friesian (all 5 IFM regions) compared with Friesian X Jersey (FxJ). Lactating dairy heifers (partition 22 to 24 months) from 0 to 160 d pp showed that the breed did not significantly affect the; number, percentage or TLS of sole or WL lesions, with the exception of 160 dpp where Friesian heifers had significantly ($P<0.05$) higher WL and sole lesions compared with FxJ. Mechanical properties (PR) of CH, declined significantly with increasing number of days post partum (dpp), while EM was significantly stiffer at 30 d pp compared with 120 dpp. PR was reduced significantly by increasing lesion score (LS), but was not confirmed by Vickers hardness and EM results. Hydration of CH significantly lowered EM compared to dried horn or horn at physiological moisture content. Short term (200 d), neither the form (zinc as salt or complexes with yeast) not level of zinc (At 1.0 or at 0.3 of NRC recommended levels (RL)) did not significantly affect; locomotion score; growth and wear rates; claw measurements and sole and WL lesions were not significantly effected by form or level of supplemental zinc up to 150 d pp. Overall, the number of days pp (dpp) significantly increased the level of sole and WL lesions, and reduced CH PR and elastic modulus (EM).

General summary

Lameness of dairy cattle is one of the main economic and welfare issues faced by the global dairy industry. The incidence of lameness has increased in recent years, affecting approximately 35%, in the UK, and 22%, in New Zealand, of dairy cattle. In the majority of cases the hind claws are affected and the main causes / types of lameness are; claw horn lesions (CHL) of the sole and white line, along with foot rot in New Zealand and digital dermatitis in the UK. This thesis aimed to apply and develop mechanical tests to determine the effect of; dietary supplements (zinc and live yeast), animal breed and number of days postpartum (dpp) on claw horn mechanical properties, along with the assessment lesion development and severity for both sole and the white line, horn growth and wear rates, and locomotion score of dairy cattle in UK and New Zealand based systems.

The rapid inclusion of the Holstein into the UK has led to substantial increases in the milk yield potential of UK dairy cattle and the need to adopt higher energy dense feeds and diets, with lower effective fibre levels. This has resulted in an increased occurrence of rumen acidosis, in particular sub-acute rumen acidosis (SARA). As a consequence, there has been a renewed interest in dietary supplements that modify rumen pH, especially naturally occurring microbial products, such as yeast. However despite the relationship between rumen acidosis and laminitis no research had been completed to assess the effect of yeast supplementation on claw horn structural integrity, CHL and lameness. In Chapter two a mixed forage diet (UK) was supplemented with live yeast and the affect on claw horn was assessed using mechanical and lesion scoring methodologies. Supplementation with live yeast significantly increased the puncture resistance (PR) of sole horn ($P<0.05$), level of mean sole hemorrhage percentage, total combined lesion score (TLS) and wear rates ($P<0.10$), while increasing daily mean milk yield, total milk fat and protein without significant increases in feed intake, providing an increase in feed conversion efficiency.

Overall, the number of days pp (dpp) significantly affected the level of sole and WL lesions, claw horn PR, growth and wear rates. Live yeast clearly offers, in some diet situations, the potential to reduce the severity of sole hemorrhaging and in improving claw horn quality and warrants further research into both the mechanism and effect of live yeast in a range of dairy cattle diets, were potential improvements in; animal productivity, feed use efficiency and animal health may be gained. The aim of this thesis was to apply and develop mechanical testing of bovine claw horn and this work showed that the use of PR was a valid indicator of dietary factors that can affect the structural integrity and strength of bovine claw horn, as was claw horn lesion scoring (CHL) and as a consequence these method were applied in New Zealand in Chapter three.

In New Zealand to facilitate 'all year round' grazing systems dairy cattle are commonly selected for lower body weight and small breeds such as Jersey and in particular the use of cross bred (Friesian x Jersey) dairy animals, including the provision of cross bred sires in the artificial insemination catalogue, has led to a high proportion of cross bred dairy cattle in the New Zealand dairy herd. There have been some reports of lower levels of lameness in Jersey and cross bred compared to Frisian dairy cattle, which has been, in part, potentially attributed to differences on claw horn colour and strength. However, there is a limited amount of detailed information that characterising the dynamics of claw horn lesion development or levels and CHL scoring, which was developed in Holstein Frisian cattle with light claw horn has never been applied to cross bred dairy cattle, which tend to commonly have dark almost back claw horn. In Chapter three both mechanical testing and lesion scoring was applied to Frisian and cross bred (Friesians x Jersey) dairy cattle during growing and first lactation phases and the international foot map (IFM) regions were used to compare claw horn strength. The growing dairy cattle (0 to 4 m of age) showed no signs of claw horn lesions, while PR differed significantly between differing regions of the IFM, with lower PR in 1, 2 (WL) compared with sole (4 and 5) while zones 4 and 5 did not differ significtly. The breed affected the PR of the claw horn, with significantly

($P<0.001$) lower PR in claw horn of Friesian (at all 5 IFM regions) compared with Friesian X Jersey (FxJ). Lactating dairy heifers (partition at 22 to 24 months age) from 0 to 160 d pp showed that the breed did not significantly affect the; number, percentage or TLS of sole or WL lesions, with the exception of 160 dpp where Friesian heifers had significantly ($P<0.05$) higher WL and sole lesions compared with FxJ. Overall, PR significantly decreased with increasing number of dpp and with increased lesion score (LS) demonstrating that the resistance to damage and thus claw horn integrity was reduced as lactation continued. The assessment of lesion score, using the existing techniques, were found to be more difficult to apply accurately in cross bred dairy cattle, potentially these methods are less suitable for application to cross bred dairy cattle (dark claw horn being most common) than to Friesian cattle (selected for white socks and thus light claw horn predominating) for which they were developed. This warrants further research, using morbid claw tissue, as would the effect of cattle breed and within breed variation, for which PR shows great potential in determining the differences and dynamics in claw horn integrity and function of differing breeds of cattle. The identification of specific genes that increase claw horn integrity and resilience, which may lead to reduced claw horn penetration and claw infection would be ideal. In this Chapter PR produced useable and repeatable data, however, material scientists favour elastic modulus (EM) as a method and while Winkler (2005) tried one method of EM, which used self tightening grips and required larger ‘dog bone’ shaped claw horn samples. This was the limiting factor, resulting in fewer suitable claw horn samples and insufficient sample size to produce sufficient data to make comparisons. As a consequence, Chapter four aims to adopt and develop a new method for EM.

In Chapter four the EM, Vickers hardness and PR tests were used, the EM test, which was recently developed, and has never been applied to bovine claw horn? The main advantages of this test were that it required only relatively small horn samples and allowed the measurement of ‘creep’ in the claw sample to be measured. The EM, Vickers hardness and PR of claw horn from two differing

periods postpartum were compared. The mechanical properties (PR) of claw horn, declined significantly with increasing number of days post partum (dpp), while EM was significantly stiffer at 30 d pp compared with 120 dpp. PR was reduced significantly by increasing lesion score (LS), but was not confirmed by the Vickers hardness and EM results. Hydration of claw horn significantly lowered EM compared to dried horn and horn at physiological moisture content. Mechanical properties tests (PR and EM) offered the potential to assess in the determination of the effect of factors such as; breed nutrition, parturition and environment that affect claw horn composition, integrity and physical function. The mechanical integrity of claw horn is particularly pertinent in grazing based dairy production systems and these mechanical tests for claw horn, and the development of others, warrant further application, research and development.

The supplementation of dairy cow diets with micronutrients has been found to be affective in reducing lameness in some situations and the use of technologies that combine micronutrients with microbial products has gained support in increasing micro-nutrient absorption by the animal. This has positive implications for reduced environmental pollution and efficacy of micronutrient use. The detailed measurement of the effect of micronutrient supplementation on claw horn integrity has not been assessed in detail using mechanical techniques. In Chapter five the short term (200 d) effect of differing levels and forms of dietary zinc are assessed, in the UK, using CHL scoring, EM and PR. The form (zinc as salt or complexes with yeast) and level of zinc (At 1.0 or at 0.3 of NRC recommended levels (RL)) supplementation did not significantly affect; locomotion score; growth and wear rates; claw measurements and sole and white line (WL) lesions were not significantly effected by form or level of supplemental zinc up to 150 d pp. Milk yield, corrected milk yield and total fat and protein yield were not significantly affected by the form of zinc offered at 1.0 RL, but the reduction in zinc levels to 0.3 RL resulting in a significant ($P<0.05$) reduction in milk yield. Overall, the number of days pp (dpp) significantly affected the level of sole and WL lesions, claw horn PR and elastic modulus (EM), growth

and wear rates. PR and EM proved to be successful in detecting changes in claw horn integrity due to increased CHL score typical to increasing number of dpp. The effect of differing forms and levels of zinc on animal health and productivity warrants further research over longer feeding periods to assess more fully the longer term effects of zinc and zinc combined with other micronutrients in the development of high quality claw horn and its effect on sole and WL lesions score and horn function. This was unfortunately beyond the level of funding and resources available in this particular case.

Key words: Claw horn, mechanical properties, lameness, dairy cattle, Zinc, Yeast.

Glossary of frequently used abbreviations

BCH	Bovine claw horn
BCS:	Body condition score
CE:	Cell envelope
CH:	Claw horn
CHD:	Claw horn disease
CHL:	Claw horn lesion
CP:	Crude protein
DD:	Digital dermatitis
d:	day(s)
DM:	Dry matter
DMI:	Dry matter intake
D pp:	Days *postpartum*
EM:	Elastic modulus
ICS:	Intracellular cementing substance
IDD:	Inter-digital dermatitis
IFAP:	Intermediate filament associated proteins
IFM:	International foot map
FCE:	Feed conversion efficiency
Fr:	Friesian
FxJ:	Friesian x Jersey
GPa:	Giga Pascal’s
LS:	Lesion score
LY:	Live yeast
MC:	Moisture content
MMP’s:	Matrixmettallonoprotinases
MPa:	Mega Pascal
MR:	Mixed ration
NC:	Net change
NEB:	Negative energy balance

N/mm^2 :	$Newton/mm^2$
NP:	Non pigmented
NLY:	No live yeast
NRL:	National recommended levels
NS:	Not significant
NZ:	New Zealand
NZF:	New Zealand Friesian
OrZn:	Organic Zinc
P:	Pigmented
pp:	*Postpartum*
PR:	Puncture resistance
RL:	Recommended levels
RH:	Relative humidity
S:	Sole
SARA:	Sub clinical acidosis
SCC:	Somatic cell count
TLS:	Total lesion score
UK:	United Kingdom
WL:	White line
VH:	Vickers hardness
WR:	Wear rate
Zn:	Zinc
ZnOX:	Zinc Oxide

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Chapter 1

Review of relevant literature

1.0 INTRODUCTION

Globally there are a number of individual conditions that cause dairy cow lameness and these can be categorised into four main types, based on the pathogenesis, which include claw, interdigital and digital (skin), “non foot” i.e. upper limb and back etc. related or cause uncertain. A summary of 18 international publications (Logue, 1999), research from New Zealand (Chesterton *et al.*, 2008; Tranter and Morris, 1991) along with data from farm recording (Kossaibati *et al.*, 1999) and surveys completed in the UK (Clarkson *et al.*, 1996a) detailing the proportion of differing lesions associated with lameness are presented in Table 1.0. Overall the main causes of lameness of dairy cattle are related to claw horn disease (CHD) of the sole and white line (WL), which account for between 0.35 and 0.76 (Logue, 1999) of lameness in the UK and 0.71 to 0.90 (Chesterton *et al.*, 2008; Tranter and Morris, 1991) in New Zealand.

It is essential that the incidence and measurement of sole and white line disorders be considered separately, due to differences in horn anatomy and factors affecting the development of these claw disorders. These differences will be explored in greater detail later in this review. While Logue (1999) did not classify sole and WL separately, other researchers have and as an instance in the UK sole disorders accounted for 0.38 (Clarkson *et al.*, 1996a) to 0.26 of lameness (Kossaibati *et al.*, 1999) while WL disorders accounted for 0.22 (Clarkson *et al.*, 1996a). However, Kossaibati *et al.* (1999) found that WL accounted for only 0.09 of lameness experienced. This may be due to the data being derived from a database of records generated by receiving animal treatments from either veterinary surgeons and (or) farmers, which would typically have led to only serve cases of lameness being detected in many instances. Whereas in the survey completed by Clarkson *et al.* (1996a) the data was collected by farm staff, foot trimmers and veterinary surgeons as soon as an animal was perceived to be lame, rather than requiring treatment.

In research completed in New Zealand sole disorders accounted for between 0.51 (Tranter and Morris, 1991) and 0.29 (Chesterton *et al.*, 2008) of lameness whereas WL disorders have remained at a constant level (over two decades) accounting for between 0.39 (Tranter and Morris, 1991) and 0.42 (Chesterton *et al.*, 2008) of the lameness. This suggests that the challenges faced by New Zealand dairy cattle had changed little up to 2008 due to the predominance of a mainly pasture based diet. This however, has change in recent years as milk prices, unreliability of summer rain fall and the use of supplementary feeds have increased in New Zealand over the last few years. Dairy producers could either choose to increase cow numbers or opt to supplement the pasture based diet with a form of fermentable carbohydrate to increase milk yield and thus financial return.

Over the past 20 years in the UK (Table 1.0) there has been a change in the main causes of lameness. While sole ulcers remain one of the main causes (Kossaibati *et al.*, 1999), digital dermatitis (DD) has increased in its importance. DD was not reported by Russell *et al.* (1982), but accounted for 0.08 of lameness in 1996 (Clarkson *et al.*, 1996a) and between 0.09 (Logue, 1999) and 0.20 (Kossaibati *et al.*, 1999) of recorded cases of lameness by 1999. The occurrence of digital and inter-digital dermatitis (IDD) has been established as one of the most important skin associated lesions (Clarkson *et al.*, 1996b). Logue (1999) reported that overall digital / inter-digital lameness was dominated by foul in the foot and DD, which vie with each other for importance and when combined together can account for up to 0.50 of lameness of dairy cattle. However, this is highly dependent on climatic, environmental conditions and individual farm management practices.

Table 1.0 Proportion of lameness of dairy cattle attributed to differing potential causes reported in globally in a review of international publications from UK and New Zealand based research

	Russell et al. (1982)	Clarkson et al. (1996b)	Logue (1999)	Kossaibati *et al.* (1999)	Tranter & Morris (1991)	Chesterton *et al.* (2008)
Total sole (S)	**0.21**	**0.38**	**-**	**0.26**	**0.51**	**0.29**
Sole ulceration / lesion	0.12	0.28	-	0.20	-	-
Sole injury / penetration	0.09	0.08	-	0.02	0.51	0.29
Under run sole	-	0.02	-	0.04	-	-
Total white line (WL)	**0.18**	**0.22**	**-**	**0.09**	**0.39**	**0.42**
White line lesion	0.14	0.22	-	-	-	-
White line disease	-	-	-	0.09	0.39	0.42
White line separation	0.04	-	-	-	-	-
Total claw disease	**0.39**	**0.50**	**0.76**	**0.35**	**0.90**	**0.71**
Total dermatitis (DD &IDD)	**-**	**0.08**	**0.19**	**0.20**	**-**	**-**
Digital dermatitis (DD)	-	0.08	-	0.20	-	-
Inter-digital (IDD)	-	<0.01	-	<0.01	-	-
Foul-in-the-foot	0.15	0.05	-	0.13	0.05	0.08
Foreign body penetration	0.05	0.05	-	0.18	-	-
Leg / hock damage	0.12	-	-	0.02	-	-
Other(s)	0.26	0.20	0.05	0.12	0.01	0.13

It seems that, interestingly, inter-digital and digital lesions have increased in importance over time (Table 1), being lower in Russell *et al.* (1982) than in 1996 Clarkson *et al.* (1996b) through to 1999 (Logue 1999; Kossaibati *et al.*, 1999), which reflects the introduction and progression of this disease. Conversely, digital dermatitis has been seen only occasionally in New Zealand, while foul accounts for between 0.05 and 0.08 of lameness (Tranter and Morris, 1991; Chesterton, 2008) where the dairy industry has been dominated by pasture based systems with limited cattle confinement. However, with the recent move towards an increased use of unroofed and roofed cattle accommodation facilities, in order to mitigate impact of weather conditions on the animal and of the animal on the environment, there may be the potential for digital dermatitis to become established.

1. 1 Development of sole and white line lesions

Claw horn lesions (CHL) of the sole and WL are caused when traumatic external compressive forces are transferred through the capsule to the dermis and in severe cases the disruption of the blood vessels (Greenough, 2007). The early pathogenesis of CHL appears to involve the distal phalanx sinking within the foot capsule, subsequently increasing pressure on the lamellae producing sole horn and the risk of sole bruising, ulceration and white line disease. The primary causal event involves increased laxity of the connective tissue and ligaments suspending the pedal bone within the foot, linked to metabolic changes associated with parturition (Tarlton and Webster, 2002).

The initial molecular changes in the dermis that are followed by functional disturbances of the sole horn have been linked to activation of matrix-metallonoproteinase (MMP) (Hendry *et al.*, 2002; Tarlton and Webster, 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) and the activation of MMP 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 resulted in a time dependent structural disintegration of the collagen network.

The activation of MMP-2 coincides 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 that an increase in the collagen cross-link ratios around parturition was associated with tissue repair and re-modelling. The damage of a limited number of collagen fibres would lead to micro-rupture and a slight increase in length of small fibre bundles, whereas the level of elongation and instability would depend on the number of fibres affected (Mulling *et al.*, 2004). MMP-9 was not found in heifers during the peri-partum period or in maiden heifers, which indicated that no inflammatory process was involved in the pathology of claw lesions in the peri-partum period (Tarlton and Webster, 2002). It was observed that blood leaked from the vessels became trapped in the newly formed claw horn and was latterly seen as the haemorrhage (Greenough, 2007). Ossent and Lischer (1998) proposed the existence of a three-stage pathogenesis involved in the development of claw horn lesions. The first stage has unknown factors that trigger 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 the separation of the dermal and epidermal layers. In the second stage, the position of the third phalanx changes in relation to the softer tissues, causing pressure induced haemorrhages and necrosis of the corium of the sole. Ischii and Nosai (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, which was probably related to compression of the dermis and epidermis of the sole. In the third stage, blood and cell debris appear within the claw horn of the sole and white line.

1.2 Sole bruising and white line haemorrhage and lesion formation

Sole bruising affects claw horn appearance and has been used as the basis of a scoring system applied to assess the level of claw health and sole damage (Leach *et al.*, 1998). The development of sole bruising and lesions has been associated with parturition (Webster, 2001; Winkler and Margerison, 2004 & 2006; Winkler *et al.*, 2002) and the number of days *postpartum* significantly affected the number, percentage, lesion intensity and thus total lesion score of both sole and white line. The research completed (Leach *et al.*, 1997; Offer *et al.*, 2003; 2000a; Winkler *et al.*, 2005) has demonstrated that sole and WL haemorrhage levels peak, in first lactation heifers, between 100 and 120 days *postpartum* and reduce in both the number and severity thereafter. Moreover, the level of sole bruising has been found to be directly related to the resistance to puncture of the sole horn (Winkler and Margerison, 2004) and strength, with increasing severity of sole lesions being correlated ($R^2 = 0.50$) with a reduction in sole horn resistance to puncture (Winkler and Margerison, 2004) and potentially wear.

The incidence and severity of sole bruising and lesions has been found to be affected by the type of housing system. The incidence and severity of claw lesions were lower in first lactation heifers that were housed on a deep litter compared with a cubicle / free stall and concrete floored housing system (Leach *et al.*, 1997; Webster, 2001). There have been some dietary factors associated with a reduction in the occurrence, severity and longevity of lesions, which include higher forage dry matter content and the dietary supplementation with micronutrients such as biotin (Hedges *et al.*, 2001; Higuchi and Nagahata, 2001), zinc (Bazle, 1993; Kessler *et al.*, 2003; Moore *et al.*, 1989; Nocek and Johnson, 2000), or mineral complexes (Nocek *et al.*, 2006; Uchida *et al.*, 2001). However, it would be better to understand and prevent both the nutritional and non-nutritional related factors that result in sole bruising.

The WL is the junction between wall and sole claw horn and this has been found to have approx. 0.20 of the strength of wall claw horn (Greenough, 2007), which was supported by the mechanical properties tests completed by Winkler and Margerison (2004), Budras *et al.* (1989) and Műlling *et al.* (1994). However, research regarding WL strength has not always been consistent as Dyer *et al.* (2004) reported two elastic modulus readings from sole horn taken from region four of the international foot map (IFM) and one of these was lower than WL and the other considerably higher (Tables 1.4 and 1.8), while Borderas *et al.* (2004) also found WL to be harder than sole claw horn. Winkler and Margerison (2004) found that WL was weaker than sole horn and the level of haemorrhage had little effect on the structural resistance of WL. This suggests that WL was consistently more susceptible to damage and separation related to external stressors. This can be correlated to research reported by Budras *et al.* (1996) who stated that WL was a result of incomplete keratinization due to high claw horn production, larger amounts of fatty intercellular cementing substance (ICS) inside a broad intercellular space and has fewer tubules than claw horn from the wall or sole, which can reach dimensions of up to 180 µm. This results in claw horn with reduced structural integrity and is more susceptible to vascular disturbance at the keratinizing portion of the epidermis (Budras *et al.*, 1996). Claw horn of reduced structural integrity is likely to be susceptible to separation and bacterial invasion/infection and the consequential pain and suffering for the lame animal.

The appearance of lesions in the white line region can be correlated to its structure and function. The juxtaposition between the claw wall and sole leads to considerable mechanical forces being transferred through the white line during locomotion. This, coupled with other factors such as walking / floor surface and animal handling (Chesterton *et al.*, 1989) can result in the breakdown of the integrity of the white line, development of lesions (Logue *et al.*,1998) and separation. The occurrence of WL lesions and or separation has been related to either external lesions or internal stress in the majority of cases. The appearance of external lesions was caused by trauma i.e. penetration by a foreign body which

could also lead to a secondary infection, inflammation and development of retroarticular or coronary band abscesses (Brizzi *et al.*, 1998). Internal stress may include previous weakening such as an episode of laminitis (Greenough, 2007), reduction in the supportive capacity of the claw wall connective tissue or the increased stress transmitted to other supportive structures predisposing the white line to separation (Tarlton and Webster, 2002).

1.3 Laminitis

Laminitis is the generic term for conditions in which the sensitive laminae of the claw are damaged (Hendry *et al.*, 1997). Hinterhofer *et al.* (2007) stated that laminitis affects the bovine claw in two distinct ways. The first is a compromised microcirculation of the dermis, which severely disturbs horn cell formation resulting in the horn becoming softer and more vulnerable to damage and with a reduced tensile strength. The second is when bioactive agents inactivate matrix metalloproteinase inhibitors, which results in the stretching of collagen fibres within the claw, especially those of the suspensory apparatus as found around first calving (Tarlton *et al.*, 2002).

Nocek (1997) has reviewed the pathophysiology of laminitis. Laminitis is instigated by the blood flow being impaired to the corium due to the action of vasoactive substances (Donovan *et al.*, 2004; Lischer and Ossent, 2002; Nocek, 1997; Socha *et al.*, 2002) e.g., histamine or endotoxins in the blood stream. The vasoactive substances create increased vascular constriction and dilation and in turn cause the development of arteriovenous shunts, further increasing the blood pressure. The increased blood pressure causes seepage through vessel walls and eventually damage and cause the walls to exude serum, which results in oedema, internal haemorrhaging of the solar corium from thrombosis and ultimately the expansion of the corium (Nocek, 1997). The separation of the strata germinativum and corium results in the breakdown of the dorsal and lateral laminar supports of the claw horn tissue and the pedal bone changes position within the corium and dorsal wall (Nocek, 1997). However, Lischer and Ossent

(2002) were not able to demonstrate a relationship between sinking and rotation of the third phalanx, separation of the dermal-epidermal junction and the occurrence of laminitis. The shift in position of the pedal bone compresses the corium in the sole and heel which can predispose the hoof to further episodes of vascular damage, haemorrhage, thrombosis, cellular inflammatory reaction and finally ischemic necrosis (Lischer and Ossent, 2002). The accumulation of exudates between the lamellae, lamellar hyperplasia, or separation at the epidermal-dermal junction causes the white line to become disrupted and become wider. The friable layers may appear at the load bearing edge of the claw and may provide a further point of entry for infection. There is an accumulation of necrotic tissue and blood at the surface of the corium, which impedes or obstructs horn production. As growth continues the detritus is incorporated into the new horn and gradually comes out to the surface and appears as red patches or a double sole or heel. An ulcer could develop when horn production has stopped (Lischer and Ossent, 2002). Kempson and Logue (1998) demonstrated that cows with sole ulcers did not show changes in the dermal epidermal junction of the wall, while cows with laminitis had severe changes in the dermal epidermal junction with no changes in the sole horn.

Subclinical laminitis is a multifactorial disease involving nutrition, management, genetic predisposition, behaviour environment and exercise (Vermunt, 2000). Subclinical laminitis is a consequence of low grade insults of laminitis and locomotion may appear unaffected. Nevertheless, there are distinct changes in the claw horn, as a result of serum seepage into the solar corium which typically causes claw horn to become softer, yellowed and waxy. Softening of the horn makes the claw prone to wear and damage, causing ulceration, heel erosion and haemorrhaging in the weight-bearing surfaces, particularly the white line and toe (Hendry *et al.*, 1997; Nocek, 1997). Acute laminitis causes severe pain from the aseptic inflammation of the dermis in a systemically sick animal which is often caused by ruminal acidosis, severe mastitis or metritis. At this stage the claw horn shows few if any visible changes (Vermunt, 2000). However, some swelling

and elevated temperature above the coronary band in the soft tissue area (Nocek, 1997) may be seen. Chronic laminitis has no systematic symptoms but several may occur localized to the claw. The growth pattern of the keratinized horn is disrupted and causes the shape of the claw to alter resulting in an elongated, flattened and broadened sole. Grooves and ridges form in the dorsal wall as a result of irregular growth (Nocek, 1997; Vermunt, 2000). Histological examination shows changes in the microvasculature of the dermal laminae resulting in oedema (Vermunt and Greenough, 1994). Hinterhofer *et al.* (2007) found that chronic bovine laminitis resulted in a clear reduction in elastic modulus in the abaxial wall, resulting in the laminitic horn exhibiting a low resistance to mechanical trauma and wear. Hinterhofer *et al.* (2007) attributed the alterations induced by chronic laminitis, especially in the abaxial wall, to afflictions of the suspensory apparatus and consequential low horn quality to a result of deformed tubular and lamellar structure of the diseased dermis and, consequently, deteriorated horn production; whereas the role played by horn tubules in moisture regulation did not appear to be disturbed. The aetiology of bovine laminitis is as multifactorial as its pathophysiology and includes nutrition, trauma, physiological alterations around parturition and the type of flooring (Bergsten, 2003).

Nutritional management has been identified as a key component in the development of laminitis, particularly the feeding of increased fermentable carbohydrate which results in an acidotic state. Metabolic and digestive disorders can predispose the cow to laminitis (Nocek, 1997). Hormonal and physiological alterations around parturition have been associated with laminitis. Pre-calving heifers with poor quality horn were more likely to develop laminitis during lactation (Kempson and Logue, 1993; Logue *et al.*, 1993). Hendry *et al.* (1997) proposed that the high incidence of bovine lameness in early lactation may stem from earlier, predisposing biochemical events, which are precipitated by parturition or changes in husbandry or animal physiology associated with parturition and lactogenesis (Kempson and Logue, 1993). Lame cattle can largely recover in later lactation and relapse in subsequent lactations, suggesting that

the endocrine and metabolic demands of pregnancy and early lactation negatively affect horn growth (Logue *et al.*, 1993) and function. The environment, such as hard floor surfaces, insufficient bedding and insufficient or excessive levels of exercise, particularly on undesirable surfaces, can predispose mechanical damage (Bergsten, 1994). Other factors, such as body condition, body weight, and feet and leg conformation, can unnaturally increase the weight load and stress on claws, exacerbating the internal mechanical damage associated with laminitis (Nocek, 1997).

1.4 Sole thickness and claw horn growth and wear rates

The sole horn offers protection to the underlying soft tissues (dermis and hypodermis) contained within the hoof capsule. The optimal protection has long been considered to require a sole thickness of 5 to 7 mm depending on region (Toussaint Raven, 1985). Without this protection trauma can occur, leading to haemorrhaging and development of abnormal distribution of weight within the claw capsule with subsequent effects on claw horn, growth, wear, quality and, thus claw conformation (van Amstel and Shearer, 2001).

There are several factors that affect the growth and wear rate of claw horn. These include the environment, stage of lactation, (Leach *et al.*, 1997), animal genetics, nutrition (Clark and Rakes, 1982) and the conformation of the claws and leg (Boelling *et al.*, 2001; Offer *et al.*, 2000a) (Table 1.1). Phillips and Schofield (1994) found cubicle housing was conducive to wear as cattle were standing for long periods on wet surfaces leading to softening of hoof horn with the abrasive surface leading to erosion of the softened horn. The simple transition from housing to pasture can have a marked effect on growth and wear rates as seen by Winkler (2005) where growth rates at housing were 6.0 mm/month compared to 7.8 mm/month at pasture and wear rates were 5.8 mm/ month at housing and 7.2 mm/ month at pasture. Other claw parameters such as claw angle of the dorsal border alters during lactation and has been found to be steeper in the first half of lactation when the claws were shorter (Offer *et al.*,

2000a). While animals that were at pasture the claws were found to be slightly longer and have a shallower angle (Boelling and Pollott, 1998; Offer *et al.*, 2000a).

Table 1.1 The effect of animal nutrition on growth and wear rates

	Rate (mm/m)			
Nutrition	Growth	Wear	Net	
With methionine	5.8-11.1	4.9-8.2	+2.9	Clark and Rakes (1982)
No added methionine	6.5-7.9	5.1-7.2	+0.7	
With biotin	4.9	3.8	+1.1	Winkler *et al.* (2005)
No added biotin	5.0	3.0	+2.0	
Hay & 1.8 kg compound				Offer *et al.* (2001)
-28 to 7 d pp	5.2	4.0	+1.2	
7 to 70 d pp	5.4	7.5	+2.1	
70 to 140 d pp	5.7	5.5	+0.2	
Grass silage				Offer *et al.* (2001)
-28 to 7 d pp	4.4	4.5	+0.1	
7 to 70 d pp	5.2	7.4	+2.2	
70 to 140 d pp	5.1	4.8	+0.3	

The changes in growth and wear rates 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 (MacCallum *et al.*, 2002; Tranter and Morris, 1992). Dietary supplements can have a positive effect on growth and wear rates as demonstrated by Clark and Rakes (1982) also reported increased growth rate in response to methionine supplementation. However, changes in nutrition and dietary supplements do not necessarily result in increased growth and decreased

wear rates over the course of the lactation. Supplementing hay with compound was found not to affect claw growth and wear rates when compared to cattle fed grass silage (Offer *et al.*, 2001). Winkler (2005) also found no significant effect of adding 20 (± 2) mg/d of biotin on growth rate, however this only research supplemented biotin up to 120 d pp which was sufficient for wall horn to be completely replaced but potentially not sufficient time for biotin to affect claw horn hardness. The dairy animal is placed under considerable strain in early lactation. As well as having just calved, the animal experiences a change in diet (dry to lactating) and increased production demands. As a result the animal's body partitions energy. Regrettably claw horn production and thus growth does not have such a high a priority as milk production and negative net growth can occur (Table 1.2).

Table 1.2 The effects of stage of lactation on growth and wear rates

Stage of lactation	Rate (mm/m)			
	Growth	Wear	Net change	
Days post partum				
-77 to -28	4.4	3.79	+0.61	Leach *et al.* (1997)
-28 to 7	4.4	3.49	+0.91	
7 to 28	4.65	6.21	- 1.56	
28 to 63	4.80	7.01	- 2.21	
63 to 98	4.88	6.29	- 1.41	
98 to 140	5.17	5.51	- 0.34	
140 to 175	5.69	7.07	- 1.38	
175 to 224	6.74	5.79	+0.95	
Days pre partum	4.5	4.2	+0.3	Livesey *et al.* (1998)
7 to 42	5.1	5.4	- 0.3	
42 to 84	6.3	4.5	+1.8	
84 to 182	6.6	5.4	+1.2	
182 to dry period	6.3	8.1	- 1.8	
Dry period	5.1	2.7	+2.4	
7 to 42 of 2nd lactation	5.4	4.5	+0.9	
Days pre partum				
-28 to 7	4.5	4.5	0.0	Livesey and Laven (2007)
7 to 42	5.6	5.7	- 0.1	
42 to 84	6.9	5.5	+1.4	
84 to 182	5.9	5.7	+0.2	

Chaplin *et al.* (2000) found significantly lower growth and wear rates in heifers in early lactation (up to 60d ± 18d) (4.17 and 6.68 mm/m, respectively) when compared to pregnant heifers (5.56 and 8.79 mm/m, respectively) with animals housed in similar conditions over the same time period. Due to the low growth rates the heifers in the study by Chaplin *et al.* (2000) experienced negative growth rates whereas the wear rates far exceeded growth. No other authors have reported this occurring in pregnant animals and normally negative net growth occurrence from one week pp according to Leach *et al.* (1997) and Livesey *et al.* (1998). However, Leach *et al.* (1997) found that negative net growth occurred up until 20 weeks pp, whereas Livesey *et al.* (1998) and Livesey and Laven (2007) found claw horn growth rates exceeded wear rates from weeks 6-26 weeks pp, but declined from 26 weeks pp until the dry period. Negative net growth could also be a result of behaviour changes in early lactation, i.e., shorter lying time.

Season (Table 1.3) has been found to affect claw horn growth and wear rates, with increased wear rate of the claw horn found to be related to the winter housing of dairy cattle in cubicles, on concrete floors, resulting in a negative net growth (Leach *et al.*, 1997; Livesey *et al.*, 1998; Offer *et al.*, 2000a; Vokey *et al.*, 2001). Clark and Rakes (1982) found increased growth rates of the claw during the spring and the summer periods and net negative growth rates during autumn and winter periods. Clark and Rakes (1982) attributed these differences to changes in the photoperiod. Winkler (2005) and Offer *et al.* (2000a) found that multiparious cow growth and wear rates varied from year to year. Offer *et al.* (2000a) reported a mean growth rate of the wall horn over five lactations of 4.5 mm/month in animals housed during the winter period and turned out to pasture during the summer. Over the five lactations claw horn growth either marginally exceed wear or wear marginally exceed growth. Therefore, the animal kept claw horn turnover balanced over the course of the lactation. Careful consideration should be taken when comparing research or even year to year data from the same herd as there is potential variation in a number of factors, i.e., forage quality, housing, genetics and environment.

Table 1.3 Effect of season, age (parity) and claw on growth and wear rates

	Rate (mm/m)			
	Growth	Wear	Difference	
Season				
Spring	6.7	4.6	+2.1	Clark and Rakes (1982)
Summer	11.1	6.9	+4.2	
Autumn	4.2	5.1	- 0.9	
Winter	6.5	6.7	- 0.2	
Autumn	6.9	9.0	- 2.1	Winkler *et al.* (2005)
Winter	8.4	6.6	+1.8	
Winter	3.2	2.5	+0.7	McCallum *et al.* (2002)
Consecutive parity				
1	5.52	5.40	+0.12	Offer *et al.* (2000a)
2	4.16	4.44	- 0.28	
3	3.80	4.56	- 0.76	
4	4.17	4.14	+0.03	
5	4.08	4.16	- 0.08	
Foot / Claw				
Hind	4.2 to 11.1	4.9 to 8.2	+2.9	Clark and Rakes (1982)
Front	4.1 to 9.0	4.4 to 7.1	+1.9	
Lateral	5.8	5.6	+0.2	Tranter and Morris (1992)
Medial	5.4	5.1	+0.3	
Claw area				
Abaxial edge	-	2.0	-	Tranter and Morris (1992)
Mid sole	-	1.3	-	
Toe	-	1.4	-	
Abaxial	5.8 to 11.1	4.4 to 8.2	+2.9	Clark and Rakes (1982)
Dorsal wall	4.2 to 8.9	3.8 to 7.0	+1.9	

Clark and Rakes (1982) reported that the hooves of the hind claws grew faster than the hooves of the front claws, while the hind claws abaxial (outer) wall grew faster than the dorsal wall. However, this difference in growth rates was not found on the front hooves. Tranter and Morris (1992) reported lateral claws grew faster than medial claws, however, lateral claws also wore faster than medial claws. The wear rates of the wall and sole horn were found to be greater on the lateral digits than on the medial digits (Offer *et al.*, 2000a; Toussaint Raven, 1985). Bovine claws are of a concave shape with the abaxial wall extending beyond the

sole and resulting in the abaxial wall being the main weight bearing surface. The abaxial wall and lateral claws sustain more weight than the dorsal wall/ medial claws, and as a consequence, both lateral and abaxial wall have a greater turnover of claw horn. Claw horn wear rate has been found to vary over the sole surface. Tranter and Morris (1992) reported that sole wear occurred more rapidly along the abaxial edge of the weight-bearing surface, was lower on the toe and heel areas and lowest on the mid-sole area. The abaxial edge is the main weight bearing area of the claw as sole wear occurred more rapidly along the abaxial edge. The mid sole area would only become weight bearing when the animal was placed in natural conditions, i.e., pasture where the surface is uneven and springy. Changes in claw shape can occur when cattle are kept on different surfaces. The sole and wall can be liable to wear flat due to the abrasive and shearing forces from walking on the concrete. The toe and abaxial wall will grow faster and wear slower than the heel and axial wall.

Hoblet and Weiss (2001) stated that it took approximately two months for the newly formed sole and heel horn to reach the weight-bearing surface. 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 (Toussaint Raven, 1985). This increased the pressure on the sole area and this 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). An imbalance between growth and wear rates could also lead to the formation of a longer anterior wall and shorter heel, altering the normal weight distribution of the claw (Toussaint Raven, 1985).

1.5 Claw horn structure

The primary role of keratin is to make the skin, hair, and horn a pliable, insoluble, and non-reactive barrier against the natural environment. Vermunt and Greenough (1995) described that the function of healthy claw horn was to protect and support to the inner structure of the digit and to assist in the dispersal of

stress and weight put upon the foot during locomotion. The biological, histological, biochemical and molecular investigations completed have clearly shown that keratin is formed through a highly specific cell process that produces proteins with specific chemical and physical properties (Dale *et al.*, 1993; Franke and Kartenbeck, 1993; Parry and Steinert, 1995; Steinert *et al.*, 1984) and that the formation of keratin proteins occur as part of a systematic process of cellular differentiation that transforms living, highly functional epidermal, cells into cornified (dead), structurally stable cells with no metabolic activity (Műlling, 2000).

Keratin is a water insoluble protein, which when organized into intermediate filaments, forms the internal skeleton of the keratinocyte. The assembly of keratin begins with the formation of a four chain complex that is arranged in two pairs, which are coiled and stabilized by hydrophobic interactions and disulfide bonds (Grosenbaugh and Hood, 1993). The alignment of the keratins within the maturing keratinocyte depends upon interactions with the intermediate filament associated proteins (IFAP) (Wang, 1985). Steinert and Roop (1988) proposed that IFAP mechanically integrate adjacent cells in the final stages of cornification and that this could be significant in cell to cell adhesion. The keratin filaments themselves are aligned in parallel to the long axis of the squame, a specialized cell which is the end product of keratinization, with disulfide cross linkages that are formed by an interaction with IFAP and cell envelope (CE) proteins. The keratinocyes only become fully differentiated when the CE proteins form rigid glutamyl-lysine bonds with the cell wall (Rice and Green, 1977). This structure gives rigidity to the cells, thus, giving keratin the mechanical strength to withstand the impacts and forces of locomotion (Műlling and Budras, 1998).

The process of keratin formation is not fully understood, but research has shown that adequate levels of calcium are required for the activity of epidermal transglutaminase and an increase in cellular cholesterol is also essential for CE formation (Rice and Green, 1977; Schmidt *et al.*, 1991). The concluding stage of

cornification is the secretion of lipids into the intercellular space by the keratinocyes, producing a lipid-rich extracellular matrix intercellular cementing substance (ICS) (Elias, 1983; Grosenbaugh and Hood, 1993; Műlling *et al.*, 1999; Műlling and Budras, 1998).

Electron microscope images have revealed a two phase organization of keratin consisting of closely packed rod like microfibrils 7.5 to 8 nm in diameter surrounded by amorphous matrix (Briggs, 1976). Each of the microfibrils is composed of a ring of nine protofibrils, with the possibility of two additional protofibrils within the circle of nine (Filsheie and Rogers, 1961). Fraser *et al.* (1962) theorized that each protofibril is a three stranded α helix. This cross linked protein matrix (due to disulphide bonds) provides mechanical continuity between the individual microfibrils forming the rigid composite, keratin (Briggs, 1976; Crewther *et al.*, 1965; Műlling and Budras, 1998).

The architecture of claw horn is determined by the surface formation of the underlying dermis (Műlling *et al.*, 1999) and the dermal papillary body not only provides mechanical support, nutrients and oxygen, but also determines the cellular composition of the claw horn. The horn tubules have corresponding horn laminae and there are two structural formations present in the papillary body, dermal papillae that are present in all regions of the claw, and dermal laminae present exclusively in the wall region. In areas with dermal papillae, the epidermis forms tubular horn; in the laminar region of the wall, horn lamellae are formed (Budras *et al.*, 1989; Budras *et al.*, 1996). The tubular horn consists of horn tubules built by the epidermis around the dermal papillae and above their tips. These tubules are connected by the inter-tubular horn between them and each horn tubule consists of an outer cortex originating from the living epidermis located around the dermal papilla and an inner medulla originating from the epidermis over the tip of the papilla. The diameter and density of tubules, as well as the ratio between cortex and medulla determine the quality of claw horn.

The medullar cells develop at the tip of the dermal papilla due to continuous proliferation in the basal layer of the keratinizing cells and are rapidly moved away from the nourishing underlying dermal blood vessels. The claw is formed by continual proliferation of keratinizing cells and this gives rise to the flaky appearance/ layers of claw horn as seen by Baillie and Fiford (1996). The mechanical strength and quality of claw horn is directly related to the dimensions of tubules, i.e., the diameter and proportion of medulla and cortex, therefore, the arrangement and spatial relationship of tubular, intertubular, and laminar horn cells (Műlling *et al.*, 1999). Baillie and Fiford (1996) state the overlapping inter-layered flakes flat surface are bonded together, which, leads to the tubules having a reinforcing effect within the claw horn. The number of tubule forming papillae is fixed during foetal development and the intertubular horn originates from the epidermis located in the spaces between the papillae and is weaker than tubular horn. Claw horn of the wall is stronger as it contains a greater density of tubules approx. 80 per mm^2 compared to 20 per mm^2 of the sole and heal (Hoblet and Weiss, 2001). The white line is composed of very soft claw horn as it has only 0.20 of the hardness of the wall (Greenough, 2007). Claw horn tubules of the WL diameter range from 120-180 μm (Budras *et al.*, 1996) compared to claw horn taken from the wall which was 20-30 μm (Baillie and Fiford, 1996).

In the WL (the junction of wall and sole horn) the connection of horn cells occurs through large amounts of fatty Intercellular cementing substance (ICS) inside broad intercellular space, which is not as mechanically stable (Budras *et al.*, 1996) and the large horn tubules with a wide medulla establish sites of potential predisposition for bacterial invasion. Once the medullar horn has fallen out of the tubules, microorganisms may invade the tubule, start horn cell destruction, and ascend within the tubule toward the inner living tissue layers, resulting in the development of white line disease (Kempson and Logue, 1993). In complicated cases, once the bacterial invasion reaches the dermis, white line abscesses, wall separation, penetration and secondary infection can develop. Many of these claw

abnormalities occur in early (<90 days pp) lactation (Green *et al.*, 2002) when WL is structurally weaker (Winkler and Margerison, 2005) as a result of nutritional deficiencies or hormonal changes occurring in dairy cattle at this stage of lactation or changes in environment.

Claw keratin is also referred to as hard keratin, which differs from soft keratin (i.e. skin cells) in that the filaments and IFAP are distinguished by a sulphur content of about 0.01 and a lipid content of about 0.04 in soft keratin and the sulphur is rather evenly distributed between cysteine and methionine. In hard keratin there are higher levels of sulphur / cysteine, approximately 0.05, primarily in the form of combined cysteine (Grosenbaugh and Hood, 1993; Parry and Steinert, 1995; Ward and Lundgren, 1954). These hard keratins form a more complex and specialized coherent structure, which has a higher tensile strength. The keratinization process involved in producing hard keratin occurs more gradually over a much wider region in which the sulphur content increases markedly (Ward and Lundgren, 1954).

The importance of the contribution made by the amorphous protein matrix to the properties of keratin is demonstrated by the elastic behaviour of keratins. Once stretched beyond its elastic region, it has a capacity for considerable creep or stress relaxation. The long term changes associated with this is an exchange of disulphide bonds within the matrix and variations in the extent of the elastic region may be due to a loss of the matrix structure (Briggs, 1976).

1.6 Factors that affect claw horn quality

The factors that determine the structure and the biomechanical properties of claw horn have been 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 (Műlling *et al.*, 1998; Patan and Budras, 2003; Pellmann *et al.*, 1993; Zaun, 1997).

The structure and quality of claw horn is ultimately dependant on physiological keratinization, as the end product can never be any better than the process that initiated in the keratinocytes (Műlling *et al.*, 1999). As the epidermis is avascular, keratinocytes are dependent on receiving oxygen and nutrients from the fine microvasculature of the corium by diffusion across the basement membrane. This diffusion can easily be disrupted, resulting in production of low quality horn (Hoblet and Weiss, 2001). Interference in the supply of nutrients to the keratinocytes can result in an inflammation in the corium caused by circulating vasoactive substances or from localized trauma (Hoblet and Weiss, 2001). In addition, damage to the keratinocytes tends to cause more poor quality horn to be produced (Nocek, 1997), but little research has demonstrated the longevity of this effect.

The rapid turnover of claw horn commonly results in incomplete keratinization, and subsequent reduction in horn quality and hardness, which allows the structure to become more susceptible to damage and vascular disturbances (Budras *et al.*, 1997, Budras *et al.*, 1996; Budras *et al.*, 1998). Damage to blood capillaries and leakage of blood content across the basement membrane separating the dermis from the epidermis (Kempson and Logue, 1993) results in the production of inferior claw horn quality. Kempson and Johnston (1990) observed a similar process in pigs with laminitis, where gaps appeared in the basement membrane and the intercellular spaces became enlarged. The inclusion of large qualities of intercellular material in claw horn and disorganized squames content results in some loss of integrity and function (Kempson and Johnston, 1990).

The external factors that have been shown to affect the structure of claw horn include high levels of humidity and chemical and microbiological factors. The

extent of the effect of the external factors was determined by the quality and integrity of the existent horn structure (Budras and Mulling, 1998). The hydration of the claw horn is likely to be controlled by the tubules (Logue, 1999; Baillie and Fiford, 1996). The effects of nutritional factors have been considered to be both an internal and external factor. The external factors are often referred to as environmental and are related to housing hygiene, such as the level of urine and manure, which can reduce the quality of healthy horn (Budras and Mulling, 1998; Kempson *et al.*, 1998).

The moisture content (MC) has been found to be affected by the micro-architecture and biochemical composition of the horn (Hendry *et al.*, 1997; Vermunt and Greenough, 1995). Mülling *et al.* (1999) stated that fatty acid metabolism affected the synthesis of intercellular cementing substance which connects horn cells and regulates permeability characteristics of the horn. Dietz and Prietz (1981) reported that the sole region has a lower density of microtubules than the wall of the claw, and that water was taken up by the intertubular material and consequently both the number of microtubules and ICS affect water uptake by the claw horn (Borderas *et al.*, 2004). Mositure content also allows for a gradient of stiffness between the rigid horn of the outer wall and the soft tissues of the dermis. Reducing the stresses between the interface between epidermis and dermis (Douglas *et al.*, 1996; Wagner and Hood, 202). This was confirmed by Kasapi and Gosline (1997) who reported that the initial elastic modulus increased from the inner (300 MPa) to the outer (560 MPa) regions of the equine claw wall. Equine dorsal outer wall (955 MPa, MC 0.28) had significantly higher elastic modulus and lower MC when compared to the dorsal inner wall (502 MPa, MC 0.35). This resulted in a significant negative correlation between the moisture content of the outer wall samples and their elastic modulus (Douglas *et al.*, 1996).

