The development of quantifiable measurements to characterise the epidermal integrity of the equine hoof capsule

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Dedication

This thesis is dedicated to the memory of my Nana, my maternal grandmother, Mrs Marjorie Wright (1907-2002), who at 90yrs old joined the University of the Third Age; Nana thank you for giving me via my mother the gift of always wanting to know more.

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Abstract

Reason for performing study: Gross defects which reduce the functional integrity of the hoof are considered to be a major cause of loss of function and suffering to the horse and therefore of financial and welfare concern. Currently the subjective visual appearance of the equine hoof wall, in terms of cracks, is used to make a clinical judgement on the quality or functionality of that hoof. Dietary intervention is recommended as a method of improving the quality of the equine hoof wall. The relationships between dietary trace element intakes, their concentration within the hoof wall tissue, the incidence of cracks and the mechanical properties of equine hoof epidermis are less well elucidated compared to other epidermal tissues. It is possible that the relationships are confounded by a lack of standardisation in material collection and objective measures. Alternatively the confounding factor could be the unique role of the ungulate hoof wall epidermis to sustain bodyweight. The ability of a structure to bear load is dependant upon the material from which it is made, its size and its geometric shape. If a certain shape of hoof results in increased stress concentrations, at specific area of the wall, then the wall might be predisposed to cracks, irrespective of any nutritional influences.

Objectives: 1.To develop objective measurements to characterise cracks and therefore the visual appearance of the hoof wall, 2. to investigate relationships between trace elements and an appropriate mechanical property, 3. to objectively describe the shape of the hoof and to investigate the relationships between all the measured parameters.

Methods: Cracks were measured in terms of their number, area and anatomical occurrence and a severity and geometric scoring system was developed. A method of collecting material from the same anatomical position for both trace element analysis and fracture toughness was developed. Trace elements were analysed from hoof wall blocks using ICP-MS and LA-ICP-MS. Trace element concentrations were compared between and within anatomical positions and the effect of two different washing techniques on sample preparation was compared. Fracture toughness was measured from the same blocks with an IZOD impact pendulum. Standardised linear and angular measurements were taken from the hoof capsule to investigate a series of ratios to describe the shape of the capsule, the same measurements were taken from photographs to compare the techniques. Statistical analysis (The ANOVA, Kruskal Wallis, 2ANOVA and t-tests) investigated the differences between the measured parameters and coefficient coefficients were calculated between the parameters.

Results: There was no difference in the distribution of cracks between anatomical positions. Simple number or area was not sufficient to establish the potential affect of cracks on function

but the geometric number score (GSNo) which is easy to measure was strongly correlated to the geometric area score,($r = 0.8$, $p < 0.0001$). Fracture toughness of the toe (28.6 ±11.9 kJ/m^2) was significantly less than that measured at the quarters, (medial 41.9 ±11.5 kJ/m^2 and 42.3 ± 11.7 kJ/m^2 lateral), $p < 0.001$. Hooves with a high crack score had low fracture toughness and higher concentration of copper, zinc and calcium compared to hooves with low or medium crack sores, ($p<0.05$). Hooves were grouped into shapes by using the ratio of capsular base length and capsular base width50% together with the vertical inclination, (medial lateral angles as measured from the frontal plane expressed as a coefficient of 90°). The ratios distinguished between hind and front feet and increased within group correlations of linear and angular measurements compared to the whole data set. Toe angle was not correlated with any other measured parameter. Relative trace elements concentration measured by LA-ICP-MS showed that trace elements differed across the hoof wall depth with the highest amounts closest to the dermis; however when measured proximo-distally down the hoof wall height, trace element concentration was less in material closest to the coronary band. Calcium concentration was correlated with crack scores but there was no correlation between fracture toughness and trace element concentration in any of the hooves.

Conclusion: This is the first time that equine hoof wall cracks have been quantified and the first time that a scoring system related to the perceived affect of the crack on function has been developed. This is the first time that ratios have been used to group hooves according to their shapes. There were no correlations between any of the measured parameters in the whole data set. When hooves were allocated to their shape groups, a number of the parameters were correlated indicting that perhaps shape is confounding the study of the relationship of the cracked hoof with other indices. This is the first time that the relative differences in trace element concentration across and down the hoof wall have been measured.

Potential Clinical Relevance: Objective, quantifiable and repeatable methods of measuring shape and cracks will aid in the investigation of factors which contribute to the functionality of the hoof wall and which to date have only been measured subjectively. These techniques can be used in epidemiological studies and can form the basis of investigations into the relationship between diet and hoof wall function.

Table of contents

'As the well- being of the feet is of first importance to horses and as nothing can well if they are amiss' Bracey Clarke,(1834).

List of Tables

List of Figures

List of Abbreviations

Please note that word[g] indicates that the word is defined in the glossary

ANOVA	Analysis of variance
Ca	Calcium
$CaCl_2$	Calcium chloride
CBmdc	Capsular base midline dead centre
CD	Capsular depth
CDWP	Capsular depth widest point
Cu	Copper
CW	Capsular width
CW50%	Capsular width 50%
CWWP	Capsular width widest point
DP	Distal perimeter
Fe	Iron
FEA	Finite element analysis
FT	Fracture toughness
ft/lbf	Foot pound force measure of energy , can be converted to joules by multiplying by 1.356
GS*	Geometric score
GSA	Total geometric area
GSAM/4	Medial quarter geometric crack area
GSAT	Toe geometric severity area
GSNo	Geometric number score
GSNoM/4	Medial quarter geometric number score
GSNoT	Toe geometric severity number
HWD	Hoof wall depth
HWH	Hoof wall height
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma mass spectrometry
IF	Intermediate filaments
IFAP	Intermediate filament associated proteins
IR	Impact resistance
IS	Impact strength
J/m	Impact strength
kJ/m^2	Impact resistance
L/4	lateral quarter
L/4 mm	Lateral quarter crack area
L/4 no	Lateral quarter crack number
L/4GSA	Lateral quarter geometric crack area
L/4GSNo	Lateral quarter geometric crack number
L/4SSNo	Lateral quarter severity crack number
LA-ICP-MS	Laser ablation inductively coupled plasma mass spectrometry
LR	Long regular
LU	Long upright
LW	Long wide
M/4	medial quarter

M/4 mm	Medial quarter crack area
M/4 SSNo	Medial quarter severity crack number
M/4GSNo	Medial quarter geometric number score
M/4no	Medial quarter crack number
MCG	Membrane coating granules
MDC	Midline dead centre
Mn	Manganese
MWP	Medial widest point
Na	Sodium
NIH	Image package for accurate digital measurements
PP	Proximal perimeter
ppm/Cppm	Amount of trace element measured in ppm from LA-ICP-MS divided by carbon
PTFE	Polytetrafluoroeythylene a specific plastic used in the manufacture of storage bottles used for chemical analysis. Very low risk of any inorganic contaminants
RR	Round regular
RU	Round upright
RW	Round wide
SL	Sole length
SS*	Severity score
SSA	Severity area score of whole hoof
SSAL/4	Severity crack area at the lateral quarter
SSAM/4	Severity area score medial quarter
SSAT	Toe severity crack area
SSNo	Severity number score of whole hoof
SSNoM/4	Severity number score medial quarter
SSNoT	Toe severity crack number
TCA	Total crack area of whole hoof
TCAM/4	Total crack area medial quarter
TCAT	Total crack area toe
TCNo	Total number of cracks on the hoof
TCNoM/4	Total crack number medial quarter
TCNoT	Total crack number toe
toe mm	Area of cracks at toe
toe no	Number of cracks at the toe
WR	Wide regular
WU	Wide upright
WW	Wide wide
Zn	Zinc

1 Introduction

'The horse's hoof has been a subject of deep study for centuries and I know of no mechanical contrivance for which the mind of man can contemplate with greater wonder and admiration. What is the use of a horse, however good or well fashioned, if it has not a sound foot to stand upon?' Duncan (1935)

1.1 Overview

The primary function of the epidermis is a barrier between the animal and its environment. Functions of keratinised structures include retention of body fluids, a barrier to external fluids, prevention of invasion by micro organisms, bacteria and other foreign matter, protection against mechanical injury, food gathering, temperature regulation and locomotion (Fraser and Macrae 1980).

The outer covering or integument of the vertebrate body consists of two layers the dermis and the epidermis. The epidermis consists of layers of cells which contain a fibrous proteinaceous material classified as keratin, (Fraser and Macrae 1980). The epidermis is formed by keratinisation, which is the process by which living epidermal cells differentiate into dead corneocytes in order to fulfil a variety of roles. The process of keratinisation and the specialised material and structural organisation of the corneocyte in the stratum medium of the equine hoof capsule are reviewed in this chapter.

In ungulates, for example the horse, modified epidermal cells have developed to provide specialised epidermal appendages. The epidermal equine hoof capsule[g] encases a single digit representational of the extreme result of digitigrade[g] evolution, (Pollitt 1998).The capsule has two main functions; the protection of the sensitive living tissues from mechanical, physical and chemical damage and the transfer of forces from the skeleton via the limb to the ground whilst the foot is at rest or in locomotion (Budras *et al.* 1989; Pollit 1992).

Word [g] indicates the word is defined in glossary

In order for the hoof wall to fulfil its purpose, the integrity[g] of the epidermis is important. At least fifteen, (15) trace elements have been recognised as having specific roles in skin differentiation and at the biochemical level imbalances of the trace elements, calcium, copper and zinc, have been shown to affect keratinisation. Copper deficiency reduced the cross-linking between high sulphur proteins, (Marston (1946); the removal of calcium from cultured skin keratinocytes prevented differentiation into corneocytes (Heenen *et al.* 1992) and diets deficient in zinc resulted in a reduction in keratin production, (Weismann *et al.* 1980). Keratinised tissues with different functions share commonality of keratin proteins, (Marshall 1986) and trace element deficiencies have similar effects on the pattern of keratinocyte differentiation across tissue types. Altering the amounts of zinc, copper or calcium has been shown to have direct affects on keratinisation, (Fell *et al.* 1985; Heenan *et al.* 1992; O'Dell 1976; Weisman *et al.* 1980) which was reflected in changes in gross appearance of the tissue, (Kapp and Simon 1980; Ray *et al.* 1997; White *et al.* 1994) or its mechanical properties (Agren and Franzen,1990; Baggott *et al.* 1988; Gillespie 1964; Reiling *et al.* 1992; Reis 1992; Hynd 2000) with either direct or indirect consequences to the function of the tissue. This chapter reviews the biochemical role of the trace elements copper, zinc and calcium within the differentiation of epidermal tissues in order to determine if there is likely to be a direct affect on keratinisation and a possible direct or indirect affect on the quality of the hoof wall.

In order to explore the term 'quality' in the concept of the hoof wall epidermis; it is first necessary to investigate how the term is currently used both in the hoof wall epidermis and in other epidermal tissues, and what inference is being drawn from the word. Secondly it is necessary to define the word quantitatively. Thirdly the relationships between factors highlighted to affect the function of epidermal tissues need investigating and finally it needs to be established if these relationships exist in the equine hoof wall epidermis.

Clinical assessment of the integrity and the functionality of the epidermal equine hoof wall is made by visual assessment and depending upon this appearance, the hoof is categorised as good or poor quality. Because the assessment is subjective it is difficult to determine if the terms good and poor quality have meaning; however gross defects which reduce the functional integrity of the hoof are considered to be a major cause of reduced function and suffering to the horses concerned, (Kempson 1990). In addition, the development of a crack on the hoof wall is assumed to result from the production of inferior horn which is then unable to cope with environmental insults, (Kempson and Logue, 1993). In other epidermal tissues it has been shown that the rate and type of keratin synthesis can be affected by differences in trace element concentrations at time of synthesis, (Marston 1946; Heenan *et al.* 1992).

Dietary intervention is recommended as a method of improving the quality of the equine hoof wall, (Comben *et al.* 1984; Kempson 1990; Kirby and Meschy 1995; Coenan and Spitzlei 1997) as measured by its visual appearance. This visual appearance of the equine hoof wall, usually in terms of cracks, is used to make a clinical judgement on the quality of that hoof, from which the functionality of that hoof is in turn inferred. The appearance of the epidermis is also used in veterinary and human medicine as a clinical indication of the health of the animal or human. An unblemished, shiny, smooth epidermis is generally associated with health, whereas a dull, brittle, dry cracked or blemished epidermis is considered an indication of ill health, whether the clinician is studying skin, coat, hair, nails or hoof. The field assessment of the equine hoof wall is based on subjective observations and common adjectives used to describe the appearance of the wall include shelly, brittle, rough and shiny. The use of these subjective adjectives to describe the appearance of the hoof wall was and is commonplace in research, (Comben *et al.* 1984; Josseck *et al.* 1995; Kempson *et al.* 1989; Kempson and Campbell 1998). This chapter reviews the current methods of visually assessing equine wall quality and highlights the need to develop a quantifiable method to measure the cracks on the hoof wall epidermis thereby providing a protocol for the clinical assessment of the visual appearance of the hoof wall. It is generally accepted that cracks occur in material whose fracture toughness has been exceeded. Chapter three describes the development of a method to measure cracks and investigates the relationship between crack severity and the measured fracture toughness of the hoof wall epidermis.