Hinterhofer *et al.* (1998) conditioned wall and sole horn samples to a relative humidity of 0.65 and the elastic modulus of conditioned samples was 1802.3

N/mm^2 for wall (MC 0.159) samples and 1673.8 N/mm^2 for sole samples (MC 0.162). Differences were not significant compared to non conditioned samples of physiological moisture content (range 1,636 and 8,650 N/mm^2). Hinterhofer *et al.* (1998) and Douglas *et al.* (1996) highlighted 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 claw 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 0.80 DM and 56 MPa for oryx horn with 0.60 DM, with increasing moisture content reducing tensile strength, while stiffness became more compliant, which could lead to softer horn.

Several authors (Baggott *et al.*, 1988; Borderas *et al.*, 2004; Collins *et al.*, 1998; Dyer *et al.*, 2004; Hinterhofer *et al.*, 1998; Hinterhofer *et al.*, 2005a; van Amstel *et al.*, 2004; Zoscher *et al.*, 2000) have observed that claw horn hardness and elastic modulus decreases with increasing moisture content. As a consequence, housing systems and the volume of slurry can lead to softer claw horn and increased wear rates (Hinterhofer *et al.*, 2005a) through reduced claw hardness. Therefore, management practices can be critical to avoid loss of structural integrity as a result of increased moisture content as demonstrated by van Amstel *et al.* (2004) and Bergsten and Petterson (1992). van Amstel *et al.* (2004) found a positive correlation between bovine sole moisture content and sole thickness and claw capsule measurements (dorsal wall length). Thin soles were softer with significantly higher moisture content when compared to normal sole thickness with lower moisture content and increasing hardness, potentially resulting in increased wear rates and higher number of CHL. Bergsten and Petterson (1992) reported a significant negative correlation between the dry matter content of the sole horn and the level of heel-horn erosion. Therefore animals may experience less heel horn erosion when claw horn has a higher dry matter content. Thus animals spending less time standing in slurry could have

higher dry matter content of claw horn than those standing for longer periods of time in slurry.

Higuchi and Nagahata (2001) suggested a relationship existed between water content, hardness, and claw health. Higuchi and Nagahata (2001) found that animals suffering from laminitis had significantly lower plasma biotin levels and higher claw horn moisture content when compared to claw horn taken from animals not suffering from laminitis. Hinterhofer *et al.* (2007) also found that the elastic modulus was lower in claw horn affected by laminitis when compared to non-affected tissue. Laminitis is known to disrupt claw horn production resulting in softer claw horn with a higher moisture content which was prone to increased number and severity of CHL and wear rates. The reduced availability of biotin could also have led to impaired keratinization and reduced claw quality. Fatty acid metabolism, biotin or both are required for synthesis of ICS that connects horn cells and regulates permeability characteristics of claw horn (Műlling *et al.*, 1999). Borderas *et al.* (2004) and Tranter *et al.* (1993) found negative correlations between measures of hardness and lameness/ lesion severity scores; the relationship between hardness and claw lesions indicates that cows with softer claws are at greater risk of lameness. Similarly, lesion scores and heel horn erosion increased significantly as heel horn hardness declined (Offer *et al.*, 2001). However, trying to determine the primary cause i.e. whether softening of the claw was either a cause of or a consequence of claw injuries is problematical.

Sole claw horn is known to have a lower number of claw horn tubules/ higher level of ICS, consequently, both the number of microtubules and ICS affect water uptake by the claw horn. Both Mulling *et al.* (1994) and Manson and Leaver (1988) have found a high negative correlation between hardness and horn structure and the sites of predilection for occurrence of lesions and locomotion score. These findings also suggest that claw horn composition significantly

affects water uptake and mechanical strength and, therefore, the claw horn ability to withstand internal and external challenges.

Location of claw sample is an important consideration when determining claw horn strength (Tables 1.4, 1.5, and 1.6) as differing locations had differing number of microtubules and ICS which both affect water uptake by the claw horn. The Shore D hardness of the dorsal wall horn and the sole horn of front claws of dairy cows were higher than the hardness of the dorsal wall horn and sole horn of the hind claws (Distl and Schmid, 1994) (Table 1.7). In dairy cows the elastic modulus of the wall horn of the toe area was found to be higher than that of the lateral wall horn, sole and WL (Franck *et al.*, 2006; Hinterhofer *et al.*, 2005b; Zoscher *et al.*, 2000) (Table 1.5). Higher elastic modulus values were also reported for the front claws when compared to the hind claws (Table 1.6) (Dyer *et al.*, 2004; Zoscher *et al.*, 2000). 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 claws (van Amstel *et al.*, 2004; Zoscher *et al.*, 2000). However, Hinterhofer *et al.* (2005b) measured the elastic modulus of bovine claw horn samples at natural physiological moisture level from the dorsal wall, abaxial wall and sole horn, but found there were no differences in the elastic modulus or moisture content between differing areas. Higher moisture content has been linked to softer claw horn. Future research could consider the affect of front and rear hooves and or lateral or medial claws as there has been no distinction made in reported data in terms of number, diameter of claw horn tubules, levels of ICS, cross linking bones and keratin filament length. Therefore, it is difficult to draw conclusions on how claw horn composition differs between front and rear claws and how it affects structural integrity.

1.7 Mechanical properties of claw horn

Claw horn is a natural biological composite of keratinized material that must be capable of accommodating and resisting high work and pressure loads without excessive deformation or catastrophic failure (Newlyn *et al.*, 1999) and as a

consequence, the important mechanical properties of interest are hardness, toughness, strength, and viscoelasticity (Baillie *et al.*, 2000; Bertram and Gosline, 1986; Bonser, 2000). These properties largely depend upon the structure and chemical composition of keratins that form the horn (Baggott *et al.*, 1988) and the horn moisture content (Baillie *et al.*, 2000; Budras *et al.*, 1996). The morphology of the material or structure being tested is at least as important as its mechanical properties Vincent (1992).

Keratin is modelled as a fiber-reinforced composite material consisting of microfibrils embedded in an amorphous, non-fibrous matrix, formed by globular proteins (Baillie and Fiford, 1996) and the matrix usually binds fibres and transfers stresses to them, which assists in withstanding the compressive forces, inhibiting cracks from propagating through the fibers (Kasapi and Gosline, 1999). The microfibrils are built by bundles of protofibrils that are formed by an α-helix of amino acids wound together. The keratinized cells are further organized into either tubular structures or intertubular material, forming a macroscale composite. The claw wall is thus considered to be a multi-level or hierarchical composite (Kasapi and Gosline, 1997) and according to Baillie and Fiford (1996), the structure of the claw horn with its overlapping fiber-reinforced flakes can also be compared to an engineered laminated composite.

1.71 Techniques for testing the mechanical properties of claw horn

The mechanical properties of claw horn in terms of hardness and stiffness (elastic modulus) have been investigated by using several different methods; Vickers harness, Shore durometer and the ball indentation methods.

1.711 Hardness

Hardness is defined as the resistance of a material to permanent deformation by a harder object. The harder the material the smaller the degree of penetration by the indenter and the smaller the size of the indentation that remains (Vincent, 1992). The deformation of the substance will cause cohesion of particles or

elements that make up a substance, as evidenced by its inflexibility or resistance to indentation or distortion. The resistance of a surface against plastic deformation is evaluated by analyzing the permanent impression of a defined indenter (Hinterhofer *et al.*, 2005a). Wear can be regarded as a process of microfracture, part of the resistance to wear is the toughness of the material and increased resistance to wear can be achieved by increasing hardness (Vincent, 1992).

There are three different techniques that have been used to measure hardness of equine and bovine claw horn, which include; the Shore durometer (Baggott *et al.*, 1988; Distl and Schmid, 1994; Hinterhofer *et al.*, 2005a; Zoscher *et al.*, 2000), ball indentation method (Hinterhofer *et al.*, 2005a; Műlling *et al.*, 1994; Pellmann *et al.*, 1993; Zoscher *et al.*, 2000) and the Vickers harness test using a micro-indenter (Hedges *et al.*, 2002). The Shore durometer, once placed flat on the materials surface, presses a spring-loaded probe into the surface with a constant force. The Shore durometer has fine a needle-like tip and small inhomogenities can lead to false results and a high variation in the results (Budras *et al.*, 1998). The ball indentation method uses a metallic sphere indenter which is pressed into a sample under a defined pressure and depth of the impression is then measured (Hinterhofer *et al.*, 2005a). The micro-indenter used a minute diamond pyramid, which is pressed into the material being investigated. The harder the material the smaller the degree of penetration of the diamond pyramid into the material, and the smaller the size of the indentation that can be measured (Vincent, 1992) and the depth of penetration decreases with increasing hardness (Vermunt and Greenough, 1995).

A high correlation has been found between the measurement of mechanical strength, through the ball impact method and histometric, histochemical and immuno-histochemical methods (Műlling *et al.*, 1994; Pellmann *et al.*, 1993). Hardness, which was measured through the ball impact method and Shore durometer, decreased from the dorsal wall towards the heel and from the

coronary border of the wall towards the weight bearing border (Hinterhofer *et al.*, 2005a). The hardness of the sole horn was significantly lower than hardness of wall horn samples, when measured through Shore durometer and ball impact methods (Table 1.4) and could indicate that the sole horn was more prone to injuries due to compressive pressures (Galbraith *et al.*, 2002; Van der Tol *et al.*, 2002). Within the sole, the hardness decreased from the sole towards the heel (Table 1.4).

Table 1.4: Hardness of the coronary wall, sole, heel and white line horn of cattle

Hardness	Area	Abaxial wall	Sole	Heel	White line	sem	Source
Shore A durometer (hu)	Toe	87.6	84.0	39.4	-	1.1	Manson & Leaver (1989)
	Mid	86.9	76.6				
Shore D durometer (hu)		65.5	43.7	31.0	-	-	Baggott *et al.* (1988)
Shore D durometer (hu)	Toe	78.4	48.7	43.0		9.5	Borderas *et al.* (2004)
	Mid	77.3			70.4		
	Abaxial				64.7		
Shore D durometer (hu)		63.9-52.5	36-49.3	-	-	-	Hinterhofer *et al.* (2005a)
Shore D durometer (hu)			49.3	58.5 to 52.5	-	-	Zoscher *et al.* (2000)
	Toe	63.2 to 62.0					
	Lateral	60.6-56.2					
Hardness (shore hu)		-	86.7	70.8	-	9.8	Webster (2001)
Ball impact (N/mm^2)		25.7	12.9	6.8 9.3‡	6.9 § 5.1†	-	Mulling *et al.* (1994)
Ball impact (N/mm^2)			10.9	15.7 to 11.2 ℓ	-	-	Zoscher *et al.* (2000)
	Toe	22.2 to 17.6					
	Lateral	17.2-15.8					
Ball impact (N/mm^2)		24.3-11.2	6.4-10.9	-	-	-	Hinterhofer *et al.* (2005a)
Vickers (Kg mm^{-2})		-	-	-	16.55		Hedges *et al.* (2002)

‡ - sole-heel junction, § -cap horn, †- terminal horn, ℓ - heel wall N.B. Zoscher *et al.* (2000) values were generated by ball impact from coronary band to border hardness units – hu A and D - Shore durometer scale used

Table 1.5 Elastic modulus, puncture resistance and moisture content of bovine coronary, sole, heel and white line horn

Area		Abaxial wall	Sole	Heel	White line	sem	Source
Elastic modulus							
Tension (N/mm^2)	Toe	613.5	134.9	-	-	147.2	Zoscher *et al.* (2000)
	Lateral	375.3					
Tension (N/mm^2)		-	102.4	-	87.9	9.8	Winkler (2005)
Tension (GPa)		-	3.02	-	-	0.58	Zhang *et al.* (2007)
Tension (GPa)		-		-		-	Dyer *et al.* (2004)
Dehydrated (MC)			3.02		1.8		
Fully-hydrated (MC)			0.106				
Tension (N/mm^2)		343.9	172.1	-	-	94.1	Hinterhofer *et al.* (2005b)
Compression/bending (MPa)		261	-	13.6	-	-	Franck *et al.* (2006)
Puncture resistance (N/mm^2)		-	9.6	-	6.1	0.29	Winkler (2005)
Moisture content (%)	Toe	25.4	31.2	-	-	2.7	Zoscher *et al.* (2000)
	Lateral	26.6					
Moisture content (%)		26.6	32.5	38.1	-	-	Baggott *et al.* (1988)
Moisture content (%)		22.6	31.7	-	-	8.2	Hinterhofer *et al.* (2005a)

N.B. Claw horn was taken from WL zone 2 for Dyer *et al.* (2004) and sole zone 4 for Zhang *et al.* (2007) and Dyer *et al.* (2004)

Table 1.6: Hardness, tensile strength, elastic modulus, puncture resistance and moisture content of the claw horn of front and hind claws of cattle

	Hind	Front	sem	Source
Hardness:				
Wall toe	4.91	4.56	-	Clark and Rakes (1982)
Coronary horn (ShD)	64.5	65.9	8.0	Distl and Schmid (1994)
Tension				
(N/mm^2)	349.5	405.5	247.0	Zoscher *et al.* (2000)
(N/mm^2)	97.0	107.8	19.43	Winkler (2005)
(GPa) Dehydrated (MC)	3.02	3.4	-	Dyer *et al.* (2004)
(GPa) Fully-hydrated (MC)	0.105	0.106		As above
Puncture resistance (N/mm^2)	9.4	9.9	0.7	Winkler (2005)
Moisture content (%)	28.7	26.9	3.7	Zoscher *et al.* (2000)
Moisture content (%)	36.8	34.1	-	Van Amstel *et al.* (2004)
Moisture content (%)	35.9	34.5	0.56	Winkler (2005)

- ShD – Shearometer unit

Diets offering higher ratios of concentrate to forage create the potential problem of acidosis which can lead to laminitis which is known to affect keratinisation by reducing blood flow and consequently, nutrients and oxygen supply which leads to inferior keratin production and reduced structural integrity. This is highlighted by Manson and Leaver (1989), where a high ratio of concentrate to forage (60:40) compared with a lower ratio (40:60) significantly decreased claw horn hardness. Offer *et al.* (2000a) also found that high dry matter unfermented diet based on straw with a concentrate mix (DM 860g/kg) resulted in lower horn hardness when at the bulb compared with a low dry matter fermented diet based

on grass silage (DM 190g/kg). However, Webster *et al.* (2001) did not find significant differences between the hardness of sole and heel horn of heifers offered low (0.25 DM) or high (0.60 DM) DM diets.

Friesian cattle tend to be more susceptible to lameness than other dairy breeds such as Jersey (Chesterton *et al.,* 2008; Pugashetti *et al.*, 2004; Peterse, 1985). There are few studies that have considered the effect of pigmentation on lameness/ claw horn hardness. Clark and Rakes (1982) found that claw horn pigmentation did not affect claw horn hardness, whereas Hepburn *et al.* (2004) reported that non-pigmented claw wall horn of cattle was significantly harder (46.5 vs. 40.3) closer (within 20 mm) to the coronary line compared with (68.5 vs. 64.8) 40 mm away from the coronary line in areas up to 4.5 cm under the coronary horn when compared to pigmented horn. As there are limited data sets available it is, therefore, difficult to draw conclusions to determine whether pigmented claw horn has superior structural integrity resulting in harder claw horn which is surmised by the lower levels of lameness reported in breeds with pigmented claw horn (Chesterton *et al.*, 2008; Peterse *et al.*, 1984; Pugashetti *et al.*, 2004).

1.721 Tensile tests

The elastic modulus is calculated by dividing the tensile stress by tensile strain and when calculating elastic modulus for materials such as claw horn, which are likely to deform when stretched, the original cross sectional area and the amount by which it has changed must also be take into account (Figure 1) (Briggs, 1976). Vincent (1992) stated that biomaterials tend to show non-linear elasticity i.e. the response shown will depend on the level of strain imposed.

The tensile tests have proved to be optimal for the testing of the stability of the intercellular connections of the horny cells (Budras *et al.*, 1998) and elastic modulus can be defined as the resistance of a material to deformation

(Hinterhofer *et al.*, 2005b). Aranwela *et al.* (1999) also recommend the use of test specimens that have a length to width ratio greater than 10.

Figure 1. The equation for the calculation of elastic modulus is given as

$$E \equiv \frac{\text{tensile stress}}{\text{tensile strain}} = \frac{\sigma}{\varepsilon} = \frac{F/A_0}{\Delta L/L_0} = \frac{FL_0}{A_0 \Delta L}$$

E is the Young's modulus

F is the force applied to the object;

A_0 is the original cross-sectional area through which the force is applied;

ΔL is the amount by which the length of the object changes;

L_0 is the original length of the object.

The claw horn behaves as a linear viscoelastic material (Collins *et al.*, 1998; Vincent, 1992) and for polymeric materials the strain rate influences the measured values of elastic modulus (Aranwela *et al.*, 1999; Baillie *et al.*, 2000). The increasing strain rates on the material may transition the material from ductile to brittle behaviour (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; Hinterhofer *et al.*, 2005b). Kasapi and Gosline (1996) tested 4 different strain rates (tests speeds of 5, 102, 1020 and 234,000 mm/min respectively) when completing tensile tests on horse wall horn samples. Initial elastic modulus, maximum stress and total energy showed significant increase with increasing strain rates and the claw wall became stiffer with increasing loading rate, being more capable of absorbing a greater amount of energy before failure, but there was no transition to brittle behaviour.

The different zones of the IFM (Shearer *et al.*, 2002) have differing structural strength of claw horn. Winkler (2005), showed a marked difference in elastic modulus and puncture resistance between the WL (zone 2), which had less structural integrity than the sole horn (zone 4), which was supported by the

mechanical tests data from Winkler and Margerison (2004); Budras *et al.* (1989); and Műlling *et al.* (1994). These findings are in contrast to Dyer *et al.* (2004) who found zones 4 and 5, have reduced elastic modulus when compared to zones 2 and 3. However, data regarding WL strength has been inconsistent as Dyer *et al.* (2004) reported two elastic modulus readings for sole taken from region 4 one of which was lower than WL and the other considerably higher (Tables 1.4 and 1.7). Borderas *et al.* (2004) also found WL to be harder than sole claw horn. Dyer *et al.* (2004) found that zone 3 had a higher elastic modulus when compared to zones 2, which is contrary to the results of Hedges *et al.* (2002). Moisture content must always be considered when interpreting results as Dyer *et al.* (2004) study used samples which had been fully dehydrated (kept at room temperature for 48 hrs), whereas Hedges *et al.* (2002) study used samples which had been put in sterile water and frozen, suggesting that the samples would have been fully hydrated. As Dyer *et al.* (2004) demonstrated, fully-hydrated claw horn has significantly reduced tensile strength/elastic modulus which is one potential reason why Hedges *et al.* (2002) results differ from Dyer *et al.* (2004). There have been no studies that measure and compare all regions (1 through to 5) of the weight bearing areas of the claw in dairy cattle at physiological moisture or samples which have been fully hydrated.

Table 1.7 Elastic modulus and puncture resistance of bovine claw horn in the different zones (1 to 6) of the international foot map (IFM)

	IFM Zone					
	2	3	4	5	6	
Elastic modulus (GPa)	1.8	1.9	1.75	1.1	1.0	Dyer *et al.* (2004)
Elastic modulus (MPa)	5.3	3.1	-	-	-	Hedges *et al.* (2002)
Elastic modulus (N/mm^2)	87.9	-	102.4	-	-	Winkler (2005)
Puncture resistance (N/mm^2)	6.05	-	9.6	-	-	Winkler (2005)

Claw horn lesions (CHL) have been found to affect the mechanical strength of claw horn in dairy cattle. Winkler (2005) found elastic modulus and PR decreased

with increasing lesion score. Structural damage of the WL significantly lowered the tensile strength (2.4 MPa) compared with undamaged tissue (4.5 MPa) (Collis *et al.*, 2004; Hedges *et al.*, 2002). Hinterhofer *et al.* (2007) also found that the elastic modulus was lower in claw horn affected by laminitis when compared to tissue non affected by laminitis. CHL are a result of inferior keratinisation where damage to corium leads to the incorporation of blood and cell debris and produces softer and more compliant claw horn which was prone to increased wear rates.

The orientation of the samples taken from the claw wall in relation to the direction of the tubules has been shown to influence test results. Dorsal wall samples, stressed parallel to the tubule orientation, were significantly stiffer (998 MPa) than samples stressed perpendicular to the tubules (912 MPa) (Douglas *et al.*, 1996). These results are in contrast to those of Hinterhofer *et al.* (2005b) who examined bovine abaxial claw and found no differences in elastic modulus between longitudinal samples (343.9 N/mm^2) when compared to the transversal samples (433.1 N/mm^2). The use of finite element analysis by Newlyn *et al.* (1999) obtained an axial-to-lateral modulus ratio lower than 2, which is small when compared to synthetic composites. However, this is dependant on the tubular and intertubular horn being the same cellular material arranged in a different way.

1.731 Puncture resistance

The puncture resistance (PR) method was developed and reported by Winkler and Margerison (2004, 2006, 2007a, 2002) to determine the mechanical properties of claw horn. This PR method had not been previously used to assess physical properties of claw horn, but has been used to determine the shear and creep behaviour and the deformation and failure properties of metals, polymers and composites, such as low-alloy steel, polymethyl methacrylate, and leaves and is used in the food industry (Aranwela *et al.*, 1999; Dobes and Milicka, 2001; Kurtz *et al.*, 2002; Lewis, 2002; Nomoto *et al.*, 2001; Satapathy and Bless, 2000).

PR is frequently used to determine mechanical properties of small or miniature specimens (Husain *et al.*, 2002; Lewis, 2002). Liu and Piggot (1998) considered the ultimate shear strength, associated with peak load, as an important parameter in 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 puncture strength when testing different dental restorative materials. The ranking order according to shear strength of these materials was consistent with their clinical performance. Aranwela *et al.* (1999) considered that the shear puncture test detected variations that correlated to physical aspects of leaf biology. The primary mode of failure in a shear puncture test performed on leaves is shearing, although tensile and compressional failures are also involved. Reppond *et al.* (1995) and Reppond and Babbit (1997) observed a linear decrease in the ultimate puncture force with increasing moisture content when testing surimi made from different types of fish. The work to failure and area under the force displacement curve up to the ultimate puncture displacement were considered as test parameters and had strong discriminating and predictive potential, which were inversely related to the wear rate of resins (Lewis, 2002), but, resulted in inconsistent measurements when used to test resins treated by a sterilization method (Lewis, 2002).

Table 1.8 Effect of time on puncture resistance and dry matter (DM) content

	Days peri-partum				
	-40	50	100	150	sem
Sole horn PR (N)	8.68^{b}	8.60^{b}	10.57^{a}	10.74^{a}	0.29
White line horn PR (N)	6.92^{a}	5.24^{c}	5.88^{bc}	6.24^{ab}	0.23
Horn DM content (%)	74.81^{a}	67.07^{b}	66.37^{b}	67.55^{b}	0.47

$^{a, b, c}$ different letters in the same row indicate values that differ significantly ($P < 0.001$). Source Winkler and Margerison (2004)

Winkler and Margerison (2007a) compared PR of sole claw horn taken from 20 heifers at 2 months before parturition (P) and 100 days postpartum and the PR force of the sole horn was significantly greater in front claws (PR: 8.2 N, -2 months pp; 11.1 N, 100d pp) compared to hind claws ($P<0.05$) (PR: 7.4 N -2 months pp, 10.3 N, 100 d pp), which was consistent with their previous research (Winkler and Margerison, 2004; Winkler *et al.*, 2002) while there was no significant difference between the inner and outer claws. Winkler and Margerison (2006) found that PR declined significantly ($P<0.001$ denoted by differing letters) as haemorrhage scores increased (0 low to 5 high) from 1 to 2 and 3 to 4 and 4 to 5 (0: 8.72^{a}, 1: 8.53^{a}, 2: 8.06^{b}, 3: 7.75^{b}, 4: 56.08^{c} and 5: 4.99^{d} Nmm^{2}) demonstrating a reduction in horn structural integrity and potential functionality as haemorrhaging increased.

Winkler and Margerison (2004) found PR of the WL was lower than that of sole horn. Decreasing significantly between 40 days pre-partum and 50 and 100 days postpartum, while PR of the sole horn decreased at 50 d pp, but had increased significantly at 100 days postpartum (Table 1.8). It was concluded by Winkler and Margerison (2007a) that mechanical tests reflected the changes in haemorrhage levels in sole horn and structural integrity of sole and WL claw horn and changes that occurred between the pre and postpartum claw horn.

The dry matter proportion (DM) of claw horn was found to vary from between 0.637 and 0.891 by Winkler and Margerison (2004), while the PR of the sole horn ranged from 6.24 to 24.66 N, while the PR of the WL horn was generally lower ranging from 2.17 to 18.60 N. The DM proportion in claw horn increased resulting in a linear increase ($P<0.01$) in the PR (N) of the sole (PR: 0.4901, DM: 0.244, ($R= 0.54$)) and the white line horn (PR: 0.4301, DM: 0.249 ($R=0.64$). Winkler and Margerison (2004) surmised that WL was weaker than sole, unless sole horn was haemorrhaged, and the greatest reduction in PR occurred in haemorrhaged sole horn during the postpartum period, while increased dry matter of horn samples changed mechanical properties from elastic to brittle,

resulting in a greater variation in PR. Collins *et al.* (1998), Hinterhofer *et al.* (1998) and Baillie *et al.* (2000) had all also found that moisture content of the claw horn influenced its mechanical properties. Hinterhofer *et al.* (1998) pointed out the importance of testing the samples at physiological moisture levels to represent the in vivo situation. In the short term, moisture loss is the factor that is likely to have the greatest effect on the mechanical properties of claw horn samples.

The prevention of moisture loss was thoroughly described by Collins *et al.* (1998) and Hinterhofer *et al.* (1998). Collins *et al.* (1998) wrapped the samples in 3 layers of wax film and stored them at 4°C while Hinterhofer *et al.* (1998) and Winkler and Margerison (2004, 2006, 2007b, 2002) kept the samples in re-sealable plastic bags at 4^0C. In one study by Winkler and Margerison (2004), samples were stored in plastic bags at 4^0C from 0 up to 8 days and tested for PR and MC, which was found not to significantly affect either PR or the moisture content of claw horn samples for the WL or sole, neither did freezing (-20 °C) of samples for up to 30 days. Winkler and Margerison (2004) found PR increased in a positive linear way ($P<0.001$) in relation to the thickness (mm) of the area tested (PR= 6.679 + 34.531 thickness, R.= 0.66) and concluded that thickness should be included as a covariant in the analysis of PR tests.

1.8 Rationale for research aims

Lameness causes significant economic losses and represents a serious welfare problems for dairy cows (Kossaibati and Esslemont, 1999). Globally, research shows that as many as 60% of dairy cows may become lame at least once a year (Vermunt, 2004). The incidence of lameness has risen from 3.88% in 1957 (Eddy and Scott, 1980) to between 5 and 55% (Clarkson *et al.*, 1996a) in the UK. In New Zealand the incidence rates has increased from 14% in 1978 (Dewes, 1978) to 20.7% in 1991 (Tranter and Morris, 1991). The main causes of lameness globally are claw horn disorders such as sole haemorrhage and ulceration, and WL disease and separation, followed by digital and inter-digital disease in housed

systems or foot rot in pasture based systems. As a consequence the study of claw horn and factors affecting claw horn disorders of the sole and WL warrant some closer investigation, potentially including the development of methods to assess these.

Claw horn is a natural biological composite formed primarily from α keratin which is capable of accommodating and resisting *in vivo* loads without excessive deformation or catastrophic failure (Newlyn *et al.*, 1999). As with most composite materials the internal structure or morphology is at least as important as the constituent materials in determining its mechanical properties (Hull and Clyne, 1996). The matrix of claw horn performs the usual range of functions in a composite, namely to bind fibres and transfer stress between them, and to help resist crack propagation (Kasapi and Gosline, 1999). Claw horn quality, in part is a reflection of its mechanical properties and is, therefore, strongly influenced by the structural factors (Műlling *et al.*, 1994; Pellmann *et al.*, 1993); intracellular factors i.e. the amount and ratio of keratin filaments IFAP's, extracelluar factors i.e. the amount and biochemical composition of ICS and connecting horn (Műlling and Budras, 1998), and architecture, i.e., arrangement and spatial relationship of tubular, intertubular and laminar horn cells, determine claw quality (Mulling *et al.*, 1999). The structure and quality of claw horn is ultimately dependant on physiological keratinisation since the quality of claw horn produced is dependant on the process that initiated in the keratinocytes (Mulling *et al.*, 1999). Since the epidermis is avascular, keratinocytes are dependent on receiving oxygen and nutrients from the fine microvasculature of the corium by diffusion across the basement membrane. This diffusion through tissue is easily disrupted and this can result in the production of low quality horn (Hoblet and Weiss, 2001). Rapid claw horn turnover commonly results in incomplete keratinization and therefore reduced horn quality and hardness, which leaves the structure more susceptible to damage and vascular disturbances (Budras *et al.*, 1997; Budras *et al.*, 1996; Budras *et al.*, 1998). Incomplete keratinization has the potential to allow greater

ingression of moisture and reduction of the essential functional characteristics of healthy claw horn and healthy claws of dairy cattle.

Logue (1999) stated that there has been an increase in lameness in the UK over the past 20 years and this could be contributed to the changes in breeding selection for higher milk yields, animal nutrition, and other management factors over this period. There are numerous studies (Bergsten and Frank, 1996; Greenough and Vermunt, 1991; Leach *et al.*, 1997; Offer *et al.*, 2000b; Winkler, 2005; Winkler and Margerison, 2004; 2007b; Winkler *et al.*, 2002) have observed the development of claw horn lesions in postpartum animals. Offer *et al.* (2003) found the peak bruising and WL damage occurred at 15 months prepartum and 26-28 months postpartum. As a consequence, nutritional management continues to be a major focal point in the attempt to reduce lameness in dairy cattle (Nocek, 1997).

The dietary supplementation with micronutrients such as biotin (Hedges *et al.*, 2001), zinc (Bazle, 1993; Kessler *et al.*, 2003; Moore *et al.*, 1989; Nocek and Johnson, 2000), and mineral complexes (Nocek and Kautz, 2006; Uchida *et al.*, 2001) have been found to reduce the levels of sole bruising and the incidence of lesions. Chesterton *et al.* (2008), Pugashetti *et al.* (2004) and Peterse (1985) have all reported that Friesian breeds tend to be more susceptible to lameness than other breeds such as the Jersey. However, there are no detailed controlled studies to assess the changes in claw characteristics of growing dairy cattle and the effect of nutrition and breed on these changes and there are few published studies regarding levels and causes of lameness in New Zealand and none of these focused on claw horn haemorrhaging and the structural strength or mechanical properties of claw horn.

Aim of PhD research

As a consequence, the aim of this PhD research was to assess the structural integrity of claw horn in both UK and New Zealand dairy cattle, using puncture

resistance and to continue to develop tensile test methodologies in conjunction with Exeter University. The data from this research program combined with other methods of claw and lameness assessment will be used to develop better data sets in order to increase the understanding of:

- The development of and changes in claw horn haemorrhaging and strength that occur in young prepartum (0 to 3 years) dairy cattle and subsequently during first lactation.
- The effect of changes during first lactation in claw horn haemorrhaging, lesion development and the integrity and structural strength of bovine claw horn
- The differences in claw horn haemorrhaging and structural integrity from differing breeds and cross breeds (Jersey x Frisian and Friesian).
- The effect of some differing diet microbial and micro-nutrient supplements have on claw horn haemorrhaging and structural strength of bovine claw horn of first lactating dairy cattle.
- To explore potential differences in the development of and changes in claw horn haemorrhaging and horn strength in first lactation cattle in differing countries (UK and NZ).

1.9 REFERENCES

Aranwela, N., G. Sanson, and J. Read. 1999. Methods of assessing leaf-fracture properties. New Phytologist. 144:369-393.

Baggott, D. G., K. J. Bunch, and K. R. Gill. 1988. Variations in some inorganic components and physical properties of claw keratin associated with claw disease in the British Friesian cow. British Veterinary Journal. 144:534-542.

Baillie, C. and R. Fiford. 1996. The three-dimensional composite structure of cow hoof wall. Biomimetics. 4:1-22.

Baillie, C., C. Southam, A. Buxton, and P. Pavan. 2000. Structure and properties of bovine hoof horn. Advanced Composite Letters. 9:101–113.

Bazle, F. K. 1993. The effect of zinc methionine in a mineral mixture on gain and incidence of footrot on steers grazing native grass pastures. Journal of Dairy Science. 76 (Suppl. 2):36. (Abstr).

Bergsten, C. 1994. Haemorrhages of the sole horn of dairy cows as a retrospective indicator of laminitis: an epidemiological study. Acta Agriculturae Scandinavica. 35: 55-66.

Bergsten, C. 2003. Causes, risk factors, and prevention of laminitis and related claw lesions. Acta Veterinaria Scandinavica. 44:157-166.

Bergsten, C. and B. Frank. 1996. Sole haemorrhages in tied primiparous cows as an indicator of periparturient laminitis: effects of diet, flooring and season. Acta Veterinaria Scandinavica. 37:383–394.

Bergsten, C. and B. Pettersson. 1992. The cleanliness of cows tied in stalls and the health of their hooves as influenced by the use of electric trainers. Preventive Veterinary Medicine. 13:229–238.

Bertram, J. E. A. and J. M. Gosline. 1986. Fracture toughness design in horse hoof keratin. Journal of Experimental Biology. 125:29–47.

Bertrand, J. E. and J. M. Gosline. 1987. Functional design of horse hoof keratin: the modulation of mechanical properties through hydration effects. Journal of Experimental Biology. 130:121-136.

Boelling, D., D. Madsen, and J. Jensen. 2001. Genetic parameters of foot and leg traits in future AI bulls – II. Correlation to body conformation traits in daughters. Acta Agriculturae Scandinavica Section A –Animal Science. 51:122-128.

Boelling, D. and G. E. Pollott. 1998. Locomotion, lameness, hoof and leg traits in cattle. I. Phenotypic influences and relationships. Livestock Production Science. 54:193-203.

Bonser, R. H. C. 2000. The Young's modulus of ostrich claw keratin. Journal of Material Science Letters. 19:1039–1040.

Borderas, T. F., B. Pawluczuk, A. M. Passille, and J. Rushen. 2004. Claw hardness of dairy cows: relationship to water content and claw lesions. Journal of Dairy Science. 87:2085-2093.

Briggs, W. D. 1976. Rigid Materials. Pages 189-191 in Mechanical design in organisms. Princeton University Press, Princeton.

Brizzi, A., C. Bergsten, R. Blowey, D. Collick, P. Greenough, D. Logue, S. Van Amstel, and J. Vermunt. 1998. Description and terminology for claw capsule lesions. Pages 98-105 in Proceedings of the Tenth International Symposium on Lameness in Ruminants, Casino Lucerne, Switzerland.

Budras, K. D., T. Hochstetter, C. Műlling, and W. Nattermann. 1997. Structure, function and horn quality in the bovine hoof: The influence of nutritional and environmental factors. Hoffmann-La Roche Ltd, Basel, Switzerland.

Budras, K. D., R. L. Hullinger, and W. O. Sack. 1989. Light and electron microscopy of keratinization in the laminar epidermis of the equine hoof with reference to laminitis. American Journal of Veterinary Research. 50:1150–1160.

Budras, K. D., C. Műlling, and H. Horowitz. 1996. Rate of keratinization of the wall segment of the hoof and its relation to width and structure of the zona alba (white line) with respect to claw disease in cattle. American Journal of Veterinary Research. 57:444–455.

Budras, K. D., C. Schiel, and C. Műlling. 1998. Horn tubules of the white line: An insufficient barrier against ascending bacterial invasion. Equine Veterinary Education. 10:81–85.

Chaplin, S. J., H. E. Ternent, J. E. Offer, D. N. Logue, and C. H. Knight. 2000. A comparison of hoof lesions and behaviour in pregnant and early lactation heifers at housing. The Veterinary Journal. 159:147-153.

Chesterton, R. N., K. E. Lawrence, and R. A. Laven. 2008. A descriptive analysis of the foot lesions identified during veterinary treatment for lameness on dairy farms in north Taranaki. New Zealand Veterinary Journal. 56:130-138.

Chesterton, R. N., D. U. Pfeiffer, R. S. Morris, and C. M. Tanner. 1989. Environmental and behavioural factors affecting the prevalence of foot lameness in New Zealand dairy herds- a case control study. New Zealand Veterinary Journal. 37:135-142.

Clark, A. K. and A. H. Rakes. 1982. Effect of methionine hydroxy analog supplementation on dairy cattle hoof and composition. Journal of Dairy Science. 65:1493-1502.

Clarkson, M. J., D. Y. Downham, W. B. Faull, W. J. Hughes, F. J. Manson, J. B. Merritt, R. D. Murry, W. B. Russell, J. Sutherst, and W. R. Ward. 1996a. Incidence and prevalence of lameness in dairy cattle. The Veterinary Record. 138:563-567.

Clarkson, M. J., W. B. Faull, W. J. Hughes, F. J. Manson, J. B. Merritt, R. D. Murray, J. Sutherst, W. R. Ward, D. Y. Downham, and W. B. Russell. 1996b. Epidemiology of lameness in dairy cattle: Description and analysis of foot lesions. The Veterinary Record. 138:586-591.

Collins, S. N., B. C. Cope, L. Hopegood, R. J. Latham, R. G. Linford, and J. D. Reilly. 1998. Stiffness as a function of moisture content in natural materials: Characterisation of hoof horn samples. Journal of Materials Science. 33:5185-5191.

Collis, V. J., L. E. Green, R. W. Blowey, A. J. Packington, and R. H. C. Bonser. 2004. Testing white line strength in the dairy cow. Journal of Dairy Science. 87:2874-2880.

Crewther, W. D., R. D. B. Fraser, F. G. Lennox, and H. Lindley. 1965. Chemistry of keratins. Advances in Protein Chemistry. 20:191-346.

Dale, B. A., R. B. Presland, P. Fleckman, E. Kamu, and K. S. Resing. 1993. Phenotypic expression and processing of filaggrin in epidermal differentiation. Pages 79–106 in Molecular Biology of the Skin: The Keratinocyte. Academic Press, San Diego, CA.

Dewes, H. F. 1978. Some Aspects of Lameness in Dairy Herds. New Zealand Veterinary Journal. 26:147-159

Distl, O. and D. Schmid. 1994. The effect of biotin supplementation on the hoof conformation, hardness and health in dairy cows. Tierarztliche Umschau. 49:581-588.

Dobes, F. and K. Milicka. 2001. Small punch testing in creep conditions. Journal of Testing and Evaluation. 29:31-35.

Donovan, G. A., C. A. Risco, G. M. DeChant Temple, T. Q. Tran, and H. H. S. van Horn. 2004. Influence of transition diets on occurrence of subclinical laminitis in Holstein dairy cows. Journal of Dairy Science. 87:73-84.

Douglas, J. E., C. Mittal, J. J. Thomason, and J. C. Jofriet. 1996. The modulus of elasticity of equine hoof wall: implications for the mechanical function of the hoof. The Journal of Experimental Biology. 199:1829-1836.

Dyer, R. M., D. Arola, D. Zhang, R. Reprogel, W. Zheng, and U. Tasch. 2004. On the mechanical behaviour of bovine hoof horn. Pages 82-84 in the thirteenth International Symposium on Lameness in Ruminants, Maribor, Solvenia.

Eddy, R. G. and C. P. Scott. 1980. Some observations on the incidence of lameness in dairy cattle in Somerset. The Veterinary Record. 106:140-144.

Elias, P. M. 1983. Epidermal lipids, barrier function and desquamation. Journal of Investigative Dermatology. 80 (suppl):44-49.

Filsheie, B. K. and G. E. Rogers. 1961. Fine structure of α– keratin. Journal of Molecular Biology. 3:784-786.

Franck, A., G. Cocquyt, P. Simoens, and N. De Belie. 2006. Biomechanical Properties of Bovine Claw Horn. Biosystems Engineering. 93:459–467.

Franke, W. W. and J. Kartenbeck. 1993. Cytokeratins. Pages 145– 148 in Guidebook to the Cytoskeletal and Motor Proteins. University Press, Oxford, UK.

Fraser, R. D. B., T. P. Macrae, and G. E. Rogers. 1962. Molecular organization in α– keratin. Nature. 193:1052-1055.

Galbraith, H., N. L. Hepburn, M. Wallace, K. A. K. Hendry, C. H. Knight, and C. J. Wilde. 2002. Sulphur amino acid supply, uptake, incorporation and DNA synthesis in bovine claw tissue cultured in vitro. Pages 209-212 in Twelfth International Symposium on Lameness in Ruminants. Orlando, Florida.

Green, L. E., J. Hedges, Y. H. Schukken, R. W. Blowey, and A. J. Packington. 2002. The impact of clinical lameness on the milk yield of dairy cows. Journal of Dairy Science. 85:2250-2256.

Greenough, P. R. 2007. Bovine laminitis and lameness. Saunders Elesevier, London.

Greenough, P. R. and J. J. Vermunt. 1991. Evaluation of subclinical laminitis in a dairy-herd and observations on associated nutritional and management factors. Veterinary Record. 128:11–17.

Grosenbaugh, D. A. and D. M. Hood. 1993. Practical equine hoof wall biochemistry. Equine Practice. 15:8-14.

Hedges, V. J., R. Blowey, A. J. Packington, C. J. O'Callaghan, and L. E. Green. 2001. A longitudinal field trial of the effect of biotin on lameness in dairy cows. Journal of Dairy Science. 84:1969-1975.

Hedges, V. J., L. E. Green, R. Blowey, A. J. Packington, and R. H. C. Bonser. 2002. Tensile strength of the white line in the claw of dairy cows supplemented with biotin. Proceedings of the Twelfth International Symposium on Lameness in Ruminants. Pages 167-170. Vol. 9-13 Jan. 2002, Orlando, Florida.

Hendry, K. A. K., C. H. Knight, H. Galbraith, and C. J. Wilde. 2003. Basement membrane integrity and keratinisation in healthy and ulcerated bovine hoof tissue. Journal of Dairy Research. 70:19-27.

Hendry, K. A. K., C. H. Knight, C. H. Rae, H. Galbraith, and C. J. Wilde. 2002. Basement membrane deterioration in hoof ulceration. Pages 171-173 in Twelfth International Symposium on Lameness in Ruminants. Orlando, Florida.

Hendry, K. A. K., A. J. MacCallum, C. H. Knight, and C. J. Wilde. 1997. Laminitis in the dairy cow: a cell biological approach. Journal of Dairy Research. 64:475-486.

Hepburn, N. L., H. Galbraith, J. R. Scaife, and L. Kinninmonth. 2004. Pigmentation, impression hardness and the presence of melanocytes in bovine claw tissue. Pages 93-94 in Thirteenth International Symposium on Lameness in Ruminants. Maribor, Slovenia.

Higuchi, H. and H. Nagahata. 2001. Relationship between serum biotin concentration and moisture content of the sole horn in cows with clinical laminitis or sound hooves. Veterinary Record. 148:209–210.

Hinterhofer, C., V. Apprich, J. C. Ferguson, and C. Stanek. 2007. Modulus of elasticity and dry-matter content of bovine claw horn affected by the changes of chronic laminitis. Veterinary Journal. 174:605-609.

Hinterhofer, C., C. Stanek, and K. Binder. 1998. Elastic modulus of equine hoof horn, tested in wall samples, sole samples and frog samples at varying levels of moisture. Berliner und Munchener Tierarztliche Wochenschrift .111:217-221.

Hinterhofer, C., M. Zoscher, C. Gabler, and C. Stanek. 2005a. The hardness of horn in different segments of the bovine claw. Berliner und Munchener Tierarztliche Wochenschrift. 118:334-340.

Hinterhofer, C. M., V. Apprich, J. C. Ferguson, and C. Stanek. 2005b. Elastic properties of hoof horn on different positions in the bovine claw. . Berliner und Munchener Tierarztliche Wochenschrift. 112:142-146.

Hoblet, K. H. and W. Weiss. 2001. Metabolic hoof horn disease – claw horn disruption. Veterinary Clinics of North America. 17:111-127.

Hull, D. and T. W. Clyne. 1996. An Introduction to Composite Materials. Cambridge University Press, UK.

Husain, A., D. K. Sehgal, and R. K. Pandley. 2002. Design of a simple, versatile, small-specimen punch test set up for determination of the mechanical behaviour of materials. Experimental Techniques September/October 2002:33-38.

Ischii, R. and K. C. Nosai. 2000. A relationship between corium lesions and location of distal phalanges in dairy cattle. Pages 179-180 in Eleventh International Symposium on Lameness of the Ruminant Digit. Parma, Italy.

Kasapi, M. and J. M. Gosline. 1996. Strain-rate dependent mechanical properties of the equine hoof wall. The Journal of Experimental Biology. 199:1133-1146.

Kasapi, M. and J. M. Gosline. 1997. Design complexity and fracture control in the equine hoof wall. The Journal of Experimental Biology. 200:1639-1659.

Kasapi, M. and J. M. Gosline. 1999. Micromechanics of the equine hoof wall: optimizing crack control and material stiffness through modulation of the properties of keratin. The Journal of Experimental Biology. 202:377-391.

Kempson, S. A. and A. M. Johnston. 1990. Changes in the hoof horn of pigs with laminitis: a scanning and transmission electron microscopic study. Advances in Veterinary Dermatology. 1:456.

Kempson, S. A. and D. N. Logue. 1993. Ultrastructural observations of hoof horn from dairy cows: changes in the white line during the first lactation. The Veterinary Record. 132:524-527.

Kempson, S. A. and D. N. Logue. 1998. Laminitis versus papillaryitis. Pages 153-154 in Proceedings of the Tenth International Symposium on Lameness in Ruminants, Casino Lucerne, Switzerland.

Kessler, J., M. I., F. A. Dufey, A. Gutzwiller, A. Stern, and H. Geyes. 2003. Effect of organic zinc sources on performance, zinc status, and carcass, meat, and claw quality in fattening bulls. Livestock Production Science. 81:161–171.

Kitchener, A. and J. F. V. Vincent. 1987. Composite theory and the effect of water on the stiffness of horn keratin. Journal of Material Science. 22:1385-1389.

Knott, L., A. J. Webster, and J. F. Tarlton. 2004. Biochemical and biophysical changes to the connective tissue of the bovine hoof around parturition. Pages 88-90 in Thirteenth International Symposium on Lameness in Ruminants. Maribor, Slovenia.

Kossaibati, M. A. and R. J. Esslemont. 1999. The incidence of lameness in a group of dairy herds in England. Page 221 in BSAS Annual Meeting. BSAS, Scarborough.

Kossaibati, M. A., R. J. Esslemont, and C. Watson. 1999. The costs of lameness in dairy herds. Page 2 in Proceedings of the National Cattle Lameness Conference. MDC. Stoneleigh,UK.

Kurtz, S. M., C. W. Jewett, J. S. Bergstrom, J. R. Foulds, and A. A. Edidin. 2002. Miniature specimen shear punch test for UHMWPE used in total joint replacements. Biomaterials. 23:1907-1919.

Leach, K. A., D. N. Logue, S. A. Kempson, J. E. Offer, H. E. Ternent, and J. M. Randall. 1997. Claw lesions in dairy cattle: Development of sole and white line haemorrhages during the first lactation. Veterinary Journal. 154:215-225.

Leach, K. A., D. N. Logue, J. M. Randall, and S. A. Kempson. 1998. Claw lesions in dairy cattle: Methods for assessment of sole and white line lesions. Veterinary Journal. 155:91-102.

Lewis, G. 2002. Key issues involved with the use of miniature specimens in the characterization of the mechanical behaviour of polymeric materials. Journal of Biomedical Materials Research. 63: 455-466.

Lischer, C. J. and P. Ossent. 2002. Pathogenesis of sole lesions attributed to laminitis in cattle. Pages 82-89 in Proceedings of the Twelfth International Symposium on Lameness in Ruminants, Orlando, Florida.

Liu, K. and M. R. Piggott. 1998. Fracture failure processes in polymers I: Mechanical tests and results. Polymer Engineering and Science. 38:60-68.

Livesey, C. T., A. M. Johnson, C. Marsh, S. A. May, and J. A. Metcalf. 1998. Hoof horn growth in Holstein cattle from first calving until six weeks after second calving. Pages 51-52 in Tenth International Symposium on Lameness in Ruminants. Casino Lucerne, Switzerland.

Livesey, C. T. and R. A. Laven. 2007. Effects of housing and intake of methionine on the growth and wear of hoof horn and the conformation of the hooves of first-lactation Holstein heifers. Veterinary Record. 160:470-476.

Logue, D. N. 1999. An overview of lameness in the dairy cow. Page 2 in Proceedings of the National Cattle Lameness Conference.

Logue, D. N., S. A. Kempson, K. A. Leach, J. E. Offer, and R. E. McGovern. 1998. Pathology of the white line. Pages 142-145 in Proceedings of the Tenth International Symposium on Lameness in Ruminants, Casino Lucerne, Switzerland.

Logue, D. N., J. E. Offer, and S. A. Kempson. 1993. Lameness in dairy cattle. Irish Veterinary Journal. 46:47-58.

MacCallum, A. J., C. H. Knight, K. A. K. Hendry, C. J. Wilde, D. N. Logue, and J. E. Offer. 2002. Effects of time of the year and reproductive state on the proliferation and keratinisation of bovine hoof cells. Veterinary Record. 151:285-289.

Manson, F. J. and J. D. Leaver. 1988. The influence of concentrate amount on locomotion and clinical lameness in dairy cattle. Animal Production. 47:185-190.

Manson, F. J. and J. D. Leaver. 1989. The effect of concentrate: silage ratio and of hoof trimming on lameness in dairy cattle. Animal Production. 49:15-22.

Moore, C. L., P. M. Walker, J. R. Winter, M. A. Jones, and J. M. Webb. 1989. Zinc methionine supplementation for dairy cows. Transactions of the Illinois State Academy of Science. 82:99–108.

Műlling, C., H. Bragulla, K. D. Budras, and S. Reese. 1994. Structural Factors Influencing Horn Quality and Sites of Predilection at the Ground Surface of the Cattle Hoof. Schweizer Archiv Fur Tierheilkunde. 136:49-57.

Műlling, C., H. Bragulla, S. Reese, K. D. Budras, and S. W. 1999. How structures in bovine hoof epidermis are influenced by nutritional factors. Anatomia Histologia Embryologia-Journal of Veterinary Medicine Series C-Zentralblatt Fur Veterinarmedizin Reihe C. 28:103–108.

Műlling, C. and K. D. Budras. 1998. Influence of environmental factors on horn quality of bovine hoof. Pages 214-215 in Proceedings of the Tenth International Symposium on Lameness in Ruminants. Casino Lucerne, Switzerland.

Műlling, C., K. D. Budras, H. Geyer, and J. Maierl. 1998. Anatomy and structure of hoof horn. Pages 176-188 in Tenth International Symposium on Lameness in Ruminants. Casino Lucerne, Switzerland.

Mulling, C., D. Frohberg-Wang, G. Ledner, and K. D. Budras. 2004. Matrix overloaded – structural alterations of claw connective tissue and their

functional implications. Pages 79-82 in Thirteenth International Symposium on Lameness in Ruminants. Maribor, Slovenia.

Műlling, C. H. 2000. The use of nutritional factors in prevention of claw diseases-Biotin as an example for nutritional influences on formation and quality of hoof horn. . Pages 78–80 in 11th International Symposium on Disorders of the Ruminant Digit. , Parma, Italy.

Mulling, C. K. W., H. H. Bragulla, S. Reese, K. D. Budras, and W. Steinberg. 1999. How structures in bovine hoof epidermis are influenced by nutritional factors. Anatomia Histologia Embryologia-Journal of Veterinary Medicine Series C-Zentralblatt Fur Veterinarmedizin Reihe C. 28:103-108.

Mulling, C. K. W. and C. J. Lischer. 2002. New aspects on aetiology and pathogenesis of laminitis in cattle. Pages 236-247 in Recent Developments and Perspectives in Bovine Medicine-XXII World Buiattrics Congress. Hanover, Germany.

Newlyn, R. A., S. N. Collins, B. C. Cope, L. Hopegood, R. J. Latham, and J. D. Reilly. 1999. Equid hoof horn a natural composite. Pages 231-240. in Proceedings of the Fifth International Conference in Deformation and Fracture of Composites. Vol. 5th, London, UK.

Nocek, J. E. 1997. Bovine Acidosis Implications on Laminitis. Journal Dairy Science. 80:1005–1028.

Nocek, J. E. and A. B. Johnson. 2000. Digital characteristics in commercial dairy herds fed metal-specific amino acid complexes. Journal Dairy Science. 83:1553-1572.

Nocek, J. E. and W. P. Kautz. 2006. Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and postpartum dairy cattle. Journal of Dairy Science. 89:260-266.

Nocek, J. E., M. T. Socha, and D. J. Tomlinson. 2006. The Effect of Trace Mineral Fortification Level and Source on Performance of Dairy Cattle. Journal of Dairy Science. 89:2679 – 2693.

Nomoto, R., T. E. Carrick, and J. F. McCabe. 2001. Suitability of a shear punch test for dental restorative material. Dental Materials. 17:415-421.

Offer, J. E., G. E. J. Fisher, S. A. Kempson, and D. N. Logue. 2001. The effect of feeding grass silage in early pregnancy on claw health during first lactation. The Veterinary Journal. 161:186-193.

Offer, J. E., K. A. Leach, S. Brocklehurst, and D. N. Logue. 2003. Effect of forage type on claw horn lesion development in dairy heifers. Veterinary Journal. 165:221-227.

Offer, J. E., D. N. Logue, and K. A. Leach. 2000a. Changes in claw conformation, growth and wear in young stock and first lactation cattle. Pages 282-284 in Eleventh International Symposium on Lameness of the Ruminant Digit. Parma, Italy.

Offer, J. E., D. N. McNulty, and D. N. Logue. 2000b. Observations of lameness, hoof conformation and development of lesions in dairy cattle over four lactations. The Veterinary Record. 147:105-109.

Ossent, P. and C. Lischer. 1998. Bovine laminitis: the lesions and their pathogenesis. In Practice. 20:415-427.

Parry, D. A. D. and P. M. Steinert. 1995. Molecular Biology Intelligence Unit. Pages 9–144 in Intermediate Filament Structure. Springer, Heidelberg, Germany.

Patan, B. and K. D. Budras. 2003. Segment specificity of the equine hoof – Part 2: correlation of horn structure and mechanical physical horn properties in the different segments of the hoof. Pferdeheilkunde. 19:177-184.

Pellmann, R., S. Reese, and H. Bragulla. 1993. Relationships between the structure and quality of equine hoof horn – A potential procedure for studying disorders of keratinization. Monatshefte fur Veterinarmedizin. 48:623-630.

Peterse, D. J. 1985. Breeding. Pages 121-123 in Cattle Foot care and Claw Trimming. E. T. T. Raven, ed. Farming Press Ltd, Ipswich.

Peterse, D. J., S. Korver, J. K. Oldenbroek, and F. P. Talmon. 1984. Relationship between Levels of Concentrate Feeding and Incidence of Sole Ulcers in Dairy-Cattle. Veterinary Record. 115:629-630.

Phillips, C. J. C. and S. A., Schofield. 1994. The effect of cubicle and straw yard housing on the behaviour, production and hoof health of dairy cows. Animal Welfare. 3:37-44.

Pugashetti, B., M. C. Shivakumar, V. S. Kulakarni, and S. V. Hosamani. 2004. Higher incidences were obIncidence of foot affections in cross-bred cows. Karnataka Journal of Agricultural Sciences. 17:901-903.

Reppond, K. D. and J. K. Babbitt. 1997. Gel properties of surimi from various fish species as affected by moisture content. Journal of Food Science. 62:33-36.

Reppond, K. D., J. K. Babbitt, S. Bernsten, and M. Tsuruta. 1995. Gel properties of surimi from Pacific herring. Journal of Food Science. 60:707-714.

Rice, R. H. and H. Green. 1977. The cornified envelope of terminally differential human epidermal kertinocyes consists of cross linked protein. Cell. 11:417-422.

Russell, A. M., G. J. Rowlands, S. R. Shaw, and A. D. Weaver. 1982. Survey of lameness in British dairy cattle. The Veterinary Journal. 111:155-160.

Satapathy, S. and S. Bless. 2000. Deep punch PMMA. Experimental Mechanics. 40:31-37.

Schmidt, R., E. J. Parish, and V. Dionisius. 1991. Modulation of cellular cholerstrol and its effects of cornified envelope function in cultured human epidermal keratinocytes. Journal of Investigative Dermatology. 97:771-775.

Shearer, J. K., E. Belknap, S. Berry, C. Guard, K. Hoblet, E. Hovingh, G. Kirksey, A. Langill, and S. R. van Amstel. 2002. The standardisation of input codes for capture of lameness data in dairy records Pages 346-349 in Proceedings of the Twelfth International Symposium on Lameness in Ruminants, Orlando, Florida.

Socha, M. T., D. T. Tomlinson, J. A.B., and L. M. Schugel. 2002. Improved claws through improved micronutrient nutrition. In 12th international symposium on lameness in ruminants. Orlando. USA.

Steinert, P. M. 1988. Molecular and cellular biology of intermediate filaments. Annual Review of Biochemistry. 57:593-675.

Steinert, P. M., J. C. R. Jones, and R. D. Goldman. 1984. Intermediate filaments. The Journal of Cell Biology. 99:22–27.

Tarlton, J. F., D. E. Holah, K. M. Evans, S. Jones, G. R. Pearson, and A. J. F. Webster. 2002. Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving. Veterinary Journal. 163:196-204.

Tarlton, J. F. and A. J. F. Webster. 2002. A biochemical and biomechanical basis for the pathogenesis of claw horn lesions. Pages 395-398 in Proceedings of the Twelfth International Symposium on Lameness in Ruminants, Orlando, Florida.

Toussaint Raven, E. 1985. Cattle footcare and claw trimming. Farming Press Ltd., Ipswich UK.

Tranter, W. P. and R. S. Morris. 1991. A Case-Study of Lameness in three Dairy Herds. New Zealand Veterinary Journal. 39:88-96.

Tranter, W. P. and R. S. Morris. 1992. Hoof growth and wear in pasture-fed dairy cattle. New Zealand Veterinary Journal. 40:89-96.

Tranter, W. P., R. S. Morris, I. R. Dohho, and N. B. Williamson. 1993. A case-control study of lameness in dairy cows. Preventive Veterinary Medicine. 15:191-203.

Uchida, K., P. Mandebvu, C. S. Ballard, C. J. Sniffen, and M. P. Carter. 2001. Effect of feeding a combination of zinc, manganese and copper amino acid complexes, and cobalt glucoheptonate on performance of early lactation high producing dairy cows. Animal Feed Science and Technology. 93:193-203.

van Amstel, S. R. and J. K. Shearer. 2001. Abnormalities of hoof growth and development. The Veterinary Clinics of North America Food Animal Practice Lameness. 17:73-91.

van Amstel, S. R., J. K. Shearer, and F. L. Palin. 2004. Moisture content, thickness, and lesions of sole horn associated with thin soles in dairy cattle. Journal of Dairy Science. 87:757-763.

Van der Tol, P. P. J., J. H. M. Metz, E. N. Noordhuizen-Stassen, W. Back, C. R. Braam, and W. A. Weijs. 2002. The pressure distribution under the bovine claw during square standing on a flat substrate. Journal of Dairy Science. 85:1476-1481.

Vermunt, J. J. 2000. Risk factors of laminitis- an overview. Pages 30-39 in The 11th international symposium on lameness in ruminants. Parma, Italy.

Vermunt, J. J. 2004. Herd lameness - a review, major causal factors, and guidelines for prevention and control. in Prophylaxis of Claw Diseases Proceedings of the 13th International Symposium and 5th Conference on Lameness in Ruminants, Maribor, Slovenija.

Vermunt, J. J. and P. R. Greenough. 1994. Predisposing Factors of Laminitis in Cattle. British Veterinary Journal. 150:151-164.

Vermunt, J. J. and P. R. Greenough. 1995. Structural characteristics of the bovine claw: horn growth and wear, horn hardness and claw conformation. British Veterinary Journal 151:157–180.

Vincent, J. F. V. 1992. Composites. Biomechanics – Materials; A Practical Approach. Oxford University Press, Oxford, UK.

Vokey, F. J., C. L. Guard, H. N. Erb, and D. M. Galton. 2001. Effect of alley and stall surfaces on indices of claw and leg health in dairy cattle housed in a free-stall barn. Journal of Dairy Science. 84:2686-2699.

Wagner, I. P. and D. M. Hood. 202. Effect of prolonged water immersion on equine hoof epidermis in vitro. American Journal of Veterinary Research. 63:1140-1144.

Wang, E. 1985. Intermediate filament associated proteins. Annals of the New York Academy of Sciences. 45:32-56.

Ward, W. H. and H. P. Lundgren. 1954. The formation, composition and properties of the keratins. Advances in Protein Chemistry. 9:243–297.

Webster, A. J. F. 2001. Effects of housing and two forage diets on the development o claw horn lesions in dairy cows at first calving and first lactation. The Veterinary Journal. 162:56-65.

Winkler, B. 2005. Mechanical properties of hoof horn, sole haemorrhage and lameness in dairy cattle. PhD dissertation. Plymouth University, Plymouth, UK.

Winkler, B. and J. K. Margerison. 2004. Mechanical properties of the sole hoof horn of heifers before and during the first lactation - a prediction of lameness susceptibility. Pages 60-62 in 13th International Symposium and 5th Conference on Lameness in Ruminants, Maribor, Slovenija.

Winkler, B. and J. K. Margerison. 2006. Mechanical properties of the hoof horn of dairy cows during lactation. Journal Animal Science. 84: Suppl. 1:415 (Abstract).

Winkler, B. and J. K. Margerison. 2007a. Effect of sample thickness, tissue moisture content and storage methods on the punch resistance and elastic modulus of the bovine hoof horn. Journal Animal Science. 85: Suppl. 1 (Abstract):419.

Winkler, B. and J. K. Margerison. 2007b. Relationships between locomotion and lesion score, punch resistance and Holstein (HUKI) conformation scores. Journal Animal Science. 85: Suppl. 1 (Abstract):423.

Winkler, B., J. K. Margerison, and C. Brennan. 2002. The testing of the mechanical properties of the hoof horn during lactation and housing. Pages 314-317 in Proceedings of the 12th International Symposium on Lameness in Ruminants, Orlando, Florida, USA.

Zaun, H. 1997. Brittle nails confirmation of diagnosis and evaluation of treatment. Hautarzt. 48:455-461.

Zoscher, M., C. Hinterhofer, C. Stanek, and C. Gabler. 2000. New aspects of mechanical properties of bovine hoof horn. Pages 91-93 in Eleventh International Symposium on Lameness of the Ruminant Digit. Parma, Italy.

Chapter 2

The effect of live yeast inclusion into mixed forage diets on milk yield, feed conversion efficiency, locomotion score, lameness and sole bruising in first lactation Holstein Friesian dairy cattle

2.0 ABSTRACT

Increased milk production potential of diary cattle and the application of energy dense diets have led to higher incidence rates of sub-clinical and clinical health problems, including laminitis/lameness. Yeast (*Saccharomyces cerevisiae*) occurs naturally and can be used as a ruminal modifier with the potential to reduce the severity and side effects of ruminal acidosis, which may include laminitis and lameness. The objective was to assess the effect of supplementing a mixed forage diet with live yeast (LY) on milk yield, sole bruising, claw horn structural strength wear and growth, and locomotion score of dairy cattle. At parturition 24 Holstein Friesian heifers were offered the total mixed ration (MR) until 120 d pp with either (n 12) the addition of live yeast (*Saccharomyces cerevisiae*) at 10 billion/h/d (LY); or (n=12) no additional live yeast (NLY). There were no differences in dry matter intake (NLY: 22.6; LY: 22.6 ±0.036 kg/day). The addition of yeast (LY) increased mean milk yield (NLY: 27.2; LY:29.5 ± 0.29 kg/day P<0.001), fat corrected yield (NLY: 24.6; LY:25.5 ±0.28 kg/day P=0.019), total milk fat (NLY: 0.95; LY:1.04 ±0.013 P<0.001), and protein yield (NLY:0.86; LY:0.94 ±0.010 P<0.001), milk urea concentration (NLY: 0.036; LY:0.038 ±0.001 P=0.001), and feed conversation efficiency (NLY: 1.03; LY: 1.12 ±0.018 kg/day P<0.001) compared with NLY. Heifers yeast had lower mean sole total haemorrhages score (LY: 16.5, NLY: 33.5 P=0.021) and higher sole puncture resistance (PR) (50 d pp: NLY: 8.82, LY 9.13 , 100 d pp: NLY: 8.63 , LY:8.72 , 150 d pp: NLY: 8.11 , LY:8.16 Nmm^2 (*P*=0.001). There was no significant difference for WL (WL) lesions or PR. Sole and WL lesion; n of claw lesions decreased significantly as number of d pp increased. In conclusion, live yeast increased milk yield, total fat, protein yield and increased claw horn puncture resistance of first lactation dairy heifers.

Keywords: Live yeast; milk yield; feed conversion efficiency; lameness.

2.1 INTRODUCTION

The substantial increase in milk yield potential of UK dairy cattle has led to the use of energy dense diets which are lower in fibre content. This has resulted in a higher incidence of ruminal acidosis and sub-acute ruminal acidosis and the use of products, such as yeast, to modify ruminal pH. The addition of yeast has been used as a 'natural' probiotic means of modifying ruminal fermentation to improve feed utilization and animal performance. Yeast is thought to stimulate cellulolytic microorganisms, increasing fibre digestion, mediating acetate to propionate acid ratio and increasing microbial protein flow to the duodenum (Williams *et al.*, 1991). The results from animal feeding experiments and meta-analysis indicate that the effects of yeast inclusion on the production performance of dairy cattle are unequivocal (Desnoyers *et al.*, 2009; Robinson and Erasmus, 2009). In studies where an animal response was observed, yeast supplementation increased feed intake rather than altering feed conversion efficiency (FCE) and only occasionally improved feed efficiency (Günther, 1989). Yeast supplemented cows milk yield was approximately 0.9 kg/d higher than non supplemented animals, however, this margin decreased proportionally as milk yield from non yeast supplemented cows increased (Robinson and Erasmus, 2009). In a recent meta–analysis Desnoyers *et al.* (2009) showed that yeast supplementation increased DMI, milk yield, ruminal pH and ruminal VFA concentrations and tended to increase milk fat percentage and decrease ruminal lactic acid concentrations. Williams *et al.* (1991) demonstrated the beneficial effects of supplementing yeast for animals offered high energy density diets, concentrated feed to forage ratio 50:50 or 60:40, finding decreased concentrations of ruminal lactic acid compared with animals offered yeast. These observations are associated with increases in ruminal pH (> 5.5), which are characteristic of stable ruminal fermentation, while a low ruminal pH (<5) has been associated with poorer claw horn quality and potentially laminitis (Nocek, 1997; Westwood *et al.*, 2003).

Lameness has become one of the most important economic issues (Bruijnis *et al.*, 2010), that reduces the welfare of dairy cattle (Whay *et al*., 1997) as incidence rates have increased considerably over the past 20 years. Clarkson *et al.* (1996) found that the mean incidence in individual herds is between 22 and 55% of cows annually. Ruminal acidosis has been recognised as a major risk factor in the development of laminitis (Nocek, 1997; Oetzel, 2000) and connection between dietary starch content and the occurrence of laminitis has been indicated (Manson and Leaver, 1988; Nocek, 1997). As dairy producers strive to meet the increasing energy requirements for dairy cattle, higher levels of rapidly fermentable carbohydrates and co-products are used in combination with decreasing levels of forage fibre and (subclinical and clinical) acidosis and laminitis become more common (Oetzel, 2000). The objective of this research was to assess the effect of the inclusion of live yeast in a mixed ration (MR) on feed intake, milk yield, sole and while line (WL) haemorrhage, claw horn characteristics, locomotion score and lameness.

2.2 MATERIALS AND METHODS

This research was completed in Devon in the UK between 1st Oct and 5 th March 2008 according to local ethical procedures and protocols approved by the Massey University Animal Ethics Committee, NZ. This study used 24 Holstein Friesian dairy heifers, which were selected at random according to calving date and in matched pairs according to calving date, breeding value, body condition score and live weight. The heifers were offered access to the same mixed ration (MR) either with the addition of live yeast (*Saccharomyces cerevisiae*) at the rate of 10 billion/h/d (LY) or without the additional live yeast (NLY), which was offered as a mixed ration (0.46 forage and 0.54 cereal based feed) detailed in Tables 2.1. An initial pre-treatment 14 d covariate milk yield measurement period was used, followed by a 14 d diet change-over period and a 150 day measurement period. All the heifers were offered MR *ad libitum* by offering 10% additional feed

calculated from the highest day intake levels on a day by day basis. MR offered and remaining was used to calculate feed intake. The heifers were housed in two separate groups, in cubicles / free stalls, within the same partitioned barn in accordance with DEFRA (2003) and BS 5052, part 40 (British Standards, 1990) standards. The passageways were scrapped manually twice daily and all lactating animals were milked twice, at 6.00 and 17.00, daily.

Table 2.1 Composition of the mixed ration (MR), compound and mineral premix

Item	Diet content
Ingredient % of dietary DM	(%)
Grass silage	48.62
Maize silage	33.46
Whole crop silage	8.25
Wheat	2.25
Soya	1.50
Mineral premix	0.06
Dairy compound	5.85
Chemical composition	
Dry Matter (%)	33.9
ME (MJ/kg DM)	11.7
Ash (%DM)	5.0
Oil-B (% DM)	3.3
NDF (%DM)	38.4
CP (%DM)	15.1
Sugars & starch (%DM)	22.1

Mineral and vitamin premix contained /kg DM basis; Cu (mg/kg) 45, Se (mg/kg) 0.6, Vit E (mg/kg) 60, Calcium, 0.21, Phosphorus, 0.07 Sodium, 0.08, Magnesium, 0.05, Vitamin A, 500000 iu, Vitamin D_3, 100000 iu, Vitamin E, 2500 iu, Mn oxide, 4500 mg Cu sulphate, 1500 mg, Protected Cu, 500 mg, Zn oxide, 3700 mg, Protected Zn, 1250 mg, Co carbonate, 72 mg, Calcium iodate, 200 mg.

2.21 Measurements

The locomotion score of all animals was assessed weekly using a 5 point scale in which 0 to 1 corresponded with non lame animals, 3 an animal showing tenderness when walking, 4 an animal that is lame and 5 was an animal that is severely lame and non weight bearing on lame limb/s (Manson and Leaver, 1988). All lame animals were examined to determine the cause of lameness and the development of each lameness case was monitored.

The hind claws of heifers were assessed for sole ulcers, sole haemorrhage and heel erosion at 50, 100, and 150 d postpartum (pp). The lesions on each foot were scored according to Leach *et al.* (1998). 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 infection. The affected areas of the sole and WL areas of the heifer's foot were outlined and subsequently photographed with a digital camera. The images were analysed for claw area, haemorrhage of WL and sole using Scion Image Analysis. The WL and sole areas of the claw horn were scored separately for number, percent and total score (percent x intensity of haemorrhage). Any other alterations present during examination, such as Digital dermatitis and heel horn erosion, were recorded.

The claw horn growth and wear rates were measured on the right rear inner claw at 50, 100 and 150 d pp. A mark was made on the claw horn wall 2 cm below the coronary border. The distance from the coronary border to the mark and from the mark to the distal end of the wall was measured and a new mark was made 2 cm below the coronary border at 50, 100 and 150 d pp. The monthly growth and wear rates were estimated from these measurements as described by Clark and Rakes (1982), and the claw angle, length of the front claw wall and height of the

heel were measured at the same time of the claw growth and wear, according to Boelling and Pollott (1998).

2.22 Collection of claw horn samples

Samples of claw sole tissue were collected from all the hind claws of all experimental heifers at 50, 100, and 150 d pp. The first outer layer of horn (1 mm) was discarded and a sample of 0.1 to 2.5 mm thickness was taken using hoof trimming knives. Samples were taken parallel to the ground surface from zones 2 and 5 of the sole surface of the claw, according to the international foot map (Shearer *et al.*, 2002). Claw horn samples from right hand lateral claws were collected, transferred immediately to a plastic bag, sealed and stored in a refrigerator at a temperature of 2 °C until analysis (Douglas *et al.*, 1996). The dry matter content of horn samples was determination using oven dry matter determination (100°C for 72 hrs) according to (MAFF, 1986).

2.23 Puncture resistance of claw horn

Claw horn samples were analysed for puncture resistance (PR) as described by Winkler (2005) using a P/2N needle probe on a TA.XT plus texture analyser with a 5 kg load cell (Stable Micro-Systems, Vienna Court, Lammas Road, Godalming, Surrey, GU7 1YL, UK). The test probe was used at a speed of 1.0 mm/sec, which enabled the material to adapt to the load (Aranwela *et al.*, 1999) and measured the force in compression in test-mode. A force-displacement curve was recorded when the test probe reached the sample and a trigger force of 5 g had been applied, the probe travelled a distance of 12.0 mm before returning to the initial position. This distance was sufficient to allow the sample to be punctured and the maximum force on puncture to be measured. The maximum puncture force values (Nmm^2) were obtained from the force-displacement curve.

A total of 5 tests were completed on the sole and WL areas of each claw in accordance with Winkler (2005), which was considered to be sufficient to detect sample differences. The thickness of the sample on the tested area was recorded

using callipers with a resolution of 0.01 mm. Each sample was scored for level of haemorrhage using a scale of 0 to 5 (0: no haemorrhage and 5: severe haemorrhage) and was used to compare the effect of haemorrhage level on PR. The mean PR data from each animal was used to compare the effect of area and individual claws of each animal.

2.24 Milk production and feed utilisation

The individual animal milk yield and milk composition was measured on one day each week using composited milk samples taken from across one evening and the following morning milking and samples were analysed using an infrared analyser (Foss Electric, Hillerod, Denmark). The live weight and body condition score (BCS) were assessed weekly. The body condition of the heifers was accessed by using 5 point scale, with half points, in which 1 corresponded to thin and 5 to fat. The dry matter intake (DMI) was calculated by difference between the amount of feed offered minus the amount of feed refused on a daily basis and feed conversion efficiency (FCE) was determined from energy corrected milk production as defined by Tyrrell and Reid (1965) (kg milk * (383 * fat % + 242 * protein % + 783.2)/3140) divided by kg DM).

2.25 Statistical Analysis

The data collection from dairy heifers was used as individual animal observations. All the data was assessed for normal distribution using the norm plot procedures in Minitab 15 (Minitab Inc., State College, PA) and found to be normally distributed. The effect of treatment diet was assessed using analysis of variance (ANOVA), general linear modelling (GLM) command in Minitab 15.0, using a confidence interval of 95%, which included diet and claw as fixed effects and animal as a random effect in the model, while horn haemorrhaging, claw horn growth and wear, milk yield, milk composition, FCE, live weight, body condition score, locomotion score were the variables to be assessed. The PR data was compared by including the thickness of the claw horn sample as a covariate in the GLM ANVOA in Minitab 15.0 with a confidence interval of 95%.

The existence of significant differences were assessed by applying Tukey's test when running the GLM ANOVA in Minitab 15.0 and statistical differences were reported when the probability value (*P*) was <0.05 and a tendency reported when *P*<0.10 in the ANOVA table. All the data was presented as mean ± standard error of the means (sem) along with the individual *P* values for each comparison of mean and individual mean standard errors.

2.3 RESULTS

Table 2.2 Overall mean for locomotion score, foot angle, dorsal border, heel depth, diagonal claw length measurements, and incidence of digital dermatitis and slurry heel in dairy cattle offered a mixed ration with no live yeast (NLY) or with live yeast (LY) between 0 and 150 d pp

	NLY	LY	SEM	P Value
Locomotion score (1 to 5)	1.30	1.40	0.051	0.087
Claw angle (mm)	49.3	48.7	0.32	0.206
Dorsal border (mm)	83.9	83.3	1.16	0.721
Heel depth (mm)	36.1	37.0	1.18	0.600
Diagonal claw length (mm)	115.4	116.6	1.41	0.559
Incidence of digital dermatitis (%)	6.9	8.3	0.989	0.423
Incidence of slurry heel (%)	9.7	9.8	0.28	0.774

There were no differences in mean locomotion score, heel depth, claw angle, dorsal border and diagonal claw length, monthly claw growth rate and wear rate or the incidence of digital dermatitis and slurry heel (Table 2.2) or wall horn growth, wear or net changes rates between 50 and 150 d pp (Table 2.3).

Table 2.3 Mean growth, wear and net changes rates of wall horn of the left hind outer claw of dairy cattle offered a mixed ration with no live yeast (NLY) or with live yeast (LY) between 0 and 150 d pp

	NLY	LY	SEM	P Value
Growth rate (mm)				
50-100 d pp	3.1	2.9	0.86	0.852
100-150 d pp	9.9	9.2	0.95	0.612
Wear rate (mm)				
50-100 d pp	10.7	4.5	2.38	0.063
100-150 d pp	1.9	0.0	2.43	0.543
Net change (mm)				
50-100 d pp	-7.7	-1.7	2.92	0.137
100-150 d pp	8.8	9.5	2.12	0.810

The heifers supplemented with live yeast (LY) had lower (P<0.05) overall mean percentage sole bruising (LY: 3.3, NLY: 5.9 P=0.041) and sole total haemorrhages score (LY: 16.5, NLY: 33.5 P=0.021) (Table 2.4).

The regression equations from the multiple regression analysis of the effect of inclusion of live yeast, post partum period (d pp), and PR including adjusted (adj) R^2 and P values for sole measurements were:

Percentage sole haemorrhage of claw horn = 8.83 -0.0028 d pp -2.63 with or without inclusion of LY, R^2 adj = 0.3. P= 0.131.
Total sole haemorrhage score of claw horn = 42.3 -17.0 or without inclusion of LY +0.0829 day pp, R^2 adj = 0.6 P=0.044.

PR of sole claw horn was: Sole claw horn PR = 9.21+ 0.146 with or without inclusion of LY +0.00123 total haemorrhage score -0.00879 day pp R^2 adj = 0.78 P<0.001.

There were no differences between; mean lesion score, number of sole and WL haemorrhages, or percentage, and total haemorrhage score or puncture resistance of the WL (Table 2.5) or claw horn DM content (NLY 90.2%; LY 90.9, SEM 1.45) between heifers offered yeast and those not offered yeast.

Table 2.4 Mean sole haemorrhages; number, percentage, total score and puncture resistance of claw horn, at 50, 100 and 150 days postpartum, of dairy cattle offered a mixed ration with either with no live yeast (NLY) added or with live yeast (LY) added

	NLY	LY	Sem	P
Sole haemorrhage (No.)				
50 d pp	1.2 [a]	0.6 [b]	0.16	0.024
100d pp	4.5	3.5	0.77	0.374
150d pp	3.8	3.6	0.78	0.883
Sole haemorrhage (%)				
50 d pp	4.4	2.5	0.86	0.141
100d pp	9.5	4.6	2.32	0.150
150d pp	3.8	2.7	0.784	0.330
Total sole haemorrhage score				
50 d pp	21.4	10.4	3.79	0.053
100d pp	46.1	23.9	11.49	0.186
150d pp	33.1	15.2	8.95	0.171
Sole puncture resistance (N mm^2)				
50 d pp	8.8 [b]	9.1 [a]	0.08	0.014
100d pp	8.63	8.69	0.022	0.100
150d pp	8.1	8.11	0.015	0.912

Table 2.5 Mean WL haemorrhages; number, percentage, total score and puncture resistance of claw horn at 50, 100 and 150 days postpartum, of dairy heifers offered a mixed ration with either with no live yeast (NLY) added or with live yeast (LY) added

	NLY	LY	Sem	P
WL haemorrhage (no.)				
50 d pp	1.0	0.8	0.286	0.685
100d pp	1.4	1.3	0.334	0.862
150d pp	0.6	0.1	0.248	0.169
WL haemorrhage (%)				
0 d pp	4.6	5.5	1.76	0.714
100d pp	10.7	11.0	2.62	0.931
150d pp	4.0	1.5	2.62	0.505
Total WL haemorrhage score				
50 d pp	21.9	42.6	19.51	0.462
100d pp	59.9	48.8	14.75	0.601
150d pp	28.0	0.0	11.61	0.807
WL puncture resistance (N mm^2)				
50 d pp	6.8	6.7	0.07	0.842
100d pp	6.5	6.5	0.04	0.907
150d pp	5.65	5.61	0.046	0.568

NLY- no inclusion of live yeast, LY- inclusion of live yeast, SEM- standard error of the mean

The number of days pp had a significant effect on the WL and sole haemorrhages (Table 2.6). Sole and WL haemorrhages number, percentage, total score (area x intensity), peaked at 100 d pp. PR in both sole and WL continued to reduce to 150 days pp.

Table 2.6 Mean sole and WL haemorrhages; number, percentage, total score and puncture resistance of claw horn, at 50, 100 and 150 days postpartum, of dairy heifers offered a mixed ration with either with no live yeast (NLY) added or with live yeast (LY) added

	50 d pp		100 d pp		150 d pp		SEM	P values		
	NLY	LY	NLY	LY	NLY	LY		d pp	Diet	d pp*Diet
Sole haemorrhage (no.)	1.4 ^{b}	0.7 ^{b}	4.5 ^{a}	3.5 ^{a}	3.8 ^{a}	3.6 ^{a}	0.648	0.001	0.253	0.812
Sole haemorrhage (%)	4.4 ^{ab}	2.6 ^{b}	9.5 ^{a}	4.6 ^{ab}	3.8 ^{ab}	2.7 ^{b}	1.503	0.201	0.036	0.411
Total Sole haemorrhage score	21.3 ^{bc}	10.3 ^{c}	46.0 ^{a}	23.8 ^{bc}	33.1 ^{ab}	15.2 ^{c}	8.692	0.096	0.019	0.810
Sole puncture resistance (N mm^2)	8.82 ^{b}	9.13 ^{a}	8.63 ^{c}	8.72 ^{bc}	8.11 ^{d}	8.16 ^{d}	0.049	0.001	0.001	0.049
WL haemorrhage (no.)	1.0 ^{ab}	0.8 ^{ab}	1.4 ^{a}	1.3 ^{ab}	0.6 ^{ab}	0.1 ^{b}	0.292	0.007	0.298	0.753
WL haemorrhage (%)	4.6 ^{b}	5.5 ^{b}	10.7 ^{a}	11.0 ^{a}	4.0 ^{b}	1.5 ^{b}	2.370	0.004	0.828	0.742
Total WL haemorrhage score	23.0 ^{bc}	41.4 ^{ab}	57.8 ^{a}	50.8 ^{ab}	28.0 ^{abc}	0.00 ^{c}	15.48	0.039	0.662	0.331
WL puncture resistance (N mm^2)	6.80^{a}	6.78 ^{a}	6.51 ^{b}	6.51 ^{b}	5.64 ^{c}	5.61 ^{c}	0.057	0.001	0.634	0.965

a,b,c,d – data in rows followed by differing superscripts differs significantly P<0.05

Table 2.8 Overall mean feed intake, feed conversion efficiency, covariate (cov.) (Milk yield week 1 and 2), milk yield, fat corrected yield, fat and protein yield, total fat and protein, lactose and urea content, live weight and body condition score of heifers offered a mixed ration with either no inclusion of live yeast (NLY) or with the inclusion of live yeast (LY)

Mean	NLY	LY	SEM	P
Feed intake (DM kg/d)	22.64	22.64	0.036	0.687
Feed conversion (kg)	1.03[b]	1.12 [a]	0.018	<0.001
Milk yield Cov. 1 (kg/d)	20.9	21.9	2.36	0.544
Milk yield Cov. 2 (kg/d)	22.8	23.9	1.91	0.639
Milk yield (kg/d)	27.2	29.6	0.29	<0.001
Fat corrected yield (kg/d)*	24.6	25.5	0.28	0.019
Fat yield (g/kg)	37.0	36.2	0.53	0.239
Protein (g/kg)	32.9	32.0	0.19	0.002
Total fat (kg/d)	0.95	1.04	0.013	<0.001
Total protein (kg/d)	0.86	0.94	0.010	<0.001
Milk lactose (g/kg)	46.2	46.2	0.08	0.918
Milk urea (%)	0.036	0.038	0.0005	0.001
Live weight (kg)	598	604	5.7	0.501
Body condition score (1 to 5)	2.4	2.4	0.04	0.877

*Fat correction to 4%,

The covariate period shows that there was no difference for mean milk yield between the two groups before the research started. Heifers supplemented with yeast (LY) had higher mean milk yield (P <0.001), fat corrected yield (P=0.019), total fat (P<0.001) and protein yield (P<0.001) and milk urea (P<0.001) concentrations compared heifers not supplemented with yeast (Table 2.8). There were no differences in DMI, live weight or body condition score between heifers offered a MR with or without the addition of live yeast (Table 2.8). However, FCE was significantly higher in the heifers supplemented with yeast (Table 2.8).

2.4 DISCUSSION

In this study, incidence and severity of sole and WL haemorrhaging reached peak levels at 100 d pp, subsiding by 150 d pp. The results corresponded with previous findings (Offer *et al.*, 2000; Winkler, 2005) where haemorrhages were found to peak between 100 and 120 d pp and to decline in both number and total score thereafter. In this study, the sole and WL PR levels illustrate the effects of increasing haemorrhage score as there was significant reduction in PR between 50 and 150 d pp. Winkler (2005) found a similar effect of stage of lactation on the mechanical properties of claw horn, reporting a decrease in PR between 30 and 160 d pp, followed by a subsequent increase at 270 d pp.

Interestingly, in this study the mean percentage and total sole haemorrhaging was greater for heifers not offered yeast compared to those offered yeast, which was reflected by a greater sole horn PR in claw horn of heifers offered yeast. Conversely, WL haemorrhaging for all parameters measured showed no significant differences between heifers offered and not offered yeast. The PR measures the peak force required to puncture the material which is a function of tensile and shear strengths. These are dependent upon both the constituent material properties and the micro-architecture. As such, changes in PR could indicate an alteration in either the constituent material or the structure of the material. In this case, the claw horn taken from yeast supplemented heifers had greater resistance to puncture than from heifers not offered yeast, suggesting the sole horn from supplemented heifers had enhanced structure and or constituent materials.

There as been no previous research reported to assess the effects of supplementing yeast (*Saccharomyces cerevisiae*) on lameness in dairy cattle. Therefore the possible aetiology is uncertain. However, yeast acts as a ruminal modifier/buffer, potentially altering the ratio of total VFA and decreasing the amount of lactic acid in the ruminal (Marden and Bayourthe, 2005) and subsequently the severity of acidosis /laminitis may be reduced. Ruminal

acidosis has been recognised as a major risk factor in the development of laminitis (Nocek, 1997; Oetzel, 2000) and connection between dietary starch content and the occurrence of laminitis has been indicated (Manson and Leaver, 1988; Nocek, 1997). Newbold *et al.* (1996) and Marden and Bayourthe (2005) have suggested that live yeast has the ability to scavenge oxygen in the rumen by consuming the traces of oxygen that enter the ruminal during the daily feed cycle in both the feed and saliva. Live yeast strengthens the anaerobic state of the ruminal milieu and stimulates the activity of cellulolytic bacteria to transform lactate into propionate, thus reducing the accumulation of lactate. Yeast supplementation may be able to prevent or reduce the severity of sub acute ruminal acidosis (SARA) by reducing or preventing the cycle which causes the release of endotoxins and vasoactive substances. This may indicate the reason why the heifers offered yeast had significantly lower levels of sole haemorrhaging.

There were no differences in locomotion score, claw angle, dorsal border, heel depth, diagonal claw length, and growth and wear rates between heifers offered and not offered yeast. The mean monthly growth and wear rates were similar to those previously reported by Leach *et al.* (1997) and Clark and Rakes (1982) and while the wear rates were not different between heifers offered and not offered yeast, the wear rate for heifers not offered yeast were higher up to 100 d pp (10.8 mm/month) compared with heifers offered yeast (4.4 mm/ month). This was reflected by a greater mean sole PR, indicative of improved claw horn quality which could be responsible for the lower wear rates. Harder claw horn is more likely to wear at a lower rate, which can be seen as an improvement in claw horn health as wear rates often can exceed growth rates (Leach *et al.*, 1997). Several factors can potentially affect growth and wear rates of claw horn: environment, stage of lactation (Leach *et al.*, 1997), genetics, nutrition (Clark and Rakes, 1982; Manson and Leaver, 1988) and the conformation of the cow. However, the heifers in this study were balanced for PIN values, calving date, live weight and BCS, housed in the same building and offered the same diet with the exception

of yeast inclusion. This may suggest that the yeast could have affected keratinisation and the quality of claw horn produced and thus wear rate. This could be through an effect on ruminal acid levels or potentially through micronutrients. Westwood *et al.* (2003) stated that changes of horn colour from white to light yellow, and a softening of the horn texture, was most likely a result of perturbed keratin metabolism. The softer claw horn in the non supplement heifers in this study therefore, could be a result of perturbed keratinisation resulting in greater wear rates.

There were no differences between treatments for claw horn DM. There has been no previously published data on DM content of claw horn taken from yeast supplemented animals. However, DM results are similar to Winkler (2005) for claw horn. Hinterhofer *et al.* (2005) proposed that correlations between elastic modulus and the DM do exist, but are not strong.

The yeast supplemented heifers had an 8% greater milk yield, when compared to heifers not offered LY and had higher concentrations of protein, fat and urea in the milk. Dawson (2000) reviewed yeast supplementation effect on milk production stating responses to supplementation were variable but ranged from a 2% to 30% increase in milk production. Moallem *et al.* (2009) found that dairy cattle supplemented with LY had increased milk yield and fat corrected milk yield, had no differences in milk fat or protein levels, but did have greater total milk fat yield than un-supplemented heifers. While Wohlt *et al.* (1991) found yeast supplementation during early lactation improved DMI, milk yield, and the digestibility of CP and ADF. In this study, the DMI did not differ between heifers offered and not offered yeast which is in agreement with a meta-analysis (Sauvant *et al.*, 2004) which showed a trend for increased milk yield with no subsequent increase in DMI, but is different from a more recent meta–analysis (Desnoyers *et al.*, 2009) showed that yeast supplementation increased DMI as well as milk yield, and tended to increase milk fat content.

The FCE for the heifers supplemented with live yeast was greater than the non supplemented heifers which support the findings of Schingoethe *et al.* (2004) where yeast supplementation increased FCE defined as kilogram of ECM/kilogram of DM intake was improved by 7% for cows fed the yeast culture. However the FCE of both treatments are below that of the normal range for dairy efficiency of 1.46 to 1.7kg ECM / kg DM (Beever and Doyle, 2007). However Quinn (2004) reported that where FCE had been determined by on farm monitoring, there had been many instances where low values had been observed (~1.1 kg ECM/kg DM). Hutjens (2001) also stated low dairy efficiency is associated with a high proportion of the herd being in first-lactation, i.e., in the growth phase. In this study, as all the experimental animals are heifers in first lactation this could also be a reason why the FCE is lower than expected. There were no differences between the NLY and LY heifers for weight and BCS which was in agreement with Erasmus *et al.* (2005).

2.5 CONCLUSIONS

In this experiment the addition of yeast reduced the incidence of mean sole haemorrhages, total haemorrhage score and puncture resistance of sole horn. This showed the potential of live yeast supplementation to reduce the severity of sole haemorrhaging and improve claw horn quality. Adding 10 billion/h/d of live yeast to a MR resulted in increased daily total milk, milk fat and protein yield without increases in feed intake, thus providing an increase in feed conversion efficiency.

2.6 FURTHER RESEARCH

Live yeast clearly offers, in some diet situations, the potential to reduce the severity of sole haemorrhaging and improve claw horn quality and warrants further research into both the mechanism and effect of live yeast in a range of dairy cattle diets where improvements in animal productivity, feed use efficiency and animal health may be gained.

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2.8 REFERENCES

Aranwela, N., G. Sanson, and J. Read. 1999. Methods of assessing leaf-fracture properties. New Phytologist. 144:369-393.

Beever, D. E. and P. T. Doyle. 2007. Feed conversion efficiency as a key determinant of dairy herd performance: a review. Australian Journal of Experimental Agriculture. 47:645-657.

Boelling, D. and G. E. Pollott. 1998. Locomotion, lameness, hoof and leg traits in cattle. I. Phenotypic influences and relationships. Livestock Production Science. 54:193-203.

British Standards. 1990. British Standard on the design and construction of cattle buildings. TSO Publications, London.

Bruijnis, M.R.N., H. Hogeveen, E.N. Stassen. 2010. Assessing economic consequences of foot disorders in dairy cattle using a dynamic stochastic simulation model. Journal of Dairy Science.93: 2419-2432.

Clark, A. K. and A. H. Rakes. 1982. Effect of methionine hydroxy analog supplementation on dairy cattle hoof and composition. Journal of Dairy Science. 65:1493-1502.

Clarkson, M. J., D. Y. Downham, W. B. Faull, R. D. Murry, J. Sutherst, W. R. Ward, and J. E. Wheeler. 1996. An epidemiological study to determine the risk factors of lameness in dairy cows. MAFF, London.

Dawson, K. A. 2000. Some milestones in our understanding of yeast culture supplementation in ruminants and their implications in animal production systems. Pages 473-486 in Proceedings of Alltech's 16th Annual Symposium on Biotechnology in the Feed Industry Nottingham University Press.

DEFRA. 2003. Code of Recommendations for the Welfare of Livestock: Cattle. Pages 17-19. Defra Publications, London.

Desnoyers, M., S. Giger-Reverdin, G. Bertin, C. Duvaux-Ponter, and D. Sauvant. 2009. Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. Journal of Dairy Science. 92:1620-1632.

Douglas, J. E., C. Mittal, J. J. Thomason, and J. C. Jofriet. 1996. The modulus of elasticity of equine hoof wall: implications for the mechanical function of the hoof. The Journal of Experimental Biology. 199:1829-1836.

Erasmus, L. J., P. H. Robinson, A. Ahmadi, R. Hinders, and J. E. Garrett. 2005. Influence of prepartum and postpartum supplementation of a yeast culture and monensin, or both, on ruminal fermentation and performance of multiparous dairy cows. Animal Feed Science and Technology. 122: 219-239.

Günther, K. D. 1989. Yeast culture's success under German dairy conditions. Page 38 in Biotechnology in the Feed Industry. Vol. 5. Alltech Technical Publications, Nicholasville.

Hinterhofer, C. M., V. Apprich, J. C. Ferguson, and C. Stanek. 2005. Elastic properties of hoof horn on different positions in the bovine claw. Berliner und Munchener Tierarztliche Wochenschrift. 112:142-146.

Quinn, E. S., C. Purcell, M. Voss, and L. Downey. 2004. Quantifying the impact of TMR feeding on dairy and beef farms. Pages 16–19 in Dairylink. Vol. 1.

Leach, K. A., D. N. Logue, S. A. Kempson, J. E. Offer, H. E. Ternent, and J. M. Randall. 1997. Claw lesions in dairy cattle: Development of sole and WL haemorrhages during the first lactation. Veterinary Journal. 154:215-225.