There is a link between the dietary intakes of zinc, copper and calcium or the concentration of these elements within cell culture mediums and the quality of keratinisation. This thesis hypothesised that the relationships between trace element concentrations, visual appearance and mechanical properties measured in other epidermal tissues would apply to the equine hoof wall. However, it is not clear if the same relationship exists between trace elements and the quality of the hoof wall epidermis.

If there is a link between nutrition and the function of the epidermis, it is important that interactions between mechanical properties, visual appearance and the affects of nutrition are measured accurately in order to elucidate the factors affecting hoof wall integrity. This in turn should help to minimise both the financial and welfare implications of a non integral epidermis.

A review highlighted that although dietary intervention has been used to improve the quality of the ungulate[g] hoof wall, there is a lack of agreement between researchers as to the affect of trace elements on the visual appearance and the mechanical properties of equine hoof epidermis and of other epidermal appendages specialised to bear weight.

It is possible that these discrepancies are due to lack of standardisation of material collection. Some researchers compare sole and wall, (Baggott *et al.* 1988), others compare different anatomical areas and attempt to correlate material properties with trace element concentration (Ley *et al.* 1998). The effect of washing techniques on trace element removal are ignored, (Chyla and Zyrnichi 2000; Harrison and Tyree 1971) and suites of trace elements are analysed without consideration as to their possible affects on keratinisation, (Kovas and Szilagyi 1973). Objective methods to describe the visual appearance of the equine hoof wall in terms of cracks are lacking, making it difficult to quantify any changes recorded, (Comben *et al.* 1984; Josseck *et al.* 1995; Kempson *et al.* 1989; Kempson and Campbell 1998). Chapter four describes the methods developed to standardise protocols for sampling hoof wall blocks, and the standardisation of techniques for the analysis of zinc, copper and calcium within the hoof wall blocks.

Alternatively one of the confounding factors may be the unique role of the ungulate hoof wall[g] epidermis to sustain the bodyweight of the horse without failing. The ability of a structure to bear load is dependant upon the material from which it is made, its size and its geometric shape, (Biewener 1992; Gordon 1978). If a certain shape of hoof results in increased stress concentrations at specific anatomical positions, due to geometric irregularities, this area of the wall maybe predisposed to cracks, irrespective of any nutritional influences. Chapter five describes the development of a method to use ratios of measurements to describe the shape of the equine hoof capsule.

The visual appearance of the hoof epidermis has been used to make judgements on the functionality of the hoof wall and dietary intervention has been recommended to improve the visual appearance without previous consideration of any of the factors above. Standardisation of sampling techniques and categorising hooves by their shape will improve the coherence in the investigations into the relationships between visual appearance, trace element content and functionality of the hoof wall epidermis. This may provide additional correlations, as found in other epidermal tissues. Chapter six investigates the relationships between all the factors measured in the experimental chapters, to see if there are any interactions between the visual appearance of the hoof wall in terms of cracks, its material property of fracture toughness, the concentrations of copper, zinc and calcium and the shape of the hoof capsule.

1.2 The epidermis

An understanding of normal structural organisation, intracellular components and relevant biochemistry is essential in explaining whether a disruption in formation, or nutritional intervention, could affect the quality and function of the horn capsule or result in changes in appearance at the gross level of the equine hoof capsule.

1.2.1 Definition

The outer layers of the body known as the integument consist of two layers. A stratified squamous, specialised tissue (epithelium) of closely adhering cells with little or no intercellular material and no blood vessels known as the epidermis and a connective tissue known as the dermis. All epidermal tissues of the integument share a similar function providing a barrier between the animal and its environment. The skin is the largest organ in the integumentary system which includes the appendages, for example: - hair, nails, beaks, antlers, claws, and hooves. The anatomy of the hoof is similar to that of the human nail, showing distinct bed and matrix regions as well as a region of perihoof which is similar to the proximal nail fold and cuticle of human nails, (Baden and Kvedar 1991). Although the epidermal hoof wall is the study of this thesis, all dermal/ epidermal tissues have a commonality of structural arrangement from the polypeptides of the keratin molecule through to the cell layers.

Commonality of keratin tissues

All epidermal cells undergo transformation from mitotic dividing cells in the basal layer to fully cornified 'dead' cells of the cornified layer. This process is similar regardless of the final function of the epidermal tissue. Despite the final function and mechanical properties of the epidermal tissues being highly diverse (Fraser and Macrae 1980; Lee and Baden 1975), commonality of the keratin proteins, in the tissues which they are found and in the process of cornification has been demonstrated. Sheep hoof plates have a similar penetration profile compared to human nail when compared in absorption tests. Hemidy *et al.* (1994) showed that

sheep hoof plates are a very simple model for human nail for studying transungal absorption due to the strong correlation between the two tissues. They also commented that the behaviour of both tissues is similar to human skin stratum corneum. Bovine hoof membrane has been proven to be similar enough to human nail plates to use as a model for testing antimycotics in drug trials, (Mertin and Lippold 1997). Lee and Baden, (1975) observed that the amino acid composition of hair and nail α protein seem similar to each other as are the low sulphur proteins from rhinoceros horn, guinea pig hair and nail. Lynch *et al.* (1986) developed antibodies to distinguish between the 'soft' and 'hard' keratins. They concluded that the low sulphur proteins of hair and nail are clearly related as they share similar X - ray diffraction patterns. In addition they noted that the amino acid sequence of hard and epidermal keratins show extensive homology. They identified intermediate filament keratins belonging to both the acidic and basic groups, which were homogenous in human hair, guinea pig, mouse and sheep and which are 'highly conserved across mammalian species'. They also concluded that the precursor cells of the hair cortex and the nail bed share a major path of epithelial differentiation. A comparison of nail, claw, hair, horn and quill led Marshall, (1986) to conclude that the tissues are similar in ultrastructure and composition and that there is an overall similarity between amino acids. Kitahara and Ogawa, (1994) had previously shown that skin and nail plate contained similar keratins, they therefore investigated to see if all three precursors were present in the nail bed and at the same time looked at bovine hoof horn. They showed that bovine hoof developed in a similar manner to that of human nail and showed three distinct types of cell populations. They concluded that bovine hoof horn cells provided an excellent model by which to investigate hair biology including hair growth. This thesis considers that the biology of keratinisation and nutritional effects on the process is similar across all epidermal tissues and therefore any discrepancies and differences between hoof wall epidermis and other epidermal tissues warrants investigation.

Normal horn development is essential in maintaining the integrity of the hoof capsule (Offer and Logue 1998) and poor quality horn in lame cows has been associated with the disruption of the synthesis and deposition of keratins explained by a change in the rate of protein synthesis or rate of cell proliferation, (Hendry *et al.* 1997). The significance of a disruption in keratinisation on the final stratum medium of the equine hoof wall may be of fundamental importance due to the highly specialised function of weight bearing. Hoof epidermis must be capable of supporting the bodyweight of the animal and withstanding the considerable forces imposed by weight bearing and locomotion, (Douglas 1996).

1.2.2 Histology

The hoof has commonly been described as modified skin, (Budras *et al.* 1989; Grosenbaugh and Hood 1992) or as a modified fingernail, (Douglas 1993) and it consists of three layers, the subcutis, the dermis and the epidermis.

The keratinocyte

The keratinocyte is the name given to the cells of the epidermal tissues. The cell contents of the keratinocyte will vary depending upon its stage of differentiation. Birbeck and Mercer, (1957) described the keratinocyte as containing fibres and an amorphous matrix. They defined the hexagonal packing of the fibres in the keratinisation zone as filaments and proposed that the hardened fibres which ultimately filled the cortex of the cell were keratin. As techniques such as antibody studies, and X ray diffraction, (Lynch *et al.* 1986), have become more sophisticated, it became increasingly easy to define rigorously, the structure of keratin. However, much of the more recent work has simply allowed researchers to elucidate the finer structures, to relate chemical composition to the structures and to confirm early results.

Keratin

Keratin is a protein, which is found within the keratinocyte, whose biochemistry, composition and structure have been extensively investigated in the literature, (Block 1939; Lee and Baden 1975; Ward and Lundgren 1954; Gillespie and Frenkel 1974; Marshall and Gillespie 1977; Gillespie 1967; Menefee 1971; Lynch *et al.* 1986; Kitahara and Ogawa 1994). In order to argue the effect of trace elements on the integrity of the epidermis, it is necessary to establish clearly what the term keratin defines and to have an understanding of its biochemistry. Different authors use the term keratin to define either a group of polypeptides or the entire contents of the keratinocyte or the whole epidermal tissue, which are summarised in *table 1.2.2A.ii page I* of the appendix. The use of different terminology makes it difficult to ascertain nutritional affects to specific aspects of formation rather than simply the final tissue.

Polypeptides

Block, (1939) isolated some of the amino acids of keratin and identified the ratios and percentages of eight amino acids from fourteen keratinised tissues. Despite this, until 1954, keratin was used to describe either the whole of the cell or indeed the entire epidermal tissue, (Ward and Lundgren 1954). It was not until 1957, that the chemical composition was being related to the differences in the structure of filaments and matrix which could be distinguished

within the keratinocyte, (Birbeck and Mercer 1957). Rogers, (1957) showed that the filaments were low in sulphur and that the cysteine rich protein could be isolated to the matrix. In addition he quantified the amino acid fractions and concluded that the microfibrils were much higher in acidic, basic and neutral amino acids compared to the matrix. He described γ keratose as the protein of the matrix; low in molecular weight, high in sulphur and incapable of being drawn into fibres. Unfortunately much of the work through the 1970s (Gillespie and Frenkel 1974; Marshall and Gillespie 1977) still described keratin in terms of the whole tissue, using terms such as 'keratins used in this study included hairs, quills, wool, horn and fur'. Lee, (1975) noted that the filament found in keratin containing tissue could be described as ordered, whereas the matrix cannot. Fraser and Macrae, (1980) described soft and hard keratin as containing filaments and matrix; indeed they assigned the material properties of keratin as being that of a composite, with fibres embedded into a matrix. As analytical techniques developed, terminology began to differentiate between the whole structures and between the keratin polypeptides, the amino acids and the specific arrangement of the keratin polypeptides into intermediate filament structures.

Keratin genes and molecular physiology

The structure of several keratin genes has been elucidated, which has contributed greatly to an understanding of their structure, (Eckert 1989). Keratin polypeptides form α helixes and have a repeating structure of seven amino acids; of these two are apolar[g] residues. Keratin molecules wrap/coil together along this unit to form a dimer which is stabilised by hydrophobic interactions. The keratin rod contains four domains with the potential to form coiled structures, separated by areas which can not coil. The rod forms the central part of all keratins (typically 45-48nm in length averaged over three hundred keratins), (Eckert 1989), and the ends of the rods determine whether the keratin is an acidic keratin or a basic keratin.

Suter *et al.* (1997) referred to the fact that the amino acid sequencing of the keratin filaments has distinguished over thirty different keratins in epithelial tissue; K9-20 are acidic and K1-8 are basic. In addition the hair follicle, (Eckert 1989) and bovine hoof, (Baden and Kvedar 1991) contain up to eight keratins; four basic and four acidic. Hoof expresses some keratins which appear to be hoof specific; keratins a1-4 are hoof specific but share epitopes common to human hair, (Baden and Kvedar 1991). Despite this elucidation some authors, (Marshall *et al.* 1991) persisted in calling the whole tissue keratin and described both filament and matrix proteins as being found in keratin and continued to call keratin a filament/matrix composite.

Keratins expand from a single gene to a multi gene family. There are 60 intermediate filament genes in the human genome and half encode for keratin with at least 16 being expressed in the

skin. Keratins are encoded by two multigene families of more than 30 members (Molloy *et al.* 1982; de Berker *et al.* 2000). There are in excess of 35 keratin genes, and these can be partitioned into two distinct sequence types, low molecular weight acidic type I keratins and high molecular weight basic type II keratins, (Morris *et al.*1985), based on their genomic structure and nucleotide sequence homology. A group of seven type I (Ha1–Ha7) and six type II (Hb1–Hb6) keratin genes are expressed in hard epithelia such as hair and nail. The specific expression of keratin genes has been largely conserved throughout evolution, suggesting the existence of a functional link between keratin proteins and the diversity of structure and function of epithelial tissues, (McGowan and Coulombe, 2000).

All functional keratin genes map to the two known keratin clusters on chromosome 12, (Hesse *et al.* 2001) and equine gene homologs mapping to human chromosome 12 have been established, (Caetano *et al.* 1999). However the pattern of keratin expression showed a difference between the nail matrix and nail bed and de Berker *et al.* (2000) described the expression of keratins in the different compartments within the nail unit demonstrating a localization of specific keratins within these compartments. The characteristics of the different keratins found at different sites is relevant to the understanding of the biology of the normal nail and changes seen in the nail with certain diseases such as psoriasis. Changes of keratin biology may result in altered morphology in a variety of keratin diseases. Mutations in genes coding for keratins K6, K16 and K17 led to thickening of the nail bed and the nail dystrophy of pachyonychia congenital, (de Berker *et al.* 2000).

Understanding how hair follicles, nail, and glands are formed during skin embryogenesis and maintained in adult life presents a formidable task (McGowan *et al.* 2002) and has implications for a variety of skin disorders. At least seven programmes of terminal epithelial differentiation are coordinated and integrated to give rise to a hair-producing follicle that undergoes a growth cycle throughout life. Complex interactions play a crucial role throughout the development and subsequent homeostasis of hair and other epithelial appendages. It is in this context that the type I intermediate filament protein keratin 17 (K17) is first synthesized within developing skin epithelia. Onset of K17 synthesis marks the adoption of an appendageal fate within embryonic ectoderm. As appendageal precursors evolve into mature hair follicles, nail, and glands, the expression of K17 becomes restricted to a subset of specialized cell types. K17 stands out among type I keratin genes in that it is expressed in both soft and hard epithelial tissues, and uses different type II keratins as polymerization partners (e.g., K6, K5, K7). In addition, the K17 gene is induced following injury or in disease contexts (e.g., viral infection, carcinoma, psoriasis).

Research has suggested that the presence or absence or mutation of certain keratin genes might be reflected in the functionality of the epidermal tissue. During the early stages of hair development, K17 appeared to be present in nearly all cells within primary hair germs (McGowan *et al.* 2002). As the hair tissue matured further, K17 protein was maintained in the outer root sheath and the matrix of the bulb but otherwise persisted in a detectable form only in the medulla of the differentiated hair shaft. McGowan *et al.* (2002) showed that its absence translated into the acute fragility of the first hair shaft produced by postnatal follicles, albeit in a strain-dependent fashion. This might point to an important contribution of the medulla compartment to the mechanical resilience of the first hair produced, or alternately, to a crucial role for K17-containing filaments in scaffolding the assembly of the differentiation-specific keratins expressed in the hair cortex and/or the inner root sheath layers. It would be interesting to speculate that hoof horn described as poor quality may have different keratins compared to horn of good quality.

The white appearance of nails in heredity white nail (Norgett *et al.* 2004) disease seems to be due to an abnormal keratinisation of cells originating from the proximal nail matrix, leading to the presence of abundant intracellular vacuoles and to a lesser compactness of keratins. Cell loss was compensated by hyper-proliferation of the distal matrix and of the nail bed keratinocytes, with persistent marked parakeratosis and loose arrangement of keratin bundles. The distal matrix and the nail bed contributed equally to formation of the lower plate. This presented the characteristics of a tissue composed of soft keratins. Genetic linkage indicated that the gene defect in the family studied resided on chromosome 12q13. As the type II keratins map within this chromosomal interval, it is possible that a mutation in one of these keratin genes may be a cause of the hereditary disease.

Keratin K2e is a type II polypeptide which is expressed in epidermis late in differentiation, (Bloor *et al.* 2003). The normal expression of K2e in the upper spinous and granular layers is increased in scars and shows distinct down regulation in psoriasis. Injury to the epidermis activates a homeostatic mechanism and the changes in gene expression that accompany re-epithelialisation are similar to those seen in other disorders of hyper-proliferation such as psoriasis, contact dermatitis and squamous cell carcinomas, (Bloor *et al.* 2003) suggesting that there is considerable overlap in the signalling cascade. It is possible that gene expression in laminitic hoof horn might differ from a non diseased hoof.

The bovine hoof has been examined as a model for the study of keratinized skin appendages. Kvedar *et al.* (1986) characterized the keratin polypeptides of hoof bed and matrix and

compared them to epidermis using two-dimensional electrophoresis and immunoblot techniques. Both hoof tissues expressed keratins 6 and 16 and b2 and a1-4 which were previously undescribed proteins unique to the bovine hoof. Keratins of hoof matrix and bed shared one or more common antigenic components as defined by immunoblot analysis. Hoof matrix expressed keratins7 and 14, which are absent in hoof bed, and a greater number of variants of keratin 6.

It is well known that cross-species sequence comparison can help highlight important functional elements such as exons, because such elements tend to be more strongly conserved by evolution than random genomic sequences. If a protein encoded by a gene is already known in one organism, it is relatively simple to search genomic DNA from another organism to identify genes encoding a similar protein, (Batzoglou *et al.* 2000). Milenhovic *et al.* (2002) showed that the number of conserved segments between horse and human increased from 43 to 113. More than half of the known keratin genes are expressed in the hair follicle and hair keratin research has made considerable progress as the entire sets of the human type I and type II hair keratin genes as well as the patterns of expression of the encoded proteins have been elucidated,(Kikkawa *et al.* 2003). Knowledge of their differential expression will help in the understanding of the mechanisms of formation and help elucidate the aetiology of diseases in keratinised tissues across species.

mK6 gene has been characterised as being 9.1kb long and expressed in hair, nail bed epithelium and dorsal tongue epithelia, (Wang *et al.* 2003). In stratified epithelia the basal keratinocytes express K5, K14, K15 and K19 as major keratins but depending upon the eventual differentiation different pairs of keratin are expressed; in skin K1/K10, in buccal epithelial K4/K13 and in cornea K3/K12 are expressed. K9 is a type I keratin expressed almost exclusively in palmo-plantar epidermis and K2 is a type II keratin expressed in oral epithelia, (Bloor *et al.* 2003). Keratins K6, K16 and K17 are associated with hyper-proliferation such as wound healing, (Takahashi and Coulombe, 1997; Takahashi *et al*, 1998). It would be interesting to study whether poor quality horn is genetic in its origin and whether there is an interaction between genome expression and nutrition.

Intermediate filaments (IF)

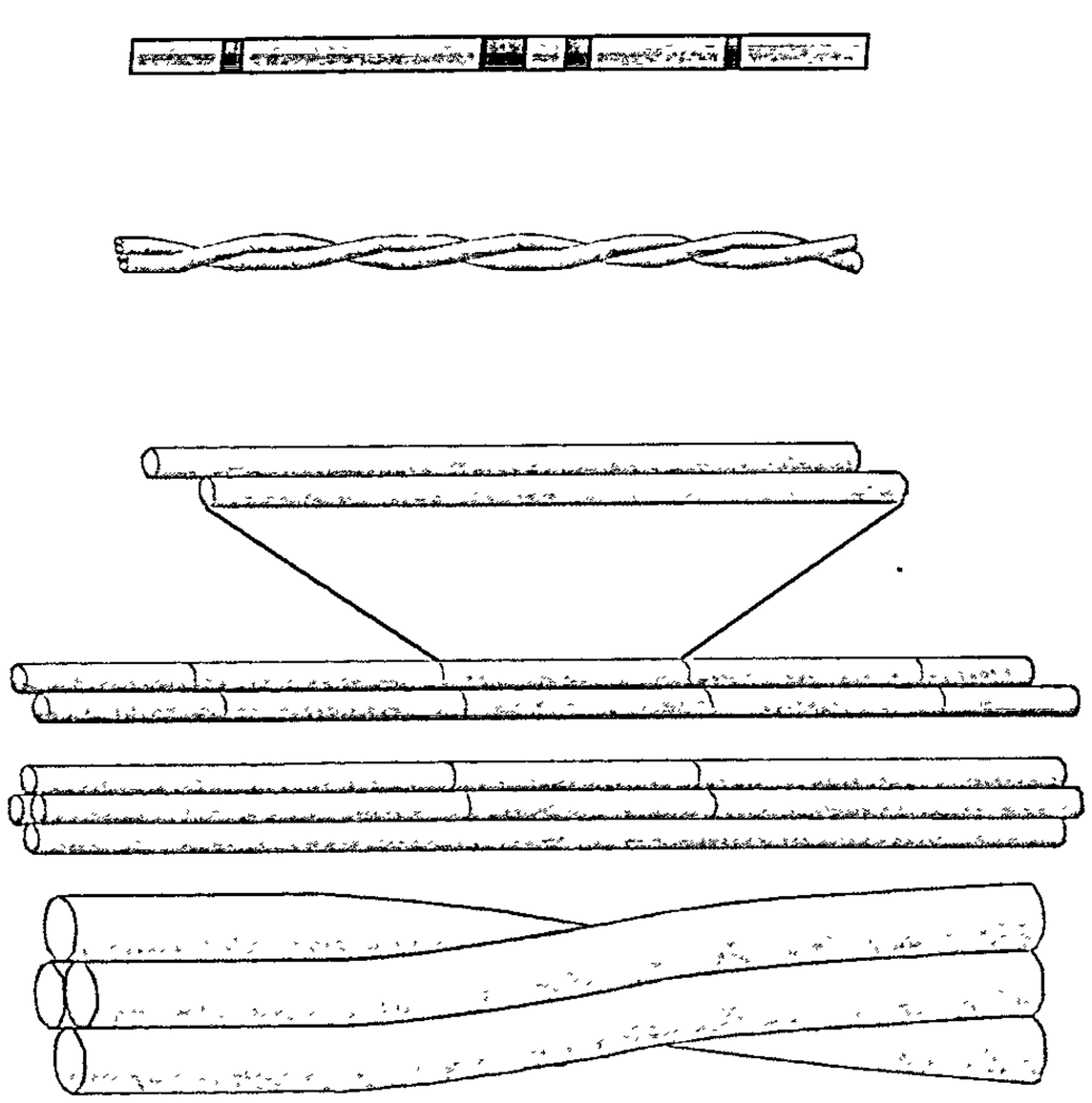
Fibrous keratin was earlier described as wispy clumps of fine filaments, (Birbeck & Mercer 1957) in the lower layer of the epidermal structure. It was recognised that the filaments were less than 100A in width, and had definite structure, being orientated parallel to the long axis of the keratinocyte. As the keratinocytes moved towards the cornified layers, the bundles rapidly increased in length and width by accretion of further filaments. Rogers, (1959) confirmed that

the microfibril was the fibrous unit of α keratin which was organised into larger bundles or macrofibrils surrounded by a matrix.

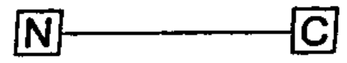
Copper has been shown to play a role in the cross linking of the keratin filaments and in the cross linking of proteins within the matrix, (Hynd 2002). Some skin literature, (Montagna and Parakkal 1974) described the fibril structures as tonofibrils and referred to the compact arrangement of fibril-matrix as the 'keratin pattern'; however a precise definition of keratin was not given. The same terms of reference are used to describe the 'keratin pattern' of hair and nail, (Montagna and Parakkal 1974). More recently it has been established that keratins belonged to a group of proteins classed as intermediate filaments, (Eckert 1989). Intermediate filaments, (IFs) comprise the cytoskeleton of numerous cells. Three filament systems are recognised, (Boden P 2003, personal communication), i) microfilaments are 6nm in diameter e.g. myosin/actin; ii) intermediate filaments are 10nm in diameter e.g. keratin and iii) microtubules are 25nm in diameter e.g. tubulin a contractile protein.

The structure of the filaments was described by Suter *et al.* (1997). An acidic keratin and a basic keratin chain pair to form a dimer. Two dimers then associate in an anti- parallel, staggered fashion to form a tetramer protofilament. Finally four protofilaments associate to form the 10nm keratin intermediate filament as shown in *plate 1.2.2.i. The helical formation of the keratin intermediate filament.*

It has been suggested that there is an association between the size and number of IFs in a keratinocyte and the complexity of the epithelium; simple epithelia express only small keratin polypeptides, whereas epithelia that require structural rigidity express a subset of larger keratin polypeptides, (Eckert 1989). The IF bundles form a complex three dimensional scaffold which is attached at the cell envelope by desmosomes; desmosomes are junctions between adjacent keratinocytes, ensuring that although the IFs do not physically continue into the neighbouring cell, there is continuity of the scaffold. In addition, it is considered that the end domains of the helixes are 'free' of the rod structure so that they can react with themselves and with the matrix associated proteins (Gillespie 1991). In human epidermis the end domains are 60-70% glycine which contributes to the insoluble but flexible characteristic of the skin. The 'hard' keratins of hoof, hair and nail have end domains which are rich in cysteine and proline; in this instance the cysteine is able to form double- double sulphur bonds with themselves or with the cysteine rich proteins of the matrix, (Marshall *et al.*1991).