Leach, K. A., D. N. Logue, J. M. Randall, and S. A. Kempson. 1998. Claw lesions in dairy cattle: Methods for assessment of sole and WL lesions. Veterinary Journal. 155:91-102.

MAFF. 1986. Analysis of Agricultural Materials RB429. ADAS, London.

Manson, F. J. and J. D. Leaver. 1988. The influence of concentrate amount on locomotion and clinical lameness in dairy cattle. Animal Production. 47:185-190.

Marden, J. P. and C. Bayourthe. 2005. Live Yeast- ruminal 02 scavenger and pH stabiliser. Feed Mix. 13:23-25.

Moallem, U., H. Lehrer, L. Livshitz, M. Zachut, and S. Yakoby. 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. Journal Dairy Science. 92:343-351.

Newbold, C. J., R. J. Wallace, and E. M. McIntosh. 1996. Mode of action of the yeast Saccharomyces cerevisiae as a feed additive for ruminants. British Journal Nutrition. 76:249-261.

Nocek, J. E. 1997. Bovine acidosis implications on laminitis. Journal Dairy Science. 80:1005–1028.

Oetzel, G. R. 2000. Clinical aspects of ruminal acidosis in dairy cattle. Pages 46-53 in 33rd Annual Convention of the American Association of Bovine Practitioners, Rapid City, USA.

Offer, J. E., D. N. McNulty, and D. N. Logue. 2000. Observations of lameness, hoof conformation and development of lesions in dairy cattle over four lactations. The Veterinary Record. 147:105-109.

Robinson, R. H. and L. J. Erasmus. 2009. Effects of analyzable diet components on responses of lactating dairy cows to Saccharomyces cerevisiae based yeast products: A systematic review of the literature. Animal Feed Science and Technology. 149:185-198.

Sauvant, D., Giger-Reverdin, S., and P., Schmidely. 2004. Rumen acidosis: Modeling ruminant response to yeast culture. Pages 221–229 in Alltech's 20th Annual Symposium Biotechnology in the Feed Industry-Reimagining the Feed Industry, Lexington, Kentucky. Nottingham University Press.

Schingoethe, D. J., K. N. Linke, K. F. Kalscheur, A. R. Hippen, D. R. Rennich, and I. Yoon. 2004. Feed Efficiency of Mid-Lactation Dairy Cows Fed Yeast Culture During Summer. Journal Dairy Science. 87:4178-4181.

Shearer, J. K., E. Belknap, S. Berry, C. Guard, K. Hoblet, E. Hovingh, G. Kirksey, A. Langill, and S. R. van Amstel. 2002. The standardisation of input codes for capture of lameness data in dairy records. Pages 346-349 in Proceedings of the Twelfth International Symposium on Lameness in Ruminants, Orlando, Florida.

Tyrrell, H. F. and J. T. Reid. 1965. Prediction of the energy value of cows milk. Journal of Dairy Science. 48:1215–1223.

Westwood, C. T., E. Bramley, and I. J. Lean. 2003. Review of the relationship between nutrition and lameness in pasture-fed dairy cattle. New Zealand. Veterinary Journal. 51:208-218.

Williams, P. E. V., C. A. G. Tait, G. M. Innes, and C. J. Newbold. 1991. Effects of the inclusion of yeast culture (Saccharomyces cerevisiae plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. Journal Animal Science. 69:3016-3026.

Winkler, B. 2005. Mechanical properties of hoof horn, sole haemorrhage and lameness in dairy cattle. PhD dissertation. Plymouth University, Plymouth.

Wohlt, J. E., A. D. Finkelstein, and C. C.H. 1991. Yeast culture to improve intake, nutrient digestibility, and performance by dairy cattle during early lactation. Journal Dairy Science. 74:1395–1400.

The work in Chapter 2 showed that the use of PR was a valid indicator of dietary factors that can affect the structural integrity and strength of bovine claw horn, as was claw horn lesion (CHL) scoring and as a consequence these methods were applied in New Zealand in Chapter three to assess and characterise the effect of breed and number of days postpartum on the strength and mechanical properties of claw horn collected from first lactation dairy heifers.

Chapter 3

The effect of breed (Jersey cross Friesian or NZ Holstein Friesian) on haemorrhage levels and mechanical properties of claw horn in growing dairy cattle and first lactation dairy heifers

3.0 ABSTRACT

The crossbreeding of dairy cattle has become more popular as a mode of increasing fitness, health and performance. However, while lameness is one of the main welfare issues faced by the dairy industry, little research has been completed to compare the effect of crossbreeding on claw characteristics, horn strength and integrity and locomotion (lameness) in dairy cattle. The objectives of this research was to determine strength and integrity of claw horn in first lactation of two year old Friesian (Fr) and Jersey cross Friesian heifers (JxFr). A total of 33 dairy calves from either Fr (15 female) or JxFr (18 female) were offered colostrum followed by whole milk at 4 l/h/d. Heifers were grazed as one group throughout and calved at 22 to 24 months of age. Calves had no visible lesions in claw horn at; 0, 14, 21, 42 or 84 days of age. Puncture resistance (PR) of sole horn was significantly affected by IFM zone of the claw and breed at 160 d pp in lactating heifers (JxFr: 0:10.4, 60: 9.2, 110: 9.4, 160:10.2, Fr: 0:10.4, 60:9.5, 110: 9.7, 160: 9.4 d pp (± 0, 0.16; 60, 0.17, 110, 0.27, 160, 0.19) (N mm^2). Breed had no significant effect the number, percentage or total score of sole or WL haemorrhages. Increasing haemorrhage score decreased PR ($P<0.001$) (0: 9.4, 8: 2.2 (Nmm^2) and pigmented (P) claw horn had a significantly higher PR compared to non pigmented (NP) claw horn (P: 9.4, NP: 9.1 ± 0.11 (Nmm^2) $P=0.038$). Claw horn dry matter content (DM) did not differ between the different breeds or pigmentation. Claw (IFM) zone significantly affected horn DM content (WL: 2: 81.1; 3: 78.3; Sole: 4: 84.3 ± 1.99 (%) ($P=0.017$). Breed did not significantly affect the number, percentage or total score of sole or WL haemorrhaging in lactating heifers. Breed wasn't found to have a significant effect on claw horn strength in lactating heifers with the exception of PR being significant higher in crossbred heifers at 160d pp when compared to NZ Friesian dairy cattle. PR significantly decreased during lactation along with lesion score, which demonstrated that claw horn of inferior integrity had been produced. Horn sample thickness significantly affected PR ($P <0.001$) and should be used as a covariate when analyzing PR.

Keywords: Lameness, horn haemorrhage, breed, puncture resistance

3.1 INTRODUCTION

Lameness in dairy cattle is one of the main welfare issues faced by the dairy industry. It has been stated that up to 60% of dairy cattle can become lame annually (Vermunt, 2004). The average incidence of lameness in NZ has risen from 14% (Dewes, 1978) to 20.7% (Tranter and Morris, 1991) in the North Island of New Zealand (NZ) and recent data implies that this level has not changed greatly in recent years (Chesterton *et al.*, 2008), which agrees with Gibbs *et al.* (2007) who stated the incidence of lameness in the South Island of New Zealand was <22%. The main causes of lameness found in NZ herds were white line (WL) disease, sole injury, axial wall lesions and foot rot (Chesterton *et al.*, 2008; Tranter and Morris, 1991).

In New Zealand (Chesterton *et al.*, 2008; Peterse, 1985) have all reported that Friesian breeds tend to be more susceptible to lameness than other breeds such as the Jersey. Vermunt and Greenough (1995) and Logue *et al.* (1994) stated the presence of pigmentation has been associated with greater resistance to lameness and with perceptions of better quality horn. Webster (1987) suggested that black claw horn was harder than white. However, research carried out using equine hooves showed that claw colour did not affect the material properties (Douglas *et al.*, 1996; Landeau *et al.*, 1983) while Runciman *et al.* (2004) found that peak extraction force and energy required for removal of claw nails from horn was variable with dark-colour compared with light-colour horn. The types of lameness associated with the differing breeds (Chesterton *et al.*, 2008) has been reported, with Friesians appearing to be much more susceptible to WL disease than Jerseys, whereas Jerseys were more likely to develop axial disease, foot rot and sole problems. Chesterton *et al.* (1989) also examined the environmental and behavioural factors associated with lameness and found a lower prevalence of lameness on farms with a high proportion of pigmented feet, such as Jersey and Jersey crossbreeds.

Overall, there have been numerous studies (Greenough and Vermunt, 1991: Leach *et al.*, 1997; Offer *et al.*, 2000) that have observed that claw horn lesions develop in dairy cattle postpartum and that these are associated with reduced claw horn strength, resistance to puncture (Winkler, 2005) and potential wear rates (Chapter 2). Chesterton *et al.* (1989) highlights the importance of the quality of walking surfaces and appropriate animal handling, particularly at milking times in minimising lameness.

The crossbreeding of Holstein and Friesian dairy cattle has been used extensively in New Zealand pasture based systems (Heins *et al.*, 2008) and has been assessed in other countries as an opportunity to increasing animal fitness, health and performance (Funk, 2006; Prendiville *et al.*, 2010). Despite the increased popularity of crossbreeding and the importance of lameness, no detailed controlled studies have been completed to measure claw horn lesions and structural strength of claw in crossbred dairy cattle. As a consequence the objectives of this research were to measure and compare the development of lameness, claw horn lesions and mechanical strength/properties of claw horn in pre-partum and post partum Holstein Friesian and Friesian cross Jersey dairy cattle.

3.2 MATERIALS AND METHODS

3.21 Animals and management

This research was completed at Massey University in New Zealand between 1st Aug 2006 and 25 th May 2009 according to local ethical procedures and protocols approved by the Massey University Animal Ethics Committee. At 48 hrs of age 33 dairy calves were selected at random from the Massey University dairy farms and allocated, according to birth date, stature and live weight, to one of two groups according to breed; NZ Friesian (Fr n=15) and 0.5 Jersey cross 0.5 Holstein-Friesian (JxFr n=18) according to sire. All calves were offered

colostrum (3 d) followed by being individually housed and offered whole milk at 4l/h/d, straw and starter diet from 4 days of age until weaning (Table 3.1). Individual animal feed intake levels were calculated, by daily measurements of feed offered and feed removed. The calves were weaned from milk and ad libitum access to starter at 9 weeks of age, after this they were maintained on pasture and the supplement limited to 2 kg/h/d up until 14 weeks of age. All the heifers were grazed in one group at pasture throughout the study and were all transferred to the dairy unit at 22 months of age.

Table 3.1. Nutrient composition of whole milk and cereal based diet fed to the calves

	Whole milk	Calf rearing supplement
Dry matter (%)	12.8	86.8
Fat (g/kg DM)	42.0	43.0
Protein (g/kg DM)	38.0	19.8
Ash (g/kg DM)	50.0	84.0
ME (MJ/kg)	22.3	12.5
NDF (g/kg DM)	-	183.0
ADF (g/kg DM)	-	102.0
Lignin (g/kg DM)	0	29.0
Ca (mg/g)	1.19	12.4
P (mg/g)	0.93	5.6

* MJ/kg DM

3.22 Lesion score and claw horn structural strength and in growing dairy cattle

There were a total of 33 dairy heifer calves of either Friesian (n=15) or Jersey cross Friesian (n=18) breed that had all hind claws assessed and the WL and sole area lesion scored according to Leach *et al.* (1998) at 30, 60 and 90 days of age, followed by at 6, 12 and 22 months of age.

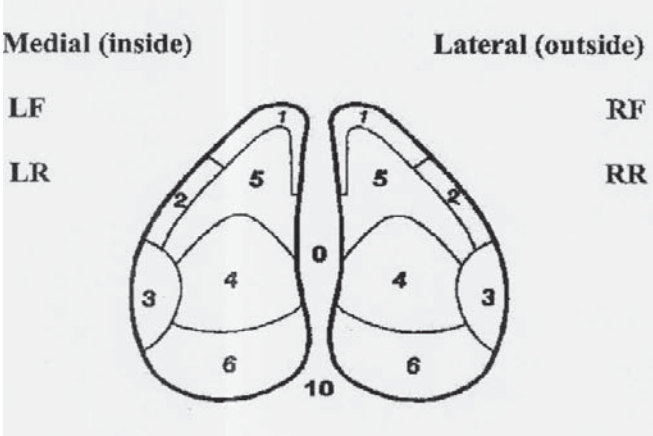

Figure 3.1 The International Foot Map (IFM) numerical representation of claw zones amended from Shearer *et al.* (2002)

3.23 Lesion score and claw horn structural strength in lactating dairy cattle

The claw and claw horn lesion score, claw horn structural strength and lameness were assessed using Fr (n =12) and JxFr (n =13) dairy cattle during first lactation at Massey University No. 4 dairy unit. The animals were assessed weekly for locomotion score, using the 5 point scoring system in which 0 to 1 corresponded with non lame animals, 3 with an animal showing tenderness when walking, 4 with an animal that is lame and 5 was an animal that is severely lame and non weight bearing on one or more limb/s (Manson and Leaver, 1988). All lame animals were examined to determine the cause of lameness and the development of each lameness case was monitored. The hind claws of all lactating heifers were assessed for sole haemorrhage and heel erosion at 0, 60, and 110 d pp. The severity of haemorrhage on each claw were scored according to (Leach *et al.*, 1998) with zero corresponding with horn without any haemorrhaging and 5 with horn showing severe haemorrhaging, while sole ulcers were graded from 6 to 8 depending on the exposure of the corium and the presence of infections. The affected areas of the sole and WL areas of each claw were marked and subsequently photographed with a digital camera and the images were analyzed to measure the size of claw area affected. WL and sole

haemorrhages were scored separately using image analysis software to calculate the number, percent and total lesion score (percent of claw affected x intensity of haemorrhage calculated from the haemorrhage score). Any other alterations present during examination were recorded.

3.24 Claw horn growth and wear rates

The claw horn growth and wear rates were measured on the right rear inner claw at 0, 60, and 110 d pp. A mark was made on the claw wall 2 cm below the coronary border. The distance from the coronary border to the mark and from the mark to the distal end of the wall was measured and a new mark was made 2 cm below the coronary border every time the hooves were measured. The monthly growth and wear rates were estimated from these measurements as described by Clark and Rakes (1982). The claw angle, length of the front claw wall and heel height were measured at the same time of the claw growth and wear, using the method according to Boelling and Pollott (1998).

3.25 Collection of claw horn samples from lactating dairy heifers

Claw sole horn tissue samples were collected from all the hind claws of all experimental heifers at 0, 60, and 110 d pp. The first outer layer of horn (1 mm) was discarded and a sample of 0.1 to 2.5 mm thickness taken using claw trimming knives. Horn samples were taken parallel to the ground surface from the right lateral sole surface of the claw from zone 4 at 0 and 60 d pp and zones 2, 3 and 4 at 110 d pp, according to the international foot map (IFM) (Shearer *et al.*, 2002) presented in Figure 3.1. Claw samples were collected, transferred immediately to plastic re-sealable bags and stored in a refrigerator at a temperature of 2 °C until analysis (Winkler and Margerison (2007).

3.26 Puncture resistance of claw horn

Claw horn samples were analyzed for puncture resistance (PR) as described by Winkler and Margerison (2007) using a P/2N needle probe on a TA.XT plus Texture Analyzer with a 5 kg load cell (Stable Micro-Systems, Vienna Court,

Lammas Road, Godalming, Surrey, GU7 1YL, UK). The test probe was used at a speed of 1.0 mm/sec, which enabled the material to adapt to the load (Aranwela *et al.*, 1999) and measured the force compression in test-mode. A force-displacement curve was recorded when the test probe reached the sample and a trigger force of 5 g had been applied, followed by which the probe travelled a distance of 12.0 mm before returning to the initial position. This distance was sufficient to allow the sample to be punctured and the maximum force on puncture to be measured. The maximum puncture force values (N) were measured from the peak of the force-displacement curve.

A total of 5 tests were completed on each category of the sole and WL horn tissue samples in accordance with Winkler and Margerison (2007), which were considered to be sufficient to detect test variations. The thickness of the sample on the tested area was recorded using callipers with a resolution of 0.01 mm. Each sample was scored for level of haemorrhage using a scale of 0 to 5 (0= no haemorrhage and 5 = severe haemorrhage). The PR of the areas was scored 1 to 5 for haemorrhage in the same region and claw and was compared to measure the effect of haemorrhage level on PR. The mean data from each animal was used to compare the effect of region and individual claws of each animal. The dry matter (DM) content of horn samples was determined using oven DM determination (100 °C for 72 hrs) according to (MAFF, 1986).

3.27 Statistical Analysis

The data collected from the dairy heifers was used as individual animal observations. All the data was assessed for normal distribution, using the norm plot procedures in Minitab 15 (Minitab Inc., State College, PA). The data that was found to be normally distributed was assessed using analysis of variance (ANOVA), general linear modelling (GLM) command in Minitab 15.0, using a confidence interval of 95%, with animal breed (Jersey X or Friesian) and postpartum period (d) as fixed effects, individual animal as a random effect and haemorrhage scoring of the sole and WL and claw characteristics as variables in

the model. The PR was analysed using the Minitab GLM command, including horn tissue sample thickness as a covariate in the analysis of covariance (ANCOVA), and using a confidence interval of 95%, with animal breed, IFM zone, sole haemorrhage score level as fixed effects, individual animal as a random effect and PR as the variable in the model. All the normally distributed data was presented as means ± standard error of the means (sem). The existence of significant differences between means was assessed using Tukey's test in the ANOVA and ANCOVA Minitab command and significant differences were reported when $P<0.05$, with a tendency being considered when $P<0.10$ and individual P values were presented for each variable assessed in the Table of data. The effect of number of days postpartum and animal breed on the mean percentage and total lesion score of the WL and sole claw horn were analysed using multiple regression analysis using least squares methods in Minitab 15.0. The overall locomotion score, claw characteristics, horn wear and growth rates sole and number of WL haemorrhages remained not normally distributed, following transformation, and these were analysed using the Kruskal- Wallis non-parametric analysis using breed as a fixed effect and this data was presented as a median with standard deviation as the error term.

3.3 RESULTS

There were no claw lesions found in the claws of growing cattle, at any of the observation points.

The sole and WL haemorrhaging severity and incidence increased up to 110 d pp and declined by 160 d pp (Table 3.2). There were no significant effects of breed on levels of sole or WL haemorrhaging, with the exception of sole haemorrhaging percentage, which was higher in Fr compared with JxFr cattle at 60 (P=0.049) and 110 (P=0.032) d pp (Table 3.2).

The regression equations from the multiple regression analysis of the effect of postpartum period (d pp) and animal breed, including adjusted (adj) R^2 and P values, for sole and WL claw measurements were:

Percentage sole haemorrhage of claw horn = - 12.5 + 0.406 d pp + 18.9 breed, R^2 adj= 0.32 (P<0.0001).
Sole total lesion score of claw horn = 9.0 + 0.755 d pp + 52.2 breed R^2 adj = 0.56 (P=0.023).

Percentage WL haemorrhage of claw horn = 97.0 + 0.244 d pp - 10.9 breed R^2 adj = 0.001 (P=0.358).
WL total lesion score of claw horn = 154 + 0.354 d pp- 25.4 breed R^2 adj = 0.003 (P=0.318).

It was found that postpartum period (time) had a significant effect on total lesion score for sole and WL haemorrhages (Table 3.3 and Figure 3.4), which was supported by the regression analysis. This demonstrates that the number of days postpartum has a significant effect on number and severity of haemorrhages of both the sole and WL regardless of breed. The highest number of lesions (2.6) (SEM 0.13, P<0.001) were found in zone 4, followed by zone 3 (1.2), zone 2 (1.5) and the lowest in zone 1 and 5 (0.7).

Table 3.2 Mean sole and white line (WL) haemorrhages (%) of claw, total lesion score and claw horn puncture resistance (PR) between 0 and 160 d pp for Jersey cross Friesian (JxFr) and Friesian (FR) heifers

		JxFr	Fr	SEM	P
Sole haemorrhage (%)	0 d pp	5.4	6.8	4.32	0.814
	60 d pp	34.1 [b]	59.7 [a]	8.85	0.049
	110 d pp	49.0 [b]	85.3 [a]	11.25	0.032
	160 d pp	61.0	78.9	14.21	0.380
Sole total lesion score	0 d pp	13.3	9.7	8.92	0.821
	60 d pp	174.2	264.9	69.68	0.269
	110 d pp	174.8	304.3	100.32	0.883
	160 d pp	133.8	123.8	33.36	0.914
Sole (zone 4) PR (Nmm^2)	0 d pp	10.4	10.4	0.16	0.923
	60 d pp	9.2	9.5	0.17	0.087
	110 d pp	9.4	9.7	0.27	0.386
	160 d pp	10.2	9.4	0.19	0.025
WL haemorrhage (%)	0 d pp	27.7	52.6	16.05	0.275
	60 d pp	121.6	114.8	25.26	0.846
	110 d pp	220.9	142.9	39.50	0.168
	160 d pp	61.7	61.6	20.93	0.998
WL total lesion score	0 d pp	38.7	75.0	20.82	0.217
	60 d pp	205.7	214.4	68.08	0.587
	110 d pp	256.3	159.4	48.77	0.131
	160 d pp	129.7	87.5	39.7	0.398
WL (zone 2) PR (Nmm^2)	0 d pp	6.8	6.5	0.34	0.484
	60 d pp	6.4	6.3	0.29	0.401
	110 d pp	6.5	6.2	0.26	0.384
	160 d pp	6.4	6.3	0.28	0.400

The breed of the animal had no significant effect on the number of sole or WL haemorrhages (JxFr: S: 0.3, WL: 1.5 (SEM 0.110); Fr: S: 4, WL: 2 (SEM 0.36). There was no significant effect of cattle breed on PR, with the exception of at 160 d pp, when JxFr cattle had significantly higher PR compared to NZ Friesian (Table 3.2). The thickness of claw horn samples had a significant effect on PR as increasing thickness would result in increased PR (mean thickness of 0.74 mm (St Dev 0.299, P <0.001)). As a result sample thickness was used as a covariate when puncture resistance data was analyzed using ACNOVA. The PR of the WL (zone 2) was weaker than that of the sole (zone 4) (PR Nmm^2, zone 2 JxFr= 6.5, Fr= 6.2, zone 4 JxFr= 9.7, Fr= 9.4, SEM 0.27, P<0.001) and was higher in pigmented claw horn than non pigmented claw horn (P=0.002), while breed of the animal had a significant interaction with pigmentation of claw horn (P=0.024) (Figure 3.3). The pigmented claw horn from Fr had the highest PR, significantly higher than pigmented horn from JxFr and non pigmented horn from both Fr and JxFr. Figure 3.3 also demonstrates that PR is significantly affected by zone of IFM P <0.001 affected however pigmentation does not significantly interact with zone of IFM (P 0.326). Overall the mean PR of pigmented claw horn was significantly higher than that of non pigmented horn (PR Nmm^2, P= 9.4; NP= 9.1, SEM 0.11; P 0.038).

There was a significant decrease (P<0.001) in PR of claw horn as haemorrhage score increased (Figure 3.2), with the exception of a haemorrhage score of 4. The PR decreased from 9.4 Nmm^2 with a score of 0 to 2.2 Nmm^2 at a haemorrhage score of 8 (adjusted geometric severity score as described by Leach *et al.* (1998)). There was no significant difference in the PR (Nmm^2) of claw horn samples taken from lateral and medial claws of hind claws (PR (Nmm^2) of, left lateral, JxFr =9.1, Fr = 9.6, left medial, JxFr = 9.6, Fr = 8.9, right medial, JxFr =8.8, Fr =9.6, and right lateral, JxFr = 9.0, Fr = 8.8; SEM 0.18.

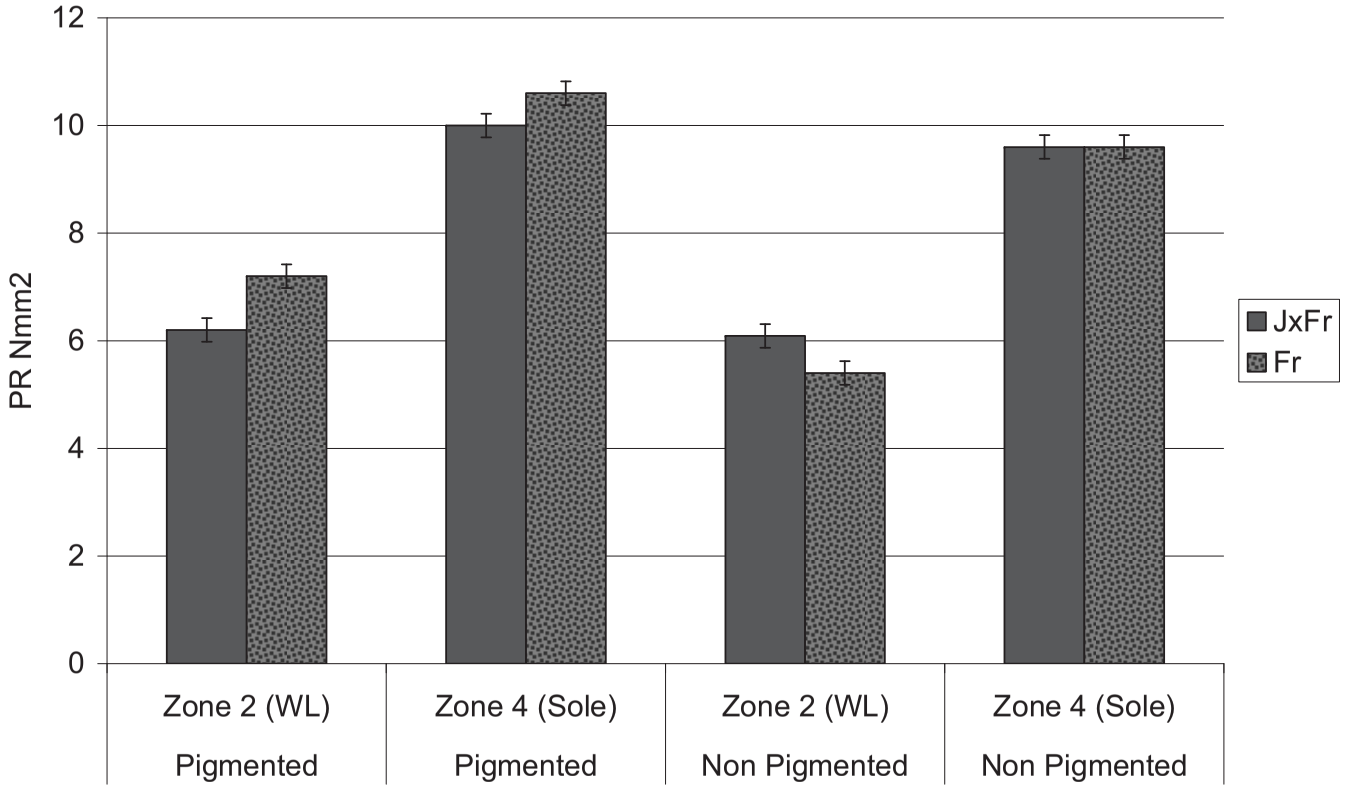

Figure 3.2 Mean PR of pigmented and non-pigmented claw horn, according to zone 2 or 4 of the international foot map from either Jersey x Friesian or Friesian breed dairy in first lactation

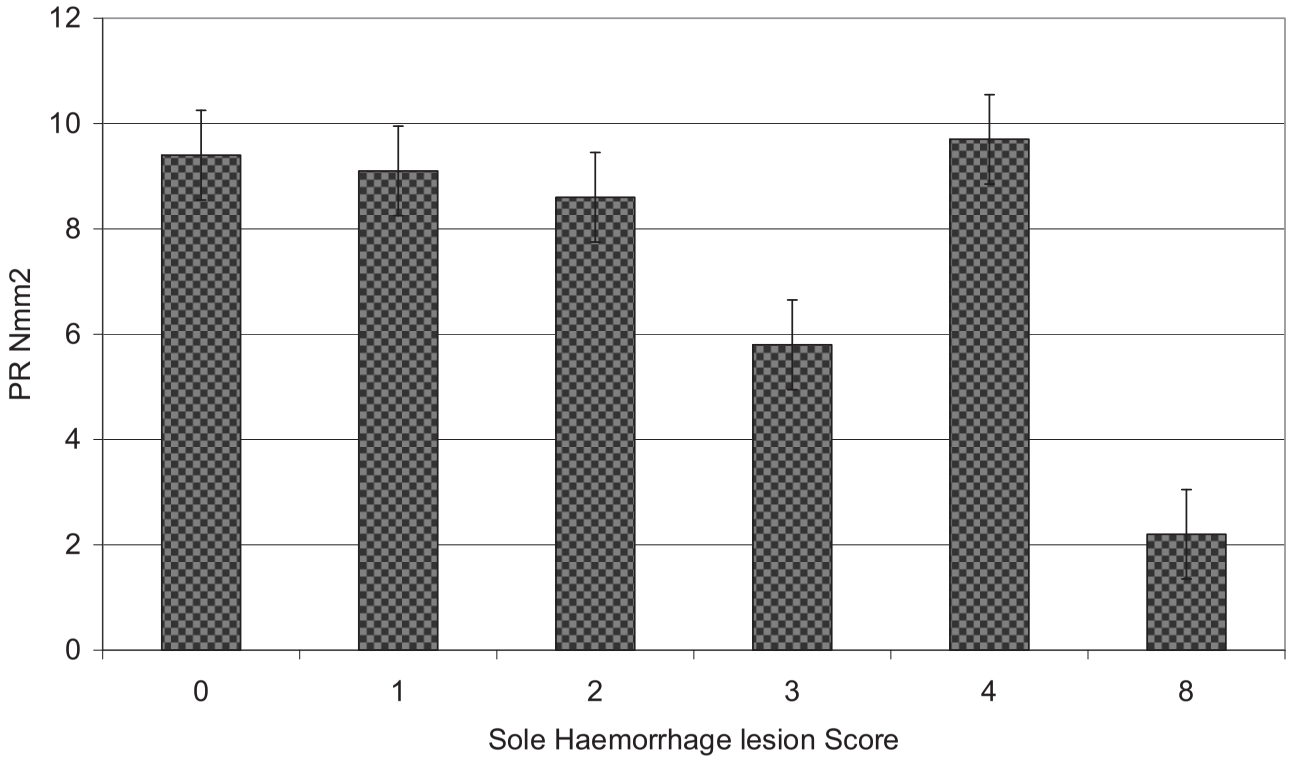

Figure 3.3 Mean puncture resistance of claw horn at differing levels of haemorrhage score taken from first lactation dairy cattle

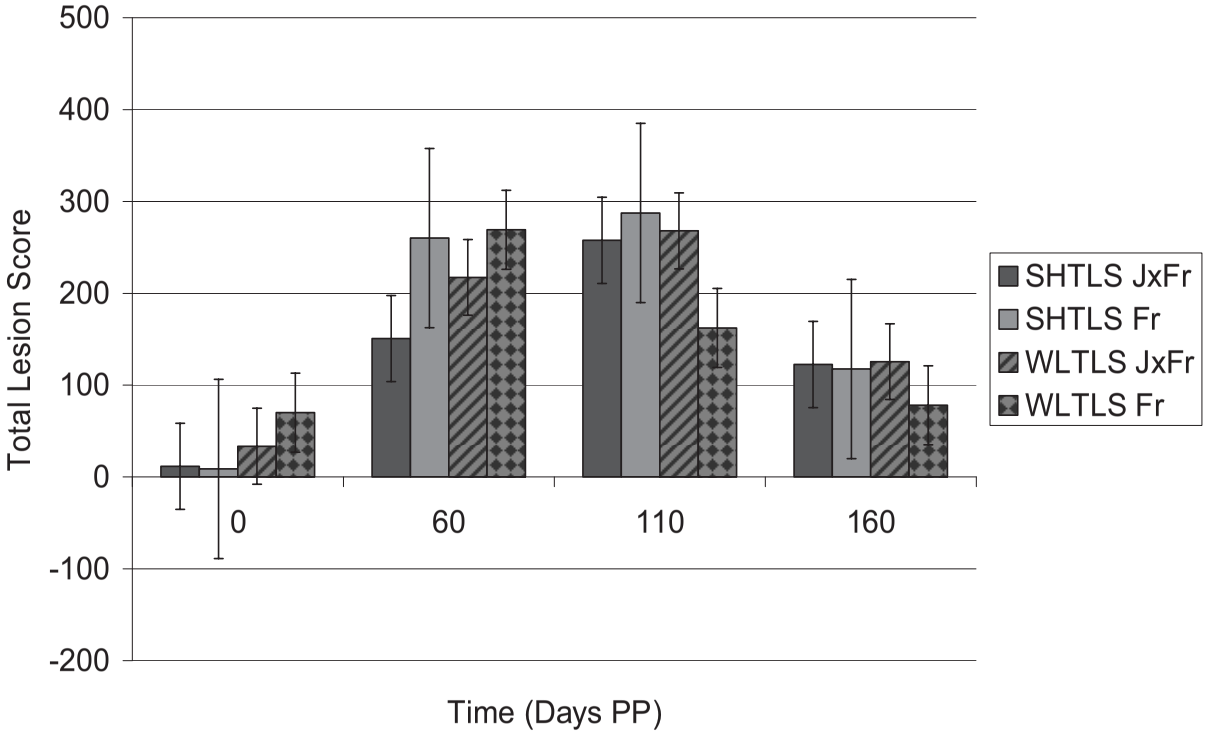

Figure 3.4 Mean total lesion score (TLS) of sole (S) and WL (WL) taken from Jersey x Friesian and Friesian dairy cattle between 0 and 160 days postpartum (PP) of the first lactation

Table 3.3 Mean total lesion score of sole and WL haemorrhages of the hind claws of first lactation dairy cattle between 0 and 160 days postpartum (d pp)

	0 d pp	60 d pp	110 d pp	160 d pp	SEM	P value
Sole total lesion score						
Jersey x Friesian	13.3 [b]	174.2[a]	174.8 [a]	133.8 [a]	46.9	<0.001
Friesian	9.7 [b]	264.9 [a]	304.3 [a]	123.8 [a]	97.6	<0.001
Overall breeds mean	11.5 [c]	219.6 [a]	239.6 [a]	128.8 [b]	33.85	<0.001
WL total lesion score						
Jersey x Friesian	38.7 [c]	205.7 [ab]	256.3 [a]	129.7 [b]	41.32	0.001
Friesian	75.0 [b]	214.4 [a]	159.4 [ab]	87.5 [b]	43.01	0.001
Overall breeds mean	56.8 [b]	210.1 [a]	207.9 [a]	108.6 [b]	29.8	0.001

[a,b,c,d] – Means in rows followed by differing superscripts differ significantly P<0.05

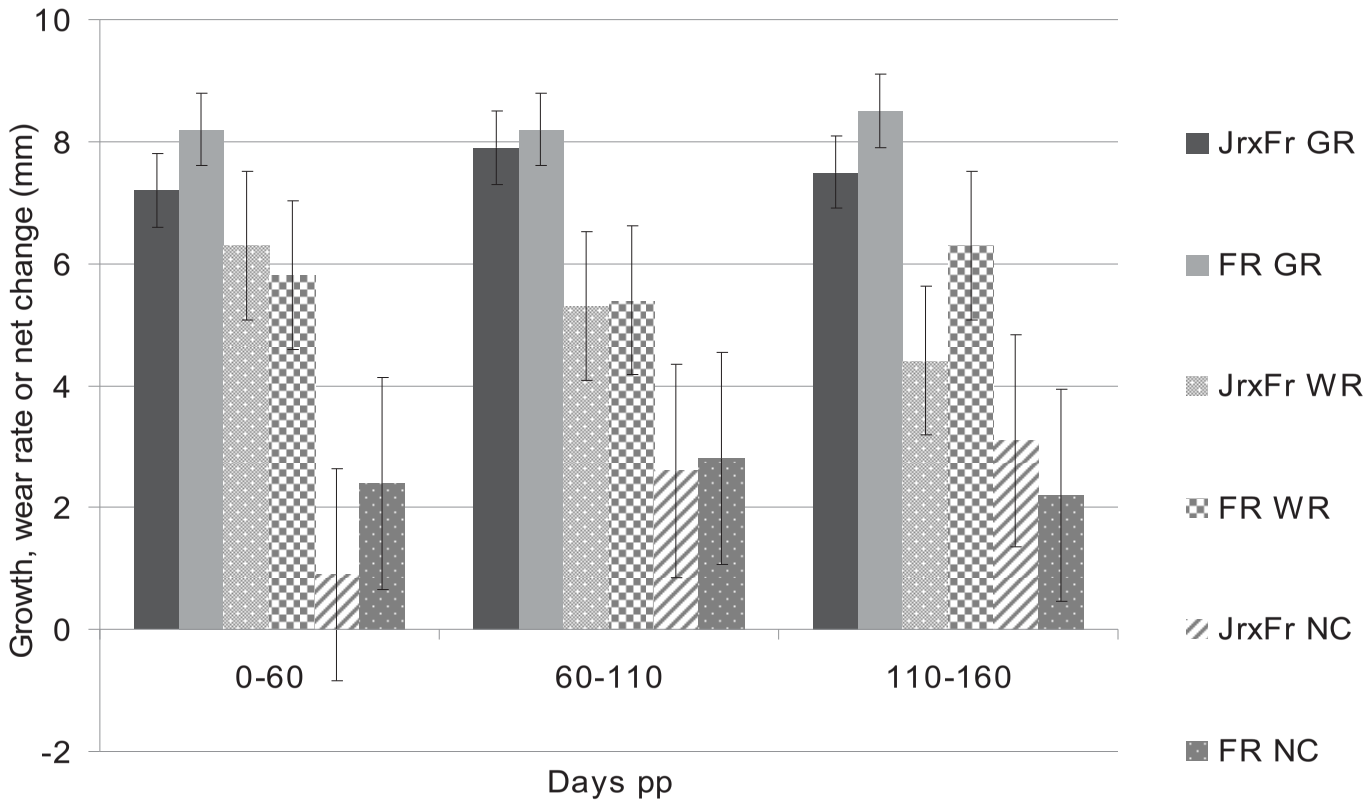

Figure 3.5 Mean growth (GR), wear rates (WR) and net change (NC) of claw wall horn, measured in first lactation Jersey x Friesian (JrxFr) and Friesian (Fr) dairy cattle between 0 and 160 d pp

There were no effects of claw horn DM between; JxFr and Fr (percentage DM, JxFr = 80.4, Fr = 82.9 (SEM 2.12)) or pigmentation (percentage DM, P = 79.6, NP = 83.7 (SEM 2.45)), differing claws (percentage DM, left, lateral= 81.8, medial = 81.4, right, medial = 80.5, lateral= 81.3 (SEM 1.99)) with the exception of IFM zone (IFM 2 =81.1, 3= 78.3, 4 = 84.3 (SEM 1.99). P=0.017).

Table 3.4 Mean claw conformation of first lactation Jersey x Friesian and Friesian dairy cattle between 0 and 160 days postpartum

	Days postpartum					
	0	60	110	160	SEM	P
Claw angle (°)						
Jersey x Friesian	43.9^{b}	43.9^{b}	43.8^{b}	45.1^{a}	0.46	0.003
Friesian	43.6^{c}	44.2^{c}	44.7^{b}	45.6^{a}	0.45	0.004
Dorsal border (mm)						
Jersey x Friesian	69.9^{b}	69.6^{b}	70.8^{ab}	72.1^{a}	0.61	0.018
Friesian	72.1^{b}	72.7^{a}	73.0^{a}	73.4^{a}	0.62	0.019
Abaxial groove height (mm)						
Jersey x Friesian	43.0^{b}	42.7^{b}	43.2^{b}	45.8^{a}	0.46	<0.001
Friesian	43.0^{b}	43.8^{b}	43.8^{b}	45.9^{a}	0.47	<0.001

^{a,b,c,d} – Means in rows followed by differing superscripts differ significantly P<0.05

There were no differences between JxFr and Fr cattle in claw angle or median locomotion score (Table 3.4 and 3.5). However, the dorsal border was significantly shorter in claws of first lactation JxFr cattle than Fr animals (SEM 0.03 P= <0.001) and the height of the abaxial groove was lower (SEM 0.022, P= 0.035) in JxFr cattle when compared to Fr animals. The monthly growth rates were significantly higher in Fr when compare to JxFr, but there were no

significant differences between breed in terms of monthly wear rate (WR) or net change (NC) in claw horn levels (Table and Figure 3.5).

Table 3.5 Median locomotion score, foot angle, dorsal border, heel depth, diagonal claw length measurements, monthly growth and wear rates for first lactation Friesian cross Jersey (JXFr) and Friesian dairy cattle

	JXFr	Fr	StDev	P
Locomotion score (1 to 5)	1	1	-	-
Claw angle (mm)	44.5	44.8	0.44	0.325
Dorsal border (mm)	7.0	7.3	0.07	<0.001
Abaxial groove height (mm)	4.36	4.43	0.048	0.035
Monthly growth rate (mm)	7.4	8.3	0.65	0.041
Monthly wear rate (mm)	5.2	5.9	1.42	0.445
Net change (mm)	2.1	1.8	1.51	0.842

3.4 DISCUSSION

In the present study there were no lesions found in the calves which corresponds to Offer *et al.* (2003) findings, where lesions were not found in growing cattle until 5 months of age and only at low levels at that age. Vermunt and Greenough (1995) did fine that lesions occurred at 6 to 7 months of age in calves reared outdoors, but this was not the case in this study. In lactating heifers PR of sole horn differed significantly at 160 d pp where JxFr cattle had higher PR than Friesian cattle. However, for both breeds PR reduced with time up until 160 d pp. This reflected the increasing in sole haemorrhaging and may be an indication that JxFr cattle are able to recover claw horn quality through improved keratinisation more rapidly than pure bred Friesian cattle. As keratinisation has never been compared between breeds, this may be a result of improved nutrient and blood flow to the keratinocytes which enables a higher quality of keratin to be produced. Decreasing nutrient supply to keratinizing epidermal cells leads to horn production of inferior quality and increased susceptibility to chemical, physical, or

microbial damage from the environment (Tomlinson *et al.*, 2004). These results may indicate some of the differences between JxFr and Friesian cattle, and contribute in part to the protective function of the claw horn and substantiate the reports of Chesterton *et al.* (2008) and Logue *et al.* (1994) where Friesian animals had higher levels of lesions and lameness, which is an indication of weaker claw horn (Winkler and Margerison, 2007).

In lactating heifers pigmentation (darkness) significantly increased the PR of the claw horn. These results are in contrast to the findings of Hepburn *et al.* (2007) which found no difference for impression hardness of coronary wall, sole and heel claw horn of pigmented or non pigmented claw horn. The PR of pigmented claw horn taken from Friesians was higher than that of JxFr cattle and non pigmented claw horn. However, Friesian dairy cattle that have been selected according to the Holstein and Friesian pedigree breed society guidelines have been selected over many generations to exhibit lower limbs with no colour pigmentation and white hair, thus these animals also have predominately non pigmented claw horn, while Jersey cross bred Friesian cattle tend to exhibit predominantly black lower limbs and hair colour, with dark pigmented claw horn. In this study while dark pigmentation on the claw horn did tend to predominate in crossbred cattle this did not coincide with increased claw horn PR, which did not support anecdotal references that suggest that JxFr cattle have harder claw horn. Interestingly, the DM was lower in pigmented claw horn and it would be expected that this would result in a lower PR as the claw horn would be softer. However, these findings are an indication of different properties of claw horn, i.e., hardness is a reflection of a material's resistance to plastic deformation and PR is a compound product of tensile and shear strengths which are dependent upon both the constituent materials' properties and the micro architecture. As a consequence, not a single parameter can describe claw horn quality as a whole and shows the need to use varying methods and mechanical tests to assess claw horn, quality and structural strength (Hinterhofer *et al.*, 2005a).

The median DM of claw horn samples taken from the heifers did not differ between breed, pigmentation or claw, however, the zone of IFM affected DM content. These results concur with the findings of Hepburn *et al*. (2007) which reported that there was no significant effect of pigmentation on DM. Zone 3 which had significantly lower dry matter content than zones 2 and 4. This may be a result of the WL in zone 3 having larger tubules (Budras *et al.*, 1996) which could result in an increased quantity of water being up taken from the environment. However, determining DM from small sample sizes and with small samples can be problematic (Hinterhofer *et al.*, 2005b), and different drying methods can cause differing rates of water and volatile loss from claw horn samples. This can make the determination of the moisture content of claw horn very variable, making comparison between results difficult. There is no agreed standard method for DM calculation (Reilly *et al.*, 2002). Therefore the large variation in DM between zones of IFM may just be due to differences in small sample sizes. DM content of claw can play an important role in claw horns structural strength and integrity. The investigation of differing methods, temperatures and drying times would be worth investigation.

The PR force required to puncture the WL horn was lower than the force required to puncture the sole horn, agreeing with the findings of Mulling *et al.* (1994) and Winkler and Margerison (2007). The data in this thesis demonstrates that the WL is weaker than the sole horn. However, while the effect of animal management on sole and WL disorders and the aetiology of sole and WL haemorrhage development has been widely researched, there are very few authors that have stated which specific region of the WL or sole from which samples, haemorrhage or lameness data corresponded to. The mean PR was 5.2Nmm^2 for region 2 and 8.5N Nmm^2 for region 5. These results are comparable with Winkler (2005) of 40 d pre-partum for Holstein Friesian dairy heifers of PR of 6.9 Nmm^2 for region 2 and 8.7 Nmm^2 for region 5. Increasing haemorrhage levels of the sole reduces the PR to levels similar to that of WL, which has consistently lower PR demonstrating its structural weakness. WL is therefore less affected by

increasing haemorrhaging levels, indicating that haemorrhage scoring of WL is a less useful measurement of the functionality, tissue damage and potential risk to lameness from separation and penetration, compared with sole bruising. WL strength, penetration and separation or striation warrant further research and any future research into lameness should consider WL and sole separately. The use of the IFM should be incorporated into research, practice and the communication of levels of differing types of lameness so clarification between researchers' findings can be clearly made.

In this study and that of Winkler (2005), increasing levels of sole bruising decreased PR of claw horn, which indicated that sole claw horn integrity had been affected by the level of haemorrhaging. With the exception of Winkler (2005), no other authors have compared mechanical properties or the effect of lesion score on claw horn structural properties. Kempson and Logue (1993) states that claw horn quality can be reduced by blood elements which have seeped out from damaged capillaries leaking across the basement membrane separating the dermis from the epidermis. Leach *et al.* (1997) found that in autumn calving heifers the combined effects of calving, housing and the lactating diet result in insults to the corium which are manifested in the form of an increased lameness and in haemorrhages of the WL and sole. Histological studies carried out on samples of horn from the animals examined showed that sole haemorrhages were virtually always accompanied by histological and morphological changes in the laminar region where the horn is generated (Leach *et al.*, 1997). The effect of increasing levels of sole bruising and decreased PR of claw horn on the wear rate has yet to be elucidated. However, increasing wear rates and the reduction in sole thickness may result in a lower ability of the claw horn to protect the lamella from compaction in the hoof capsule. This may in turn be a contributory factor in increased haemorrhage levels, particularly in pasture based systems where dairy cattle walk longer distances.

The lameness experienced by the lactating heifers in the present study was a result of either sole or WL haemorrhaging. With the exception of percentage of sole haemorrhaging, levels of haemorrhaging (number and TLS) did not differ between breed. These findings correspond with those of Dewes (1978), Tranter and Morris (1991) and Chesterton *et al.* (2008) who found that WL disease and sole injury accounted for two out of the four main causes of lameness in NZ. Chesterton *et al.* (2008) found that Jersey cattle had higher levels of sole injury compared to Friesians whereas Friesians had higher levels of WL disease. However, this was not observed in the this study where there were no differences for haemorrhaging of the WL for either percentage or total score or total score for sole lesions. Friesians WL haemorrhaging peaked at 60 d pp compared to 110 d pp for JxFr cattle and then declined. Friesians also had significantly higher sole haemorrhaging percentage than the JxFr.