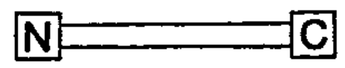


Monomer



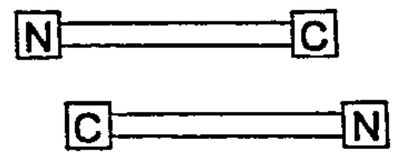
Polypeptide of keratin

Parallel Dimer



Interaction between an acidic & a basic polypeptide

Anti-Parallel Tetramer

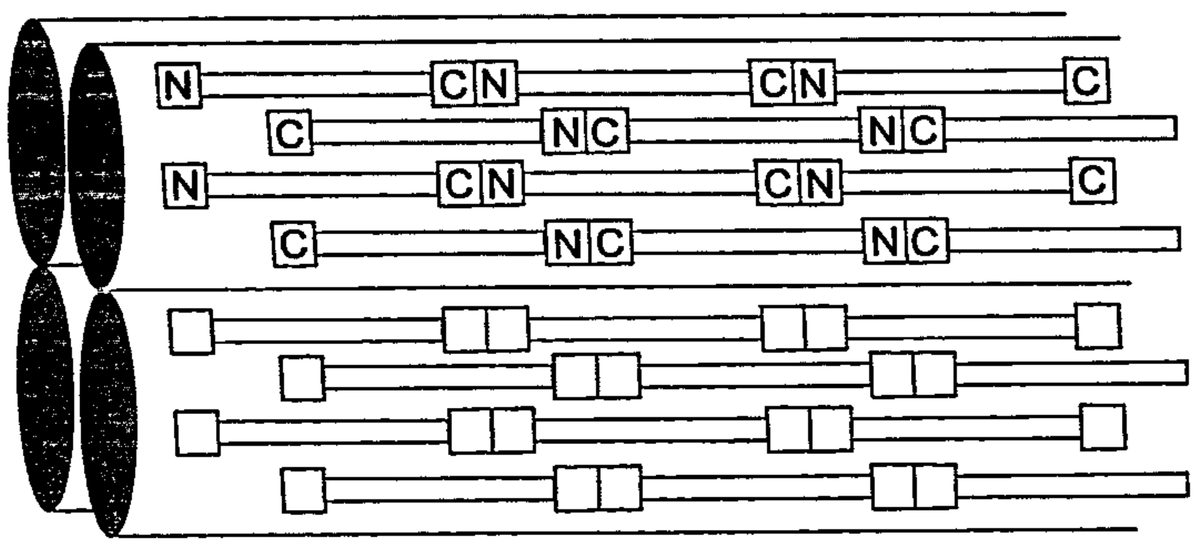


Two dimers associate in an anti-parallel to form a protofilament

Protofilament

Protofibril

Intermediate Filament



Four protofilaments form the 10nm diameter keratin intermediate filament

Artwork © Dave Gibson

Plate 1.2.2.i The helical formation of the keratin intermediate filament

Note 1 adapted from Suter *et al* (1997)

The matrix

The matrix surrounds the keratin intermediate filaments, within the keratinocyte and was described as the denser material between the filaments, (Birbeck & Mercer 1957). It was considered to function as adhesive cement. Even by 1974, Montagna still described the cytoplasm as an amorphous matrix, which is high in cysteine and sulphur. Lynch *et al.* (1986), using antibodies, identified a group of high sulphur amino acids, which were smaller than the 40-70 K range of IFs and lacked the epitope[g] recognised by the IF antibody. They classified them as proteins of the matrix and defined them as 'intermediate filament associated proteins, (IFAPs). O'Guin *et al.* (1989), using monoclonal antibodies showed that the IFAPs were produced later in hair follicle development and did not appear until after the zone of elongation. Dale *et al.* (1989) concluded that the matrix high sulphur proteins may bind copper via their cysteine residues. Suter *et al.* (1997) also distinguished the matrix from keratin by stating that cells stop making keratin protein and start to produce interfilamentous associated proteins (IFAPs) such as filaggrin[g] once they reach the granular layer. Filaggrin and the interfilamentous proteins act as glue for the keratin filaments and allow bundling of the intermediate filaments into macrofilaments.

The cell envelope

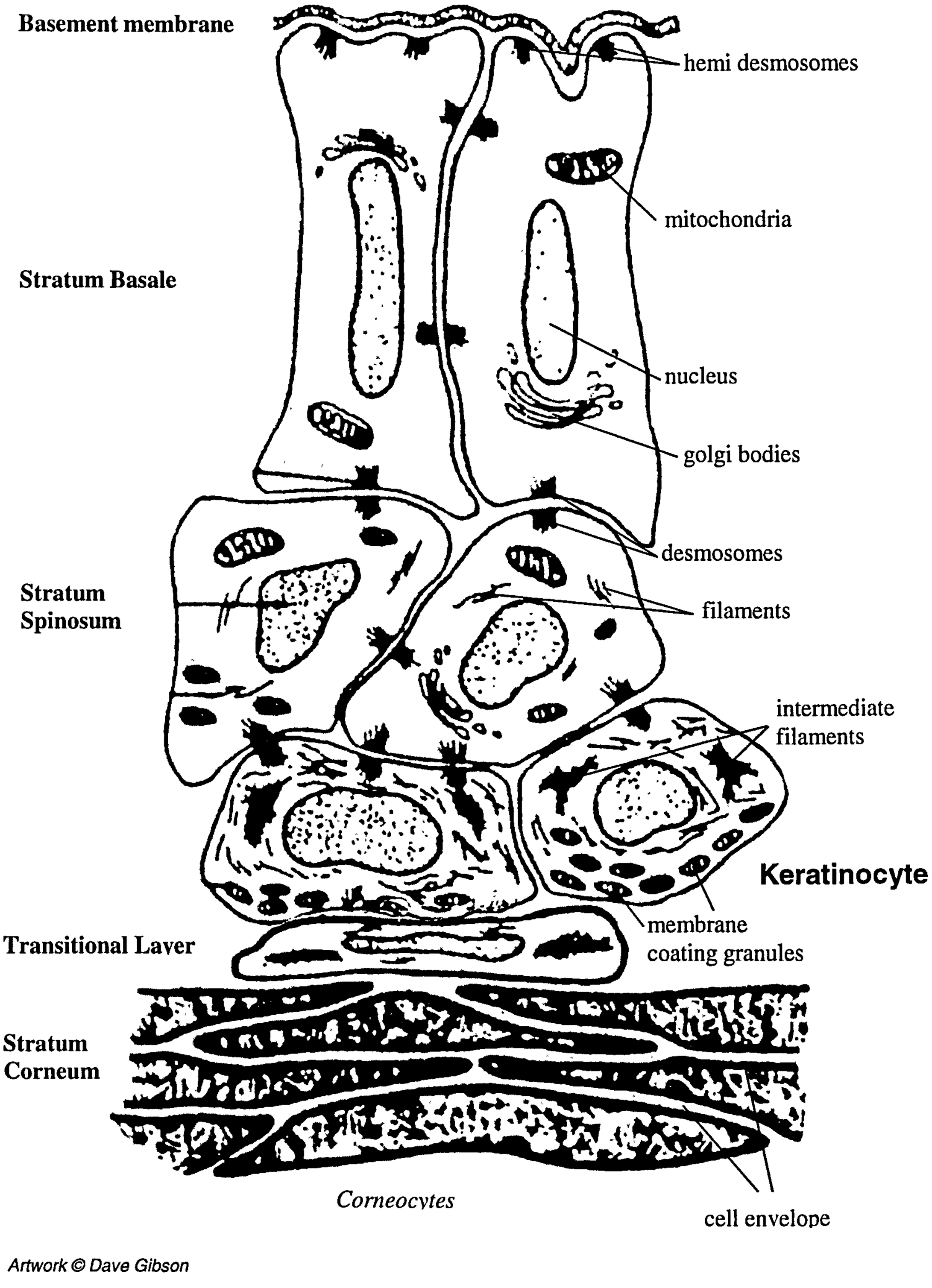
The cell envelope does not form around the keratinocyte until the termination process is almost completed. Its formation is dependant upon the calcium concentration within the cell. The presence of a resistant cell membrane consisting of two lightly stainable β segments with a central δ segment of intercellular cement had been discovered with the electron microscope in the 1970s, (Peters and Bradbury 1976),although its components were not known. Peters and Bradbury, (1976) proposed that the membrane contained high levels of lysine, making it resistant to chemicals and hydrophobic. Reichert *et al.* (1993) described the cell envelope as a stabilised protein envelope to which was attached ceramides and fatty acids originating from lamellar bodies. The envelope accounts for about 7-10% of the dry matter of the stratum corneum and is 90% protein and 10% lipid, making it the most insoluble part of the corneocytes, Reichert *et al.* (1993). Cross linking of up to 18% of the lysine by ε- (γ- glutamyl) lysine bonds and the bis (γ- glutamyl) polyamine cross linking of glutamine residues contributes significantly to the stability of the envelope. Involucrin , a calcium dependant enzyme, is found in human keratinocytes and is thought to serve as a scaffold for the ordered cross linking between the different proteins by exposing glutamine residues to transglutaminases, like a zip, (Eckert 1989).

The original plasma membrane of the keratinocyte disintegrates as it matures, to be replaced by a highly cross linked, insoluble outer membrane which is about 15nm thick and is known as the cornified or cell envelope, (Suter *et al.* 1997). The envelopes are extremely resistant to alkali, detergents and reducing agents and they confer a high level of rigidity and stability to the terminated keratinocyte, (Eckert 1989), once the termination process is completed and the keratinocyte has been cornified it is defined as a corneocyte. Leeder, (1986) considered that the strength of the wool was dependant upon the adhesive connections and therefore that the cell envelope/ cell membrane complex had an effect on the mechanical and chemical properties of wool which is out of proportion to the small amount present. Leeder, (1986) described the cell membrane complex as being 6% of the weight of the whole wool fibre; in his definition he included the resistant membranes, (cell envelope), the lipids and the intercellular cement, all of which are responsible for the adherence of the corneocytes to each other.

For the purpose of this thesis, and in line with the more recent literature, keratin is defined as the polypeptides, (categorised genetically into acidic and basic groups), which form the intermediate filaments of the keratinocyte and corneocyte. This allows discussion regarding relationships between trace element concentrations and keratin and other proteins within the epidermis.

1.2.3. The process of keratinisation and cornification

As well as containing similar cell types, all stratified epidermal tissues are composed of a series of distinguishable layers, through which the keratinocyte differentiates from a relatively undifferentiated, rapidly reproducing stem cell of the basal layer and terminates into a non-reproducing, non living corneocyte, *plate 1.2.3.i., The differentiation of the keratinocyte into the corneocyte*. Trace elements may influence the differentiation of any layer within the epidermal structure.



Artwork © Dave Gibson

Plate 1.2.3.i The differentiation of the keratinocyte into the corneocyte

Note 1 x10,000
Note 2 adapted from Eckert, 1989

Basement membrane

The basement membrane is the junction between the dermis and the epidermis. It is only 40-60nm thick and cannot be seen by the light microscope, (Suter *et al.* 1997). It is not cellular but is composed of extracellular matrix components mostly anchorage proteins. These proteins are responsible for the anchorage of the first layer of epidermal cells which are the basal cells. In addition, the basal cells have hemidesmosomes and focal adhesions on their base to ensure attachment to the basement membrane. The basement membrane follows the contours of the dermis which, in the equine hoof is divided into three regions; the coronary corium, the laminae corium and the solar corium. In each region the dermis is increased in surface area; by papillae at the coronary band and at the sole and by interdigitation in the longitudinal axis similar to the human nail bed, (Baden & Kvedar 1991).

Stratum Basale or Basal cell layer

The keratinocyte starts as a columnar cell, which is attached by papillae to the basement membrane (Birbeck & Mercer 1957). The basal keratinocytes synthesis K14 (type I acidic) and K5 (type II basic) keratins, form small intermediate filaments, which aggregate around the nucleus or attach to the hemidesmosomes and the desmosomes at the cell membrane, (Suter *et al.* 1997). Skin calcium binding proteins are present to prevent premature differentiation of the basal keratinocyte.

Stratum spinosum

As the keratinocyte migrates to the next layer it develops numerous desmosomes in the cell membranes which gives it a spine like appearance hence its name. The cells start to synthesise a new and larger molecular mass keratin, (Eckert 1989) and contain filament bundles. The keratin composition of the keratinocyte changes as the cells migrate though the layers and differentiate. As the cell moves into the spinous and germinating layers, the smaller keratins are replaced by K1 and K10, much larger keratins, which are considered to be markers of differentiation and are only ever expressed in the upper layers of the epidermis (Reichert *et al.* 1993). The development of the cell envelope is dependant upon involucrin and transglutaminase, both of which are present in only very small amounts in the basal cells. The keratinocyte synthesises large quantities of both in the spinous layer once it is ready to start the process of cross- linked envelope formation.

Stratum granulosum

The epidermis of the hoof wall does not contain a granular layer (Pollit 1992), however lamellar bodies and lipids are apparent in cultured hoof cells, (Hendry *et al.* 1995). The process of differentiation of the equine hoof epidermis differs only from other epidermal tissues by the

absence of keratohyalin granules. Keratohyalin is associated with more pliable epidermal tissues which undergo desquamation, (Leach 1980), such as the skin, but is also absent in the nail epidermis. As the cells move towards the stratum corneum, the keratinocyte synthesises profilaggrin which is a precursor of the calcium dependant filaggrin which aids in the aggregation of the keratin filaments, (Eckert 1989), into macrofilaments. Filaggrin is only found in the upper cell layers.

Membrane coating granules (MCG) are formed in association with the Golgi apparatus[g], (Montagna and Parakkal 1974) and migrate towards the plasma membrane to release their lipid contents into the intercellular space. The dense material lies in discrete intercellular domains, greatly expanding the surface area, (Elias 1981).

Stratum corneum

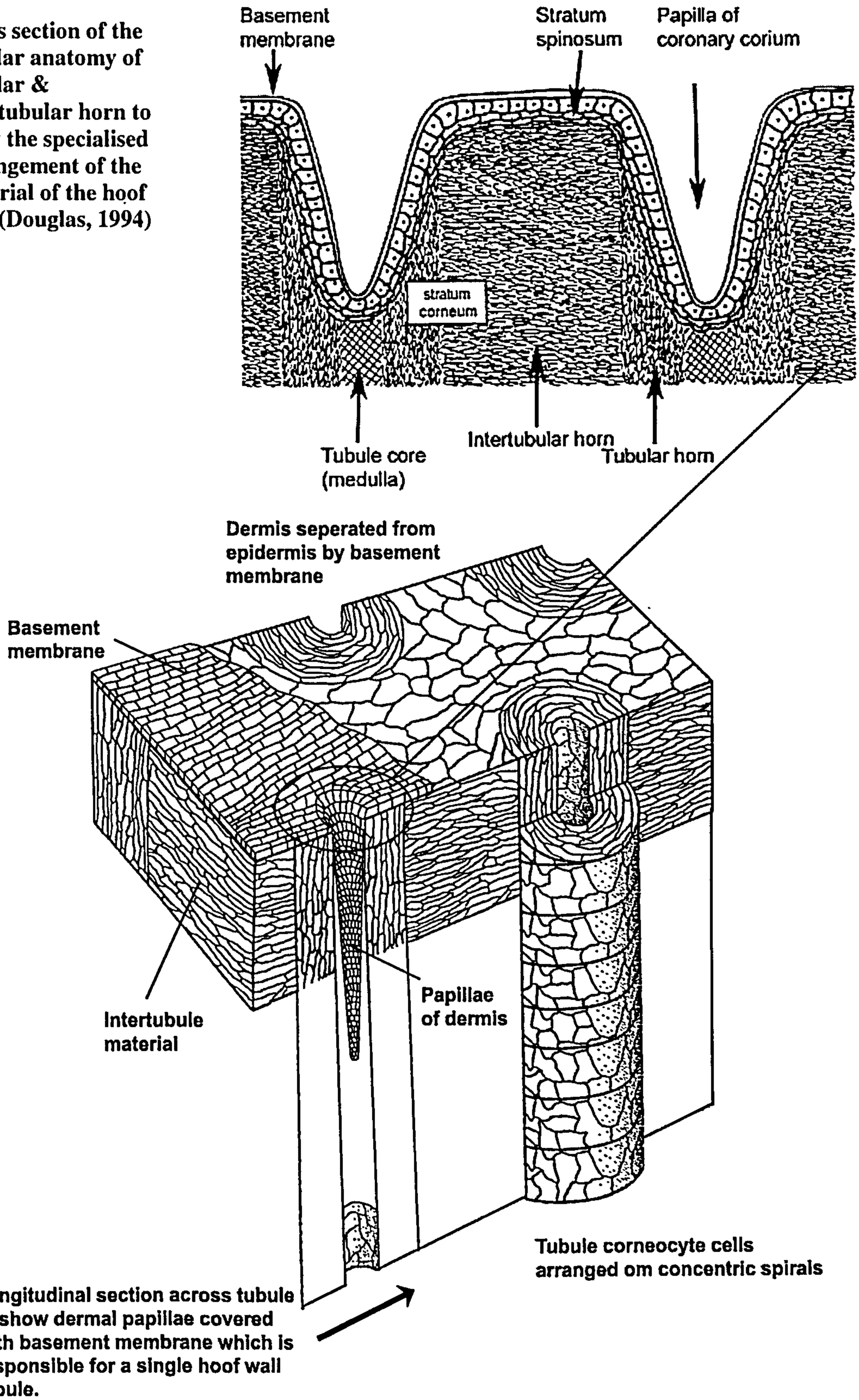
The plasma membrane starts to disintegrate and intercellular calcium enters the cell, which triggers the development of the cell envelope. At the same time proteolytic enzymes destroy the cell organelles which exit the cell as the cell envelope gradually thickens and develops underneath the plasma membrane. The membrane coating granules release their lipid contents into the intercellular spaces and fuse with the membrane. It is thought that these lipids play an important role in the water barrier of the cornified cells, (Eckert 1989) as well as acting as cement, which together with the desmosomes hold the corneocytes together.

By the time the keratinocyte has finally migrated to this layer, it has undergone intense enzymatic activity and restructuring. All its cell organelles have been destroyed by lipases and at the same time the lamella granules fuse with the cornified envelope and release their lipid contents into the intercellular space. The cell dehydrates, loses 70% of its dry weight and its cycle of differentiation is terminated, (Eckert 1989). The corneocytes contain keratin macrofilaments, a matrix of associated proteins and some remnants of membranes and cell organelles which were not completely digested. It is therefore possible that some of the trace elements that are not incorporated into specific proteins may still remain within the corneocyte.

1.2.4 The structure of the equine hoof wall

The hoof wall structure is a complex modification of stratum corneum as shown in *plate 1.2.4.i. The arrangement and structure of the stratum medium of the equine hoof wall epidermis.* The modification is necessary so that the epidermis and the dermis stay attached and can withstand the forces imposed by the weight of the horse during movement. The dermis folds into papillae which increases its surface area. The dermis is covered with a basement membrane which produces the epidermis; the membrane covering the folds of the papillae produce cells which differentiate into the material which forms the tubules, the tip of the papillae forms the medulla of the tubule and the epidermis between the papillae forms the intertubular horn. The cornified structure of the hoof wall is the stratum medium and is considered a composite with the tubules acting like reinforcing rods and the intertubule material as concrete. The tubules run proximo-distally[g] from the coronary band to the sole, *plate 1.2.4.i.* parallel to the dorsal[g] surface of the wall. The function of the hoof wall depends upon both the combined properties of the composite nature of its material, which is the keratin intermediate filaments embedded in the matrix, and the composite nature of its structure, which are the tubules embedded in the intertubule material, (Kasapi and Gosline 1996; 1997; 1999). Alterations to the keratinocyte because of changes in nutrient supply during keratinisation may affect the function of the material of the hoof wall.

Cross section of the cellular anatomy of tubular & intertubular horn to show the specialised arrangement of the material of the hoof wall (Douglas, 1994)



Section through stratum medium of the hoof wall to show structure of the equine hoof wall epidermis, (*adapted from Bertram and Gosline, 1986*) *Artwork © Dave Gibson*

Plate 1.2.4.i The structure of the hoof wall epidermis

1.2.5 The shape of the equine hoof wall

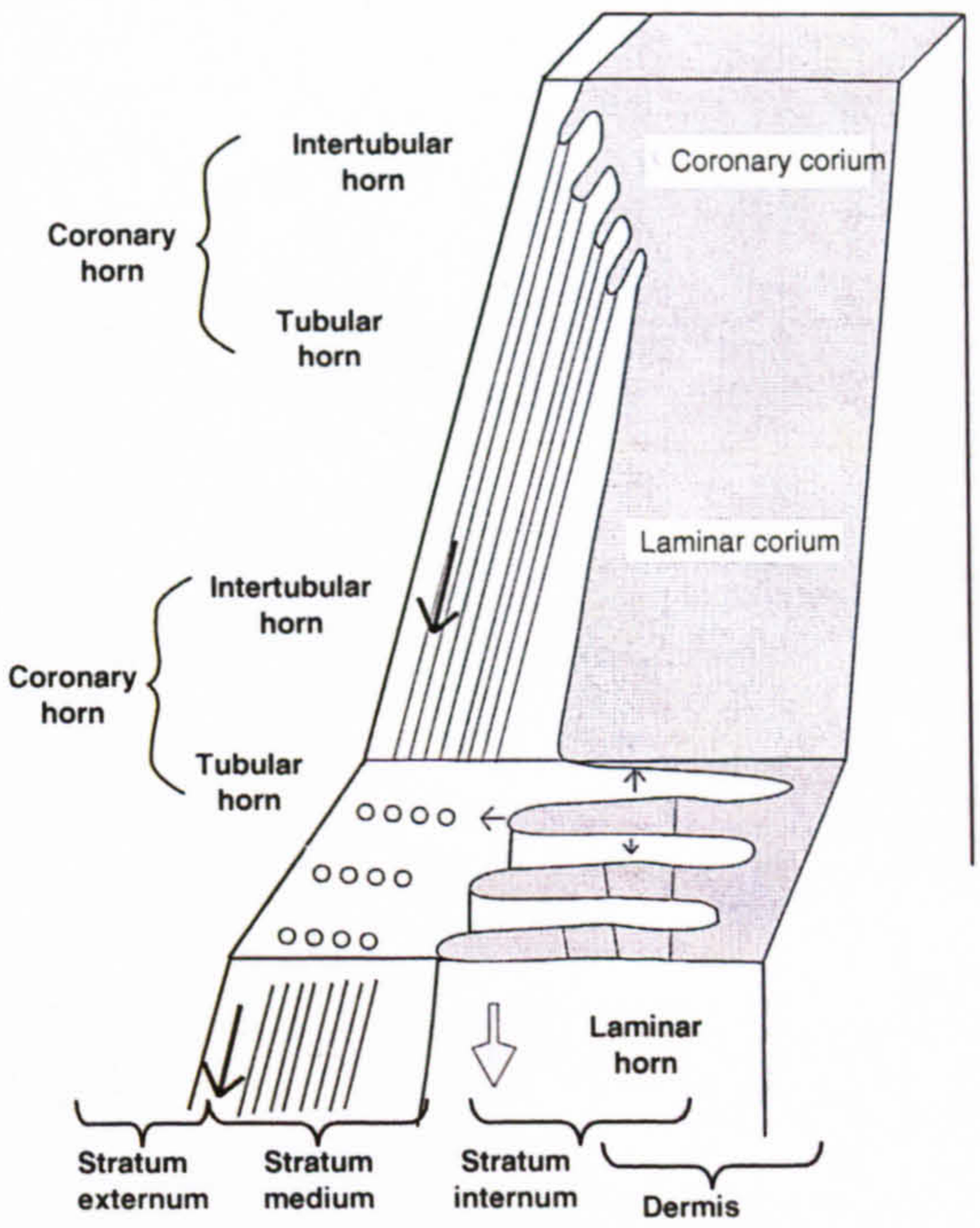
The material of the hoof wall forms the hoof capsule. Stratum medium, (corneum) is produced by the dermis around the coronary band, *plate 1.2.5.i The relationship between the structures and shape of the equine hoof wall capsule* and the wall grows from the coronary band, new material is continuously being produced at a rate of 0.164mm/day, (Reilly *et al.* 1998). It would be natural to assume that the shape of the hoof wall is dependant upon the coronary band, however as discussed in chapter 5, there are numerous factors which contribute to the shape of the wall and capsule and there is still debate as to whether the hoof wall is a cone or a cylinder.

The ability of a structure to bear load is in part dependant upon its shape. In the case of the hoof changes in shape have been shown to alter stress concentrations as reviewed later in this chapter which may affect crack incidence independent of the material formed by keratinisation or as a combination of both factors.