The number of days post partum significantly affected the number, percentage and TLS for both sole and WL as peak haemorrhaging occurred at 110 d pp and had declined by 160 d pp. This concurs with findings of Leach *et al.* (1997), Offer *et al.* (2000) and Winkler (2005) showing haemorrhages peak between 100 and 120 d pp and reduces in number and severity thereafter. Though not significant, zone 4 had the highest number of lesions compared with the other zones. Haemorrhaging in zone 4 is a sign of potential compression of the dermis by traumatic external compression. This can be caused by displacement of the pedal bone causing injury to vascular structures and blood being incorporated into the claw horn as keratinization occurs (Greenough, 2007). Zones 2 and 3 also had high levels of lesions, with low levels of lesions in zones 1 and 5. Greenough (2007) stated that the impact from each stride is greatest at heel/sole junction (zones 3 and 4) as a result the digital cushion expands sideways when compressed under weight causing pressure to be exerted on zone 3. Zone 3 is the broadest part of the WL with the highest rate of horn production; as a result it is particularly susceptible to alterations of vascular system and disruption to nutrition and is an indicator of subclinical laminitis. No other peer reviewed paper

has shown results in terms of number, or severity of lesions by IFM. It is difficult therefore to determine whether this is a normal pattern of results for dairy cattle or whether it is specific to NZ or just this farm.

Friesians had a significantly longer dorsal border when compared to JxFr cattle which has been seen in Friesian cattle of pasture based systems in the UK (Boelling and Pollott, 1998; Offer *et al.*, 2000). Abaxial groove height was significantly lower in JxFr cattle when compared to the Friesians. This was unexpected as dorsal length was significantly shorter for JxFr cattle when compared to Friesians. Previous research shows shallower heel depth corresponds with increased dorsal border length when cattle were at pasture (Smit *et al.*, 1986; Offer *et al.*, 2000) and on earthen-surfaced corrals (Vermont, 1990). This may be due to the cattle reversing out of the rotary shed. Increased wear to the heels of cattle occurs when twist/turning once out of the shed. This appears to affect crossbred cattle more than Friesians. There is no published data on claw horn conformation for New Zealand dairy cattle making it difficult to determine whether the results from this study are “normal” JxFr cattle in a pasture based in New Zealand and also to determine the effects of differing milking shed systems on claw horn conformation.

Friesian had greater wall horn growth rate than JxFr cattle. However, wear and net change of claw did not significantly differ between breeds. The growth and wear rates of the Fr and JxFr were similar to those published in data from the Northern hemisphere (Clark and Rakes, 1982; Livesey *et al.*, 1998; Offer *et al.*, 2000) However, wall horn growth rates were higher than those stated by Tranter and Morris (1992) with research carried out in the same region of NZ as the current study. However, growth and wear rates tend to vary with season and from year to year in the same location (Offer *et al.*, 2000; Winkler, 2005) as the quality of pasture management practice and climate are different between years.

3.5 CONCLUSIONS

No haemorrhaging was found in growing cattle at 14 weeks of age. Breed did not affect number or total score of sole or WL haemorrhaging in lactating dairy heifers. It was with one exception where the PR was higher in JxFr heifers at 160 d pp when compared to Friesian dairy cattle, there were no differences for PR of sole and WL between breeds. The horn PR decreased over time and with lesion score which intimates that claw horn of inferior structural strength had been produced. The thickness of the claw horn sample affected PR ($P<0.001$) and should always be used as a covariate when analyzing PR of claw horn.

3.6. FURTHER RESEARCH

The assessment of lesion score using the existing techniques developed for white claws was applied and lesions were detected in both JxFr and Friesian cattle. However, lower levels of sole lesions were found, while WL lesions were more apparent. Changes in lesion score were more apparent WL and sole. There may be difficulties in accurately applying this technique in crossbred dairy cattle as potentially these methods are less suitable for application to cross bred dairy cattle due to dark claw horn being most common. As this method was developed using Friesian cattle selected for white socks, where light claw horn predominates. This warrants further research, using morbid claw tissue as the effect of cattle breed and within breed variation could be assessed, for which PR shows great potential in determining the differences and dynamics in claw horn integrity and function of differing breeds of cattle. The identification of specific genes that increase claw horn integrity and resilience, which may lead to reduced claw horn penetration and claw infection.

3.7 ACKNOWLEDGEMENTS

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3.7 REFERENCES

Aranwela, N., G. Sanson, and J. Read. 1999. Methods of assessing leaf-fracture properties. New Phytologist. 144:369-393.

Boelling, D. and G. E. Pollott. 1998. Locomotion, lameness, hoof and leg traits in cattle. I. Phenotypic influences and relationships. Livestock Production Science. 54:193-203.

Budras, K. D., C. Műlling, and H. Horowitz. 1996. Rate of keratinization of the wall segment of the hoof and its relation to width and structure of the zona alba (white line) with respect to claw disease in cattle. American Journal of Veterinary Research. 57:444–455.

Chesterton, R. N., K. E. Lawrence, and R. A. Laven. 2008. A descriptive analysis of the foot lesions identified during veterinary treatment for lameness on dairy farms in north Taranaki. New Zealand Veterinary Journal. 56:130-138.

Chesterton, R. N., D. U. Pfeiffer, R. S. Morris, and C. M. Tanner. 1989. Environmental and behavioural factors affecting the prevalence of foot lameness in New Zealand dairy herds- a case control study. New Zealand Veterinary Journal. 37:135-142.

Clark, A. K. and A. H. Rakes. 1982. Effect of methionine hydroxy analog supplementation on dairy cattle hoof and composition. Journal of Dairy Science. 65:1493-1502.

Dewes, H. F. 1978. Some aspects of lameness in dairy Herds. New Zealand Veterinary Journal. 26:147-159.

Douglas, J. E., C. Mittal, J. J. Thomason, and J. C. Jofriet. 1996. The modulus of elasticity of equine hoof wall: implications for the mechanical function of the hoof. The Journal of Experimental Biology. 199:1829-1836.

Funk, D. A. 2006. Major advances in globalization and consolidation of the artificial insemination industry. Journal of Dairy Science. 89:1362-1368.

Gibbs, J., J. Laporte-Uribe, C. Trotter, and J. Noel. 2007. Rumen function and lameness in pasture based dairy cows of the South Island of New Zealand. Pages 903-903 in Joint Annual Meeting of the American-Dairy-

Science-Association/Poultry-Science-Association-Asociacion, San Antonio, USA.

Greenough, P. R. 2007. Bovine laminitis and lameness. Saunders Elesevier, London.

Greenough, P. R. and J. J. Vermunt. 1991. Evaluation of subclinical laminitis in a dairy-herd and observations on associated nutritional and management factors. Veterinary Record. 128:11–17.

Heins, B. J., L. B. Hansen, A. J. Seykora, D. G. Johnson, J. G. Linn, J. E. Romano and A. R. Hazel 2008. Crossbreds of Jersey × Holstein Compared with Pure Holsteins for Production, Fertility, and Body and Udder Measurements During First Lactation. Journal of Dairy Science. 91:1270–1278.

Hepburn, N. L., L. Kinninmonth, and H. Galbraith. 2007. Pigmentation, impression hardness and the presence of melanosomes in bovine claw tissue. Journal of Agricultural Science. 145:283–290.

Hinterhofer, C., M. Zoscher, C. Gabler, and C. Stanek. 2005a. The hardness of horn in different segments of the bovine claw. Berliner und Munchener Tierarztliche Wochenschrift. 118:334-340.

Hinterhofer, C. M., V. Apprich, J. C. Ferguson, and C. Stanek. 2005b. Elastic properties of hoof horn on different positions in the bovine claw. Berliner und Munchener Tierarztliche Wochenschrift. 112:142-146.

Kempson, S. A. and D. N. Logue. 1993. Ultrastructural observations of hoof horn from dairy cows: changes in the WL during the first lactation. The Veterinary Record 132:524-527.

Landeau, L. J., D. J. Barrett, and S. C. Batterman. 1983. Mechanical properties of equine hooves. American Journal of Veterinary Research. 44:100–102.

Leach, K. A., D. N. Logue, S. A. Kempson, J. E. Offer, H. E. Ternent, and J. M. Randall. 1997. Claw lesions in dairy cattle: Development of sole and WL haemorrhages during the first lactation. Veterinary Journal. 154:215-225.

Leach, K. A., D. N. Logue, J. M. Randall, and S. A. Kempson. 1998. Claw lesions in dairy cattle: Methods for assessment of sole and WL lesions. Veterinary Journal. 155:91-102.

Livesey, C. T., A. M. Johnson, C. Marsh, S. A. May, and J. A. Metcalf. 1998. Hoof horn growth in Holstein cattle from first calving until six weeks after second calving. Pages 51-52 in Tenth International Symposium on Lameness in Ruminants. Casino Lucerne, Switzerland.

Logue, D. N., J. E. Offer, and J. J. Hyslop. 1994. Relationship of diet, hoof type and locomotion score with lesions of the sole and white line in dairy-cattle. Animal Production. 59:173-181.

MAFF. 1986. Analysis of Agricultural Materials RB429. ADAS, London.

Manson, F. J. and J. D. Leaver. 1988. The influence of concentrate amount on locomotion and clinical lameness in dairy cattle. Animal Production. 47:185-190.

Műlling, C., H. Bragulla, K. D. Budras, and S. Reese. 1994. Structural Factors Influencing Horn Quality and Sites of Predilection at the Ground Surface of the Cattle Hoof. Schweizer Archiv Fur Tierheilkunde. 136:49-57.

Offer, J. E., K. A. Leach, S. Brocklehurst, and D. N. Logue. 2003. Effect of forage type on claw horn lesion development in dairy heifers. Veterinary Journal. 165:221-227.

Offer, J. E., D. N. McNulty, and D. N. Logue. 2000. Observations of lameness, hoof conformation and development of lesions in dairy cattle over four lactations. The Veterinary Record. 147:105-109.

Peterse, D. J. 1985. Breeding. Pages 121-123 in Cattle Foot care and Claw Trimming. Farming Press Ltd, Ipswich.

Prendiville, R., K. M. Pierce, F. Buckley 2010. A comparison between Holstein-Friesian and Jersey dairy cows and their F_1 cross with regard to milk yield, somatic cell score, mastitis, and milking characteristics under grazing conditions Journal of Dairy Science. 93:2741-2750.

Reilly, J. D., H. Newlyn, B. Cope, R. J. Latham, S. Collins, and L. Hopegood. 2002. A comparison of different moisture loss methods for measuring hoof

wall content. Page 193 in Proceedings of the Twelfth International Symposium on Lameness in Ruminants, Orlando, Florida.

Runciman, C. J., J. J. Thomason, G. Springett, S. Bullock, and W. Sears. 2004. Horseshoe fixation versus hoof colour, a comparative study. Biosystems Engineering. 89:377–382.

Shearer, J. K., E. Belknap, S. Berry, C. Guard, K. Hoblet, E. Hovingh, G. Kirksey, A. Langill, and S. R. van Amstel. 2002. The standardisation of input codes for capture of lameness data in dairy records Pages 346-349 in Proceedings of the Twelfth International Symposium on Lameness in Ruminants, Orlando, Florida.

Smit, H., B., Verbeek, D.J., Peterse, J., Jansen, B.T., McDaniel and R.D., Politiek. 1986b. The effect of herd characteristics on claw disorders and claw measurements in Friesians. Livestock Production Science 15: 1-9.

Tomlinson D.J., C.H. Mülling, and T.M. Fakler. 2004.Invited review: formation of keratins in the bovine claw: roles of hormones, minerals, and vitamins in functional claw integrity. Journal dairy science. 87:797-809.

Tranter, W. P. and R. S. Morris. 1991. A Case-Study of Lameness in three Dairy Herds. New Zealand Veterinary Journal. 39:88-96.

Tranter, W. P. and R. S. Morris. 1992. Hoof growth and wear in pasture-fed dairy cattle. New Zealand Veterinary Journal. 40:89-96.

Vermunt, J. J. 2004. Herd lameness - a review, major causal factors, and guidelines for prevention and control in Prophylaxis of Claw Diseases Proceedings of the 13th International Symposium and 5th Conference on Lameness in Ruminants, Maribor, Slovenija.

Vermunt, J. J. and P. R. Greenough. 1995. Lesions associated with subclinical laminitis of the claws of dairy calves in two management-systems. British Veterinary Journal. 151:391-399.

Webster, J. 1987. Understanding the dairy cow. BSP Professional Books, Oxford.

Winkler, B. 2005. Mechanical properties of hoof horn, sole haemorrhage and lameness in dairy cattle. PhD dissertation. Plymouth University, Plymouth.

Winkler, B. and J. K. Margerison. 2007. Effect of sample thickness, tissue moisture content and storage methods on the punch resistance and elastic modulus of the bovine hoof horn. Journal Animal Science. 85: Suppl. 1 (Abstract):419.

Zoscher, M., C. Hinterhofer, C. Stanek, and C. Gabler. 2000. New aspects of mechanical properties of bovine hoof horn. Pages 91-93 in Eleventh International Symposium on Lameness of the Ruminant Digit. Parma, Italy.

In the previous Chapters PR produced useable and repeatable data, however, material scientists favour elastic modulus (EM) as a method and while Winkler (2005) tried one method of EM, which used self tightening grips and required larger 'dog bone' shaped claw horn samples. Requiring larger sample size was the limiting factor, resulting in fewer suitable claw horn samples and insufficient sample size to produce sufficient data to make comparisons. As a consequence, Chapter four aims to adopt and develop a new method for EM.

Chapter 4

Assessment of the mechanical properties of claw horn using Tensile, Vickers hardness and puncture resistance tests

This chapter has been submitted to the Journal of Dairy Science for publication

4.0 ABSTRACT

Lameness is one of the greatest welfare issues of the dairy industry and the majority of cases are associated with claw horn disorders and penetration. Bovine claw horn (BCH) is known to develop haemorrhages following parturition, reducing claw horn quality, which affects mechanical and functional properties of BCH. This aim of this study was to compare tensile testing of elastic modulus (EM), Vickers hardness (VH) and puncture resistance (PR) as methods of assessing changes in the mechanical properties BCH. Two experiments were completed, 1) BCH samples were collected from 5 animals slaughtered at a local abattoir to assess the effect of ambient relative humidity (RH) and hydration on the EM of BCH. 2) BCH samples were collected from the sole (international foot map (IFM) 5) and white line (IFM 2) of 36 first lactation dairy heifers at 30 and 120 d pp and with differing levels of haemorrhage score (0 – non; 5 – high) to assess changes in the mechanical properties of BCH. Relative humidity did not significantly affect the elastic modulus of sole claw horn, while a relatively short 3 h period of soaking of BCH in distilled water significantly reduced claw horn EM (0 h: 726.5 MPa; 3 h: 263.9 MPa). In lactating animals between 30 and 120 d pp there was a significant reduction in the EM of sole horn (30 d pp: 872.4; 120 d pp: 518.9 MPa), PR of sole (30 d pp: 0.98; 120 d pp: 0.63 (±0.45) log ^{10}N) and white line (30 d pp 0.90; 120 d pp: 0.82 log^{10} (±0.45), and a reduction in VH of the white line (30 d pp: 131.4; 120 d pp: 126.5 (±1.76) MPa), but not the sole (30 d pp: 135.3; 120 d pp: 133.4 (± 1.76) MPa). The PR of BCH was consistently significant lower in white line compared with sole and was lower in sole horn with LS of 3. There was no significant difference in EM or VH between sole and white line, or with increasing levels of lesion score. The mechanical tests were considered valid methods to determine some of the mechanical properties of claw horn, each elucidating differing properties of BCH, however PR was the method that most frequent indicator of changes in the mechanical properties of BCH.

Keywords: Mechanical properties, claw horn, dairy cattle

4.1 INTRODUCTION

Lameness causes significant economic loss and is a serious welfare problem for dairy cattle (Kossaibati and Esslemont, 1999) and research indicates that as many as 60% of cows in a herd may become lame annually (Vermunt, 2004). The overall incidence of lameness has risen from 3.88% in 1957 (Eddy and Scott, 1980) to 55% (Clarkson *et al.*, 1996) in the UK, which is most frequently associated with claw horn disease which is derived, in part, from a deterioration of the mechanical competence of bovine claw horn (BCH) (Zoscher *et al.*, 2000). The specific mechanical properties that change and result in lameness are largely unknown (Zhang and Arola, 2007) and while puncture resistance has been elucidated as a useful measurement (Winkler *et al.,* 2005), the assessment of horn hardness and elasticity, sometimes referred to as stiffness, may also be indicative of changes in mechanical integrity of BCH.

In mechanical terms, bovine claw horn is a natural biological composite formed in the main from α keratin which is capable of accommodating and resisting in vivo loads without excessive deformation or catastrophic failure (Newlyn *et al.*, 1999). As with most composite materials the internal structure or morphology is at least as important as the constituent materials in determining its mechanical properties (Hull and Clyne, 1996). Keratin is formed at the supramolecular scale from microfibrils which are bundles of protofibrils themselves formed by the α-helix amino acid chains. Microfibrils are roughly aligned together within cells, which are further organised into either tubular structures or intertubular material, forming a multihierarchical composite (Kasapi and Gosline, 1997). This matrix performs the usual range of functions in a composite, namely to bind fibres and transfer stress between them, and to help resist crack propagation (Kasapi and Gosline, 1999). Claw horn quality, in part a reflection of its mechanical properties, is therefore influenced strongly by the following structural factors (Műlling *et al.*, 1994); intracellular factors i.e. the amount and ratio of keratin filaments and intermediate filament associated proteins (IFAP's), extracelluar factors i.e. the amount and biochemical composition of intercellular cementing substance (ICS), connecting

horn, and architecture i.e. arrangement and spatial relationship of tubular, intertubular and laminar horn cells (Mulling *et al.*, 1999). The structure and quality of claw horn is ultimately dependant on physiological keratinisation since the quality of claw horn produced is dependent on the process that initiated in the keratinocytes (Mulling *et al.*, 1999)

Since the epidermis is avascular, keratinocytes are dependent on receiving oxygen and nutrients from the fine microvasculature of the corium by diffusion across the basement membrane. Diffusion is easily disrupted and results in the production of low quality horn (Hoblet and Weiss, 2001). Poor quality horn formed after damage to the keratinocytes tends to cause more poor quality horn to be produced (Nocek, 1997). Rapid claw horn turnover commonly results in incomplete keratinization and therefore reduced horn quality and hardness, which leaves the structure more susceptible to damage and vascular disturbances (Budras *et al.*, 1996). Claw horn containing large qualities of ICS and disorganized squames content results in some loss of integrity and function (Kempson and Johnston, 1990). Reduced horn quality can also be a result of blood elements which have seeped out from damaged blood capillaries and leaks across the basement membrane separating the dermis from the epidermis (Kempson and Logue,1993). Numerous studies such as Greenough and Vermunt, (1991) and Leach *et al.*, (1997) have observed the development of claw horn lesions in postpartum animals; as a result the claw horn quality of these animals may have been reduced.

There is a wide range of mechanical property values, specifically elastic modulus and hardness, reported in the literature. Unfortunately, many of the authors did not state the location of the claw from which horn sample had been taken or the number of d pp, however where this information had been stated the data has been included to allow more direct comparisons. Hedges *et al.*, (2002) found that the elastic modulus of white line, zone 3 (3.1 MPa) was significantly weaker than the zone 2 (5.3 MPa). Zoscher *et al.*, (2000) found the elastic modulus of the sole

(toe) to be 134.9 MPa whereas Winkler (2005) found elastic modulus (in tension) ranged from 71.49-151.6 MPa in sole horn whereas white line claw horn ranged from 77.8-104.1 MPa over a period of 150 days postpartum. In contrast Zhang and Arola (2007) stated elastic modulus (in tension) in zone 4 of sole horn was 30200 MPa. Mulling (1994) using ball impact found the hardness of sole horn to be 12.9 MPa and Zoscher *et al.*, (2000) found it to be 10.9 MPa. Hedges *et al.*, (2002) found no significant effect of biotin supplementation on white line strength using the Vickers micro-hardness (supplemented 157.4 MPa, vs unsupplemented 157.5 MPa) and there were no significant differences in the white line strength (zone 2) between medial (162.3 MPa) and lateral claws (152.6 MPa). Winkler (2005) found puncture resistance (PR) ranged from 8.6-10.7 N mm^2 in sole whereas, white line claw horn ranged from 5.24-6.9 N mm^2. In heifers claw horn strength was significantly affected by time, at 40 days prepartum PR was 8.68 N mm^2 and the elastic modulus (EM) (in tension) was 88.76 MPa, decreasing at 50 days post partum (d pp) (PR 8.6 N mm^2 and EM 71.5 MPa) and increased at 150 dpp (PR 10.74 N mm^2 and EM 151.6 MPa). It is worth noting that puncture resistance is a complex compound measure, which includes effects deriving from tensile and compressive strength and post yield properties.

The importance of the integrity of claw horn and the variation in mechanical properties reported, warrants further investigation of claw horn structural properties and the development of methods to access this. As a consequence, the objective of this research was to establish a method for determining elastic modulus in tension, to compare it to Vickers hardness and PR, and to investigate whether these mechanical tests can detect the differences in the mechanical competence of horn that are known to change between 30 and 120 d pp and the differences in mechanical competence of horn from sole and / or white line horn, which are also known to differ. A robust reliable mechanical test that can differentiate between healthy and pathological horn material would be useful in developing the understanding and prevention of lameness.

4. 2 MATERIALS AND METHODS

4.21 Claw horn samples

The claw horn samples use in this study were collected from research studies completed between the 1st Oct 2007 and 1 June 2008 according to local ethical procedures and protocols approved by the Massey University Animal Ethics Committee.

4.22 Morbid tissue samples

The claw horn samples (n= 25) used to test for the effect of relative humidity and water content on claw horn integrity were collected from 5 animals slaughtered for other purposes than research at a nearby abattoir, in Devon UK. This approach to the assessment of the effect of relative humidity (RH) and the extension of water content of the claw horn, was applied by Winkler (2005), in which the effect of RH on the PR of claw horn was fully elucidated. In this study the same methods were applied to assess the effect of similar levels of RH on EM.

4.23 Lactating animals

There were 36 Holstein Friesian heifers that were selected at random from a dairy herd of 120 dairy cattle, based at Bridgewater College, Sommerset, UK and were matched according to calving date, breeding value, and body condition score (BCS). All the heifers were offered adlibitum access to the same grass silage and forage maize silage diet (Table 4.1 and 4.2) and were housed in straw bedded yards (7.5 m^2 / animal) prepartum and in cubicles / free stalls postpartum in accordance with DEFRA (2003) and BS 5052, part 40 (BS, 1990) standards. The passageways were scraped manually twice daily and all lactating animals were milked twice daily at 05.30 and 17.00 h.

Table 4.1 Composition of the mixed ration (MR) and compound, and mineral premix

Item Ingredient % of dietary DM	Diet content (%)
Maize silage	30.8
Grass silage	25.3
Protein blend	20.9
Permeate	2.6
Compound	9.3
Hay	3.3
Rolled wheat	5.5
Megalac	1.6
Mineral premix †	0.6
Chemical composition	
Dry Matter (%)	40.8
ME (MJ/kg DM)	12.4
NDF (%DM)	34.7
CP (%DM)	16.3
Sugars (%DM)	8.9
Starch (%DM)	14.9

† The mineral and vitamin premix contained (/kg DM basis); macronutrients (%), Salt, 10.0, Calcium, 16.0, Phosphorus, 8.0 , Magnesium , 14.0, Sodium, 4.0; vitamins (iu/kg), Vit A 3000000, Vit D3 60000, Vit E 3250; micronutrients (mg/kg), Copper, 3000.0, Manganese, 1000.0, Cobalt, 34.65 ,Iodine, 300.0, Selenium, 50.0.

The claw horn samples (n= 32) used to compare differing mechanical tests were collected from the left lateral claw of first lactation heifers at 30 and 120 days postpartum. The samples were taken from the distal surface of the claw parallel to the ground surface from zones 2 and 5, according to the International Foot

Map (IFM) (Shearer *et al.*, 2002). The first outer layer of horn (1 mm) was discarded and a sample of 0.1 to 2.5 mm thickness taken using claw trimming knives (variation in thickness occurred as a result of animal movement), transferred immediately to plastic bags, sealed and stored in a freezer for 10 d at a temperature of -20 °C until analysis. The dry matter content of horn samples was determined using oven dry matter (DM) determination (100 °C for 72 hrs) according to (MAFF, 1986). The individual animals had claw horn samples (n= 32) scored for the level of hemorrhaging, using a scale of 0 to 5 (0: No haemorrhage and 5: Severe haemorrhage) according to Leach *et al.*, (1997), but no tissue became available that matched the 4 and 5 on this scale and as such no data will be presented for the lesion score 4 or 5. The number of observations with each lesion score are as follows: LS 0= 100, LS 1= 70, LS 2= 50, LS 3= 20). The claw horn samples were collected and tested using elastic modulus (EM), Vickers hardness (VH) and puncture resistance (PR) tests were ever possible.

4.24 Effect of relative humidity on elastic modulus

The humidity of the air for each specified RH was generated in a large airtight cabinet by pumping air vigorously through porous artificial stones immersed in water. The claw horn samples (taken from morbid tissue) were tested in a second smaller chamber connected to the large chamber via suitable tubing. This smaller chamber was used to enclose the claw horn sample during the completion of the mechanical tests. The RH inside the humidity cabinet and the smaller test chamber was kept constant (±1 %) between ambient and 75 % RH. Claw horn samples from the sole (zone 5 of IFM) were collected from lateral and medial claws from 5 animals (4 samples from each claw were measured (replicated) 5 times) were tested at 25%, 55% and 75% RH, and were left to equilibrate for half an hour prior to testing at that environmental RH according to Winkler (2005) .

4.25 Effect of soaking claw horn samples in distilled water for different periods of time on elastic modulus

As an alternative method to alter the water content of the claw horn samples of the sole (zone 5 of IFM) of 5 different morbid claws (testing of 5 horn samples from each claw were repeated 6 times) were tested in tension after collection and then placed in distilled water. The six samples from each claw were then placed in distilled water for 3, 6, 12, 24, 48 and 72 hours, following which, samples were tested again. The effect of RH on VH was not assessed as it had previously been demonstrated that testing of claw horn samples was to be applied at natural physiological moisture.

4.26 Vickers hardness test

Hardness is measured as the permanent deformation 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). Claw horn specimens were taken from surplus claw horn samples collected from a previous research. Trial slivers approximately 10 mm in diameter were fixed in resin (Struers Rotopol- 22 Durofit- 2 kit), polished with various grades of glass paper until the surface finish reached a standard were the indentations could clearly be seen and measured to 1 micron. Samples were tested using a Future Tech microhardness tester (FM-IE) in a Vickers diamond test at a load of 100 g force, dwell time of 15 seconds. Claw horn (n= 32) samples were tested in zones 2 and 5 of the IFM (Shearer *et al.*, 2002); the hardness being the mean of 5 indentations at random points in each of the zones in a accordance with Johnnson and Rapoff (2007). All samples were kept in the same conditions at all times to avoid any differences in conditioning.

4.27 Puncture resistance of claw horn

Claw horn samples (n= 32) were analysed for puncture resistance (PR) as described by Winkler *et al.* (2002) using a P/2N needle probe on a TA.XT plus Texture Analyser with a 30 kg load cell (Stable Micro-Systems, Vienna Court,

Lammas Road, Godalming, Surrey, GU7 1YL, UK). The rate of displacement of the test probe was 1.0 mm/sec, which enabled the material to undergo some stress relaxation known as 'adapting to the load' (Aranwela *et al.*, 1999). A force-displacement curve was recorded when the test probe came into contact with the sample and a trigger force of 5 g had been reached, following which the probe was driven 12.0 mm into and through the sample before returning to the initial position. This distance was sufficient to allow the sample to be punctured and the maximum force during puncture measured. The maximum puncture force values (N/mm^2) were obtained from the peak values in the force-displacement curve. A total of 5 tests were repeated on each claw horn sample from the sole and white line areas of each claw in accordance with Winkler (2005), which was considered to be sufficient to establish test repeatability and variability. The thickness of the sample in the loaded area was measured using callipers with a resolution of 0.01 mm.

4.28 Tensile Testing

The claw horn samples (n = 32) which were collected from zone 5 of the IFM (Shearer *et al.*, 2002) were cut into approx 1mm by 1mm by 10mm length sections and three replicates from each sample were then tested. Two small strips of carbon copy paper (typically 0.3 mm long 30 µm wide) required for strain measurement were temporarily adhered onto the surface of the samples, each of which was placed one third of the way along the length of each of the sample, from each end of the sample. The samples were then gripped by clamps in a universal testing machine with LVDT and video extensometer, which was a specialist machine, custom built specifically for the measurement of tensile characteristics of composite materials, such as claw horn, by Prof Smith at Exeter University, UK). An overhead microscope was used to check sample alignment and measure strain in test.

The mechanical testing was carried out as previously described by Smith *et al.*, (2000) and in accordance with ASTM D3039/D3039m-07. The samples were

tested in tension as per Smith et al (2000). Strain was measured via the overhead microscope using an edge following system (videoextensometer, Messphysik GmbH, Austria). The system edge follows the surface markers and reports their separation along the sample approx' every 200 milliseconds. This data was used to calculate the strain. Load data from the testing machine were also recorded every 100 miliseconds. The specimens were deformed in tension cyclically by 0.01 mm at a rate of 0.001mms^{-1} for five cycles, with measurements being taken from the fifth cycle.

The separation between markers was used to calculate engineering strain (ε) as:

$$\varepsilon = \frac{\Delta l}{l_0} \quad (1)$$

where Δl is the change in distance between the two markers and l_0 is the original distance between the two markers. The load data from the test machine software were used to calculate the stress (σ) as:

$$\sigma = \frac{P}{A_0} \quad (2)$$

where P is the force on the specimen and A_0 is its cross-sectional area (calculated as the product of width measured using callipers and thickness measured in a scanning electron microscope).

The slope of the stress vs strain graph was taken as the elastic modulus (E). Also known as the Young's modulus, E was calculated from experimental data using a least-squares fit to a straight line over the rising portion of the fifth cycle of the stress versus strain data (Smith *et al.*, 2000).

4.29 Statistical Analysis

The mechanical properties techniques used different units of scale and as such the data for each technique and as such data was compared in MPa, by multiplying the GPa from the VH test by 1000 to make this into MPa. This data

was assessed for normal distribution and found to be normally distributed, with the exception of PR which was log 10 transformed and then found to be normally distributed, using the norm plot procedures in Minitab 15 software (Minitab Inc., State College, PA). The data was analysed by analysis of variance (ANOVA) for EM and VH, while PR was analysed by analysis of covariance (ANCOVA) by including horn sample thickness as a covariate in the model, using the general linear model command in Minitab 15 (Minitab Inc., State College, PA). In the model IFM zone (2 or 5), d pp (30 and 120), lesion score (0, 1, 2 and 3), RH and time soaked in distilled water were included as fixed effects in the models, animal was included as a random effect and the variables assessed in the model were EM, VH and PR. A confidence interval of 95% was applied in all the models and comparisons were made using Tukey's test in the GLM command. The data was normally distributed and was presented as mean ± standard error of the means, with significantly statistical differences were reported when $P<0.05$ and a tendency being considered when $P<0.10$. The existence of relationships between mechanical properties (PR, VH and EM) and lesions scores were assessed using Pearson's correlation technique in Minitab 15.0 (Minitab Inc., State College, PA).

4.3 RESULTS

The number of days postpartum was found to influence the elastic modulus significantly; specifically the mean elastic modulus of claw horn samples taken from zone 5 (IFM) at 30 and 120 d pp were found to be significantly different (P 0.009). The Vickers hardness tests showed a significant difference between zones 2 and 5 (IFM) (P 0.005) but not between samples from 30 and 120d pp. PR results also showed that there were significant differences between zones 2 and 5 (IFM) (P 0.015) and d pp (P 0.003) (Table 4.2)

Table 4.2 Mean Elastic modulus, Vickers hardness and puncture resistance of white line (IFM 2) and sole (IFM 5) claw horn taken from of hind claws of lactating dairy heifers at 30 and 120 days postpartum (d pp)

	White line		Sole			P	
d pp	30	120	30	120	SEM	d pp	IFM
Elastic modulus (E MPa)†	-	-	872.4	518.7	113.26	0.009	-
Vickers hardness (MPa)	131.4 [b]	126.5 [c]	135.3 [a]	133.4 [a b]	1.76	0.005	NS
PR (Log10 $Nmm^{2)}$	0.90 [b]	0.82 [c]	0.98 [a]	0.63 [d]	0.045	0.001	0.015

[a,b,c,d] – Data in rows followed by differing superscripts differs significantly $P<0.05$

†(in tension)

The EM of S and PR of both the WL and S horn declined significantly between 30 and 120 d pp and the PR of WL was significantly lower than that of S at 30 d pp. However, the greatest reduction in PR was found in sole between 30 and 120 d pp. The VH of sole horn showed no significant reduction between 30 and 120 d pp, while the VH of the WL did show a significant reduction. The Pearson's correlations found elastic modulus to be significantly ($P<0.001$) negatively correlated to lesion score -0.313. The PR data for haemorrhage score (Table 4.3) was significantly different between scores 1 and 3, there were no horn samples that were haemorrhaged to scores of 4 or 5.

There was no significant difference found for the DM of the claw horn samples taken at 30 (93.8%) or 120 days (88.4%) with a standard error of the mean (SEM) of 2.73. The thickness of the horn sample had a significant effect on PR ($P<0.001$), increasing the PR as the thickness of the sample increased. The mean thickness was 1.49mm.

The mean elastic modulus of horn taken from sole was not found to be significantly affected by RH (Figure 4.1). There was also no significant difference found in the DM (96.3 and the SEM was 1.16) of the claw horn samples used for the relative humidity mechanical testing.

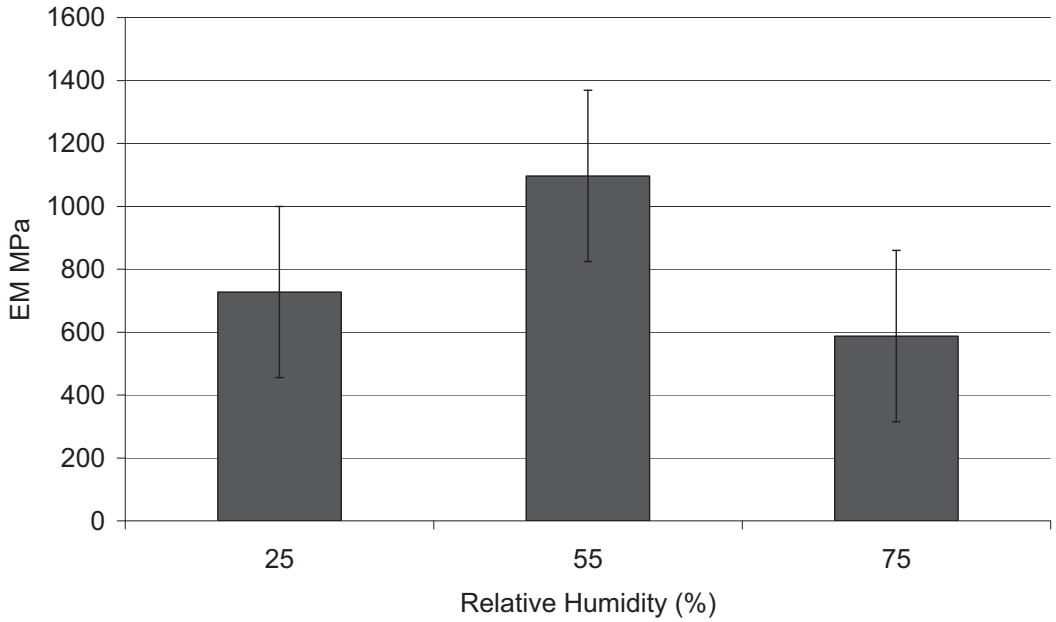

Figure 4.1 Mean elastic modulus of morbid sole horn at 25, 55 and 75 % relative humidity

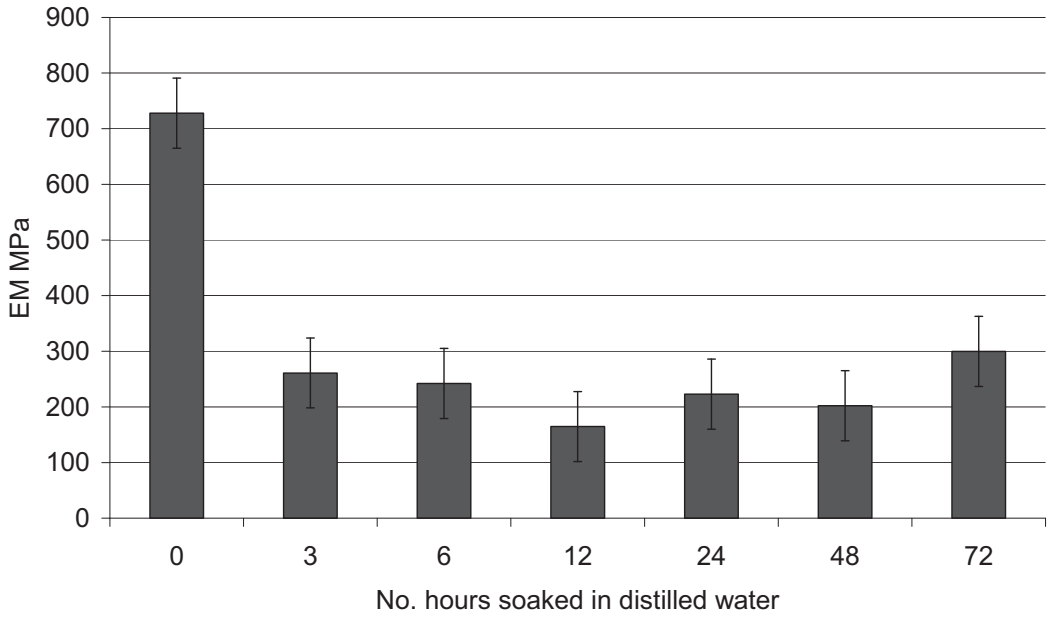

Figure 4.2 Mean elastic modulus of morbid sole horn following soaking in distilled water for between 0 and 72 h

The mean elastic modulus of horn taken from zones 5 of the IFM was not significantly affected by exposure time to distilled water, at least up to 72 hrs (Figure 4.2). However, elastic modulus of soaked claw horn was significantly reduced when compared to claw horn which had not been soaked in distilled water.

Table 4.3 Mean elastic modulus, Vickers hardness and puncture resistance of bovine claw horn taken from the sole and white line of lactating dairy heifers and scored for the level of haemorrhage (lesion score)

	Lesion Score (LS)								SEM	P value	
	0		1		2		3				
	WL	Sole	WL	Sole	WL	Sole	WL	Sole		LS	WL/Sole
Elastic modulus (E MPa)†	-	899.0	-	639.4	-	431.4	-	812.3	193.46	NS	-
Vickers hardness (MPa)	124.5	137.3	129.4	135.3	123.6	124.5	130.4	129.4	4.41	NS	NS
PR (Log10 $Nmm^{2)}$	0.82 ^{b}	0.87 ^{a}	0.64 ^{c}	0.97 ^{a}	-	1.01 ^{a}	0.35^{d}	0.67 ^{bc}	0.18	0.001	0.014

NS- Not significantly different

^{a,b,c,d} – Data in rows followed by differing superscripts differs significantly $P<0.05$

† - Measured in tension

4.4 DISCUSSION

The elastic modulus showed a significant difference in claw horn properties between measurement periods, i.e. the claw horn increased in elasticity as the elastic modulus declined between 30 and 120 d pp. PR also showed a significant reduction between 30 and 120 d pp. This corresponds with the research of Winkler (2005) who found a decrease in claw horn elastic modulus and PR until 150 d pp. The Vickers hardness results did not reveal significant differences in claw horn properties, the decrease in hardness coincided with the decrease in EM. Very few authors have observed the effect of stage of lactation on the mechanical properties of claw horn, Winkler (2005) found a significant decrease in PR from 30 d pp to 160 d pp reflecting a similar scenario to the one presented in the current study.

Parturition, lactation, housing and environment, age and season have been found to correlate highly with lameness (Logue, 1999). The samples used for this research were taken from heifers that were housed over winter in the same building and fed the same pre and post partum diet to try to limit the number of factors that were going to impact on the level of lameness experienced. All the heifers in this study also underwent parturition and the hormonal changes associated with parturition that are believed to be major contributory factor in the development of sole and white line lesions (Holah *et al.*, 2000; Tarlton *et al.*, 2002). The heifers may also have experienced some level of negative energy balance, as dry matter intake can decrease by approximately 10 to 30 % in late pregnancy and early lactation. This is related to the mobilisation of body reserves (Hoblet and Weiss, 2001) which are known to be significantly correlated with locomotive problems during lactation (Collard *et al.*, 2000). Any one of these factors could have resulted in significant decreases in elastic modulus, PR and the non-significant reduction in hardness.

The findings from three methods used to test the mechanical properties of claw horn are determined by the constituent materials and or its structure, however these methods are considering measuring different aspects. The elastic modulus (stiffness/ flexibility) is a product of the constituent materials (keratin and ICS in this case) and the cellular and intercellular microarchitecture (Mulling *et al.*, 1999). Therefore elastic modulus is a measure of the bonds and transfer of stress within the microarchitecture, which will help determine the probability of crack propagation (Kasapi and Gosline, 1999). However, hardness is a reflection of a material's resistance to plastic deformation and is in the main determined by the constituent materials' innate plasticity. Therefore formation of the horn tubules, size, density and the function in moisture regulation also influence the resistance of the material to wear and can be measured by hardness (Hinterhofer *et al.*, 2007). Harder claw horn is likely to wear at a slower rate which can be seen as an improvement to claw horn health as often wear rates can exceed growth rates (Leach *et al.*, 1997). Whereas PR considers the peak force required to puncture the material and may be thought of as a compound product of tensile and shear strengths, which are dependent upon both the constituent materials' properties and the microarchitecture. As such, changes in PR as a result of either changes in the constituent materials or the structure could indicate the likelihood of stone / foreign body penetration of claw horn.

There was no significant difference in the elastic modulus or Vickers hardness of claw horn samples taken from the white line or sole across a range of haemorrhage scores. This suggests that the lesions do not cause or indicate any deterioration in gross mechanical competence in contrast to Winkler (2005) who found that haemorrhage score was significantly negatively correlated to stiffness. The elastic modulus results did show that with increasing lesion score the stiffness of the claw decreased with the exception of lesion score 3; however, Pearson's correlation found it to be negatively correlated to stiffness. This could be partly due to the low number of samples with this lesion score (which could be due to using 1st lactation animals which don't tend to have as severe bruising as

multiparous animals) and how much the haemorrhaging has affected the quality of the claw horn. However, there was a significant decline in PR correlated with increasing haemorrhage score. Winkler (2005) also found that an increasing haemorrhage score decreased the PR of claw horn. With the exception of Winkler (2005), no other authors have compared mechanical properties and haemorrhage score. Haemorrhages found in inferior claw horn production are caused when the epidermis, (thus keratinocytes) do not receive enough oxygen and nutrients from the fine microvasculature of the corium by diffusion across the basement membrane. This diffusion can easily be disrupted. Interference in the supply of nutrients to the keratinocytes can result in an inflammation in the corium caused by circulating vasoactive substances or from localized trauma (Hoblet and Weiss, 2001). Kempson and Logue (1993) stated that claw horn quality can be reduced by blood elements which have seeped out from damaged capillaries leaking across the basement membrane separating the dermis from the epidermis. Leach *et al.* (1997) found that in autumn calving heifers, the combined effects of calving, housing and the lactating diet resulted in insults to the corium which manifested in the form of increased levels of lameness and haemorrhages of the white line and sole. Histological studies carried out by Leach *et al.* (1997) on samples of horn from the animals examined showed that sole haemorrhages were virtually always accompanied by histological and morphological changes in the laminar zone where the horn of the white line is generated. Despite these findings of changes induced by lesions, it seems that the lesions are not correlated, at least in this study, with deterioration in mechanical properties of claw horn. Thus further, studies were a greater number of samples were to be obtained with higher levels of haemorrhaging potentially from multiparous animals (with a known history of lameness) could yield results to determine whether haemorrhaging causes deterioration in mechanical properties of claw horn.

The range of elastic modulus results of the sole horn, 431 to 899 MPa, fit within the ranges reported by Zoscher *et al.* (2000) and Winkler (2005), but are lower

than those reported by Zhang and Arola (2007) and Dyer *et al.* (2004) of 3000 MPa. The difference between these reported values for elastic modulus could be due to the different test speeds, sample size and levels of conditioning (e. g. whether the sample has been left to acclimatise to the RH of the room or is in its "natural" physiological state as taken from the animal) of claw horn used by the various authors as suggested by Hinterhofer *et al.* (2005b). The Vickers hardness data showed that the claw horn samples for white line were slightly lower than those reported by Hedges *et al.* (2002). These differences could be as a result of the variation between animals, diet, and housing (as obviously the same animals, diet location were not used for both studies). The number of tubules, tubule size (dimensions can reach 180 μm) or larger amounts of fatty ICS (Budras *et al.*, 1996) would also potentially cause variations in WL hardness. Therefore the indenter could have been penetrating the softer ICS or into the centre of a large tubule. The PR data from this research has reported a larger range for both sole (7.7 to 16.8 MPa) and white line (4.7 to 11.6 N MPa) than those stated by Winkler (2005) of 8.6 to 10.7 MPa in sole and 5.24 to 6.9 MPa. However, there is a large SEM (4.85) on the PR data suggesting a large amount of variability within the claw horn samples; which would largely be due to the varying thickness of the claw horn samples. This may have resulted in a larger range of results than previously found by Winkler (2005). Thickness was used as a covariate when completing statistical analysis as it was found to significantly affect the PR of claw horn samples, as also found by Winkler (2005). Unfortunately, due to the nature of working with a natural product and live animals, some variation in sample thickness was unavoidable. Some animals were quiet which enabled a thinner sample to be taken but if an animal was moving around it made it more difficult to take samples and generally resulted in a thicker sample being taken. Sample thickness ranged from 0.45 to 2 mm from sole horn and 0.3 to 1.75 for white line horn which were collected using a hoof knife. Winkler (2005) used a wood plane to collect horn samples on some experiments, but came to the conclusion that for PR the inclusion of sample thickness as a covariate in the analysis of variance was more effective. While PR

is significantly affected by the variation in thickness of claw horn sample, sample thickness is used successfully as a covariate in ANCOVA when analysing PR data as found by Winkler (2005).

When comparing the results from the three different mechanical parameters, EM data produced the largest range of values and subsequent higher SEM than Vickers hardness and PR methods produced more consistent values with lower SEM Suggesting potentially that the latter tests produce more reliable results. This could be due to these tests considering the deformation and penetration of the samples whereas EM is considering the stiffness/flexibility of the sample. However, the mechanical parameters are considering different aspects of claw horn quality and strength and as a result no one test can describe claw horn quality as a whole (Hinterhofer *et al.*, 2005a). As stated above all the data produced from the mechanical tests were found to be in the range produced by current research (Hedges *et al.*, 2002; Winkler, 2005; Zhang and Arola, 2007; Zoscher *et al.*, 2000) with similar SEM (Hedges *et al.*, (2002) and Dyer *et al.*(2004) did not report SEM values). Therefore all the mechanical parameters should be considered when determining claw horn quality and strength.