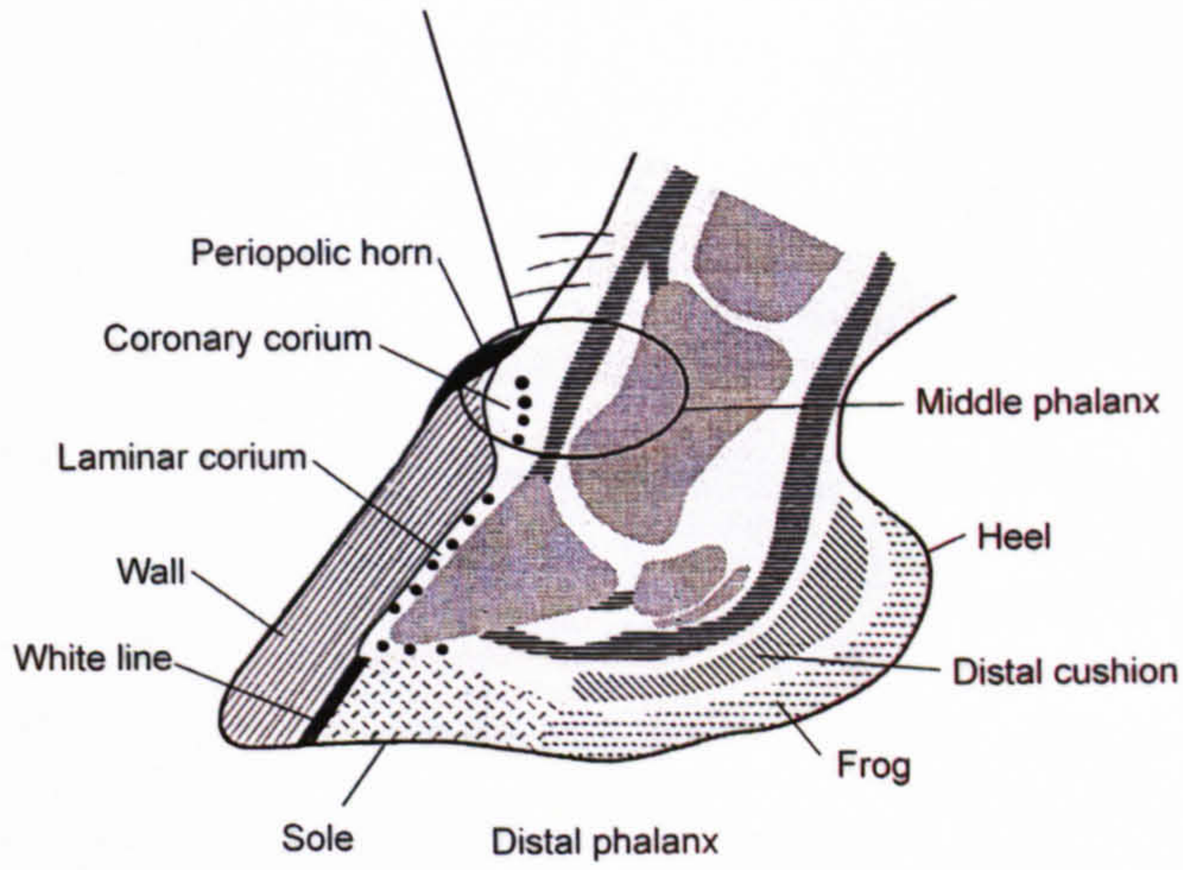
1.3 The role of trace elements in keratinisation

It is clear that nutrition can affect the process of keratinisation. Certain trace elements are involved in normal keratinisation and cornification. Altering the levels of these trace elements may have an affect on keratinisation. The effects of trace elements on keratin and keratin containing tissues are likely to be similar. Illustrations from a wide breadth of epidermal tissues can be used to hypothesis the possible influence of dietary trace elements on keratinised equine hoof wall when, experimental results are not available. It is important to distinguish between trace elements and minerals which have an indirect effect on keratinisation as a result of reduced dietary intake or impaired metabolism, from those which have a direct effect and are involved in the metabolism of the keratinisation, (Hynd 2000). If trace elements were simply being used as co-catalysts then to assume that the trace element would be found in the keratinised material would be naïve.

Transverse & longitudinal hoof wall block section from the proximal hoof wall, (adapted from Leach, 1996)



Sagittal section to show the relationship between structure & shape (Kempson, 1994)



Gross shape of the hoof wall epidermis

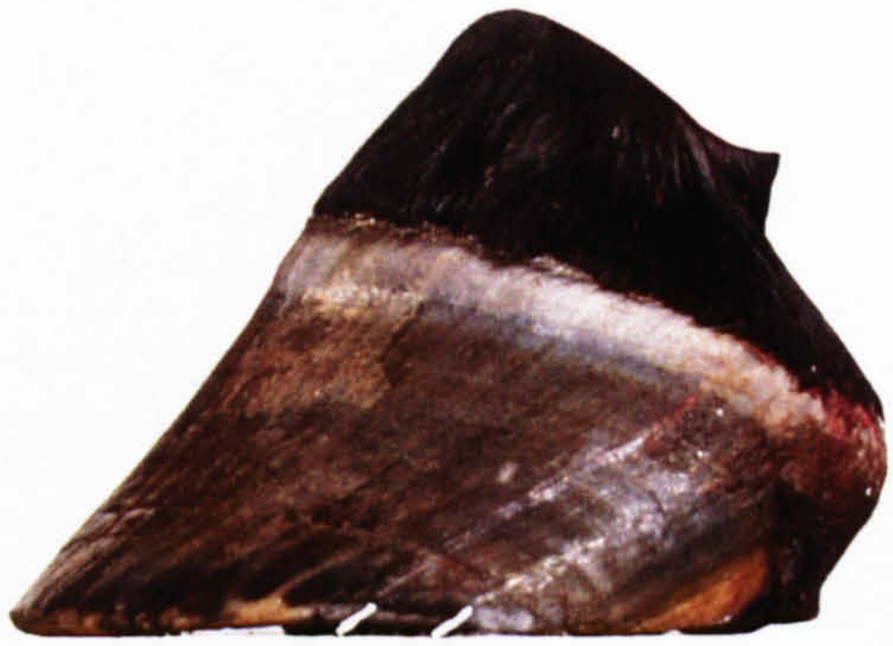


Plate 1.2.5.i **The relationship between the structures and shape of the equine hoof wall capsule.**

1.3.1 Copper

It is likely that the mechanism behind copper deficiency in keratinogenesis is similar to that in collagen and elastin formation because of the involvement of copper with the cross linking enzyme lysyl oxidase as reviewed below. If the effect that copper has on the microfibril and matrix formation of elastic tissue is similar to that in keratinisation, then copper deficiency in equine hoof horn may affect the IFAPs not the keratin.

Elastic tissue contains microfibrils and during elastogenesis, elastin is organised amongst the microfibrils. The microfibrils bond with elastin to form fibres, which in turn form bundles and in mature elastic tissue, the microfibrils are found in the interstices of the amorphous matrix, (Howell 1987). Similar to keratinisation, elastogenesis involves a high level of cellular activity, as well as vesicles which release their contents to 'coat' the fibres. In copper deficiency, elastic tissue degenerates in three stages; i) swelling of elastic fibrils, ii) partial dissolution and vacuolation of the elastic tissue matrix and iii) total dissolution of the matrix and swelling of fibres, (Gawthorne 1987). Culturing cells from copper deficient chicks resulted in; reduced lysyl oxidase activity, reduced oxidative deamination, reduction of lysine being converted to desmosine and reduced cross linking in elastin tissue, and a reduction in elasticity of the original tissues,(Hill *et al.* 1967)

Adding copper, or copper bound to albumin, to the medium of previously copper deficient cells induced lysyl oxidase, (Rayton and Harris 1979). By using labelled copper and lysine, Rayton and Harris, (1979) showed that copper became bound to a newly synthesised protein fraction, and concluded that is likely that the induction by copper resulted in enzyme synthesis rather than enzyme activation. In addition it was shown that when protein synthesis was blocked, the copper did not bind to any proteins, suggesting that the inclusion of copper into lysyl oxidase must happen post- translation. Assuming the mechanism for activating lysyl oxidase [g] is similar to that in keratinisation, then the amine oxidase required for cross linking of the keratin IFs and the IFAPs will not be formed without adequate copper. What is still unclear is where the amine oxidase[g] goes once it has activated the cross linkages and whether analysing the cornified tissue is likely to be indicative of a copper deficiency.

Other effects of induced copper deficiency on cell integrity have been illustrated in cattle pancreatic tissues, (Fell *et al.* 1985). The sacrificed cattle showed a reduction in the collagen of

the basement membrane which resulted in a loss of cell adhesion. The authors reported dissociation and disorganisation between individual cells in histological samples and one has to assume that these were not sampling anomalies. The authors concur that the deficiency is likely to be affecting the glycoproteins and sulphation[g] rather than the cross linkages.

Some of these affects may well occur in the keratinising cells of the equine hoof wall. It is clear that copper is involved in the cross linking of collagen, elastin, (O'Dell 1976); it is also clear that copper plays a similar role in keratinisation. Marston, (1946) measured the amount of free sulphydryl groups in the keratinising layers of epidermal tissues from sheep fed copper deficient and normal diets. Alkaline nitroprusside, an alkalising agent which reacts with thiol (-SH) groups to form a purple complex, was applied to fresh skin or wool fibres. The basal cells through to the granular layer of keratinising tissue stained very lightly; the granular layer through to the fully cornified layer was intensely stained and the staining stopped abruptly in the fully cornified wool. When applied to copper deficient wool, the intense purple colour extended ten times further, indicating that the –SH groups had not been oxidised to S-S. In normal epidermis, the oxidation of –SH to S-S is catalysed by copper within 8-12hrs and the fibre is 'set'. In copper deficient sheep, this oxidation is still incomplete after three days.

Patients suffering from Menkes[g] syndrome have pathological symptoms in both collagen, elastin and hair tissue, (Tsukahara *et al.* 1994) similar to that described in the literature above. The syndrome is a result of a mutation in a gene, (ATP7A) which determines a membrane copper binding protein, (MNK) which is found in the Golgi apparatus of most cells. The mutation results in MNK failing to supply copper to copper dependant enzymes as they pass through the secretory pathway. The pathological signs in this disease have been explained by isolating the gene responsible, thus showing that the deficiency signs are linked to a failure to transport copper, (Dagenais *et al.* 2001). The pathology is not just in connective tissue but also epidermal tissues of hair and skin because of a secondary deficiency of the copper requiring enzymes in collagen and keratin , (Suzuki and Gitlin 1999). A similar pattern of reduced S-S groups and increased -SH has been shown in the hair of people suffering from Menkes disease. 'An enzyme responsible for forming the disulphide bonds has still not been identified and the possibility that it is a direct effect of copper still exists',(Danks 1991). Thus it does not seem unreasonable to hypothesise that the pathological effects of copper deficiency due to failure of cross linking are similar across the tissue types.

1.3.2 Zinc

In cell culture divalent[g] ions stimulated the polymerisation of keratin polypeptides[g] into IFs *in vitro*, however ten times more $CaCl_2$ was required than $ZnCl_2$ (Fukuyama *et al.* 1980). Even at lower molar concentrations; (0.4-0.5mM Zn compared to 1-3mM Ca), the zinc stimulated the formation of filaments and bundles much more rapidly than calcium. These differences led the authors to indicate that there may be specific binding sites for zinc in keratin molecules.

Evidence that zinc is needed for keratinisation is offered by Lansdown and Sampson, (1997) who showed that the concentration of skin zinc increases by at least 15% above normal during the inflammatory and proliferative phases of wound healing. They also compared zinc concentrations in epithelial tissues of the dog, comparing tissues with high levels of proliferative activity, (the tongue) with areas of intermediary prolific activity (skin) and low proliferative activity, (the footpad). The concentration of zinc was highest in the tissues with the greatest rates of epithelial proliferation and metabolic activity.

Researchers have studied the effect of zinc deficiency on the ultra structure of epidermal cells under the electron microscope, (Weismann *et al.* 1980). Skin from three groups of rats was studied: - i) zinc deficient with adlib[g] energy; ii) supplemented zinc and iii) pair fed[g]. Throughout the epidermis, zinc deficient rats had keratinocytes which were oedematous[g], especially at the edge of the cytoplasm; the mitochondria were cystic and lacked DNA granules. The Golgi apparatus were small and primary lysosomes were present. In the basal and spinous layers only, the keratinocytes contained numerous ribosomes and mitochondria. The mitochondria had no DNA granules and IFs were sparse. In addition, the desmosomes were poorly developed and some degenerate keratinocytes were present; but the filaments were located in middle of cell and the IFs were at the periphery. The authors concluded that zinc deficiency depressed DNA synthesis in epidermal cells.

Ultra structural changes in pig epidermis showed similar results, (Kapp and Simon 1980); in addition, the IFs lost attachment with the desmosomes and free desmosomes appeared in the intracellular space. The gross lesions measured had their origins in the germinating layers with the main effect on the keratin filaments. The lesions were most noticeable in areas of the skin exposed to mechanical stress. Thus it appears that zinc affects those cells that are rapidly differentiating and the changes are reflected in the final tissue.

Whilst it is well accepted that zinc deficiency results in skin disorders and is implemented in the keratinocyte growth mechanism, the exact mechanism has yet to be elucidated. Zinc has a duplicitous effect on the growth rate of keratinocytes; (Bourdeau and Isbisch 1998). At low concentrations, (0.3 – 3.0 X 10^{-6} g/ml) zinc increased the growth rate of keratinocytes by up to 20%, at high concentrations, (30 X10^{-6} g/ml) zinc decreased the growth rate of keratinocytes by 33%. Growth rate was measured by labelling IF. The toxicity of zinc at high concentrations was more apparent when zinc was added to a culture of carcinoma cells; growth rate was decreased by 94%. Excess zinc may well have similar gross effects on keratogenic material as a deficiency of zinc. The effect of zinc may not be primary but may be secondary because it is competing with other cations for binding sites on the protein.

Zinc is associated with proteins in both a catalytic and structural role. Because of the number of enzymes associated with zinc, the effect of a deficiency or excess of the ion is unlikely to be restricted to only one aspect of keratinisation. Zinc deficiency is associated with hyperplasic[g] epithelium, (Said Al-Naief and Ashrafi 1995). Characteristics of the hyperplasic epidermis included an increase in membrane coating granules, (MCG) in the granular layer of cells and the presence of MCG in the cytoplasm of the stratum corneum. Cell nuclei were also present in the stratum corneum. In 'normal' stratum corneum, MCGs would not be present having expelled their contents intercellularly before cornification. The lipid and hydrolytic enzymes which are 'extruded' from the MCGs help desquamation at the surface of the stratum corneum. The lack of extrusion from the MCGs may explain the thickened skin in parakeratosis, a sign of Zn deficiency but the biochemical link is still unclear.

Others, (Calvin and Bleau 1974), presented evidence to show that zinc–thiol complexes have a widespread structural role within keratinised tissue. Zinc cannot be extracted from rat sperm epidermis either by washing with Tris buffers or by sonification indicating its location within highly stable cell structures. Incubation with sulphydryl reagents and EDTA did release the zinc. In addition the majority of zinc in rat sperm is localised within a cysteine rich protein fraction. The primary role of zinc in nutrition appears to be the fundamental process of cell replication and gene expression. The concept that cells require a precise quantum of zinc before embarking on cell division is consistent with near normal zinc content of most tissues even in severely zinc deficient animals, (Purser 1979). Proteins containing both zinc and copper can be isolated and extracted from rat epidermal cells and are proposed to be involved in the regulation of certain enzymic functions which regulate the last stage of epidermal cell differentiation, (Ito *et al.* 1984). It is clear that zinc is essential for keratinocyte maturation into the corneocyte.

1.3.3 Calcium

Calcium plays an important role in keratinocyte differentiation, (Eckert 1989). Using keratinocyte cell cultures, the calcium content of the cell medium can be manipulated and the effect on the keratinocytes monitored. Reducing calcium in cell culture medium from 0.1mM to 0.05mM resulted in the keratinocytes remaining in an undifferentiated single layer, (Hennings *et al.*1980; Heenen *et al.*1992). When the calcium concentration was increased to 1.2mM, the cells stopped cell division, developed desmosomes and within a few days, stratified and developed cell envelopes, (Heenen *et al.* 1992). Hennings *et al.* (1980) recorded an inhibition in DNA synthesis and a reduction in RNA and protein synthesis soon after calcium was increased in the medium. Perhaps indicating that induction of termination by calcium is accompanied by a complete ending of proliferation. Calcium also appears to trigger synthesis of new keratins. Heenen *et al.* (1992) showed that cells kept in low calcium medium only contained keratins associated with reduced differentiation, similar to those found in the basal layers; increasing the calcium stimulated the synthesis of the differentiation specific keratins, K1 and K10 within a few hours. Most *in vitro* research has looked at the effect of intercellular calcium; however Pruche *et al.* (1996) showed that the increase of calcium in the cytosol of the cell is due to both internal store mobilisation and influx.

Work with disrupted keratinocytes indicated that the precursor protein needed for cell envelope formation was present in the spinous layer in the cell cytoplasm, (Rice and Green 1979) and the cross linking of the protein was prevented by chelating calcium with EDTA. The authors were in debate as to whether the calcium required to stimulate the cross linking was intra or intercellular. They proposed that the keratinocytes have large amounts of sequestered calcium in organelles such as mitochondria. Since terminal differentiation affects both organelles and cell membrane, the calcium necessary for transglutamase may come from internal or external sources. If terminal differentiation is dependant upon external sources, then dietary intake would be of relevance as intake may affect plasma levels, (external source) of calcium.

The keratinocytes require calcium to activate the epidermal transglutamase which catalyses the formation of the ε - (γ- glutamyl) lysine bonds between the glutamyl groups of involucrin[g], (the amine[g] acceptor) and the primary amino group of other proteins, (Eckert 1989). The presence of

calcium also removes up to six other soluble proteins as they are incorporated into the cell envelope, (Eckert 1989).

Involucrin is an elongated length protein molecule, with over 40% of glutamine and glutamic acid residues, which is incorporated into the cell envelope, (Eckert *et al.* 1993). It has been shown that the presence of calcium induces not only transglutamase but also mRNA, (Ng *et al.* 1996). Transcription studies were carried out on convoluted keratinocytes in mediums with different concentrations of calcium, (Ng *et al.* 1996). It was shown in all cases that calcium increased the number of involucrin mRNA in a dose dependant manner, with the greatest effect in the medium with the highest calcium; the increase was specific. Ng *et al.* (1996) also identified the exon[g] segment along the involucrin molecule, which is responsible for basal calcium transcription activity and by removing it, showed that calcium could not switch on the transcription.

Using skin biopsies from mice, Menon *et al.* (1992) studied calcium distribution in the epidermis by fixing the calcium with an aldehyde fixative and viewing under the electron microscope. Calcium containing bundles appeared as discrete bundles or aggregates within the dermal tissues. In the basal cells of the epidermis there was very little calcium in the intercellular precipitates, (the calcium in this layer is contained within the nuclei and other organelles), but this increased progressively towards the stratum corneum with the highest concentration in the granular layer. The same pattern was seen for intracellular calcium with most of the calcium in the cytosol. In the lower layers, the calcium was sequested in cell organelles. Skin calcium binding proteins have been isolated in the basal cells of epidermis and it has been proposed that the sequestering of calcium at this stage stops premature differentiation of the cells, (Eckert 1989). There is little or no calcium in the stratum corneum, (Menon *et al.*1992). However when the stratum corneum was purposely disrupted with either acetone or cellotape, the intra and inter-cellular precipitates of calcium were immediately depleted and large clusters and precipitates of calcium appeared in the stratum corneum. When the stratum corneum had recovered by 10%, calcium started to reappear in the lower layers. It has been shown in psoriasis that the stratum corneum contains intercellular calcium aggregates, (Menon *et al.* 1992). It is possible that hoof wall containing higher than average calcium concentration may be an indication of disruption during differentiation.

As well as appearing to be the trigger for termination of keratinisation, other proteins are dependant upon calcium. Loricrin, a major component of the cornified envelope is calcium dependant, (Reichert *et al.* 1993) and the expression of its mRNA is dependant *in vitro* on calcium levels above 0.1mM. Cadherins are calcium dependant cell-cell adhesion molecules

found in squamous stratified epithelium and in adheren junctions. They are responsible for the adhesion of keratinocytes, (Suter *et al.* 1997) throughout the whole of the epidermal tissue.

Bernards and Korge, (2000) showed that a reduction in calcium in a monoculture of cells taken from patients suffering from an autosomal dominant inherited blistering disorder of the skin results in the loss of keratin binding to the desmosomal plaque. In addition, Sprecher *et al* (2001) have shown that pathogenic mutations in nineteen different keratin genes have been identified in the tail of the keratin rod. The tails of the rod are recognised as being the zone of overlap between adjacent keratin intermediate filaments. Structural analysis of the mutated keratins showed a failure of the intermediate filaments to bundle and a failure of the binding of the filaments to the desmosomes. The role of calcium in keratin tissue may be more fundamental than previously considered and may be influencing the keratin differentiation at molecular/genetic level by having an effect on the proteins being transcribed.

Finally it has been shown that profilaggrin, trichohyalin and reptin, which bind the intermediate filaments and are associated with lateral association of the IFs, contain calcium binding domains similar to the other calcium binding proteins(known as S100 proteins) found in epidermal tissues, (Donato 1999). These proteins are responsible for the disulphide cross links between IFs to make macrofilaments, an event which is facilitated by high calcium concentrations. Donato, (1999), indicated that upon calcium binding the S100 dimers cross-bridge two homologous (IFs or involucrin) target proteins.

The above review highlighted that copper, zinc and calcium are directly involved in the metabolism of keratin and altering their concentrations has a direct effect on keratinisation. If the affect that these trace elements have on keratinisation results in changes in the function of the epidermal tissue, then it might be useful to be able to accurately measure the concentration of zinc, copper and calcium to enable investigation into relationships between the two factors.

1.4 The effect of dietary intake of trace elements on the properties of epidermal tissues

It needs to be appreciated that trace element concentrations of tissues very rarely reflect dietary intakes. For example if a trace element deficiency results in a reduced growth rate of a tissue, then although the total trace element is reduced, the concentrations may not be significantly lowered. The type of epidermal tissue, interactions with other trace elements, exogenous contaminations and other nutrients will all affect the concentrations of trace elements within a tissue, (Cousins 1985). In addition, absolute values of trace elements and ratios to one another within epidermal tissues will vary considerably depending upon the state of health of the individual, their age, their sex, hair colour, external contamination and interactions with other minerals, (Combs 1987). Therefore correlations between dietary intakes and levels in tissues should be interpreted with caution.

The physiological observations of trace element deficiencies cannot always be directly linked to involvement in keratinisation. One way to assess the biological function of the trace element is to review papers, where researchers have isolated cells or cellular material from tissue which is deficient in the specific trace elements and looked at aspects of cell structure, enzymic activity, and processes whose biological pathways are known. This can be further substantiated when a controlled comparison with tissue that is not deficient allowed researchers to shed light as to whether the deficiencies or excesses are of functional significance. When reviewing the biochemistry of calcium, copper or zinc in the keratinisation process and the effect at macroscopic level, only controlled experimental designs for example, pair feeding[g], use of de ionised water, fibreglass and plastic feedbins, plastic coated pens, wooden floors and analysed feed were considered for inclusion. Alternatively experimental design such as match pairing[g] was considered because this minimised other variables and meaningful comparisons could be drawn from the literature.

1.4.1 Effects on visual appearance of the epidermis

Changing dietary intakes of zinc or copper has been shown to have an association with the visual appearance of epidermal tissues in several species. Zinc deficiency is clinically recognised by lesions of the integument and its outgrowths, hair, wool and feathers, (Underwood and Suttle 1999) and is associated with an inhibition of wool growth and loss of fleece, (Reis 1989). Dietary zinc methionine has been shown to improve the visual scores of dairy cow claws compared to an unsupplemented group, (Moore *et al.* 1989), as well as significantly improving both macroscopic and microscopic appearance of horn quality in beef cattle. Dietary deficiency of zinc in pigs resulted in the development of parakeratosis[g] and hyperkeratosis[g] and skin lesions over the entire integument, (Kapp and Simon 1980). Zinc deficiency in goats resulted in alopecia with remaining hair coarser than the controls. Microscopic examination of the goats' hair showed excess keratin formation, retained nuclei in the stratum corneum and a reduced width in the stratum granulosum, (Ray *et al.* 1997). Clinical observations of three field cases of sheep and goats suffering from zinc deficiency highlighted the pathologies associated with this deficiency in ruminants, (Nelson *et al.* 1984). All external epidermal tissues were affected. There was loss of wool and crimp; wool fibres became brittle and atrophy of wool follicles resulted in alopecia. Skin became thickened and scurfy, (parakeratosis) hoof walls buckled, became ridged and had deep transverse ridges running lateral medially and there were signs of excessive hoof growth, (hyperkeratosis).

Clinical signs of copper deficiency are associated with changes in the appearance of wool and the most striking feature of copper deficiency in the sheep is deterioration of crimp[g], (Lee 1956). Clinical signs in cattle include rough hair coats and a spectacled appearance around the eyes as hair loses pigment and in lambs a loss of wool crimp and reduced growth rate, (Suttle 1983). If copper depletion is longer than one hundred and seventy days then cows develop a stilted gait due to splaying of hooves, (Mills *et al.* 1976).

Although there appears to be an effect of trace elements on the visual appearance of epidermal tissues, a relationship cannot be easily monitored unless objective visual measurements are adopted as discussed in chapter 3.

1.4.2 Effects on mechanical properties of the epidermis

The supply of many minerals affects wool growth by influencing feed intake and metabolism, but only zinc and copper are implemented in having an effect on the mechanical properties of wool, (Reis and Salhu 1994). Normally the longitudinal strength of a single wool fibre is measured by determining the load required to break the fibre or by comparing its stress/extension curves, (Reis 1992). Effects of trace elements on feed intake need to be separated from the direct effect of trace elements on keratinisation. A large reduction in the supply of nutrients fed to sheep will reduce wool fibre diameter, simply because there will be less keratin material, (Reis 1992), thus confounding true trace element effects with those of a general nutrition deficiency.

The tensile strength of copper deficient fibres is less and elastic properties are abnormal compared to normal wool fibres, (Marston 1946). Nutritional copper deficiency may lead to incomplete cross linking and mechanically weak fibres, (Gillespie 1964). Wool from copper deficient sheep has a reduced affinity for dyes, abnormal elastic properties, reduced strength and a tendency to become permanently set when stretched, (Gillespie 1964).