It is well known that the mechanical properties of keratinous and other biological materials are strongly influenced by their state of hydration. The moisture content of claw horn has been found to affect the values of hardness, elastic modulus, bending stiffness and fracture toughness of claw horn (Collins *et al.*, 1998; Hinterhofer *et al.*, 1998; Baillie *et al.*, 2000; Winkler, 2005). These findings suggest that as moisture content of claw horn increases it becomes more compliant (a reduction in elastic modulus) and therefore wear rate could increase. For example as a result of prolonged exposure to slurry i.e. in winter housing systems the moisture content of claw horn could increase and thus result in increased wear rates. Bonser *et al.* (2003) reported that the increase in the moisture content of the claw horn lead to an increase of the rate of wear of the horn on rough surfaces. On smooth surfaces the rate of wear decreased with

higher moisture contents of the horn. Those findings show a complex interaction between the mechanical properties of the claw horn, moisture content, the friction and wear caused by different surfaces, indicating the importance of controlling environmental moisture levels when cattle are housed on hard surfaces such as concrete floors.

There were no significant differences found for the DM of the claw horn samples taken at 30 or 120 days. Winkler (2005) also reported no significant differences in the dry matter content of the claw horn between 50, 100 and 150 days postpartum. There was also no significant difference in DM of the samples used for the relative humidity mechanical testing. Hinterhofer *et al.* (2005b) propose that correlations between elastic modulus and the DM do exist, but are not strong. However, determining DM from small sample sizes and with small amounts of tissue sample can be problematic (Hinterhofer *et al.*, 2005b), and different drying methods can cause differing rates of water and volatile loss from claw horn samples. This can make the determination of the moisture content of claw horn variable, thus making detection of small differences, that may affect claw horn strength, difficult due to the larger number of claw horn samples required to find potential significant differences. Furthermore, there is no agreed specific standard method for DM calculation (Reilly *et al.*, 2002) of claw horn.

The changes in the elastic modulus of sole horn taken from zone 5 of the IFM, and soaked in distilled water for various periods up to 72 hrs was found not to be significant. However, comparing the elastic modulus of claw horn tested at its physiological moisture content with the claw horn that has been soaked in distilled water there is a significant difference in elastic modulus. The elastic modulus values from these tests fall in the same range as found by Collins *et al.* (1998) for fully hydrated samples. Winkler (2005) found a lower elastic modulus for hydrated samples, whereas Zoscher *et al.* (2000) found a higher range of values for elastic modulus for toe, lateral wall, sole horn of front and hind claws. Winkler (2005), who used a type of EM test on one occasion, found no significant

difference for elastic modulus of claw horn soaked in distilled water between 3 and 72 hours, this study repeated the soaking of claw horn in distilled water as the study was using a different technique for calculating EM, while the VH method was designed to test samples at natural physiological moisture content.

The changes in the elastic modulus of sole horn tested over a range of RH were found not to be significant (water content of 4% at ambient RH), unlike Hinterhofer *et al.* (1998), who found samples conditioned at a RH of 65 % to have elastic modulus of 1673.8 ± 557.8 MPa. However, non conditioned wall and sole samples had a moisture content of 31.5%, and elastic modulus of 230 ± 92.4 MPa; this result was found to be significantly different by Hinterhofer *et al.* (1998). The data from this research shows there were no significant changes in claw horn stiffness with RH. However, the moisture content of Hinterhofer *et al.* (1998) showed claw horn samples at ambient RH did significantly reduce the elastic modulus and the mechanical properties of the claw horn, and surmised the probable increased risk of the dairy cow becoming lame.

The research by Winkler (2005) demonstrated that PR from claw horn can produce reliable reproducible results using a relatively small number of replicates. Husain *et al.* (2002) and Lewis (2002) also stated that PR is frequently used to determine mechanical properties of small or miniature specimens. Aranwela *et al.* (1999) confirmed the repeatability of PR to detect the fracture properties in leaves.

Elastic Modulus and Vickers hardness were also used as comparative approaches to determine the mechanical properties of bovine claw horn. As previously utilised by researchers (Dyer *et al.*, 2004; Hinterhofer *et al.*, 2005b; Winkler, 2005 and Zoscher *et al.*, 2000). The technique employed in this research to calculate elastic modulus however, uses a videoextensometer to record the movement of the markers on the sample. This was deemed to be important in the assessment of claw horn due to its elastic and composite nature

and has not previously been used for the assessment of bovine claw horn. This technique has been used by Smith *et al.* (2000) to determine the mechanical properties of the hind wings of a locust, confirming the method's repeatability and suitability for use on small samples.

As there are no recognised standard methods specifically dedicated for claw horn ASTM 3039D303M-07 the standard test method for tensile properties of polymer matrix composite materials was used. As claw horn is a natural biological composite formed in the main from α keratin which is capable of accommodating and resisting *in vivo* loads without excessive deformation or catastrophic failure (Newlyn *et al.*, 1999). The Vickers hardness test results in this thesis were comparable to those of Hedges *et al.* (2002). However, a test load of 100g rather than 20g was used as the residual impression were larger which enabled greater precision and repeatability when measuring the residual impressions (Johnson and Rapoff, 2007), but had not been previously applied to bovine claw horn.

4.5 CONCLUSIONS

The results from this research indicate that the mechanical properties of claw horn especially elastic modulus are significantly affect by stage of lactation as it was significantly stiffer at 30 days than 120 days postpartum. Lesion score was found to significantly affect the PR of claw horn but not Vickers hardness and elastic modulus. Hydrated claw horn had significantly lower elastic modulus when compared to dry or physiological moisture content of claw horn. Mechanical properties such as elastic modulus, hardness and PR offer approaches to help determine whether claw horn is capable of withstanding changes brought about by nutrition, parturition and environment which affect its composition, structure and thus mechanical integrity.

4.6 FURTHER RESEARCH

PR of claw horn continued to be a useable method, but varying mechanical properties of claw horn may be affected by environmental and animal factors

such as parturition, lactation and genetics etc and as such there should be continued research to assess and apply PR, Vickers hardness and elastic modulus tests using relatively small claw horn samples.

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4.8 REFERENCES

Aranwela, N., G. Sanson, and J. Read. 1999. Methods of assessing leaf-fracture properties. New Phytologist. 144:369-393.

Baillie, C., C. Southam, A. Buxton, and P. Pavan. 2000. Structure and properties of bovine hoof horn. Advanced Composite Letters. 9:101–113.

Bonser, R. H. C., J. W. Farrent, and A. M. Taylor. 2003. Assessing the Frictional and Abrasion-resisting Properties of Hooves and Claws. Biosystems Engineering. 86:253-256.

British Standards. 1990. British Standard on the design and construction of cattle buildings. TSO Publications, London.

Budras, K. D., C. Műlling, and H. Horowitz. 1996. Rate of keratinization of the wall segment of the hoof and its relation to width and structure of the zona alba (white line) with respect to claw disease in cattle. American Journal of Veterinary Research. 57:444–455.

Clarkson, M. J., D. Y. Downham, W. B. Faull, W. J. Hughes, F. J. Manson, J. B. Merritt, R. D. Murry, W. B. Russell, J. Sutherst, and W. R. Ward. 1996. Incidence and prevalence of lameness in dairy cattle. The Veterinary Record. 138:563-567.

Collard, B. L., P. J. Boettcher, J. C. M. Dekkers, D. Petitclerc, and L. R. Schaeffer. 2000. Relationships between energy balance and health traits of dairy cattle in early lactation. Journal of Dairy Science. 83:2683-2690.

Collins, S. N., B. C. Cope, L. Hopegood, R. J. Latham, R. G. Linford, and J. D. Reilly. 1998. Stiffness as a function of moisture content in natural

materials: Characterisation of hoof horn samples. Journal of Materials Science. 33:5185-5191.

DEFRA. 2003. Code of Recommendations for the Welfare of Livestock: Cattle. Pages 17-19. Defra Publications, London.

Dyer, R. M., D. Arola, D. Zhang, R. Reprogel, W. Zheng, and U. Tasch. 2004. On the mechanical behaviour of bovine hoof horn. Pages 82-84 in The thirteenth International Symposium on Lameness in Ruminants, Maribor, Solvenia.

Eddy, R. G. and C. P. Scott. 1980. Some observations on the incidence of lameness in dairy cattle in Somerset. The Veterinary Record. 106:140-144.

Greenough, P. R. and J. J. Vermunt. 1991. Evaluation of subclinical laminitis in a dairy-herd and observations on associated nutritional and management factors. Veterinary Record. 128:11–17.

Hedges, V. J., L. E. Green, R. Blowey, A. J. Packington, and R. H. C. Bonser. 2002. Tensile strength of the white line in the claw of dairy cows supplemented with biotin. Proceedings of the Twelfth International Symposium on Lameness in Ruminants. Pages 167-170. Vol. 9-13 Jan. 2002, Orlando, Florida.

Hinterhofer, C., V. Apprich, J. C. Ferguson, and C. Stanek. 2007. Modulus of elasticity and dry-matter content of bovine claw horn affected by the changes of chronic laminitis. Veterinary Journal. 174:605-609.

Hinterhofer, C., C. Stanek, and K. Binder. 1998. Elastic modulus of equine hoof horn, tested in wall samples, sole samples and frog samples at varying levels of moisture. Berliner und Munchener Tierarztliche Wochenschrift. 111:217-221.

Hinterhofer, C., M. Zoscher, C. Gabler, and C. Stanek. 2005a. The hardness of horn in different segments of the bovine claw. Berliner und Munchener Tierarztliche Wochenschrift. 118:334-340.

Hinterhofer, C. M., V. Apprich, J. C. Ferguson, and C. Stanek. 2005b. Elastic properties of hoof horn on different positions in the bovine claw. Berliner und Munchener Tierarztliche Wochenschrift. 112:142-146.

Hoblet, K. H. and W. Weiss. 2001. Metabolic hoof horn disease – claw horn disruption. Veterinary Clinics of North America. 17:111-127.

Holah, D. E., K. M. Evans, G. R. Pearson, J. F. Tralton, and A. J. F. Webster. 2000. The histology and histopathology of the support structures in the laminated zone of the bovine hoof in maiden heifers and around the time of first calving. Pages 94-96 in The Eleventh International Symposium on Lameness of the Ruminant Digit, Parma, Italy.

Hull, D. and T. W. Clyne. 1996. An Introduction to Composite Materials. Cambridge University Press, UK.

Husain, A., D. K. Sehgal, and R. K. Pandley. 2002. Design of a simple, versatile, small-specimen puncture test seup for determination of the mechanical behaviour of materials. Experimental Techniques September/October 2002:33-38.

Johnson, W. M. and A. J. Rapoff. 2007. Microindentation in bone: Hardness variation with five independent variables. Journal of Material Science. 18:591–597.

Kasapi, M. and J. M. Gosline. 1997. Design complexiy and fracture control in the equine hoof wall. The Journal of Experimental Biology. 200:1639-1659.

Kasapi, M. and J. M. Gosline. 1999. Micromechanics of the equine hoof wall: optimizing crack control and material stiffness through modulation of the properties of keratin. The Journal of Experimental Biology. 202:377-391.

Kempson, S. A. and A. M. Johnston. 1990. Changes in the hoof horn of pigs with laminitis: a scanning and transmission electron microscopic study. Advances in Veterinary Dermatology. 1:456.

Kempson, S. A. and D. N. Logue. 1993. Ultrastructural observations of hoof horn from dairy cows: changes in the white line during the first lactation. The Veterinary Record. 132:524-527.

Kossaibati, M. A. and R. J. Esslemont. 1999. The incidence of lameness in a group of dairy herds in England. Page 221 in BSAS Annual Meeting. BSAS, Scarborough.

Leach, K. A., D. N. Logue, S. A. Kempson, J. E. Offer, H. E. Ternent, and J. M. Randall. 1997. Claw lesions in dairy cattle: Development of sole and white line haemorrhages during the first lactation. Veterinary Journal. 154:215-225.

Lewis, G. 2002. Key issues involved with the use of miniature specimens in the characterization of the mechanical behaviour of polymeric materials. Journal of Biomedical Materials Research. 63: 455-466.

Logue, D. N. 1999. An overview of lameness in the dairy cow. Page 2 in Proceedings of the National Cattle Lameness Conference.

MAFF. 1986. Analysis of Agricultural Materials RB429. ADAS, London.

Műlling, C., H. Bragulla, K. D. Budras, and S. Reese. 1994. Structural factors influencing horn quality and sites of predilection at the ground surface of the cattle hoof. Schweizer Archiv Fur Tierheilkunde. 136:49-57.

Mulling, C. K. W., H. H. Bragulla, S. Reese, K. D. Budras, and W. Steinberg. 1999. How structures in bovine hoof epidermis are influenced by nutritional factors. Anatomia Histologia Embryologia-Journal of Veterinary Medicine Series C-Zentralblatt Fur Veterinarmedizin Reihe C. 28:103-108.

Newlyn, R. A., S. N. Collins, B. C. Cope, L. Hopegood, R. J. Latham, and J. D. Reilly. 1999. Equine hoof horn a natural composite. Pages 231-240 in Proceedings of the Fifth International Conference in Deformation and Fracture of Composites. Vol. 5th, London, UK.

Nocek, J. E. 1997. Bovine Acidosis Implications on Laminitis. Journal Dairy Science. 80:1005–1028.

Reilly, J. D., H. Newlyn, B. Cope, R. J. Latham, S. Collins, and L. Hopegood. 2002. A comparison of different moisture loss methods for measuring hoof wall content. Page 193 in Proceedings of the Twelfth International Symposium on Lameness in Ruminants, Orlando, Florida.

Shearer, J. K., E. Belknap, S. Berry, C. Guard, K. Hoblet, E. Hovingh, G. Kirksey, A. Langill, and S. R. van Amstel. 2002. The standardisation of input codes for capture of lameness data in dairy records Pages 346-349 in Proceedings of the Twelfth International Symposium on Lameness in Ruminants, Orlando, Florida.

Smith, C. W., R. Herbert, R. J. Wootton, and K. E. Evans. 2000. The hind wing of the dessert locust (Schistocerca Gregaria Forskal) II. Mechanical properties and funtioning of the membrane. The Journal of Experimental Biology. 203:2933–2943.

Tarlton, J. F., D. E. Holah, K. M. Evans, S. Jones, G. R. Pearson, and A. J. F. Webster. 2002. Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving. Veterinary Journal. 163:196-204.

Vermunt, J. J. 2004. Herd lameness - a review, major causal factors, and guidelines for prevention and control. In Prophylaxis of Claw Diseases Proceedings of the 13th International Symposium and 5th Conference on Lameness in Ruminants, Maribor, Slovenija.

Vincent, J. F. V. 1992. Composites. Biomechanics – Materials. A Practical Approach. Oxford University Press, Oxford.

Winkler, B. 2005. Mechanical properties of hoof horn, sole haemorrhage and lameness in dairy cattle. PhD dissertation. Plymouth University, Plymouth.

Winkler, B., J. K. Margerison, and C. Brennan. 2002. The testing of the mechanical properties of the hoof horn during lactation and housing. Pages 314-317 in Proceedings of the 12th International Symposium on Lameness in Ruminants, Orlando, Florida, USA.

Zhang, D. and D. Arola. 2007. A method for characterizing the mechanical behaviour of hoof horn. Journal Material Science. 42:1108-1115.

Zoscher, M., C. Hinterhofer, C. Stanek, and C. Gabler. 2000. New aspects of mechanical properties of bovine hoof horn. Pages 91-93 in Eleventh International Symposium on Lameness of the Ruminant Digit. Parma, Italy.

The mechanical properties tests (PR and EM) developed for bovine claw horn in Chapter 4 offered the potential to assess in the determination of the effect of factors such as; nutrition, parturition and environment that affect claw horn composition, integrity and physical function. These tests were applied in this Chapter, Chapter 5.

Chapter 5

The effect of zinc source and level (oxide or organic) on lameness parameters in Holstein Friesian dairy heifers.

The data in this chapter has been submitted to the Journal of Dairy Science for publication.

5.0 ABSTRACT

Zinc (Zn) is recognised as one of the micro-nutrients that play a vital role in maintaining normal cellular metabolism in all animals and is an essential micro-nutrient which is vital in the keratinisation process and production of healthy claw horn. Dairy cattle diets have traditionally been supplemented with inorganic mineral salts, which tend to interact with other nutrients reducing absorption rates and thus organic minerals have been developed to reduce nutrient interactions and increase mineral absorption. This research aims to assess the effect of the form (organic or inorganic) and level of Zn on locomotion score, claw horn puncture resistance (PR), claw horn damage (sole (S) and white line (WL)), condition score, milk yield and composition. At 21 d pre to 150 d pp 36 Holstein Friesian heifers were offered compound feed supplemented with either (n=12) Zn oxide at 1.0 (1 ZnOx), (n=12) organic Zn at 1.0 (1 OrZn) or (n=12) organic Zn at 0.3 (0.3 OrZn) of NRC (2001) recommended levels (RL). There was no significant effect of Zn supplementation on median locomotion score, S bruising, WL damage, claw horn PR of S or WL, horn DM content, wall horn growth or wear rates, DM intake, plasma Zn or iron levels, mean live weight, body condition, milk fat, protein, lactose concentrations (g/kg) or somatic cell count levels (SCC) (00,000/ml). Mean milk yield and fat corrected yield were significantly higher ($P<0.001$) for animals offered Zn at 1.0 (ZnOx and OrZn) compared with OrZn at 0.3 of NRC RL. Total milk fat and protein yields were not significantly different between cattle offered Zn at NRC RL (organic and Zn oxide) or differing levels of organic Zn (1.0 or 0.3 of NRC RL). However, cattle offered Zn Oxide at the NRC RL ($P<0.05$) had significantly higher total milk fat and protein yields than those offered organic Zn at 0.3 of NRC (2001) RL. In conclusion supplementing Zn as either oxide or organic at NRC RL and organic Zn at 0.3 NRC RL had no significant effect on locomotion score or claw horn characteristics from 21 pre to 150 d post partum. Fat corrected milk yield and total fat and protein yield were lower in heifers offered organic Zn at 0.3 NRC RL, compared with Zn oxide at NRC RL.

Key words: Zinc, Dairy Cattle, Claw horn

5.1 INTRODUCTION

Lameness of dairy cattle causes significant economic losses and represents a serious welfare problem (Kossaibati and Esslemont, 1999). The incidence of lameness has risen from 3.88% in 1957 (Eddy and Scott, 1980) to 55% (Clarkson *et al.*, 1996) in the UK and research indicates that as many as 60% of a herd may become lame at least once annually (Vermunt, 2004). Studies of lameness (Greenough and Vermunt, 1991; Leach *et al.,* 1997) have observed that claw horn haemorrhages develop in dairy catttle postpartum. During early lactation and periods of dietary nutrient insufficiencies, essential nutrients and minerals are be mobilised from body tissues to support milk production which ultimately affects the quality and quantity of milk as well as reproduction (Manspeaker *et al.*, 1987). In particular, Zn has been established as an essential micro-nutrient required for the maintenance of health and performance of dairy cattle and was identified as playing a vital role in the processes of keratinisation (Műlling *et al.*, 1999, Műlling, 2000) and thus claw horn production. Zn is involved in the process of keratin synthesis and in collagen and skin nucleic acid synthesis. Compromising the supply of this vital nutrient to keratin-forming cells results in inferior keratinized tissue production, which may lead to increased susceptibility to claw disorders and ultimately lameness (Műlling *et al.*, 1999).

In blood plasma, Zn is mostly (0.75) bound as plasma protein, erythrocytes (0.22) and leucocytes (0.03) (Hosnedlová *et al.*, 2005). Zn supplementation can reduce iron and copper status; the negative effects of iron supplementation on indices of Zn and copper status have also been reported (Sandström, 2001). Numerous studies have investigated the influence of organic Zn compounds on the metabolic profile. Several studies (Kessler *et al.*, 2003; Malcolm-Callis *et al.*, 2000; Spears, 1989) have found that the Zn concentration in cattle plasma have not been affected by the Zn source, whether organic or inorganic. However, these differ from the findings of Rojas *et al.* (1995) where Zn source affected Zn concentration in sheep plasma.

Blood plasma samples are frequently used to assess micro nutrient status as micro nutrients are significantly correlated to nutritional status (Mills, 1987) and this method is less invasive than liver sampling. However, there are disadvantages, as red blood cells in cattle have a life span of about 160 d (Schalm, 1980), the concentrations of minerals in whole blood often change slowly and blood samples need to be handled carefully to prevent hemolysis and contamination of plasma (Kincaid, 1999).

The nutrition of lactating dairy cattle continues to be one of the focal points of lameness research (Nocek, 1997) and the use of supplements such as biotin (Hedges *et al.*, 2001), Zn (Bazle, 1993; Kessler *et al.*, 2003) and mineral complexes (Nocek *et al.*, 2006) have been found to reduce sole bruising and the incidence of sole haemorrhages. Cattle diets have traditionally been supplemented with inorganic minerals, as these salts are hydrolysed in the digestive tract to form free ions and are absorbed. However, free ions can form complexes with other dietary molecules, which can make them difficult to absorb. The availability of trace minerals varies considerably and under extreme conditions may be unavailable. Chelated mineral complexes are more stable in the digestive tract and protected from forming complexes with other dietary components that would otherwise inhibit absorption (Spears, 1996), making complexes more bioavailable / bioactive providing animals with a metabolic advantage that often results in improved performance. These mineral complexes may theoretically be included at lower levels without compromising performance, thus minimising nutrient excretion and environmental pollution, resulting in reduced economic and environmental costs (Close, 2002; Paik, 1999). As a consequence, there is growing interest in organic either proteinated or chelated trace minerals (Close, 2002; Paik, 1999). Manspeaker *et al.* (1987) demonstrated that supplemental chelated amino acids were more bio-available than inorganic salts and resulted in improved reproductive performance. Studies have shown improved growth, milk yield, reproductive performance, and/or immune response in ruminants offered diets containing organic trace minerals

(Gunter *et al.*, 1999; Spears, 1996). However, studies researching absorption of trace elements from organic compounds Boland *et al.* (1996), Manspeaker *et al.*, (1987), Stanton *et al.* (2000) have not always been unequivocal and have shown differences in absorption levels. However, increasing the bioavailability of trace minerals, particularly Zn, may improve absorption and utilization rates, thus increasing the integrity of keratinised tissues (Ballantine *et al.*, 2002). As a consequence, the objective of this research was to assess the effect of the form of dietary Zn source (inorganic or organic) and level on locomotion score, bruising and damage of the sole and white line, claw horn puncture resistance, growth and wear rate, condition score, milk yield, milk composition and health of dairy cattle.

5.2 MATERIALS AND METHODS

5.21 Animals, management and feeding

The claw horn samples use in this study were collected from research studies completed between the 1st Oct 2007 and 1 June 2008 according to local ethical procedures and protocols approved by the Massey University Animal Ethics Committee. A total of 36 heifers were selected at random from the dairy herd of 120 dairy cattle at Rodway Farm (Bridgwater College), Cannington, Somerset, UK and allocated according to calving date, breeding value, and body condition score (BCS) to one of three treatments (n =12). The heifers were offered a grass silage and forage maize silage diet (18.2 kg DM /h/day) (Table 5.1). In addition to the basal diets zn levels, the heifers were offered supplementary compound feed (maximum of 2 kg/animal/day) with either; Zn oxide (1 ZnOx) or organic Zn at 1.0 (1 OrZn) equivalent to 18.33 mg/kg DM or 0.3 (0.3 OrZn) equivalent to 6.11 mg/kg DM of NRC (2001) recommended levels (RL) from 21 d prepartum, until 150 d postpartum. This feed was introduced through an out of parlour (shed) feeder (Titan, Fullwood, UK), which allowed measurement of individual feed intake by means of weigh cells located in the bins and ear tag transponders. The diets were formulated in Ultamix professional, to be appropriate and adequate for

nutrient, macro and micro nutrient status. The heifers (from all treatments) were housed together either in straw bedded yards (7.5 m^2 / animal) prepartum and in cubicles / free stalls postpartum in accordance with DEFRA (2003) and BS 5052, part 40 (BS, 1990) standards. The passageways were scraped manually twice daily and all lactating animals were milked twice daily.

Table 5.1 Composition of the mixed ration (MR), compound, and mineral premix

Item	Diet content
Ingredient % of dietary DM	(%)
Maize silage	30.8
Grass silage	25.3
Protein blend	20.9
Permeate	2.6
Compound	9.3
Hay	3.3
Rolled wheat	5.5
Megalac	1.6
Mineral premix†	0.6
Chemical composition	
Dry Matter (%)	40.8
ME (MJ/kg DM)	12.4
NDF (%DM)	34.7
CP (%DM)	16.3
Sugars (%DM)	8.9
Starch (%DM)	14.9
Zn (mg/kg DM)	31.0

† The mineral and vitamin premix contained (/kg DM basis); macronutrients (%), Salt, 10.0, Calcium, 16.0, Phosphorus, 8.0 , Magnesium , 14.0, Sodium, 4.0; vitamins (iu/kg), Vit A 3000000, Vit D3 60000, Vit E 3250; micronutrients (mg/kg), Copper, 3000.0, Manganese, 1000.0, Cobalt, 34.65 ,Iodine, 300.0, Selenium, 50.0.

5.22 Milk yield and milk composition, live weight and body condition

Heifers were milked twice daily at approximately 0600 h and 1700 h. Milk yield and milk composition were measured weekly and composite milk samples were taken at 2 consecutive milking's (pm and am), which were analyzed using an Infrared Analyzer (Foss Electric, Hillerod, Denmark). The live weight, body condition and locomotion score were assessed weekly using 5 point scale scoring systems, with half points, in which 1 corresponded to thin and 5 to fat (Anon, 2001).

5.23 Locomotion score and claw assessment

The locomotion score of all animals was assessed weekly using a 5 point scale in which 0 and 1 corresponded with non lame animals, 3 an animal showing tenderness when walking, 4 an animal that is lame and 5 was an animal that is severely lame and non weight bearing on lame limb/s (Manson and Leaver, 1988). All lame animals were examined to determine the cause of lameness and the development of each lameness case was monitored.

The hind claws of heifers were assessed for sole ulcers, sole haemorrhage and heel erosion at -50, 0, 30, 60, 120, and 150 d pp. The haemorrhages on each foot were scored according to (Leach *et al.*, 1998). Haemorrhages were scored from 0 to 5, zero being a horn with no presence of haemorrhage and 5 being a horn presenting with of severe haemorrhage. Sole ulcers were graded from 6 to 8, depending on the exposure of the corium and the presence of infections. The affected areas of the sole and WL areas of the heifer's foot were outlined and subsequently photographed with a digital camera. The images were analysed for claw area, haemorrhage of WL and sole using Scion Image Analysis. The WL and sole areas of the claw horn were scored separately for number, percent and total score (percent x intensity of haemorrhage). Any other alterations present during examination, such as Digital dermatitis and heel horn erosion, were recorded.

The claw growth and wear rates were measured on the right rear inner claw at 0, 30, 60, 120, and 150 d pp. A mark was made using a soldering iron on the claw wall 2 cm below the coronary border. The distance from the coronary border to the mark and from the mark to the distal end of the wall was measured and a new mark was made 2 cm below the coronary border every time the hooves were measured. The monthly growth and wear rates were estimated from these measurements as described by (Clark and Rakes, 1982) and the claw angle, length of the front claw wall and height of the heel were measured at the same time of the claw growth and wear, according to (Boelling and Pollott, 1998).

5.24 Collection of claw horn samples

Samples of claw sole tissue were collected from all the hind left lateral claw of all experimental heifers at 0, 30, 60, 120, and 150 d pp. The samples were taken from the distal surface of the claw parallel to the ground surface from zones 2 and 5, according to the International foot map (IFM) (Shearer *et al.*, 2002). The first outer layer of horn (1 mm) was discarded and a sample of 0.1 to 2.5 mm thickness taken using claw trimming knives (variation in thickness occurred as a result of animal movement), transferred immediately to plastic bags, sealed and stored in a freezer for 1 month until analysis. The dry matter content of horn samples was determined using oven dry matter (DM) determination (100 °C for 72 hrs) according to (MAFF, 1986).

Prior to testing claw horn samples for PR or EM each sample was scored for level of haemorrhage, using a scale of 0 to 5 (0: No haemorrhage and 5: Severe haemorrhage according to the Leach *et al.*, (1997). The mean data from each animal was used to compare the effect of claw region of IFM from each animal on each mechanical test.

5.25 Puncture resistance of claw horn

Claw horn samples (n= 36) were analysed for puncture resistance (PR) as described by Winkler (2005) using a P/2N needle probe on a TA.XT plus Texture Analyser with a 5 kg load cell (Stable Micro-Systems, Vienna Court, Lammas Road, Godalming, Surrey, GU7 1YL, UK). The test probe was used at a speed of 1.0 mm/sec, which enabled the material to adapt to the load (Aranwela *et al.*, 1999) and measured the force in compression in test-mode. A force-displacement curve was recorded when the test probe reached the sample and a trigger force of 5 g had been applied; following this the probe travelled a distance of 12.0 mm before returning to the initial position. This distance was sufficient to allow the sample to be punctured and the maximum force on puncture to be measured. The maximum punch force values (Nmm^2) were obtained from the force-displacement curve. A total of 5 tests were completed on the sole (region 5 IFM) and white line (region 2 IFM) areas of each sample in accordance with Winkler and Margerison (2007). This was considered to be sufficient to detect test variations. The thickness of the sample on the tested area was recorded using callipers with a resolution of 0.01 mm.

5.26 Tensile Testing (elastic modulus)

The claw horn samples (n = 32, 32 out of the 36 samples collected in total were used because the other 4 samples were not large enough to obtain a suitable length section for tensile testing) were collected from region 5 of the IFM (Shearer *et al.*, 2002) and were cut into approx 1mm by 1mm by 10mm length sections and three replicates from each sample were then tested. Two small strips of carbon copy paper (typically 0.3 mm long 30 µm wide) required for strain measurement were temporarily adhered onto the surface of the samples, each of which was placed one third of the way along the length of each sample, from each end of the sample. The samples were then gripped by clamps in a universal testing machine with LVDT and video extensometer, which was a specialist machine, custom built specifically for the measurement of tensile characteristics of composite materials, such as claw horn, by Prof Smith at

Exeter University, UK). An overhead microscope was used to check sample alignment and measure strain in test.

The mechanical testing was carried out as previously described by Smith *et al.*, (2000) and in accordance of ASTM D3039/D3039m-07. The samples were tested in tension as per Smith et al (2000). Strain was measured via the overhead microscope using an edge following system (videoextensometer, Messphysik GmbH, Austria). The system edge follows the surface markers and reports their separation along the sample approx. every 200 milliseconds. This data was used to calculate the strain. Load data from the testing machine were also recorded every 100 miliseconds. The specimens were deformed in tension cyclically by 0.01 mm at a rate of 0.001mms^{-1} for five cycles, with measurements being taken from the fifth cycle.

The separation between markers was used to calculate engineering strain (ε) as:

$$\varepsilon = \frac{\Delta l}{l_0} \quad (1)$$

where Δl is the change in distance between the two markers and l_0 is the original distance between the two markers. The load data from the test machine software were used to calculate the stress (σ)as:

$$\sigma = \frac{P}{A_0} \quad (2)$$

where P is the force on the specimen and A_0 is its cross-sectional area (calculated as the product of width (measured using callipers) and thickness (measured in a scanning electron microscope)).

The slope of the stress vs. strain graph was taken as the elastic modulus (E). Also known as the Young's modulus, E was calculated from experimental data using a least-squares fit to a straight line over the rising portion of the fifth cycle of the stress versus strain data (Smith *et al.*, 2000).

The horn sample periods of 30 and 120 d pp were chosen to be PR and tensile tested because at 30 d pp greater number of samples of a suitable section size can be obtained and at 120 d pp because peak claw horn hemorrhaging in heifers has been reported to occur between 100- 120 d pp (Leach *et al.*, 1997; Offer *et al.*, 2003; 2000a; Winkler *et al.*, 2005).

5.27 Plasma mineral levels

Blood plasma samples were taken pre supplementation and 5 months after the start of supplementation. Blood plasma samples were collected by peripheral venepuncture to assess blood plasma mineral Fe and Zn levels pre and post experimental compound feeding. Colour metric analysis (Olympus AU 400) was used to calculate the blood plasma samples for Zn and blood plasma iron levels were determined by flame AA – atomic absorption by spectrum photometer at Veterinary Laboratories Agency Shrewsbury, UK.

5.28 Statistical Analysis

The data collection from dairy heifers was used as individual animal observations. All the data was assessed for normal distribution using the norm plot procedures in Minitab 15 (Minitab Inc., State College, PA) and found to be normally distributed, with the exception of locomotion score which remained not normally distributed and as such was analysed by Man Whitney, non parametric methods, using a confidence interval of 95%. The normally distributed data was used to assess the effect of treatment diet using analysis of variance (ANOVA), general linear modelling (GLM) command in Minitab 15.0, using a confidence interval of 95%, which included diet and IFM as fixed effects and animal as a random effect in the model, while horn or sole haemorrhaging, claw horn growth and wear, milk yield, milk composition, live weight and body condition score were applied as variables to be assessed. The PR data was compared by including the thickness of the claw horn sample as a covariate in the GLM ANVOA in Minitab 15.0 with a confidence interval of 95%. The existence of significant differences were assessed by applying Tukey's test when running the GLM

ANOVA in Minitab 15.0 and statistical differences were reported when the probability value (*P*) was <0.05 and a tendency reported when *P*<0.10 in the ANOVA table. All the data was presented as mean ± standard error of the means (sem) along with the individual *P* value.

5.3 RESULTS

Plasma Zn levels tended to increase in the post partum period, compared with pre-partum plasma levels, but neither the level of plasma Zn nor the increase in plasma Zn differed significantly between the differing levels or sources of Zn offered (Table 5.2). Plasma iron levels remained similar during the pre and post partum periods and there were no differences between plasma iron levels in plasma from animals supplemented with differing Zn sources or levels either pre or postpartum (Table 5.2).

Table 5.2 Plasma iron and zinc levels of cattle pre and post supplementation of zinc oxide (1 ZnOx) or organic zinc at 1.0 (1 OrZn) or at 0.3 (0.3 OrZn) of NRC (2001) recommended levels,

	1 ZnOx	1 OrZn	0.3 OrZn	SEM	P
Plasma iron levels (umol/l)					
Prepartum	27.8	26.5	22.4	3.03	0.439
Postpartum	29.1	25.6	29.6	2.97	0.598
Difference (+/-)	1.3	-0.9	7.2	3.44	0.262
Plasma zinc levels (umol/l)					
Prepartum	9.9	8.5	7.2	1.87	0.295
Postpartum	13.6	12.8	12.6	0.82	0.651
Difference (+/-)	3.7	4.3	5.4	1.107	0.557

There were no differences for median locomotion score between the different forms of Zn or levels of supplementation. All groups started with locomotion score of 1, which peaked at locomotion score of 3 at 70 d pp (Zn Oxide) 89.6 d pp (1 OrZn) and 52.5 d pp (0.3 OrZn). There were no differences in the sole and white

line haemorrhage number, percentage of area or total lesion score for white line haemorrhaging in hind claws of heifers offered different forms of Zn or levels of supplementation between 50 d pre up to 150 d postpartum (Tables 5.3 and 5.4).

Table 5.3 Number of haemorrhages, percentage of claw area affected by haemorrhages and total haemorrhage score of the white line (WL) in hind claws of cattle offered zinc oxide (1 ZnOx) or organic zinc at 1.0 (1 OrZn) or at 0.3 (0.3 OrZn) of NRC (2001) recommended levels

	1 ZnOx	1 OrZn	0.3 OrZn	SEM	P
White line (WL) haemorrhaging					
Number of WL haemorrhages					
-50 d (peripartum)	0.4	0.0	0.3	0.19	0.353
0 d	0.1	0.2	0.1	0.125	0.998
30 d	0.3	1.4	2.1	0.685	0.141
60 d	2.3	2.9	3.4	0.99	0.698
120 d	2.3	2.1	3.7	0.89	0.388
150 d	2.3	3.0	3.4	0.98	0.733
Percentage of WL haemorrhage					
-50 d (peripartum)	2.8	0.0	4.7	2.11	0.262
0 d	1.3	1.1	1.6	1.39	0.960
30 d	6.2	34.8	65.7	17.16	0.057
60 d	44.9	59.5	70.0	21.48	0.677
120 d	59.2	56.7	80.6	23.53	0.744
150 d	60.3	72.7	87.9	24.85	0.705
Total WL haemorrhage score					
-50 d (peripartum)	2.8	0.0	4.7	2.11	0.262
0 d	1.3	1.1	1.6	1.39	0.981
30 d	8.0	36.9	55.5	17.6	0.143
60 d	50.8	70.3	70.0	23.1	0.808
120 d	85.3	84	103.7	37.9	0.925
150 d	84.2	81.7	111.2	33.5	0.801

Table 5.4 Number of haemorrhage, percentage of claw area affected by haemorrhages and total haemorrhage score of sole haemorrhage in hind claws of cattle offered zinc oxide (1 ZnOx) or organic zinc at 1.0 (1 OrZn) or at 0.3 (0.3 OrZn) of NRC (2001) recommended levels

	1 ZnOx	1 OrZn	0.3 OrZn	SEM	P
Sole (S) haemorrhaging					
Number of S haemorrhages					
-50 d (peripartum)	0.2	0.0	0.5	0.25	0.352
0 d	0.4	0.2	0.2	0.28	0.824
30 d	1.2	1.2	1.1	0.44	0.991
60 d	2.4	2.7	2.3	0.49	0.837
120 d	4.0	5.0	4.5	0.56	0.396
150 d	3.9	5.6	4.6	0.69	0.199
Percentage of S haemorrhage					
-50 d (peripartum)	0.1	0.0	1.6	0.53	0.329
0 d	0.1	0.9	0.3	0.41	0.291
30 d	3.7	4.2	7.6	1.97	0.343
60 d	13.7	18.0	15.3	4.88	0.804
120 d	39.8	41.9	40.0	16.71	0.996
150 d	45.2	60.8	68.4	16.57	0.602
Total S haemorrhage score					
-50 d (peripartum)	0.1	0.0	1.1	0.53	0.329
0 d	0.1	0.9	0.3	0.42	0.305
30 d	7.5	9.1	7.6	4.14	0.956
60 d	36.8	42.1	21.7	15.26	0.603
120 d	86.0	117.1	99.3	25.58	0.644
150 d	77.4	116.6	85.6	20.12	0.335

The mean growth, wear and net change in claw horn were not different between animals offered Zn oxide and organic Zn at differing levels.

There were significant (P<0.001) increases in the sole and white line haemorrhage numbers, percentage of area or total score for sole haemorrhaging of hind claws between 50 days prepartum and 150 days postpartum (Table 5.6).

Table 5.5 Post partum (pp) growth, wear and net change rates of wall horn of hind left lateral claw of cattle offered zinc oxide (1 ZnOx) or organic zinc at 1.0 (1 OrZn) or at 0.3 (0.3 OrZn) of NRC (2001) recommended levels

	1 ZnOx	1 OrZn	0.3 OrZn	SEM	P
Growth (mm)					
0 to 30 d pp	4.4	5.1	3.7	0.60	0.601
30 to 60 d p	2.5	2.0	2.4	0.44	0.667
60 to 120 d p	2.3	3.4	2.9	0.50	0.260
120 to 150 d p	1.8	0.8	1.7	0.60	0.410
Wear (mm)					
0 to 30 d pp	4.5	4.8	4.0	0.61	0.145
30 to 60 d p	0.6	1.3	2.2	0.63	0.220
60 to 120 d p	1.6	2.1	1.8	0.95	0.685
Net change (mm)					
0 to 30 d pp	-0.1	0.3	-0.3	2.18	0.299
30 to 60 d p	1.9	0.7	0.2	0.63	0.170
60 to 120 d p	0.2	-1.3	-0.1	0.58	0.917

Table 5.6 Effect of days pp on mean number of sole and white line haemorrhages, percentage of claw affected by haemorrhages, total haemorrhage score, claw horn growth and wear rate in hind claws between 50 d pre partum and 150 d post partum

	Days peripartum						sem	P Value
	-50	0	30	60	120	150		
Sole								
Haemorrhages (No.)	0.2 ^{c}	0.3 ^{c}	1.1 ^{c}	2.4 ^{b}	4.5 ^{a}	4.7 ^{a}	0.28	<0.001
Haemorrhage (%)	0.4 ^{b}	0.4 ^{b}	5.2 ^{b}	15.7 ^{b}	40.6 ^{a}	58.1 ^{a}	5.65	<0.001
Total score	0.4 ^{b}	0.4 ^{b}	8.0 ^{b}	33.5 ^{b}	100.8 ^{a}	93.2 ^{a}	8.29	<0.001
White line								
Haemorrhages (No.)	0.2 ^{b}	0.1 ^{b}	1.3 ^{b}	2.8^{a}	2.7^{a}	2.9^{a}	0.43	<0.001
Haemorrhage (%)	2.5 ^{b}	1.3 ^{b}	35.5 ^{ab}	58.1 ^{a}	65.5 ^{a}	73.6 ^{b}	10.07	<0.001
Total score	25 ^{c}	1.3 ^{d}	33.5 ^{c}	63.7 ^{b}	91.0 ^{a}	92.3 ^{a}	13.37	<0.001
Claw horn								
Growth rate (mm)			4.4 ^{a}	2.3 ^{b}	2.9 ^{b}	1.4 ^{c}	0.37	<0.001
Wear rate (mm)			3.0	3.0	3.8		0.83	0.812
Net change (mm)			1.4	-0.7	-0.9		0.904	0.141

$^{a, b, c}$ - Means followed by differing superscript letters differ significantly

Table 5.7 Mean mechanical properties of claw horn taken from regions 2 and 5 of the International foot map (IFM) at 30 and 120 days postpartum of dairy cattle offered zinc oxide (1 ZnOx) or organic zinc at 1.0 (1 OrZn) or at 0.3 (0.3 OrZn) of NRC (2001) recommended levels

	Time d pp			Treatment				P value	
	30	120	sem	1 ZnOx	1 OrZn	0.3 OrZn	sem	Trt	d pp
Sole									
Punch resistance (N mm^2)	15.9	13.3	1.75	18.0	13.6	12.0	2.31	0.146	0.324
Elastic modulus (E MPa)	872.4	518.7	113.26	745.1	661.2	631.3	151.2	0.859	0.009
White line									
Punch resistance (N mm^2)	9.8	6.9	1.14	9.3	7.9	7.9	1.75	0.683	0.093
Dry matter (%)	92.6	90.9	1.22	92.1	90.2	93.1	1.45	0.334	0.576

Table 5.8 Mean heel depth and claw diagonal and dorsal border size, and claw angle of hind outer claws of dairy cattle offered zinc oxide (1 ZnOx) or organic zinc at 1.0 (1 OrZn) or at 0.3 (0.3 OrZn) of NRC (2001) recommended levels

	1 ZnOx	1 OrZn	0.3 OrZn	SEM	P
Heel depth (mm)					
0 d pp	36.3	36.4	33.4	1.53	0.335
30 d pp	34.4	36.0	33.2	1.12	0.195
60 d pp	33.9	34.8	32.7	1.31	0.489
120 d pp	34.6	37.3	35.2	1.91	0.563
150 d pp	38.2	43.2	40.9	1.91	0.165
Diagonal measure (mm)					
0 d pp	123.8	126.4	124.8	2.296	0.684
30 d pp	124.5	125.5	123.8	2.002	0.817
60 d pp	122.5	125.3	122.5	2.102	0.588
120 d pp	126.4	128.8	127.2	1.65	0.541
150 d pp	128.4	128.2	127.5	1.63	0.952
Claw angle (degrees)					
0 d pp	45.5	47.0	46.0	0.57	0.157
30 d pp	47.0	47.7	48.3	0.61	0.315
60 d pp	47.7	47.7	47.6	0.51	0.992
120 d pp	49.4	47.8	48.2	0.91	0.408
150 d pp	47.7^{b}	49.2^{ab}	50.0^{a}	0.53	0.021
Dorsal border (mm)					
0 d pp	88.4	88.2	89.2	2.41	0.968
30 d pp	88.6	85.2	83.3	2.28	0.242
60 d pp	87.7	88.2	86.8	1.71	0.933
120 d pp	89.3	86.7	90.1	2.51	0.593
150 d pp	87.8	87.1	86.7	2.53	0.976

The wall horn growth rate was significantly affected by the number of days postpartum (Tables 5.6, 5.7 and 5.9), while the wear rates were not significantly affected by postpartum period, which was equivalent to a net loss 3.2 mm of claw horn between 60 to 150 days in lactation (Table 5.6).

There was no significant difference between animals offered Zn oxide or differing levels of organic Zn in relation to mean foot angle, heel depth, dorsal border length, diagonal claw length or claw horn dry matter content, elastic modulus or PR of claw horn from either the sole or white line (Tables 5.7, 5.8 and 5.9). The number of day's pp did significantly increase claw angle and heel depth (table 5.9). The elastic modulus reduced significantly with increasing number of days postpartum, while the PR did not change significantly with increasing number of days postpartum. Elastic modulus and PR decreased with the level of haemorrhaging (with the exception of a score of 3 for EM and 1 for PR) however, not significantly (Score, EM MPa, 0 = 899.0, 1= 639.4, 2= 431.4; 3 = 812.3, sem 193.46; PR N mm^2, 0= 11.4, 1=14.7, 2= 4.1, 3= 2.9; sem 7.69). Claw horn sample thickness had a significant effect on PR ($P < 0.001$) mean thickness being 1.49 mm.

The mean milk yield and fat corrected yield (Table 5.10) were significantly higher ($P<0.001$) for cattle offered Zn oxide and organic Zn at 1.0 of NRC (2001) RL compared to those offered organic Zn at 0.3 of NRC (2001) RL. While there were no significant differences in milk fat, protein, lactose concentrations (g/kg) or somatic cell count levels (SCC) (00,000/ml) between differing sources and levels of Zn. The total milk fat and protein yields were not significantly different between cattle offered Zn at NRC RL (organic and Zn oxide) or differing levels of organic Zn (1.0 or 0.3 of NRC RL). However, cattle offered Zn Oxide at the NRC RL ($P<0.05$) had significantly higher total milk fat and protein yields than those offered organic Zn at 0.3 of NRC (2001) RL.

Table 5.9 Effect of number of days pp (-50 to 150 d pp) and Zn supplementation zinc oxide (1 ZnOx) or organic zinc at 1.0 (1 OrZn) or at 0.3 (0.3 OrZn) of NRC (2001) recommended levels on mean number, percentage and total haemorrhage score of sole and WL haemorrhages, hoof conformation and claw horn growth & wear rate in hind claws

	Days peripartum								
	-50			0			30		
	1 ZnOx	1 OrZn	0.3 OrZn	1 ZnOx	1 OrZn	0.3 OrZn	1 ZnOx	1 OrZn	0.3 OrZn
S haemorrhage									
Number	0.2 f	0.0 f	0.5 f	0.4 f	0.2 f	0.2 f	1.2 f	1.2 f	1.1 f
(%)	0.14 d	0.0 d	1.06 d	0.07 d	0.87 d	0.31 d	3.69 d	4.25 d	7.57 d
Total score	0.14 e	0.0 e	1.06 e	0.07 e	0.93 e	0.31 e	7.45 e	9.06 de	7.57 e
WL									
Number	0.4 c	0.0 c	0.3 c	0.1 c	0.2 c	0.1 c	0.3 c	1.4 b	2.2 ab
(%)	2.78 d	0.0 d	4.66 d	1.29 d	1.12 d	1.64 d	6.1 d	34.76 cd	65.72 ab
Total score	2.78 d	0.0 d	4.68 d	1.29 d	1.12 d	1.64 d	8.01 d	36.91 c	55.48 b
Diagonal (mm/m)				123.8	126.4	124.8	124.5	125.5	123.8
Claw angel (^{0})				45.50 ed	47.0 c	46.0 d	47.0 c	47.7 c	48.3 bc
Heel depth (mm/m)				36.30 cde	36.44 cde	33.40 e	34.36 e	36.00 cde	33.18 ef
Dorsal border (mm/m)				88.4	88.2	89.2	88.6	85.2	83.3
Growth rate (mm/m)							4.41 ab	5.15 a	4.00 bc
Wear rate (mm/m)							4.55	4.83	3.51
Net change (mm/m)							-0.14	0.32	-0.03

Table 5.9 continued.

	Days peripartum									sem	P Value	
	60			120			150					
	1 ZnOx	1 OrZn	0.3 OrZn	1 ZnOx	1 OrZn	0.3 OrZn	1 ZnOx	1 OrZn	0.3 OrZn		Trt	Days
Sole haemorrhage												
Number	2.4^{de}	2.7^{cd}	2.3^{de}	4.0^{b}	5.0^{ab}	4.5^{ab}	3.9^{bc}	5.6^{a}	4.6^{ab}	0.674	0.378	<0.001
(%)	13.67^{d}	18.02^{d}	15.29^{d}	39.78^{c}	41.94^{bc}	39.99^{c}	45.22^{bc}	60.80^{ab}	68.41^{a}	9.641	0.649	<0.001
Total score	36.77^{cd}	42.09^{c}	21.70^{cde}	86.00^{b}	117.14^{a}	99.33^{ab}	77.44^{b}	116.55^{a}	85.64^{b}	14.146	0.222	<0.001
White line												
Number	2.2^{ab}	2.9^{ab}	3.4^{a}	2.3^{ab}	2.1^{b}	3.7^{a}	2.3^{ab}	3.0^{a}	3.4^{a}	0.774	0.171	<0.001
(%)	44.87^{bc}	59.4^{ab}	70.04^{ab}	59.22^{ab}	56.67^{ab}	80.64^{ab}	60.31^{ab}	72.66^{ab}	87.86^{a}	17.954	0.082	<0.001
Total score	50.81^{b}	70.3^{abc}	70.04^{abc}	85.30^{ab}	83.98^{ab}	103.75^{a}	84.18^{ab}	81.67^{ab}	111.15^{a}	23.321	0.363	<0.001
Diagonal (mm/m)	122.2	125.3	122.5	126.4	128.8	127.2	128.4	128.2	127.5	3.219	0.147	0.069
Claw angel (^{0})	47.7^{c}	47.7^{c}	47.6^{c}	49.4^{ab}	47.8^{c}	48.3^{bc}	47.7^{c}	49.2^{ab}	50.0^{a}	0.641	0.339	<0.001
Heel depth (mm/m)	33.92^{ef}	34.83^{def}	32.73^{f}	34.58^{def}	37.33^{cd}	35.18^{c}	38.25^{bc}	43.17^{a}	40.91^{ab}	1.607	0.034	<0.001
Dorsal border (mm/m)	87.7	88.2	86.8	89.3	86.7	90.1	87.8	87.1	86.7	2.309	0.621	0.561
Growth (mm/m)	2.5^{ef}	2.0^{f}	2.4^{e}	2.3^{e}	3.4^{cd}	2.9^{de}	1.8^{f}	0.8^{g}	1.7	0.39	0.771	<0.001
Wear (mm/m)	0.6	1.3	2.2	1.6	2.1	1.8				2.115	0.585	0.812
Net change (mm/m)	1.9	07	0.2	0.7	1.3	-0.1				1.05	0.421	0.141

$^{a, b, c}$ - Means followed by differing superscript letters differ significantly

Table 5.10 Mean milk yield, fat corrected yield, milk composition and somatic cell count (SCC) of cattle offered zinc oxide (1 ZnOx) or organic zinc at 1.0 (1 OrZn) or at 0.3 (0.3 OrZn) of NRC (2001) recommended levels

	1 ZnOx	1 OrZn	0.3 OrZn	SEM	P
Milk yield (kg/h/d)	28.06[a]	27.35[a]	26.43[b]	0.323	0.001
Fat corrected yield (kg/h/d) †	25.6[a]	25.0[a]	24.2[b]	0.34	0.006
Fat yield (g/kg)	36.9	36.7	36.7	0.41	0.973
Protein (g/kg)	31.9	31.6	31.4	0.23	0.637
Milk lactose (g/kg)	46.2	46.2	46.3	0.14	0.978
Total fat (kg/d)	1.02 [a]	1.00 [a b]	0.96 [b]	0.014	0.011
Total protein (kg/d)	0.89 [a]	0.86 [a b]	0.83 [b]	0.011	0.001
SCC (00,000 cells / ml)*	58.8	57.3	59.3	16.38	0.997
Live weight (kg)	534.7	537.6	531.5	3.30	0.847
Body condition score (1 to 5)	2.09	2.14	2.11	0.022	0.637

* Geometric Mean

† Corrected to 40.0 g/kg of milk fat.

[a, b, c] - Means followed by differing superscript letters differ significantly

The mean live weight and body condition score (BCS) were not significantly different for animals offered organic Zn compared to the Zn Oxide and organic Zn at 0.3 NRC (2001) RL (Table 5.10).

5.4 DISCUSSION

Blood plasma Zn levels were low prior to the research taking place, despite the heifers receiving a diet with Zn mineral supplementation. Plasma Fe levels were in the normal range for bovine blood plasma and were therefore not sufficiently high enough to affect the absorption/ bioavailability of Zn. The blood plasma samples taken at the end of the research showed that plasma Zn had increased though not significantly and the heifers were no longer deficient and plasma Fe levels remained within the normal parameters for iron levels meeting the animal

requirements during the research period (Kincaid, 1999). These results show that the supplementation forms and levels were sufficient to increase the plasma Zn. However, there were no significant differences between treatments for plasma Fe or Zn levels which coincides with previous findings (Kessler *et al.*, 2003; Malcolm-Callis *et al.*, 2000; Spears, 1989) stating that plasma Zn levels were not affected by Zn source. This did not support the theory that inorganic salts are hydrolysed in the digestive tract to form free ions which are very reactive and can form complexes with other dietary molecules (Close, 2002). Whereas chelated micronutrient are suggested to be more stable in the digestive tract due to both chemical charge (electron neutral, ligand and metal make up) and physical structure (size and ligand source) thus protected from forming complexes with other dietary components that would otherwise inhibit its absorption (Paik, 1999, Spears, 1996). This is then further influenced by the animals' age, sex, stage of growth, pregnancy, lactation, nutritional status, disease, gastrointestinal secretions and microflora as well as gastrointestinal transit time (Fairweather-Tait, 1996; Johnson, 1989). The use of chelated minerals in previous research has also shown unequivocal findings (Boland *et al.*, 1996; Manspeaker *et al.*, 1987; Stanton *et al.*, 2000). However, Kessler *et al.* (2003), Bazle (1993) and Moore *et al.* (1988) have all found improvements in claw horn quality or lower cases of claw horn diseases when the cattle were supplemented with organic sources of Zn.

Supplementing cattle diets with organic forms of zinc has been demonstrated by Stern *et al.*, (1998) to improve clinical claw status, horn quality and tensile strength scores of beef cattle when compared to supplementing diets with zinc oxide. In this research there were no significant differences between supplementing the heifers' diets with either Zn oxide or organic Zn at either 1.0 or 0.3 NRC (2001) RL for median locomotion score; mean sole and white line haemorrhaging up to 150 d pp. Toni *et al.* (2007) also found no significant differences between locomotion scores of animals offered either inorganic minerals or organic trace minerals. Nocek *et al.* (2006) found solar

haemorrhaging was not affected by trace minerals (Zn, Mn, Cu, and Co) fortification level (at 0.75 to 1.0 of NRC recommended levels) or source (inorganic or organic). EM was higher in sole and PR was higher for both sole and white line claw horn from heifers supplemented with Zn oxide compared to the heifers offered organic Zn supplementation but these differences were not significant. These results do not coincide with those of Stern *et al.* (1998) which showed a significant improvement in clinical claw status, microscopic horn quality of the coronary band, and tensile strength scores of cattle offered Zn amino acid and Zn polysaccharide. The research of Kessler *et al.* (2003) suggested that, even though not significantly improved, bulls offered Zn proteinate tended to have a better histological score and higher tensile strength of claw horn compared to the other Zn sources. Therefore the results from measurements taken to evaluate lameness and claw horn strength did not reflect previous indications, as Zn has a critical role in forming the horn of the claw and has been found to increase claw integrity by increasing wound healing, the rate of epithelial tissue repair and maintenance of cellular integrity (Smart and Cymbaluk, 1997). Zn is also known to be involved in the process of keratin generation and in collagen and skin nucleic acid synthesis. Moreover, when nutrient supply to keratin-forming cells is compromised or completely interrupted, inferior keratinized tissue, i.e., horn, is produced, which may lead to increased susceptibility to claw disorders and ultimately to lameness (Műlling *et al.*, 1999). However, micronutrient status within the animal can be slow to respond to dietary micronutrient supplementation, while significant improvements in claw health and horn quality in response to micronutrients has been found to take as long as 15 months (Hedges *et al.*, 2001). As a consequence longer term study of the effect of zinc warrants consideration.

The PR data from this research has showed a greater range for both sole (7.7 to 18.0 MPa) and white line (4.7 to 11.6 N MPa) PR than those stated by Winkler (2005) of 8.6 to 10.7 MPa in sole and 5.24 to 6.9 MPa. However, there is a large sem (4.85) on the PR data suggesting variability within the claw horn samples.

This could be due to varying thickness of the claw horn samples, however sample thickness was used as a covariate when completing statistical analysis as it was found to significantly affect the PR of claw horn samples, as also found by Winkler (2005).

Unfortunately, due to the nature of working with a natural product and live animals, some variation in sample thickness was unavoidable. Some animals were quiet which enabled a thinner sample to be taken but if an animal was moving around it made it more difficult to take samples and generally resulted in a thicker sample being taken. Sample thickness ranged from 0.45 to 2 mm from sole horn and 0.3 to 1.75 for white line horn which were collected using a hoof knife. Winkler (2005) used a wood plane to collect horn samples on some experiments, but came to the conclusion that for PR the inclusion of sample thickness as a covariate in the analysis of variance was more effective. While PR is significantly affected by the variation in thickness of claw horn sample, sample thickness is used successfully as a covariate in ANCOVA when analysing PR data as found by Winkler (2005). A different texture analyser was used in this research. Unfortunately it would not load the macro used for previous PR research and as a result a different macro was used to determine initial peak PR. This may have resulted in a larger range of results than previously found by Winkler (2005). Thickness of samples was found to significantly affect the PR of claw horn samples, which was also as found by Winkler (2005).

At 150 d pp haemorrhage scores had not started to decline, as 150d pp was the last observation point, it remains unknown whether 150d pp was the peak in haemorrhage score. This observation is unusual as Leach *et al.* (1997), Offer *et al.* (2000), and Winkler (2005) have shown that haemorrhaging peaked in first lactation dairy heifers between 100 and 120 days postpartum and reduces in number and severity thereafter. In this research, the number of days postpartum resulted in a significant increase in the total sole haemorrhage score (-50 d pp: 0.4, 150d pp: 93.2 (± 8.29)) and white line total haemorrhage score (-50d pp: 25,

150 d pp: 92.3 (± 13.37). Both the elastic modulus and PR declined significantly between 30 and 120 d pp. This corresponds with the research of Winkler (2005) who found a decrease in claw horn EM and PR up to 150d pp. PR and elastic modulus also decreased, though not significantly, with increasing haemorrhage score, which are similar to the results of Winkler (2005) who had higher haemorrhage scores and found that increasing haemorrhage score significantly decreased the PR of claw horn. With the exception of Winkler (2005) no other authors have compared mechanical properties or the effect of haemorrhage score on these properties. Kempson and Logue (1993) stated that claw horn quality can be reduced by elements of blood that seep out from damaged capillaries leaking across the basement membrane separating the dermis from the epidermis. Leach *et al.* (1997) found in autumn calving heifers that the combined effects of calving, housing and the lactating diet resulted in a combination of insults to the corium, which manifested in the form of haemorrhages of the white line and sole and increased locomotion score and lameness. Histological studies carried out on samples of claw horn showed that sole haemorrhages were virtually always accompanied by histological and morphological changes in the laminar region where the horn of the white line is generated (Leach *et al.*, 1997).

Parturition, lactation, housing and environment, age and season have been found to be highly correlated with lameness (Logue, 1999). The samples used for this research were taken from heifers that were housed over winter in the same building and fed the same pre and post partum diet to try to limit the number of factors that were going to impact on the level of lameness experienced. All the heifers in this study had also undergone parturition and the hormonal changes associated with parturition that are believed to be major contributory factor in the development of sole and white line lesions (Holah *et al.*, 2000; Tarlton *et al.*, 2002). The heifers may also have experienced some level of negative energy balance, as dry matter intake can decrease by approximately 10 to 30 % in late pregnancy and early lactation, that is related to the mobilisation of

body reserves (Hoblet and Weiss, 2001) which is known to be significantly correlated with locomotive problems during lactation (Collard *et al.*, 2000). Any one of these factors could have resulted in decreases in elastic modulus and PR.

The findings from the methods used to test the mechanical properties of claw horn are determined by the constituent materials and or the structure. However, these methods are considering different aspects. The elastic modulus (stiffness/ flexibility) is a product of the constituent materials (keratin and ICS in this case) and the cellular and intercellular microarchitecture (Mulling *et al.*, 1999). Therefore elastic modulus is a measure of the bonds and transfer of stress within the microarchitecture. That will help determine the probability of crack propagation (Kasapi and Gosline, 1999). Whereas PR considers the peak force required to puncture the material and may be thought of as a compound product of tensile and shear strengths, which are dependent upon both the constituent materials' properties and the microarchitecture. As such, changes in PR as a result of either changes in the constituent materials or the structure could indicate the likelihood of stone / foreign body penetration of claw horn. As such, changes in PR and EM could indicate that something in either the constituent materials or the structure has altered. The most likely reason for a lack of significant difference in the PR compared with EM is a combination of the lower amplitude of change in PR and the greater variability found in PR.

Longer term duration experiments are more likely to observe improvements in claw horn health i.e. reduction in haemorrhages or claw horn integrity. Hedges *et al.* (2001) undertook research for 18 months on Biotin based on going beyond the complete horn renewal at approximately 15 months. While Nocek *et al.* (2006) did not find a reduction of sole and white line haemorrhages until the 2nd lactation when trace minerals were supplemented as organic complexes or inorganic forms. Thus treatment period in this research of approximately 5-6 months may not have been sufficient to detect an effect of Zn oxide Vs organic Zn on locomotion scores, claw haemorrhaging and structural integrity. Toni *et al.*

(2007) calculated that the horn capsule of the claw is a composite of horn produced over the past 12 to 30 months. The research of Greenough (1997) showed that the dorsal surface of the claw grows at an average rate of 2.5 mm/month in beef cattle and 5 to 6 mm/month in intensively fed cattle and the length of the dorsal wall of the medial claw is approximately 7.5 cm. Wall horn growth rates in dairy cattle have been found to be around 5.0 mm/month (Table 1.1 and 1.2) with greater variation in wear rate during lactation (4.0 to 7.5) than growth rate. This can result in a negative net equity of claw horn when wear exceeds growth, resulting in thin soles more prone to damage i.e. stone penetration.

Changes in the shape of the claw after the start of the lactation have been established by Winkler (2005) and Offer *et al.* (2000). There were no significant differences for claw conformation measurements of the heifers supplemented with either Zn oxide or organic Zn at either 1.0 or 0.3 NRC (2001) RL. However, in the current study there was a significant increase in the lateral heel depth and the claw angle of hind hooves during the postpartum period. Offer *et al.* (2000) and Winkler, (2005) have both reported that 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. These changes were considered to be probably related to changes in the growth and wear rates (Offer *et al.*, 2000).

Level and form of Zn supplementation did not affect claw horn growth and wear rates of the heifers. Livesey and Laven (2007) also found no effect on growth and wear rates when supplementing heifers with 1.15 of the dietary requirement for Zn with Zn methionine compared to the un-supplemented diet which provided 0.95 of the Zn dietary requirement. In this research the mean monthly growth and wear rates were similar to those previously reported by Leach *et al.* (1997); and Clark and Rakes (1982). However, the number of days post partum did have a significant effect on the growth rate of the heifers claw horn, which is similar to Livesey and Laven (2007) who found that growth rate was affected by time after

calving. Although Offer *et al.* (2001) and Leach *et al.* (1997) reported the mean growth rate was not significantly affected by time after calving. However, in this study growth rate did not exceed wear and negative net growth was still occurring at 120 d pp, which is comparable to Offer *et al.* (2001) observations of negative net growth until 10 weeks pp and Leach *et al.* (1997) until 20 weeks pp. However, both Offer *et al.* (2001) and Leach *et al.* (1997) connected the negative net growth back to increased wear rates and the changes the animals experienced due to calving and housing. However, Livesey and Laven (2007) found that net growth rates return to pre-partum levels by 6 weeks pp and suggested the possible differences between wear rates between experiments could be due to differences in the environment, for example, in the roughness of the concrete in yards and standings, the amount of standing water, the stocking density, previous exposure to concrete and the distances walked each day by the cows. Several factors can potentially affect the rate of growth of horn, genetics, nutrition (Clark and Rakes, 1982), wear rate (Manson and Leaver 1988) and the conformation of the cow. All of which along with accuracy when measuring growth and wear rates, could have resulted in differences between experiments. The heifers in this research were balanced for PIN values, calving date, and BCS, housed in the same building and offered the same diet with the exception of the form and level of zinc supplemented. As the supplementation source or level did not affect growth and wear rates it would suggest that that the negative net growth rates experienced in this research are a product of all the aforementioned factors (genetics, nutrition, environment); as no one factor can be singled out as the cause of the claw horn wear exceeding growth resulting in negative net change in claw horn.

Smith *et al.* (1999) demonstrated that feeding supplemental Zn methionine to Holstein cows increased production of 3.5% fat-corrected milk however, the feed already contained four times the NRC (2001) Zn RL. In the current research milk yield, corrected milk yield total fat and protein results show that there was no difference between the Zn Oxide and organic supplemented at 1.0 NRC RL

however, there were significantly lower results for the organic Zn supplemented at 0.3 NRC RL which suggests that the Zn levels were not sufficient to meet the heifer's requirements. This differs from Cope *et al.*, (2009) findings which showed Zn fed organic form at the NRC (2001) had higher milk production than those fed zinc oxide NRC (2001) RL and organic Zn at a lower level than NRC (2001) RL. Cope *et al.*, (2009) also found no significant differences in milk composition between levels and forms of Zn. Zinc requirements for dairy cows vary by stage of lactation and milk production creates a significant drain on zinc stores, thus zinc requirements are highest in early lactation (NRC, 2001). During periods of dietary mineral insufficiencies, essential minerals could be mobilised from body tissues to support milk production, which ultimately affects the quality and quantity of milk as well as reproduction (Manspeaker *et al.*, 1987). Whitaker *et al.* (1997) also found that there were no significant differences for milk yield, milk composition and SCC between the heifers receiving organic Zn or inorganic form of Zn. Spain *et al.* (1993) again found milk production and SCC did not differ between cows fed Zn oxide and those offered 0.50 of their supplemental Zn from the proteinate form and the remaining 0.50 from oxide. However, Nocek *et al.* (2006) reported an increased milk production in animals receiving diets containing organically complexed minerals and a mixture of inorganic and organically complexed minerals. However, the diets in Nocek *et al.* (2006) research were supplemented with a mixture of complexed minerals and supplemented in excess of NRC (2001) requirements, and one specific mineral cannot be acknowledged for the enhanced milk yield. Ashmead *et al.* (2004) found that there was not a significant difference in milk yield between inorganic and organic mineral supplementation until the third lactation where there was a 11.5% difference in favour of the organic mineral supplementation ($P<.005$). Average daily milk production increased 10.9% from the first lactation to the third lactation in the inorganic mineral group compared to 23.3% in the organic mineral group.

Ashmead *et al.* (2004) research found cattle offered organic minerals had significantly higher BCS when compared to cattle offered inorganic minerals. The findings in this research have shown that supplementary Zn form and level had little effect on BW or BCS in dairy heifers. As live weight and BCS were slightly higher for the organic Zn supplement at 1.0 NRC RL compared to the Zn Oxide and organic Zn 0.3 NRC RL but not significantly. Whitaker *et al.* (1997) also found no significant differences for live weight and BCS between the heifers receiving differing forms of Zn (Zn proteinate or inorganic Zn). Similarly, Nocek *et al.* (2006) and Cope *et al.*, (2009) reported no significant effect when Zn was fed as either as inorganic or organically chelated on BW or BCS. Many of the studies (Ashmead *et al.*, 2004, Manspeaker *et al.*, 1987, Nocek *et al.*, 2006, Uchida *et al.*, 2001) have focused on a combination of minerals rather than one specific mineral i.e. Zn and have found significant reductions in haemorrhages and improved production and reproduction. Supplementation for multiple lactations (Ashmead *et al.*, 2004, Nocek and Johnson, 2000, Nocek *et al.*, 2006) has also shown significant improvements in claw health and production. Therefore, focusing on longer supplementation of minerals or by using a combination of minerals may conceivably better suit today's dairy cow's requirements.

5.5 CONCLUSIONS

Neither the level nor the form of Zn supplementation significantly affected the locomotion score, claw horn characteristics, sole or white line haemorrhaging or wall horn growth and wear rates, indicating no promotion of claw health or prevention of lameness from offering organic minerals or disadvantage of offering 0.3 of NRC RL of Zn, compared with NRC RL levels of Zn oxide from 21 d pre-partum till 150 d postpartum. However, the length of the period of supplementation can affect the effect of micronutrients on claw horn integrity and haemorrhaging (Ashmead *et al.*, 2004, Nocek and Johnson, 2000, Nocek *et al.*, 2006) and it is recommended that further research be completed over a more extended supplementation and animal observation period of approximately 15

months is recommended and as such larger groups of animals would be required, to allow for infertility / empty rates and delayed conception, and ensure that sufficient animals are available for claw examination during the subsequent lactation.

Animals offered Zn at 1.0 (full) NRC (2001) recommended levels from either Zn oxide or organic Zn sources produced significantly higher mean milk yield, corrected milk yield and total fat and protein yields than animals offered organic Zn at 0.3 of NRC (2001) requirements, which supports the current RL for Zn and that Zn levels should not be lowered to 0.3 of NRC RL when this type of organic Zn is being offered to lactating dairy cattle.

This research illustrates the changes which occur over the postpartum period in claw horn in terms of increasing numbers, percentage and total score of sole haemorrhage and white line haemorrhages and the decrease in claw horn strength (puncture resistance and elastic modulus) and claw horn growth rate. These results indicate a reduction in claw horn mechanical strength, health and potential alterations in the constituent materials or the structure during the postpartum period, which has significant implication for claw horn function and penetration. However, greater variability in the PR data in this particular experiment, compared to other experiments, made it difficult to assess and compare changes in EM and PR during the postpartum period. Further research to assess and compare PR and EM would be useful to allow mechanical testing to be developed to assess changes in claw horn strength and function. This research would facilitate the assessment and selection of factors that increase and maintain claw horn strength and function and reduce lameness during the lactation of dairy cattle.

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5.7 REFERENCES

Anon. 2001. Condition scoring of dairy cattle PB6492 .Defra Publications, London.

Aranwela, N., G. Sanson, and J. Read. 1999. Methods of assessing leaf-fracture properties. New Phytologist. 144:369-393.

Ashmead, H. D., S. D. Ashmead, and R. A. Samford. 2004. Effects of metal amino acid chelates on milk production, reproduction, and body condition in holstein first calf heifers. International Journal Applied Research Veterinary Medicine. 2:252-260.

Ballantine, H. T., M. T. Socha, D. J. Tomlinson, A. B. Johnson, A. S. Fielding, J. K. Shearer, and S. R. van Amstel. 2002. Effects of feeding complexed zinc, manganese, copper and cobalt to late gestation and lactating dairy cows on claw integrity, reproduction and lactation performance. The Professional Animal Scientist. 18:211–218.

Bazle, F. K. 1993. The effect of zinc methionine in a mineral mixture on gain and incidence of footrot on steers grazing native grass pastures. Journal of Dairy Science. 76 (Suppl. 2):36 (Abstr).

Boelling, D. and G. E. Pollott. 1998. Locomotion, lameness, hoof and leg traits in cattle. I. Phenotypic influences and relationships. Livestock Production Science. 54:193-203.

Boland, M. P., G. O'Donnell, and O. C. D. 1996. The contribution of mineral proteinates to production and reproduction in dairy cattle. Pages 95–103. in Alltech's 12th Annual Symposium Biotechnology in the Feed Industry. Nottingham University Press, Lexington, Kentucky.

British Standards. 1990. British Standard on the design and construction of cattle buildings. TSO Publications, London.

Clark, A. K. and A. H. Rakes. 1982. Effect of methionine hydroxy analog supplementation on dairy cattle hoof and composition. Journal of Dairy Science. 65:1493-1502.

Clarkson, M. J., D. Y. Downham, W. B. Faull, W. J. Hughes, F. J. Manson, J. B. Merritt, R. D. Murry, W. B. Russell, J. Sutherst, and W. R. Ward. 1996.

Incidence and prevalence of lameness in dairy cattle. The Veterinary Record. 138:563-567.

Close, W. H. 2002. Trace mineral nutrition in pigs: working within the new recommendations Pages pp.401-406 in Nutritional biotechnology in the food and feed Industries; Alltech's 18th Annual Symposium Nottingham University Press.

Collard, B. L., P. J. Boettcher, J. C. M. Dekkers, D. Petitclerc, and L. R. Schaeffer. 2000. Relationships between energy balance and health traits of dairy cattle in early lactation. Journal of Dairy Science. 83:2683-2690.

Cope, C. M., A. M. Mackenzie, D. Wilde, and L. A. Sinclair. 2009. Effects of level and form of dietary zinc on dairy cow performance and health. Journal of Dairy Science. 92:2128–2135

DEFRA. 2003. Code of Recommendations for the Welfare of Livestock: Cattle. Pages 17-19. Defra Publications, London.

Eddy, R. G. and C. P. Scott. 1980. Some observations on the incidence of lameness in dairy cattle in Somerset. The Veterinary Record. 106:140-144.

Fairweather-Tait, S. J. 1996. Bioavailability of dietary minerals. Biochemical Society Transactions. 24:775–780.

Greenough, P. R. 1997. Basic concepts of bovine lameness. in Lameness in Cattle. 3rd ed. A. D. Weaver, ed. W. B. Sanders Co, Philadelphia, PA.

Greenough, P. R. and J. J. Vermunt. 1991. Evaluation of subclinical laminitis in a dairy-herd and observations on associated nutritional and management factors. Veterinary Record. 128:11–17.

Gunter, S. A., E. B. Kegley, G. C. Duff, and D. Vermeire. 1999. The performance by steers fed different zinc sources before and during receiving at a New Mexico feedlot. Journal Animal Science. 77 (Suppl. 1).19 (Abstract).

Hoblet, K. H. and W. Weiss. 2001. Metabolic hoof horn disease – claw horn disruption. Veterinary Clinics of North America. 17:111-127.

Holah, D. E., K. M. Evans, G. R. Pearson, J. F. Tralton, and A. J. F. Webster. 2000. The histology and histopathology of the support structures in the

laminated region of the bovine hoof in maiden heifers and around the time of first calving. Pages 94-96 in The Eleventh International Symposium on Lameness of the Ruminant Digit, Parma, Italy.

Hedges, V. J., R. Blowey, A. J. Packington, C. J. O'Callaghan, and L. E. Green. 2001. A longitudinal field trial of the effect of biotin on lameness in dairy cows. Journal of Dairy Science. 84:1969-1975.

Hosnedlová, B., M. Šoch, and T. Trávníček. 2005. Effect of the additive intake of zinc on its content in blood plasma of dairy cows. in XIIth ISAH Congress on Animal Hygiene. Vol. 49-Animals and environment Volume 1 Warsaw Poland.

Johnson, P. E. 1989. What can in vitro methods tell us about mineral availability? Biological Trace Element Research. 19:3–10.

Kempson, S. A. and D. N. Logue. 1993. Ultrastructural observations of hoof horn from dairy cows: changes in the white line during the first lactation. The Veterinary Record. 132:524-527.

Kessler, J., M. I., F. A. Dufey, A. Gutzwiller, A. Stern, and H. Geyes. 2003. Effect of organic zinc sources on performance, zinc status, and carcass, meat, and claw quality in fattening bulls. Livestock Production Science. 81:161–171.

Kincaid, R. L. 1999. Assessment of trace mineral status of ruminants: A review. Pages 1-10 in American Society of Animal Science. Indianapolis, Indiana, USA.

Kossaibati, M. A. and R. J. Esslemont. 1999. The incidence of lameness in a group of dairy herds in England. Page 221 in BSAS Annual Meeting. BSAS, Scarborough.

Leach, K. A., D. N. Logue, S. A. Kempson, J. E. Offer, H. E. Ternent, and J. M. Randall. 1997. Claw haemorrhages in dairy cattle: Development of sole and white line haemorrhages during the first lactation. Veterinary Journal 154:215-225.

Leach, K. A., D. N. Logue, J. M. Randall, and S. A. Kempson. 1998. Claw haemorrhages in dairy cattle: Methods for assessment of sole and white line haemorrhages. Veterinary Journal. 155:91-102.

Livesey, C. T. and R. A. Laven. 2007. Effects of housing and intake of methionine on the growth and wear of hoof horn and the conformation of the hooves of first-lactation Holstein heifers. Veterinary Record. 160:470-476.

Logue, D. N. 1999. An overview of lameness in the dairy cow. Page 2 in Proceedings of the National Cattle Lameness Conference.

MAFF. 1986. Analysis of Agricultural Materials RB429. ADAS, London.

Malcolm-Callis, K. J., G. C. Duff, S. A. Gunter, E. B. Kegley, and D. A. Vermeire. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing beef steers. Journal Animal Science. 78:2801-2808.

Manson, F. J. and J. D. Leaver. 1988. The influence of concentrate amount on locomotion and clinical lameness in dairy cattle. Animal Production. 47:185-190.

Manspeaker, J. E., M. G. Robl, G. H. Edwards, and L. W. Douglass. 1987. Chelated minerals: Their role in bovine fertility. Veterinary Medicine. 82:951–956.

Mills, C. F. 1987. Biochemical and physiological indicators of mineral status in animals: copper, cobalt and zinc. Journal Animal Science. 65:1702-1711.

Műlling, C., H. Bragulla, S. Reese, K. D. Budras, and S. W. 1999. How structures in bovine hoof epidermis are influenced by nutritional factors. Anatomia Histologia Embryologia-Journal of Veterinary Medicine Series C-Zentralblatt Fur Veterinarmedizin Reihe C. 28:103–108.

Műlling, C. H. 2000. The use of nutritional factors in prevention of claw diseases-Biotin as an example for nutritional influences on formation and quality of hoof horn. Pages 78–80 in 11th International Symposium on Disorders of the Ruminant Digit. Parma, Italy.

Mulling, C. K. W., H. H. Bragulla, S. Reese, K. D. Budras, and W. Steinberg. 1999. How structures in bovine hoof epidermis are influenced by

nutritional factors. Anatomia Histologia Embryologia-Journal of Veterinary Medicine Series C-Zentralblatt Fur Veterinarmedizin Reihe C. 28:103-108.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th ed. National Academy Press, Washington DC.

Nocek, J. E. 1997. Bovine Acidosis Implications on Laminitis. Journal Dairy Science. 80:1005–1028.

Nocek, J. E. and A. B. Johnson. 2000. Digital characteristics in commercial dairy herds fed metal-specific amino acid complexes. Journal Dairy Science. 83:1553-1572.

Nocek, J. E., M. T. Socha, and D. J. Tomlinson. 2006. The Effect of Trace Mineral Fortification Level and Source on Performance of Dairy Cattle. Journal of Dairy Science. 89:2679 – 2693.

Offer, J. E., G. E. J. Fisher, S. A. Kempson, and D. N. Logue. 2001. The effect of feeding grass silage in early pregnancy on claw health during first lactation. The Veterinary Journal. 161:186-193.

Offer, J. E., D. N. McNulty, and D. N. Logue. 2000. Observations of lameness, hoof conformation and development of haemorrhages in dairy cattle over four lactations. The Veterinary Record. 147:105-109.

Paik, I. K. 1999. Strategies to reduce environmental pollution from animal manure: Nutritional management option - Review. Asian-Australasian Journal of animal sciences. 12:657-666.

Rojas, L. X., L. R. McDowell, R. J. Cousins, F. G. Martin, N. S. Wilkinson, A. B. Johnson, and J. B. Velasquez. 1995. Relative bioavailability of two organic and two inorganic zinc sources fed to sheep. Journal Animal Science. 73:1202–1207.

Sandström, B. 2001. Micronutrient interactions: effects on absorption and bioavailability. British Journal Nutrition. 85:Suppl 2:S181-185.

Schalm, O. W. 1980. Blood and blood-forming organs. Page 791 in Bovine Medicine and Surgery II. H. E. Amstutz, ed. American Veterinary Publication, Santa Barbara, CA.

Shearer, J. K., E. Belknap, S. Berry, C. Guard, K. Hoblet, E. Hovingh, G. Kirksey, A. Langill, and S. R. van Amstel. 2002. The standartisation of input codes for capture of lameness data in dairy records Pages 346-349 in Proceedings of the Twelfth International Symposium on Lameness in Ruminants, Orlando, Florida.

Smart, M. and N. F. Cymbaluk. 1997. Role of nutritional supplements in bovine lameness in Lameness in Cattle 3rd ed. P. R. Greenough and A. D. Weaver, ed. W. B. Sanders Co, Philadelphia, PA.

Smith, C. W., R. Herbert, R. J. Wootton, and K. E. Evans. 2000. The hind wing of the dessert locust (Schistocerca Gregaria Forskal) II. Mechanical properties and funtioning of the membrane. The Journal of Experimental Biology. 203:2933–2943.

Smith, M. B., H. E. Amos, and M. A. Froetschel. 1999. Influence of Ruminally Undegraded Protein and Zinc Methionine on Milk Production, Hoof Growth and Composition, and Selected Plasma Metabolites of High Producing Dairy Cows. The Professional Animal Scientist. 15:268–277.

Spain, J. N., Hardin. D., B. Steevans, and J. Thome. 1993. Effect of organic zinc supplementation on milk somatic cell count and incidence of mammary gland infections of lactating dairy cows. Journal Dairy Science. 76 (Suppl. 1): (abstr).

Spears, J. W. 1989. Zinc methionine for ruminants: relative bioavaiability of zinc in lambs and effects of growth and performance of growing heifers. Journal Animal Science. 67:835–843.

Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. Animal Feed Science Technology. 58:151–163.

Stanton, T. L., J. C. Whitter, T. W. Geary, C. V. Kimerling, and A. B. Johnson. 2000. Effects of trace mineral supplementation on cow – calf performance, reproduction, and immune function. The Professional Animal Scientist. 16:121-127.

Stern, A., H. Geyer, I. Morel, and K. J. 1998. Effect of organic zinc on horn quality in beef cattle. Pages 233–235 in 10th International Symposium on Lameness in Ruminants. P. Ossent, Ed, Lucerne, Switzerland.

Tarlton, J. F., D. E. Holah, K. M. Evans, S. Jones, G. R. Pearson, and A. J. F. Webster. 2002. Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving. Veterinary Journal. 163:196-204.

Toni, F., L. Grigoletto, C. J. Rapp, M. T. Socha, and D. J. Tomlinson. 2007. Effect of Replacing Dietary Inorganic Forms of Zinc, Manganese, and Copper with Complexed Sources on Lactation and Reproductive Performance of Dairy Cows. The Professional Animal Scientist. 23:409–416.

Uchida, K., P. Mandebvu, C. S. Ballard, C. J. Sniffen, and M. P. Carter. 2001. Effect of feeding a combination of zinc, manganese and copper amino acid complexes, and cobalt glucoheptonate on performance of early lactation high producing dairy cows. Animal Feed Science and Technology. 93:193-203.

Vermunt, J. J. 2004. Herd lameness - a review, major causal factors, and guidelines for prevention and control. in Prophylaxis of Claw Diseases Proceedings of the 13th International Symposium and 5th Conference on Lameness in Ruminants, Maribor, Slovenija.

Whitaker, D. A., H. F. Eayres, A. K., and J. M. Kelly. 1997. No Effect of a Dietary Zinc Proteinate on Clinical Mastiffs, Infection Rate, Recovery Rate and Somatic Cell Count in Dairy Cows. The Veterinary Journal. 153:197-204.

Winkler, B. 2005. Mechanical properties of hoof horn, sole haemorrhage and lameness in dairy cattle. PhD dissertation. Plymouth University, Plymouth.

Winkler, B. and J. K. Margerison. 2007. Relationships between locomotion and haemorrhage score, punch resistance and Holstein (HUKI) conformation scores. Journal Animal Science. 85: Suppl. 1 (Abstract):423.

Chapter 6.0

Final discussion

6.0. FINAL DISCUSSION

The aims of this thesis were to use existing methods to monitor lameness and claw health by using standard methods of locomotion scoring, claw assessment techniques, and to assess the structural integrity of claw horn using mechanical techniques such as puncture resistance, Vickers hardness and elastic modulus. To increase the reproducibility/reliability of elastic modulus results from bovine claw horn the tensile testing equipment located at Exeter University was used. A number of experiments were completed to assess factors that could potentially affect claw health, structural integrity and lameness in growing and first lactation dairy cattle.

Throughout the animal based research animals were assessed for milk yield and composition and lameness using locomotion score and lesion score. The mechanical tests were then used to establish the structural integrity of growing cattle, the effects of nutrition in the form of supplementation of live yeast (at 10 billion/h/d), and zinc (zinc oxide or zinc organic at the NRC recommended levels for zinc and zinc complexed zinc at 0.3 of the NRC), parturition, IFM, haemorrhaging, severity of haemorrhaging and consequential changes. The rear claws were assessed and claw horn, haemorrhage (sole and white line), claw growth and wear rate, claw measurements and horn sample DM content were measured.

6. 1 Factors affecting mechanical tests

The research by Winkler (2005) demonstrated that PR from claw horn can produce reliable reproducible results using a relatively small number of replicates. Husain *et al.* (2002) and Lewis (2002) also stated that PR is frequently used to determine mechanical properties of small or miniature specimens. Aranwela *et al.* (1999) confirmed the repeatability of PR to detect the fracture properties in leaves. The research from this PhD has also produced the same reliable replicable work, which corresponded with the findings of Winkler's research into the mechanical properties of claw horn. The reliability of each

method was determined by taking repeated measurements (using each method i.e. PR) and then the difference between each repeat measurement was used to determine how repeatable the method was.

Elastic Modulus and Vickers hardness were also used as comparative approaches to determine the mechanical properties of bovine claw horn, as previously utilised by researchers (Dyer *et al.*, 2004; Franck *et al.*, 2006; Hinterhofer *et al.*, 2005b; Winkler, 2005 and Zoscher *et al.*, 2000). The technique employed in this research to calculate elastic modulus however, uses a videoextensometer to record the movement of the markers on the sample. This was deemed to be important in the assessment of claw horn due to its elastic and composite nature and has not previously been used for the assessment of bovine claw horn. This technique has been used by Smith *et al.* (2000) to determine the mechanical properties of the hind wings of locust, confirming the method's repeatability and suitability for use on small samples. As there are no recognised standard methods specifically dedicated for claw horn ASTM 3039D303M-07 the standard test method for tensile properties of polymer matrix composite materials was used. As claw horn is a natural biological composite formed in the main from α keratin which is capable of accommodating and resisting *in vivo* loads without excessive deformation or catastrophic failure (Newlyn *et al.*, 1999). The Vickers hardness test results in this thesis were comparable to those of Hedges *et al.* (2002). However, a test load of 100g rather than 20g was used as the residual impression were larger which enabled greater precision and repeatability when measuring the residual impressions (Johnson and Rapoff, 2007), but had not been previously applied to bovine claw horn.

The claw horn specimen size and thickness is an important consideration when implementing techniques to determine the mechanical properties of any material and bovine claw horn is no exception. Unfortunately, due to the nature of working with a natural product and live animals, some variation in sample thickness was unavoidable. Some animals were quiet which enabled a thinner

sample to be taken but if an animal was moving around it made it more difficult to take samples and generally resulted in a thicker sample being taken. Sample thickness ranged from 0.45 to 2 mm from sole horn and 0.3 to 1.75 for white line horn which were collected using a hoof knife. Winkler (2005) used a wood plane to collect horn samples on some experiments, but came to the conclusion that for PR the inclusion of sample thickness as a covariate in the analysis of variance was more effective. While PR is significantly affected by the variation in thickness of claw horn sample, sample thickness is used successfully as a covariate in ANCOVA when analysing PR data as found by Winkler (2005). This is further substantiated by the research in this thesis. This approach reduces sample preparation time and has given reliable and repeatable results.

The ASTM 3039D303M-07 has set guidelines to ensure the production of reliable data, to test the significance of treatment differences and the calculation for elastic modulus uses specimen size to determine the resulting elastic modulus. The claw horn samples were therefore cut to produce a 1 mm by 1 mm to 10mm specimen or a specimen to fit within those ratios. Vickers hardness samples required the samples to be thick enough to ensure that the indenter would not penetrate the resin which holds the specimens in place and thus produce false results. As a consequence, samples were between 5 to 10 mm in width and at least 1 mm in thickness prior to mounting and testing for Vickers hardness. However, despite these efforts EM and Vickers hardness in this research did not produce more repeatable results than PR, but demonstrated the need for a range of mechanical properties that need to be considered to characterise the complex nature of bovine claw horn.

6.2 Claw horn moisture content

The mechanical properties of keratinous and other biological materials are strongly influenced by the state of hydration. The moisture content of claw horn has been found to affect the values of hardness, elastic modulus, bending stiffness, puncture resistance and fracture toughness of claw horn (Collins *et al.*,

1998; Hinterhofer *et al.*, 1998; Baillie *et al.*, 2000; Winkler, 2005). The moisture content of claw horn varied between the chapters/experiments in this thesis; Chapter 5 /zinc: 90.2 to 93.1 (%); Chapter 3: Lactating heifers; 85.1 to 81.5 (%), dairy breed; 80.4 to 82.9 (%); horn pigment; 79.6 to 83.7 (%); Chapter 4: Mechanical tests; 88.4 to 93.8 (%) and the majority of these fell into ranges previously found (Baggott *et al.*, 1988; Hinterhofer *et al.*, 2005b; Winkler and Margerison, 2004; Zoscher *et al.*, 2000). Variation in moisture content can be a result of the drying process as determining DM from small sample sizes can be problematic (Hinterhofer *et al.*, 2005b), as drying can cause differing rates of water and volatile loss from claw horn samples. This can make the determination of the moisture content of claw horn very variable, making comparison between results difficult. There is also no agreed standard method for DM calculation (Reilly *et al.*, 2002). Therefore a standardised method for dry matter determination and the effect of differing drying temperatures, times and methods could be considered a valuable area to be for further research.

The results from chapter 4 on the mechanical properties of claw horn show that there is a strong relationship between claw horn moisture content and elastic modulus, as fully hydrated samples were significantly less stiff than those of claw horn at natural physiological moisture content. This corresponds with Dyer *et al.* (2004) findings that there was a significant difference between the elastic modulus of dry claw horn and fully hydrated claw horn. These results show that hydration of claw horn decreases stiffness, making it potentially more susceptible to increased wear rates, sole penetration and bruising from external trauma. Therefore dairy producers should consider whether herd management practices could result in cows with high claw horn moisture and as a result increased lameness levels or factors that could prevent moisture penetration of claw horn and subsequent reductions in structural strength, as demonstrated in Chapter 4. This research shows there were no significant changes in claw horn stiffness with changing RH which is consistent with the findings of Hinterhofer *et al.* (1998). Moisture content was shown to be more important than RH by Hinterhofer *et al.*

(1998), as the elastic modulus of conditioned claw horn wall and sole samples (at a relative humidity of 65 %) were not statistically significant to those of non conditioned samples. 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. Therefore storage after sample collection until analysis is very important to maintain the correct physiological moisture and to prevent unnecessary inaccuracies in data produced from mechanical property tests. The prevention of moisture loss was thoroughly described by Collins *et al.* (1998) and Hinterhofer *et al.* (1998) and methods of prevention were described in their methodology. Collins *et al.* (1998) wrapped the samples in three layers of para film and stored them at 4^{o}C and Hinterhofer *et al.* (1998) and Winkler and Margerison (2004, 2006, 2007, 2002) kept the samples in re-sealable plastic bags at 4^{o}C. Winkler and Margerison (2004) study showed that storing samples in plastic bags a at 4^{o}C for up to 8 days did not result in significant changes of the samples moisture content and freezing for up to 30 days had no significant effect on the DM and PR of the sole or white line horn. In the short term, moisture loss is a key factor that is likely to have the greatest effect on the mechanical properties of claw horn samples, while the ability of claw horn to resist moisture penetration is likely to be a key issue in the retention of structural integrity and function thus affecting horn wear rates, claw horn puncture and penetration, and subsequent lameness. This is likely to be an important factor which could result in higher levels of claw infection and lameness in situations where claw horn is subjected to long periods in a high moisture environment, e.g. during housing, while on stand off and pasture in high rain fall periods. The increase in lameness in moist environmental conditions could be related to a number of factors, including infection, high claw wear rates, potentially thin sole and poor protective ability of the sole, WL and sole penetration.

6.3 Sole and white line haemorrhages

The findings from Chapters 2, 3, 4 and 5 shows that haemorrhage levels significantly reduce claw horn integrity. PR decreases with increasing lesion

score with the exception of score 4 (0, 9.4; 1, 9.1; 2, 8.6; 3, 5.3; 4, 9.7; 8, 2.2; sem 0.85), which demonstrates the reduced ability of claw horn to resist the stresses and strains of animal locomotion and claw function. However the elastic modulus and Vickers hardness results did not show a significant decrease where the PR showed a significant reduction in lesion score in Chapters 4 and 5. Winkler (2005) found that lesion score was significantly negatively correlated to stiffness. In this thesis the elastic modulus showed that with increasing lesion score claw horn stiffness decreased significantly (with the exception of lesion score 3) and Pearson's correlation found it to be significantly negatively correlated to stiffness. With the exception of Winkler (2005), no other authors have compared mechanical properties or the effect of lesion score on claw horn stiffness and the method used in this thesis has never been applied to bovine claw horn. Borderas *et al.* (2004) found negative correlations between various hardness measurements and claw health scores, stating that 'sound' cows had harder claws than cows with injured claws. However, a definition of sound or injured claws was not related to lesion score. This research demonstrated the reduction in PR with increasing number of day's pp, peaking at 50 to 110 d pp. In this thesis, further to any previous research, lower levels of haemorrhage were found in Jersey cross bred cattle and concurrent higher levels of claw horn PR. Similarly, Friesian cattle offered yeast in mixed rations had lower haemorrhage and concurrent higher levels of claw horn PR. Both these areas warrant further research to be completed on the effect of these (yeast and effect of breed and individual animals) on claw horn haemorrhage and lameness.

Leach *et al.* (1997) found that in autumn calving heifers, the combined effects of calving, housing and the lactating diet resulted in insults to the corium and that these were manifested in the form of an increase in locomotion score and haemorrhages of the white line and sole. Claw horn quality can be reduced by blood elements which have seeped out from damaged capillaries leaking across the basement membrane separating the dermis from the epidermis manifesting as sole horn lesions (Kempson and Logue, 1993). Despite these findings of

lesions and reduced claw horn quality induced by insults to the corium as a result of factors that occur pre and post partum e.g. nutrition, environment, and claw growth/wear etc. (Leach *et al.*, 1997; Livesey and Laven, 2007; Webster, 2001) further work with different techniques to determine the mechanical properties of claw horn are still required, with a further undertaking of histological research into the changes in keratin composition with increasing lesion score to determine whether lesion score can be correlated more closely with deterioration in mechanical properties of claw horn.

6.4 White line and sole claw horn

The results from the collection of papers presented in this thesis show that white line claw horn is structurally weaker than sole claw horn. With typical white line (zone 2) PR scores ranging from 5.64 to 6.8 N mm^2 compared with sole (zone 4) which ranged from 8.1 to 10.4 N mm^2 showing the PR force required to puncture or penetrate claw horn from the white line was lower than the force required for sole horn, agreeing with the findings of Mulling *et al.* (1994) and Winkler and Margerison (2004). In contrast to Borderas *et al.* (2004) and Dyer *et al.* (2004) where WL was stronger than sole claw horn. The results in this thesis demonstrate that claw horn from the white line is structurally weaker than the sole horn. The horn of the white line is a result of high horn production resulting in soft horn with higher levels of lipids in the medullary cells, abundant amounts of ICS with high lipid content and inside a broad intercellular space, which is not as mechanically stable (Budras *et al.*, 1996) as sole horn.

Very few authors who have stated which specific region of the white line or sole in terms of IFM the claw horn samples have been taken from (Tables 1.4, 1.5 and 1.6), which makes it difficult to compare results as the zone which claw horn is taken from significantly affects its mechanical properties. The research of Winkler (2005) corresponds to the research in this thesis showing that zone 2 is has less structural integrity than the claw sample taken from zone 4, which is contrast to Dyer *et al.* (2004) where zones 4 and 5 EM was lower when compare

to zones 2 and 3. Dyer *et al.* (2004) also found that zone 3 had a higher elastic modulus when compared to zones 2, which is contrary to the results of Hedges *et al.* (2002). In assessing claw horn the MC must always be considered when interpreting results as Dyer *et al.* (2004) used claw horn samples that had been fully dehydrated (kept at room temperature for 48 hrs) whereas Hedges *et al.* (2002) used samples that had been hydrated (placed in sterile water and frozen) suggesting that the samples would have been fully hydrated. As Dyer *et al.* (2004) demonstrated fully hydrated claw horn has significantly reduced tensile strength and elastic modulus which is one potential reason for the differences between results from Hedges *et al.* (2002) and Dyer *et al.* (2004). Therefore there is still conflicting results being produced regarding the structural integrity of white line and sole claw horn. However it would be easier to make more informed conclusions when researchers used the zones of the IFM when stating where claw samples were collected. Further potential research could be completed to assess the mechanical properties of claw horn taken from all regions of the IFM at physiological moisture content and after samples were fully hydrated.

6.5 The number of days postpartum

The number of days postpartum had a significant effect on structural integrity of claw horn as confirmed in all the chapters presented in this thesis. This showed that there was a reduction in structural integrity up to and between 100 to 150 d pp for both elastic modulus and PR. The elastic modulus showed a decline in modulus between 30 and 120 d pp. PR results also showed a significant reduction between 30 and 120 d pp in Chapter 4 and 50 to 150 d pp in Chapter 2. This corresponds with the research of Winkler (2005) who found a decrease in claw horn stiffness and PR up to 150 d pp. The Vickers hardness tests did not show significant differences in claw horn properties, though there was a decrease in hardness common with the decline in stiffness. Very few authors have observed the effect of stage of lactation on the mechanical properties of claw horn. Winkler (2005) found a significant decrease in PR from 30 d pp to 160 d pp before increasing at 270 d pp.

This research has shown that the days postpartum significantly affected the number, percentage and total lesion score for both sole and white line at peak haemorrhaging as shown by Chapter 3 (NZ) where peak lesions occurred at 110 d pp and had declined by 160 d pp and Chapter 2 (UK) showed a similar pattern where peak lesions were seen at 100 d pp and levels had reduced by 150 d pp. Chapter 5 (UK) differed in that d pp significantly affected lesion score but at 150 d pp lesion scores had not started to decline and the last observation point was 150 d pp so in this experiment it remains unknown whether 150 d pp was the peak in lesion score. This observation is unusual as Leach *et al.* (1997), Offer *et al.* (2000b), and Winkler (2005) have shown haemorrhaging levels peak in lactating dairy heifers between 100 and 120 days *postpartum* and reduces in number and severity thereafter which concurs with Chapters 2 and 3. While absolute comparison cannot be made across years it is useful to compare the results of differing chapters and experiments (Fig 6.1).

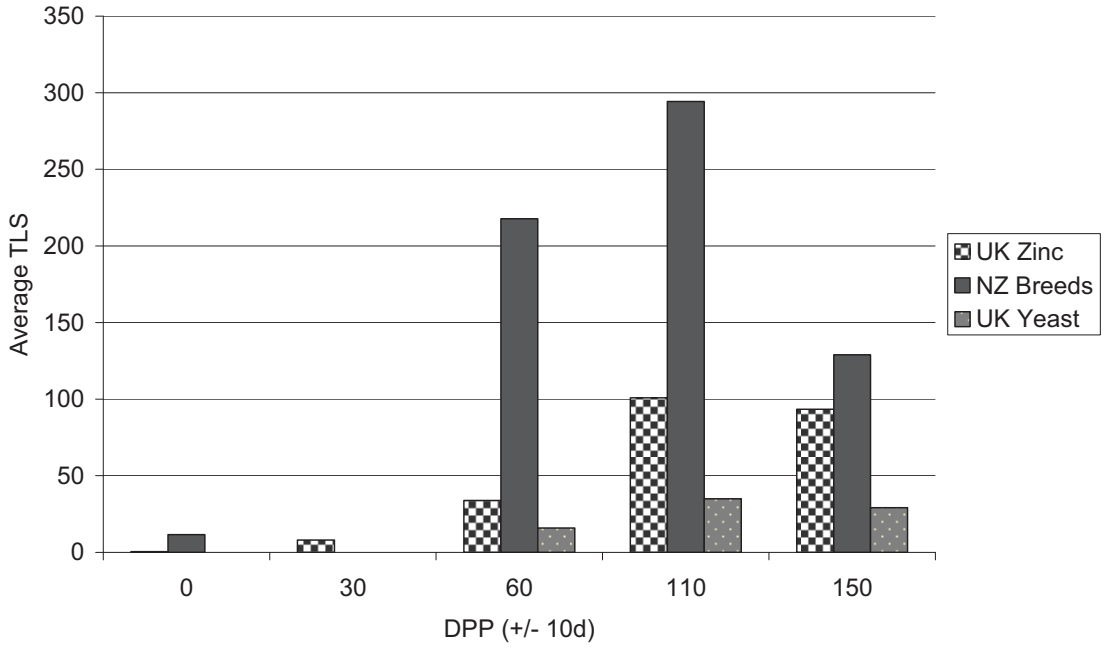

Figure 6.1 Average total lesions score (TLS) of sole lesions for the research undertaken in Chapters 2 (yeast), 3 (NZ breeds) and 5 (zinc).

A similar pattern (Figs 6.1 and 6.2) for TLS to peak approx 110 to 120 days occurs irrespective of location. However the UK based research in Chapters 2 and 5 have far lower TLS for both sole and white line lesions that were found in the research completed in NZ (Chapter 3) by the same observer and method. This could be a result of the effects of parturition, nutrition, environment or claw growth/wear (Leach *et al.*, 1997; Livesey and Laven, 2007; Webster, 2001) and of animals walking long distances or the maintenance of cow tracks and / or animal handling (Chesterton *et al.*, 1989).

There are a limited number of published papers detailing the incidence and prevalence of lameness in NZ (Chesterton *et al.*, 2008). Current research is being completed by a number of researchers in New Zealand. However, at present there are no published data on the number, percentage and severity of CHL according to differing areas of the claw during the postpartum period. It is very difficult therefore to determine the 'normality' of the pattern and levels of CHL and whether under further investigation into aetiology and pathogenesis of lameness in cattle under New Zealand conditions show similarities or differences from the UK/ Northern hemisphere. It is likely that there will be key differences that need to be elucidated to ensure a complete understanding and potential reduction of lameness of dairy cattle in New Zealand.

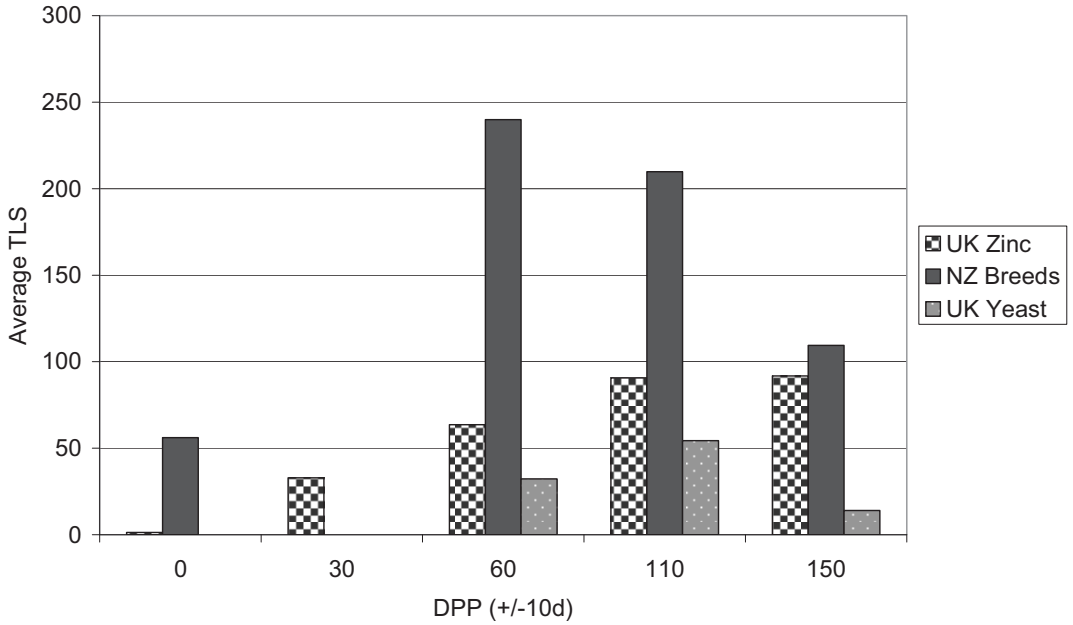

Figure 6.2 Average total lesions score (TLS) of white line lesions for the research undertaken in Chapters 2 (yeast), 3 (NZ breeds) and 5 (zinc)

6.6 Locomotion score

Locomotion score did not indicate any significant lameness problems in any of the experiments in Chapters 2, 3 or 5. The median locomotion score of 1 with a peak score for chapter 5 was 2 which occurred at 71 d pp. The mean locomotion score of 1.35 with a peak lesion score for chapter 2 was 4 which occurred at 84 d pp. However, there were cases of digital dermatitis which could result in a high locomotion score. Chapter 3 showed a median locomotion score of 1, peaking at locomotion score of 4 at 98 d pp. Locomotion score has been shown to reflect a similar trend to lesion score increasing with d pp until peaking between 100 to 120 days pp (Leach *et al.*, 1997, Offer *et al.*, 2000a, Vermunt and Greenough, 1996, Winkler, 2005). Parturition and the hormonal changes associated with parturition are believed to be major contributory factors in the development of sole and white line lesions. As hormonal changes cause the suspensory structure i.e. collagen fibres which connect to the pedal bone to the horn capsule which allows compression of the corium by the pedal bone (Holah *et al.*, 2000, Tarlton

et al., 2002). The heifers may also have experienced some level of negative energy balance (NEB), as dry matter intake can decrease by approximately 10 to 30 % in late pregnancy and early lactation. This is mediated by hormones and neuropeptides which is related to the mobilisation of body reserves (Hoblet and Weiss, 2001) and can result in dairy animals going into a NEB known to be significantly correlated with locomotive problems during lactation (Collard *et al.*, 2000).

The inability to detect dietary effects on locomotion score could be a result of the low incidence levels of lameness detected and/ or due to the subjective nature of locomotion score (Toni *et al.*, 2007). Locomotion score combined with the lesion score data demonstrates the resistance of dairy cattle to limp/show poor locomotion, potentially due to innate survival mechanisms. Therefore it is important to maintain the same observer recording locomotion to ensure no discrepancies in data collection and ensure any fine changes in locomotion are observed, which could be the case when multiple observers are used. However, despite this locomotion score and early detection of lameness (locomotion score 3 on a scale of 1 to 5) rather than locomotion score 4 is of key importance in practical dairy management on farm in reducing the economic and welfare impacts of lameness. It was particularly important to check locomotion score data for normality as this data may not always be normally distributed. As a result the data may have to be transformed to become normally distributed so it could be analysed using a parametric analysis e.g. ANOVA or if still not normally distributed it would then have to be analysed using a non parametric analysis i.e., Kruskal-Wallis. It also highlights the need for the measurement of factors such as lesion score (sole and WL) and PR etc over postpartum period to assess claw horn factors that are related to the development of lameness. The assessment of locomotion score using mechanical methods has received research attention and the continued development of automated systems to detect changes in locomotion is of great value to the dairy industry and animal welfare.

6.7 Growth and wear rates

The supplementation of yeast (Chapter 2) showed that neither supplemented or non supplemented heifers had claw horn growth and wear rates that were significantly different (mean growth rate NY 5.9 mm/mo. and LY 6.1 mm/mo.). However, despite the rates not being significantly different, the non supplemented heifers wear rate was higher (10.8 mm/mo.) than the LY supplemented heifers of (4.4 mm/mo.) ($P<0.10$) up to 100 days pp suggesting for that period that the LY supplemented heifers had greater structural integrity and harder claws shown by the higher PR required to puncture the claw horn of LY supplemented heifers.

Growth and wear rates were again not significant as shown in Chapter 5 (mean growth rate mm 1ZnO 2.7, 1 OZn 2.8, 0.3 OZn 2.6; mean wear rate mm 1ZnO 3.1 OZn 3.9, 0.3 OZn 2.8). Neither the PR nor EM differed significantly between zinc source or level which were reflected in similar wear rates as all treatments had similar structural integrity. Overall, there were also no significant relationships found between claw horn growth and wear rates, TLS, d pp and PR.

The regression equation for the effect of PR, days pp and TLS on average claw horn growth rates were:

Mean horn growth rates were affected by PR, days pp and TLS = 5.98 - 0.386 Sole PR + 0.0324 days pp + 0.0033 TLS Sole, R^2 (adj) = 0.15 P= 0.402.

The regression equation for the effect of net change of claw horn, PR, day's pp and TLS on average claw horn wear rates was:

Mean horn wear rates were affected by PR, days pp and TLS = 9.52 - 0.458 Sole PR + 0.0173 days pp- 0.0112 TLS Sole, R^2 (adj) = 0.34 P= 0.600.

The regression equation for the effect of net change of claw horn, PR, day's pp and TLS on average claw horn wear rates was:

Mean horn wear rates were affected by net change of claw horn, PR, days pp and TLS = - 11.2 + 0.244 Sole PR + 0.0396 days pp+ 0.0303 TLS Sole, R^2 (adj) = 0.126 P= 0.573).

However, this would not correspond with the theories that suggest inferior claw horn (which has lower mechanical properties than sound claw horn) wears at a greater rate as keratinisation as been disturbed (Hinterhofer *et al.*, 2007, Hinterhofer *et al.*, 2005a, Műlling *et al.*, 1999).

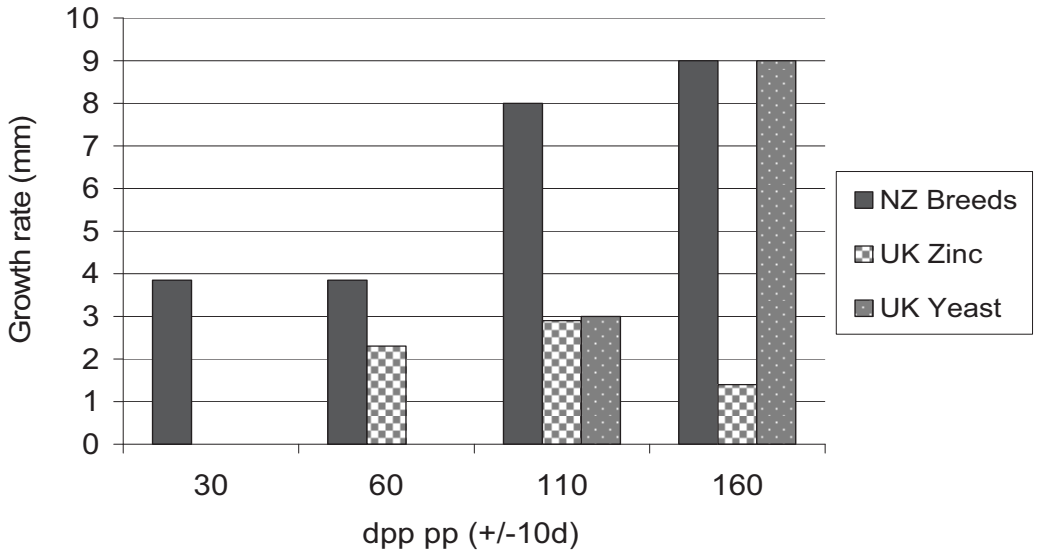

Figure 6.3 Average growth rates for the research undertaken in Chapters 2 (yeast), 3 (NZ breeds) and 5 (zinc).

Figures 6.3-6.5 Mean growth, wear and net change in growth rates have been calculated using the mean for all the animals in each experiment, because the results are being used as a comparison between time periods for each experiment rather than looking at the treatments within an individual experiment.

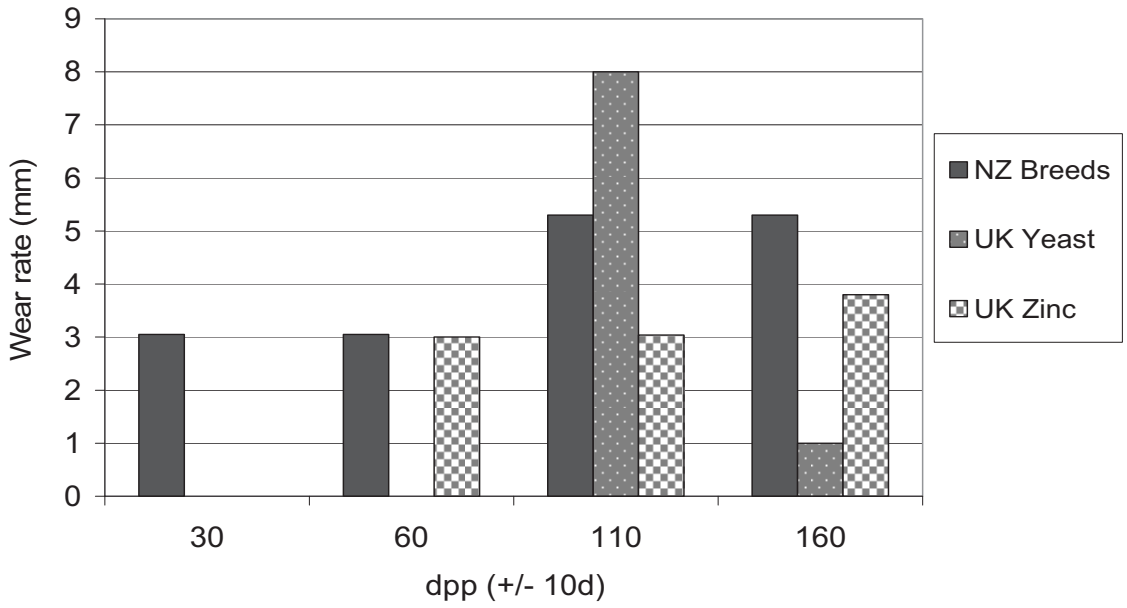

Figure 6.4 Average wear rates for the research undertaken in Chapters 2 (yeast), 3 (NZ breeds) and 5 (zinc).

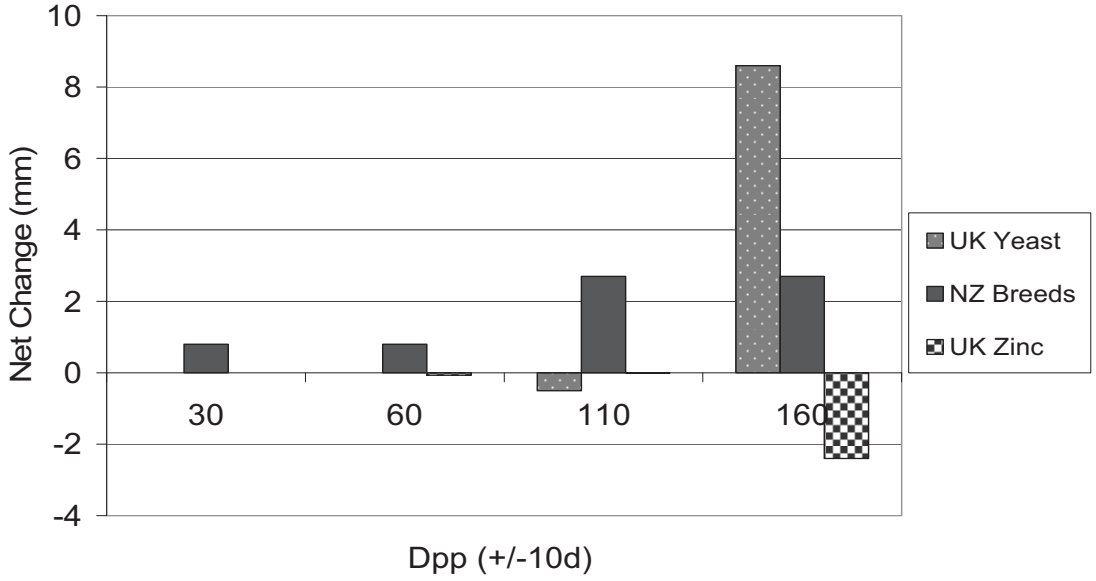

Figure 6.5 Net changes in claw average growth for the research undertaken in Chapters 2 (yeast), 3 (NZ breeds) and 5 (zinc).

The monthly horn growth rates were generally higher in (Chapter 3) the NZ system when compared to the 2 experiments (Chapters 2 and 5) completed in the UK (Figure 6.3) which were similar to the published data from the Northern hemisphere (Clark and Rakes, 1982; Livesey *et al.*, 1998; Offer *et al.*, 2000b) and New Zealand (Tranter and Morris, 1992). Growth rate seems to be more consistent across experiments (Tables 1.1, 1.2 and 1.3) and the limited data on season suggest that there may be seasonal differences (Table 1.3) and higher growth rate in spring and summer (Chapter 3; NZ experiment) compared to winter Autumn and winter (Chapter 2 and 5; UK experiments).

The claw horn wear rates (Figure 6.4) were higher in NZ when compared with the UK research with the exception of Chapter 2 at 100 d pp. This is most likely to be a result of the distances that NZ dairy cattle walk to pasture, but could be affected by a number of other factors including diet, yard surface conditions and the maintenance of farm tracks which can be very abrasive when stones are exposed due to soil erosion which is likely to increase claw wear rates (Chesterton *et al.*, 1989; Tranter and Morris, 1992). Net change (figure 6.5) in growth resulted in NZ dairy cattle having a positive net change whereas dairy cattle in the UK based research resulted in negative equity which continued up until 150 d pp. As the research finished at this point it is not known at what point growth then exceeded wear. Conversely the study carried out in the UK by Livesey and Laven, (2007) found positive net growth was achieved by 6 weeks pp and as previous research has shown, there are many factors such as parturition, genetic predisposition, claw and leg conformation, diet, season and housing that need to be considered which interact to affect the growth and wear rates of the claws (Livesey *et al.*, 1998; Livesey and Laven, 2007; Offer *et al.*, 2000a). The horn growth and wear rates can vary from year to year in the same animals in the same location/ housing (Winkler, 2005) therefore variability needs to be considered when assessing claw horn and nutrient supplementation periods. Increased wear rate of the claw horn has been found to be related to winter housing (cubicles, concrete floors), the amount of standing water, the

stocking density, previous exposure to concrete, cow tracks and the distances walked each day by the cows (Livesey and Laven, 2007; Tranter and Morris, 1992) resulting in a negative net growth rate (Leach *et al.*, 1997; Livesey *et al.*, 1998; Offer *et al.*, 2000b; Vokey *et al.*, 2001). Vokey *et al.* (2001) found that claw horn growth was influenced by rate of wear and MacCallum (2002) found that the pressure on the horn stimulated the claw horn growth rate to increase. Therefore it is very difficult to pin point the exact cause of positive or negative net growth and consequently direct comparisons cannot be made. However, a period of negative claw horn accumulation (Table 1.2) has been seen at differing times during the postpartum period and the extent and effect of these on distal claw thickness could be a potential cause of a reduction in protective ability of the claw capsule for corium, potential weakening of the sole or white line and increased possibility of claw penetration. Thus increased lameness occurred, particularly in NZ based systems where cows walk long distances. This area of claw health and function warrants further research.

6.8 Claw shape

There were no differences for the claw measurements from Chapter 2 or 5, which showed similar measurements for housed animals for claw angle, heel depth, dorsal border, diagonal claw measurement which correspond to (Boelling and Pollott, 1998; Offer *et al.*, 2000b). The (NZ) heifer in Chapter 3, which had maintained a shallower claw angle, shorter dorsal border and higher heel height than the UK heifers which resulted in a smaller more compact foot and lower wearing surface. The shorter dorsal border of the NZ heifers did not correspond with some observations that animals that are grazed have longer dorsal borders (Boelling and Pollott, 1998; Offer *et al.*, 2000b). However the dorsal borders and heel depth from Chapters 2 and 5 based in the UK were longer and shallower than those in Chapter 3 (NZ heifers) but foot angle was steeper than NZ. There is however no other published data to compare the NZ results to determine whether these are normal results for the area and typical differences between the UK and NZ.

6.9 Supplementation of diet

Nutritional management continues to be one of the major focal points in the attempt to reduce lameness in dairy cattle (Nocek, 1997). Dietary supplementation with micronutrients such as biotin (Hedges *et al.*, 2001), zinc (Kessler *et al.*, 2003; Moore *et al.*, 1989; Nocek and Johnson, 2000), and mineral complexes (Nocek *et al.*, 2006; Uchida *et al.*, 2001) have been found to reduce the levels of sole bruising and the incidence of lesions.

In Chapter 2 it showed that supplementing a MR diet with 10 billion/h/d live yeast significantly reduced the level of mean sole haemorrhage percentage, total score and increasing PR. This illustrated that there was an improved claw quality/ integrity and less severe haemorrhaging as a result of LY supplementation. No other study has assessed the effects LY supplementation on haemorrhaging and the potential effects which may be able to mediate the effects of acidosis and laminitis which are growing welfare issues of high yielding dairy cows. Therefore the possible aetiology is uncertain. Conversely yeast acts as a rumen modifier/ buffer potentially decreasing the amount of lactic acid in the rumen (Marden and Bayourthe, 2005) and subsequently the severity of acidosis /laminitis may be reduced. Thrune *et al.* (2009) found that supplementing dairy cows with active dry yeast increased the mean, minimum and maximum ruminal pH, decreased time spent in sub-acute rumen acidosis, and tended to decrease total VFA concentration in the rumen compared with un-supplemented cows. Rumen acidosis has been recognised as a risk factor in the development of laminitis (Nocek, 1997; Oetzel, 2000) and connection between dietary starch content and the occurrence of laminitis has been indicated (Manson and Leaver, 1988; Nocek, 1997). Newbold *et al.* (1996) and Marden and Bayourthe (2005) have suggested that live yeast has the ability to scavenge oxygen in the rumen by consuming the traces of oxygen that enter the rumen during the daily feed cycle in both the feed and saliva. Live yeast strengthens the anaerobic state of the ruminal milieu and stimulates the activity of cellulolytic bacteria to transform

lactate into propionate, thus reducing the accumulation of lactate. Yeast supplementation may be able to prevent or reduce the severity of sub acute ruminal acidosis (SARA), by reducing or preventing the cycle which causes the release of endotoxins and vasoactive substances. This may indicate the reason why the heifers offered yeast had significantly lower levels of sole haemorrhaging. While there is a reasonable level of information demonstrating the effect of yeast on the rumen environment, further research is required to demonstrate the effect of yeast on the rumen environment, microflora and fauna, plasma metabolites and inflammatory responses that may help to understand the mechanisms involved in reducing the claw horn haemorrhage levels of heifers offered yeast.

The level and form of zinc supplementation from 50 d pre to 150 postpartum did not significantly affect plasma iron or zinc levels, locomotion score; claw measurements and either sole or WL haemorrhaging throughout the experimental period. Growth and wear rates did not differ due to level or form of zinc supplementation. Failure to detect a treatment effects may be due to an insufficient duration of treatment to observe improvements in claws. Cope *et al.* (2009) also undertook research comparing inorganic and organic forms of zinc and found no significant differences in locomotion score between treatments after 14 weeks. Hedges *et al.* (2001) undertook research for 18 months on Biotin based on going beyond the complete horn renewal at approximately 15 mo. Whereas Toni *et al.* (2007) calculated that the horn capsule of the claw is a composite of horn produced over the past 12 to 30 mo. From Greenough (1997) research shows that claw horn on the dorsal surface of the claw grows at an average rate of 2.5 mm/mo in beef cattle and 5 to 6 mm/mo in intensively fed cattle and the length of the dorsal wall of the medial claw is approximately 7.5 cm. Nocek *et al.* (2006) research supplementing trace minerals as complexes or inorganic forms did not find a reduction of sole and white line haemorrhages until the 2nd lactation. Thus treatment period in this research of approximately 5-6 months may not have been sufficient to detect an effect of Zn oxide compared to

Organic Zn on locomotion scores, claw haemorrhaging and structural integrity. Many studies (Ashmead *et al.*, 2004; Manspeaker *et al.*, 1987; Nocek *et al.*, 2006; Uchida *et al.*, 2001) have focused on mineral complexes rather than one specific mineral (i.e. zinc) and have found significant reductions in haemorrhages and improved milk production and reproduction. Therefore perhaps the mineral complexes approach better suits today's dairy cow's requirements. Any research on the effect of micronutrient supplementation on claw horn health should be funded adequately to be completed over an extended period of 15 to 18 months.

6.10 Recommendations for further research

Further knowledge (histological and mechanical properties) of how claw keratin changes over the pre and postpartum period may allow the development of improved, nutrition, animal husbandry and housing to reduce the levels of lameness experience.

Although the research undertaken in this thesis did not show any significant benefits of supplementing complexes of micronutrients over traditional forms of mineral supplementation, very little research has been undertaken and further investigation into mineral complexes and environmental based research into levels of minerals excreted may provide the pros and cons of supplementation of complexes of micronutrients as the merits of product cannot be determined in one piece of research.

Researchers are still uncertain of the mode of action of yeast in the rumen in terms of how it increases rumen fluid pH. However one theory is live yeast scavenges oxygen and as a result maintains an anaerobic environment and promotes activity of cellulolytic bacteria to transform lactate into propionate thus reducing the accumulation of lactate. Further research in this area could confirm this theory or find a different mode of action. This in turn could establish the aetiology of how LY supplementation reduces lameness in cattle.

There are still very few studies concerning the prevalence and causes of lameness in NZ. More detailed research to determine what are "normal" claw measurements and the main lesions experienced by dairy cattle in pasture based systems according to the IFM would help to establish further husbandry recommendations for dairy producers in NZ to reduce the cost of lameness in terms of profit and animal welfare.

6.11 REFERENCES

Aranwela, N., G. Sanson, and J. Read. 1999. Methods of assessing leaf-fracture properties. New Phytologist. 144:369-393.

Ashmead, H. D., S. D. Ashmead, and R. A. Samford. 2004. Effects of metal amino acid chelates on milk production, reproduction, and body condition in Holstein first calf heifers. International Journal Applied Research Veterinary Medicine. 2:252-260.

Baggott, D. G., K. J. Bunch, and K. R. Gill. 1988. Variations in some inorganic components and physical properties of claw keratin associated with claw disease in the British Friesian cow. British Veterinary Journal. 144:534-542.

Baillie, C., C. Southam, A. Buxton, and P. Pavan. 2000. Structure and properties of bovine hoof horn. Advanced Composite Letters. 9:101–113.

Boelling, D. and G. E. Pollott. 1998. Locomotion, lameness, hoof and leg traits in cattle. I. Phenotypic influences and relationships. Livestock Production Science. 54:193-203.

Borderas, T. F., B. Pawluczuk, A. M. Passille, and J. Rushen. 2004. Claw hardness of dairy cows: relationship to water content and claw lesions. Journal of Dairy Science. 87:2085-2093.

Budras, K. D., C. Műlling, and H. Horowitz. 1996. Rate of keratinization of the wall segment of the hoof and its relation to width and structure of the zona alba (white line) with respect to claw disease in cattle. American Journal of Veterinary Research. 57:444–455.

Chesterton, R. N., K. E. Lawrence, and R. A. Laven. 2008. A descriptive analysis of the foot lesions identified during veterinary treatment for lameness on dairy farms in north Taranaki. New Zealand Veterinary Journal. 56:130-138.

Chesterton, R. N., D. U. Pfeiffer, R. S. Morris, and C. M. Tanner. 1989. Environmental and behavioural factors affecting the prevalence of foot lameness in New Zealand dairy herds- a case control study. New Zealand Veterinary Journal. 37:135-142.

Clark, A. K. and A. H. Rakes. 1982. Effect of methionine hydroxy analog supplementation on dairy cattle hoof and composition. Journal of Dairy Science. 65:1493-1502.

Collard, B. L., P. J. Boettcher, J. C. M. Dekkers, D. Petitclerc, and L. R. Schaeffer. 2000. Relationships between energy balance and health traits of dairy cattle in early lactation. Journal of Dairy Science. 83:2683-2690.

Collins, S. N., B. C. Cope, L. Hopegood, R. J. Latham, R. G. Linford, and J. D. Reilly. 1998. Stiffness as a function of moisture content in natural materials: Characterisation of hoof horn samples. Journal of Materials Science. 33:5185-5191.

Cope, C. M., A. M. Mackenzie, D. Wilde, and L. A. Sinclair. 2009. Effects of level and form of dietary zinc on dairy cow performance and health. Journal of Dairy Science. 92:2128-2135.

Douglas, J. E., C. Mittal, J. J. Thomason, and J. C. Jofriet. 1996. The modulus of elasticity of equine hoof wall: implications for the mechanical function of the hoof. The Journal of Experimental Biology. 199:1829-1836.

Dyer, R. M., D. Arola, D. Zhang, R. Reprogel, W. Zheng, and U. Tasch. 2004. On the mechanical behaviour of bovine hoof horn. Pages 82-84 in The thirteenth International Symposium on Lameness in Ruminants, Maribor, Solvenia.

Franck, A., G. Cocquyt, P. Simoens, and N. De Belie. 2006. Biomechanical Properties of Bovine Claw Horn. Biosystems Engineering. 93:459–467.

Greenough, P. R. 1997. Basic concepts of bovine lameness. in Lameness in Cattle. 3rd ed. W. B. Sanders Co, Philadelphia, PA.

Hedges, V. J., R. Blowey, A. J. Packington, C. J. O'Callaghan, and L. E. Green. 2001. A longitudinal field trial of the effect of biotin on lameness in dairy cows. Journal of Dairy Science. 84:1969-1975.

Hedges, V. J., L. E. Green, R. Blowey, A. J. Packington, and R. H. C. Bonser. 2002. Tensile strength of the white line in the claw of dairy cows supplemented with biotin. Proceedings of the Twelfth International Symposium on Lameness in Ruminants. Pages 167-170. Vol. 9-13 Jan. 2002, Orlando, Florida.

Hinterhofer, C., V. Apprich, J. C. Ferguson, and C. Stanek. 2007. Modulus of elasticity and dry-matter content of bovine claw horn affected by the changes of chronic laminitis. Veterinary Journal. 174:605-609.

Hinterhofer, C., C. Stanek, and K. Binder. 1998. Elastic modulus of equine hoof horn, tested in wall samples, sole samples and frog samples at varying levels of moisture. Berliner und Munchener Tierarztliche Wochenschrift. 111:217-221.

Hinterhofer, C., M. Zoscher, C. Gabler, and C. Stanek. 2005a. The hardness of horn in different segments of the bovine claw. Berliner und Munchener Tierarztliche Wochenschrift. 118:334-340.

Hinterhofer, C. M., V. Apprich, J. C. Ferguson, and C. Stanek. 2005b. Elastic properties of hoof horn on different positions in the bovine claw. Berliner und Munchener Tierarztliche Wochenschrift. 112:142-146.

Hoblet, K. H. and W. Weiss. 2001. Metabolic hoof horn disease – claw horn disruption. Veterinary Clinics of North America. 17:111-127.

Holah, D. E., K. M. Evans, G. R. Pearson, J. F. Tralton, and A. J. F. Webster. 2000. The histology and histopathology of the support structures in the laminated region of the bovine hoof in maiden heifers and around the time of first calving. Pages 94-96 in The Eleventh International Symposium on Lameness of the Ruminant Digit, Parma, Italy.

Husain, A., D. K. Sehgal, and R. K. Pandley. 2002. Design of a simple, versatile, small-specimen puncture test setup for determination of the mechanical behaviour of materials. Experimental Techniques September/October 2002:33-38.

Johnson, W. M. and A. J. Rapoff. 2007. Microindentation in bone: Hardness variation with five independent variables. Journal of Material Science. 18:591–597.

Kempson, S. A. and D. N. Logue. 1993. Ultrastructural observations of hoof horn from dairy cows: changes in the white line during the first lactation. The Veterinary Record. 132:524-527.

Kessler, J., M. I., F. A. Dufey, A. Gutzwiller, A. Stern, and H. Geyes. 2003. Effect of organic zinc sources on performance, zinc status, and carcass, meat, and claw quality in fattening bulls. Livestock Production Science. 81:161–171.

Leach, K. A., D. N. Logue, S. A. Kempson, J. E. Offer, H. E. Ternent, and J. M. Randall. 1997. Claw lesions in dairy cattle: Development of sole and white line haemorrhages during the first lactation. Veterinary Journal. 154:215-225.

Lewis, G. 2002. Key issues involved with the use of miniature specimens in the characterization of the mechanical behaviour of polymeric materials. Journal of Biomedical Materials Research. 63: 455-466.

Livesey, C. T., A. M. Johnson, C. Marsh, S. A. May, and J. A. Metcalf. 1998. Hoof horn growth in Holstein cattle from first calving until six weeks after second calving. Pages 51-52 in Tenth International Symposium on Lameness in Ruminants. Casino Lucerne, Switzerland.

Livesey, C. T. and R. A. Laven. 2007. Effects of housing and intake of methionine on the growth and wear of hoof horn and the conformation of the hooves of first-lactation Holstein heifers. Veterinary Record 160:470-476.

MacCallum, A. J., C. H. Knight, K. A. K. Hendry, C. J. Wilde, D. N. Logue, and J. E. Offer. 2002. Effects of time of the year and reproductive state on the

proliferation and keratinisation of bovine hoof cells. Veterinary Record. 151:285-289.

Manson, F. J. and J. D. Leaver. 1988. The influence of concentrate amount on locomotion and clinical lameness in dairy cattle. Animal Production. 47:185-190.

Manspeaker, J. E., M. G. Robl, G. H. Edwards, and L. W. Douglass. 1987. Chelated minerals: Their role in bovine fertility. Veterinary Medicine. 82:951–956.

Marden, J. P. and C. Bayourthe. 2005. Live Yeast- ruminal 02 scavenger and pH stabiliser. Feed Mix. 13:23-25.

Moore, C. L., P. M. Walker, J. R. Winter, M. A. Jones, and J. M. Webb. 1989. Zinc methionine supplementation for dairy cows. Transactions of the Illinois State Academy of Science. 82:99–108.

Műlling, C., H. Bragulla, K. D. Budras, and S. Reese. 1994. Structural Factors Influencing Horn Quality and Sites of Predilection at the Ground Surface of the Cattle Hoof. Schweizer Archiv Fur Tierheilkunde. 136:49-57.

Műlling, C., H. Bragulla, S. Reese, K. D. Budras, and S. W. 1999. How structures in bovine hoof epidermis are influenced by nutritional factors. Anatomia Histologia Embryologia-Journal of Veterinary Medicine Series C-Zentralblatt Fur Veterinarmedizin Reihe C. 28:103–108.

Newbold, C. J., R. J. Wallace, and E. M. McIntosh. 1996. Mode of action of the yeast Saccharomyces cerevisiae as a feed additive for ruminants. British Journal Nutrition. 76:249.

Newlyn, R. A., S. N. Collins, B. C. Cope, L. Hopegood, R. J. Latham, and J. D. Reilly. 1999. Equine hoof horn a natural composite. Pages 231-240. in Proceedings of the Fifth International Conference in Deformation and Fracture of Composites. Vol. 5th, London, UK.

Nocek, J. E. 1997. Bovine Acidosis Implications on Laminitis. Journal Dairy Science. 80:1005–1028.

Nocek, J. E. and A. B. Johnson. 2000. Digital characteristics in commercial dairy herds fed metal-specific amino acid complexes. Journal Dairy Science. 83:1553-1572.

Nocek, J. E., M. T. Socha, and D. J. Tomlinson. 2006. The Effect of Trace Mineral Fortification Level and Source on Performance of Dairy Cattle. Journal of Dairy Science. 89:2679 – 2693.

Oetzel, G. R. 2000. Clinical aspects of ruminal acidosis in dairy cattle. Pages 46-53 in 33rd Annual Convention of the American Association of Bovine Practitioners, Rapid City, USA.

Offer, J. E., D. N. Logue, and K. A. Leach. 2000a. Changes in claw conformation, growth and wear in young stock and first lactation cattle. Pages 282-284 in Eleventh International Symposium on Lameness of the Ruminant Digit. Parma, Italy.

Offer, J. E., D. N. McNulty, and D. N. Logue. 2000b. Observations of lameness, hoof conformation and development of lesions in dairy cattle over four lactations. The Veterinary Record. 147:105-109.

Reilly, J. D., H. Newlyn, B. Cope, R. J. Latham, S. Collins, and L. Hopegood. 2002. A comparison of different moisture loss methods for measuring hoof wall content. Page 193. Vol. 9-13 Jan. 2002, Orlando, Florida.

Smith, C. W., R. Herbert, R. J. Wootton, and K. E. Evans. 2000. The hind wing of the dessert locust (Schistocerca Gregaria Forskal) II. Mechanical properties and functioning of the membrane. The Journal of Experimental Biology 203:2933–2943.

Tarlton, J. F., D. E. Holah, K. M. Evans, S. Jones, G. R. Pearson, and A. J. F. Webster. 2002. Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving. Veterinary Journal. 163:196-204.

Thrune, M., A. Bach, M. Ruiz-Moreno, M. D. Stern, and J. G. Linn. 2009. Effects of Saccharomyces cerevisiae on ruminal pH and microbial fermentation in dairy cows Yeast supplementation on rumen fermentation. Livestock Science. 124:261-265.

Toni, F., L. Grigoletto, C. J. Rapp, M. T. Socha, and D. J. Tomlinson. 2007. Effect of Replacing Dietary Inorganic Forms of Zinc, Manganese, and Copper with Complexed Sources on Lactation and Reproductive Performance of Dairy Cows. The Professional Animal Scientist. 23:409–416.

Tranter, W. P. and R. S. Morris. 1992. Hoof growth and wear in pasture-fed dairy cattle. New Zealand Veterinary Journal. 40:89-96.

Uchida, K., P. Mandebvu, C. S. Ballard, C. J. Sniffen, and M. P. Carter. 2001. Effect of feeding a combination of zinc, manganese and copper amino acid complexes, and cobalt glucoheptonate on performance of early lactation high producing dairy cows. Animal Feed Science and Technology. 93:193-203.

Vermunt, J. J. and P. R. Greenough. 1996. Sole haemorrhages in dairy herds managed under different underfoot and environmental conditions. British Veterinary Journal. 152:57-73.

Vokey, F. J., C. L. Guard, H. N. Erb, and D. M. Galton. 2001. Effect of alley and stall surfaces on indices of claw and leg health in dairy cattle housed in a free-stall barn. Journal of Dairy Science. 84:2686-2699.

Webster, A. J. F. 2001. Effects of housing and two forage diets on the development o claw horn lesions in dairy cows at first calving and first lactation. The Veterinary Journal. 162:56-65.

Winkler, B. 2005. Mechanical properties of hoof horn, sole haemorrhage and lameness in dairy cattle. PhD dissertation. Plymouth University, Plymouth.

Winkler, B. and J. K. Margerison. 2004. Mechanical properties of the sole hoof horn of heifers before and during the first lactation - a prediction of lameness susceptibility. Pages 60-62 in 13th International Symposium and 5th Conference on Lameness in Ruminants, Maribor, Slovenija.

Winkler, B. and J. K. Margerison. 2006. Mechanical properties of the hoof horn of dairy cows during lactation. Journal Animal Science. 84: Suppl. 1:415 (Abstract).

Winkler, B. and J. K. Margerison. 2007. Effect of sample thickness, tissue moisture content and storage methods on the puncture resistance and

elastic modulus of the bovine hoof horn. Journal Animal Science. 85: Suppl. 1:419 (Abstract).

Winkler, B., J. K. Margerison, and C. Brennan. 2002. The testing of the mechanical properties of the hoof horn during lactation and housing. Pages 314-317 in Proceedings of the 12th International Symposium on Lameness in Ruminants, Orlando, Florida, USA.

Zoscher, M., C. Hinterhofer, C. Stanek, and C. Gabler. 2000. New aspects of mechanical properties of bovine hoof horn. Pages 91-93 in Eleventh International Symposium on Lameness of the Ruminant Digit. Parma, Italy.

Chapter 7.0

Publications

Lethbridge, L.A., Margerison, J.K., Reynolds, G.W., Laven, R., Brennan, C.S. 2008. Puncture force resistance of hoof horn from New Zealand (NZ) Friesian and Jersey cross NZ Friesian dairy. Journal of Dairy Science. 91, Suppl. 1,

Lethbridge, L.A., Margerison, J.K. 2008. The puncture force of hoof horn from New Zealand Friesian and Jersey Cross Friesian dairy cattle breeds. Page 7 in Proceedings of the British Society of Animal Science, Scarborough, UK.

Lethbridge, L.A., Margerison, J.K. 2008. Effect of the inclusion of a live yeast culture into mixed forage diets on milk yield, locomotion score, lameness and sole bruising in first lactation Holstein Friesian dairy cattle. Page 117 in Proceedings of the British Society of Animal Science, Scarborough, UK.

Margerison, J.K., Brennan, C.S., Lethbridge, L.A., Laven, R., Reynolds, G.W. 2008. Effect of Dairy Cattle Breed on the Strength of Hoof Horn Taken From New Zealand Friesian and Jersey Cross Friesian Dairy Cattle. Page 355 -358 in 15th International Symposium and 7th Conference on Lameness in Ruminants, Kuopio, Finland.

Margerison, J.K., Lethbridge, L.A., Laven, R., Reynolds, G.W., Brennan, C.S. 2007. Effect of Milk Level and Plant Extracts on Hoof Health and Horn Structural Strength of New Zealand Friesian and Jersey Cross Friesian Dairy Heifers. Pages 359 -362 in 15th International Symposium and 7th Conference on Lameness in Ruminants, Kuopio, Finland.

Margerison, J.K., Lethbridge, L.A., Hollis, J., Snell, A., Stephens, G., Winkler, B. 2006. Effect of locomotion core and cause of lameness on feed intake, feeding behaviour, live weight change, milk yield, milk let down, milking duration of Holstein Friesian dairy cattle. Pages 165-166 in 14th International Symposium and 6th Conference on Lameness in Ruminants – Uruguay.

Lethbridge, L. A., Margerison, J. K, Parfitt, D. 2006. Effect of yeast in mixed grass, forage maize and whole crop silage diets on locomotion score, lameness and sole bruising in first lactation Holstein Friesian dairy cattle. 9394 in 14th International Symposium and 6th Conference on Lameness in Ruminants - Uruguay

Lethbridge, L.A., Margerison, J.K., Reynolds, G.W., Laven, R., Brennan, C.S. Chrenkova, M., Hentenyi, L. 2006. Genetic determinism and QTL mapping of plant parameters involved in the efficient and sustainable utilisation of forage maize in animal nutrition. Journal of Dairy Science. 89, Suppl. 1, 254

Lethbridge, L. A., Margerison, J. K. 2006. Comparison of two differing five point locomotion scoring methods. Pages 163-164 in 14th International Symposium and 6th Conference on Lameness in Ruminants – Uruguay.