The changes seen in steely wool are thought to be due to defective cross linking associated with increased free sulphydryl[g] groups and concomitant decreases in disulphide bonds[g] (Hynd 2000). There were very significant differences between the amino acid composition of the high sulphur proteins of a control compared to steely wool. Steely wool contained less *S*-carboxymethylcysteine and more aspartic acid, leucine and phenylalanine compared to the control, (Gillespie 1964). These changes are thought to be associated with delayed or impaired keratinisation due to copper's involvement in the oxidation of thiol[g] groups to form the disulphide links, (Danks 1991). Many authors, (Danks 1991; Gillespie 1964; Hynd 2000) continue to quote the original work of (Marston 1946) who provided evidence of the effect of copper on the process of keratinisation, *section 1.3.1*. The plastic condition, loss of crimp and the mechanical properties of copper deficient wool was restored, (Marston 1946), after it had been treated with alkyl halides to reduce the S-S bonds. Zinc deficiency has been shown to reduce the breaking strength of skin incisions, (Agren and Franzen 1990).

The effects of calcium deficiency on the mechanical properties of keratinised epidermal tissues appear not to have such as direct effect as copper and zinc. The calcium content of nail has in

the past been linked to its hardness and the effect of calcium was considered analogous to the matrix of mineralization which calcium provides in both bone and enamel, (Forslind 1970; Pautard 1963). *Table 1.4* summarises the effects of calcium, zinc and copper in keratinised tissues.

1.4.3 Effect of dietary manipulation of trace elements on their concentration in epidermal tissue

Sheep fed very zinc deficient diets had significantly less zinc in their wool compared to control diets, (White *et al.* 1994), however the relationship between dietary intakes of zinc and the concentration of zinc in the wool was not linear. Diets deficient in zinc were reflected in a reduction in cutaneous zinc concentration of rats, (Kapp and Simon 1980) and goats on zinc deficient diets had considerably less zinc in their hair compared with controls, (Ray *et al.* 1997).

In addition to the clinical signs, various researchers have looked at the histological effects of zinc deficiency on keratinised tissues. Sheep wool growth has been shown to be significantly and rapidly retarded on zinc deficient diets, (White *et al.* 1994). Pair fed sheep grew significantly more wool than zinc deficient sheep, but body growth stopped in both groups. The deficient wool fibres were incompletely keratinised and had retained cell nuclei, more follicles and remained immature for longer in Zn deficient sheep. Histology showed cortical cell volume was reduced as were their widths but not cell length. The authors suggested that these changes were indicative of an affect on protein (keratin) synthesis because there was no affect on mitosis but a reduction in cell size. These changes were reflected by a reduction in wool zinc content in the most zinc deficient sheep but the authors showed no correlation between dietary zinc intakes and wool or plasma levels of zinc.

It has been shown in psoriasis that the stratum corneum contains intercellular calcium aggregates, (Menon *et al.* 1992). In normal skin, calcium is rarely found in the stratum corneum. High levels of calcium are found in the stratum corneum of dry, flaky skin, (Forslind 2002). Forslind, (1970) and Pautard, (1963) analysed the nail for calcium and found levels. Pautard, (1963) showed by X ray diffraction that particles of calcium salt were grouped around the tubular horn in cattle claws although it is not clear from where the samples were taken. The hypothesis that keratin contained hydroxyapatite was disproved, (Forslind 1970), but in doing so it was shown that calcium can be detected in keratinised stratum corneum.

In cattle a reduction in copper below minimum recommended intakes was reflected in a decrease in hair copper values by 39% compared to the starting values. The relationship was linear with an R of 0.89; a similar correlation with R = 0.95 was seen in blood plasma. A linear correlation with hypocupraemia[g] and fleece cooper was seen in the fleece of lambs, with a R = 0.83, (Suttle 1983); the decreases were associated with clinical signs of copper deficiency. The effect of a dietary deficiency of copper was associated with a reduction in copper concentration in hair across more than one species, indicating that copper does remain within the corneocyte.

In order to investigate if copper enzymes played a role in the development of the epidermal tissue, researchers, (Mills *et al.* 1976), measured the activity of several enzymes including cytochrome oxidase,(oxidative reactions) and monoamine oxidase (collagen and elastin), as well as whole blood copper concentration at different levels of copper intakes. Blood copper and the copper enzymes decreased in line with decreased copper intakes. They argued that by the time gross manifestations such as deterioration of hair coat occurred; other tissues will have developed lesions. This hypothesis was confirmed by post mortem degenerative changes in elastin[g], collagen[g] and osteoblasts[g]. The changes were attributed to low lysyl oxidase activity, which resulted in defective cross linking in collagen and elastin, (Mills *et al.* 1976).

It is important to sample hoof from the same place to analyse trace elements and mechanical properties when investigating associations between the two factors. The reduced copper intake may have been transitional and therefore cracks in the hoof wall may not appear until the tissues formed at the time of deficiency have been exposed for a period of time to loading and environmental conditions. If the hoof is sampled in different places, the dietary deficiency may have been addressed in the tissue sampled; although the cracked material is still growing out.

1.4.4 Effects of trace elements on the epidermal integrity of the hoof wall

Copper, zinc or calcium affect both the microscopic and macroscopic structure of epidermal tissue. Because of the similarity in the biochemistry of epidermal tissue formation, it is hypothesised that a similar association would be found in the equine hoof wall. However the association is less clear in the epidermis of the ungulate hoof.

A survey of piglets and swine on similar dietary intakes, (Kovacs and Szilagyi 1973), found that the sole contained a significantly higher concentration of calcium compared to wall and frog but less copper and zinc. Similarly in horses, wall horn had higher zinc than sole horn and this was

related to hardness with the wall being harder than sole, (Coenan and Spitzlei 1997). It should be noted that if true comparisons are to be made, it is important that samples are taken from the same anatomical regions. Care should be taken in interpreting such results. Sole and horn are different anatomically and the differences in hardness may have been due to the differences in tubular/intertubular ratios, or to the difference in moisture content or to the difference in protein content, all of which have been shown to correlate with differences in hardness, (Forslind 1980). In addition, the sole tissue is renewed every two months whereas the wall takes 270-365 days for complete renewal, (Pollitt 1990).

Equine hoof horn of poor quality as measured by the Shore D hardness test was found to be low in zinc, (Coenan and Spitzlei 1997). Dietary zinc intake had no affect on the hoof hardness of dairy cows measured using a Rockwell metal hardness tester, (Moore *et al.* 1989), however no analysis was taken of the horn itself, so it is not appropriate to compare the two results with each other. An increase in horn hardness has been related to increased levels of copper, calcium and zinc in the claw of the dairy cow, (Baggott *et al.* 1988). Claw was measured first for hardness and then samples for trace elements were taken from the same anatomical positions, ensuring that material of the same anatomical age was tested. No standardised washing techniques were described nor duplicate sampling or spiking of solutions and this lack of standardisation may have introduced experimental variation. The differences in zinc between the sole and the wall were significant, $p<0.01$ (Baggott *et al.* 1988) and the differences in hardness significant <0.001. There had been no attempt to investigate the relationship between the two parameters, thus no defence could be made as to whether the association was significant. Care must be taken if implying a cause and affect especially as the harder horn had significantly lower water content compared to the sole, ($p<0.001$) and water is likely to have a greater influence on hardness than the amounts of trace elements. It has been shown that hardness can be directly correlated to water content, (Forslind 1980).

An increase in zinc concentration in equine hoof wall was established after feeding zinc to horses with poor quality hooves, (Coenen and Spitzlei 1996) with an improvement in the mechanical properties. Seasonal differences in mineral intakes and the effect of changes in diet were also reflected in changes in mineral content of equine hoof horn, (Ley *et al.* 1998), but the changes were not associated with any changes in mechanical properties. Ley *et al.* (1998) sampled from different places to obtain samples for either the trace element analysis or the mechanical testing. There was not enough detail to be clear as to where Coenen and Spitzlei, (1996) sampled hoof, but samples for zinc analysis were collected during normal hoof care suggesting that clippings were used. They noted in the results that poor quality horn had higher

water content and as indicated above this was likely to influence hardness tests far more than zinc concentration in the horn. The fact that only one group showed a relationship between the mechanical tests and the trace element concentrations may also be explained by the fact that Ley *et al.* (1998) tested the material for tensile strength and elasticity, rather than hardness. Neither of these mechanical properties are related to hardness and thus there was no reason to expect consistency of results. The usefulness of hardness testing is further discussed in chapter three.

The discrepancy of results within the literature is confounded because frequently sampling was either from different anatomical sites or from the same anatomical sites but from material of different age. Methods should be developed to test material properties and trace element concentrations from the same anatomical positions and from material of the same age, whenever possible and these techniques are described in chapters two and three. Comparisons between different mechanical properties should also be interpreted with care, especially as the hoof is made from complex, isotropic composite material whose mechanical properties differ depending upon whether material is tested parallel or at 90° to the tubules, (Bertram and Gosline, 1986; Kasapi and Gosline, 1997; 1999).

Table 1.4 Summary of the role of copper, zinc and calcium in keratinised tissues

Trace element	Biochemical involvement	Gross or histological changes
Copper	Co enzyme for lysyl-and amine oxidases. Required for cross linking of keratin IFs to IFAPs; oxidises thiol groups within 8-12hrs	Deterioration of crimp May have a greater affect on IFAPs rather than IF. Therefore a decrease in copper may result in a reduction in elasticity.
Zinc	Stimulates formation of filaments and bundles. IFs lose their attachment in deficiency. Excess zinc decreases growth of neoplastic cells	Lesions most noticeable in areas exposed to mechanical stress. MCGs do not extrude in Zn deficiency, lack of lipids, therefore 'dry' keratinised tissue
Calcium	Induces termination and stops proliferation. Stimulates K1 and K10 keratins as part of differentiation Catalyst for cell envelope formation Ca disulphide stimulates cross links between IFs to form macrofilaments, (keratin fibres)	Increase in calcium may effect differentiation

1.5 Limitations of current methods used to assess the appearance of the equine hoof wall

In the equine, the effect of nutrition on keratinisation has been measured by recording the appearance of the hoof wall, using subjective adjectives such as shelly, brittle, rough and shiny (Comben *et al.* 1984; Josseck *et al.* 1995; Kempson 1987; Kempson and Campbell, 1998; Kempson and Robb, 2004). This appearance is used to determine the quality of the epidermal structure by designating the hooves as good or poor quality. The adjectives are routinely used clinically and in research. It is reasonable to suggest that these adjectives make it difficult to follow a repeatable or reproducible study of the wall thus preventing any meaningful correlations with gross appearance, function and any aspect of nutrition.

For example, Comben *et al.* (1984) and Kempson, (1990) assessed the change in gross appearance of hoof epidermis over twelve months in response to dietary treatment. The gross changes over time were described subjectively; "new, healthy horn was observed, crumbling around nail holes had virtually stopped and there was an increased strength in the wall". None of these observations were sufficiently tightly defined as to allow the experiment to be reproduced if a comparison was required over a different time period. In addition, no attempt had been made to statistically evaluate the findings. No attempt was made to quantify the degree of defect, no scoring system was used and changes were not quantified. The measurements were so subjective, that one would have to question if comparable indices were being measured.

Josseck *et al.* (1995) studied the effect of biotin on the macroscopic, (gross) appearance of horses' feet by developing a system to score the bearing border[g] and sole of the hoof. The bearing border is not an ideal site for sampling as it is exposed to contamination from the ground, it is altered by farriery intervention and it is twelve months removed from any dietary effects. A 'map' of the sole was shown to illustrate the areas, but it was not delineated and whilst the sampling was repeatable, it would be difficult to reproduce from the information provided. The regions were classed according to their elasticity, firmness, hardness, smell and compactness; none of these attributes were tested for, thus the scoring system was subjective. Each anatomical region was severity scored as to whether the defect affected usage. Whilst there had been an attempt to use an objective measure of severity, the results were discussed

subjectively using adjectives such as 'brittle, chipped, thin, soft and crumbling, foetid smell. When describing changes, the authors referred to the coronary border becoming smoother, an adjective which was not used in the method section. This type of reporting does not allow reproducibility in other trials.

Use of visual assessment to obtain material for histological investigation

Visual assessment will only be of use, if it has some relationship to underlying histological pathology or material property or can be used as a predictor of disease aetiology.

Geyer and Schulze, (1994) investigated the histology of horses' hooves which had been first scored according to their appearance. The description of the appearance was semi-subjective: - crumbly horn, cracks in the coronary horn, chips in the weight bearing border and decayed horn were assessed and a score given to their changes. Histological samples were taken but the sampling areas were not tightly defined and a subjective score was made on the histological specimens. There was no presentation of means graphically, nor any indications of standard deviations, there was no statistical treatment of the data. Despite a semi subjective score system being described in the methods, there was limited reference to it in the results; instead the subjective statements such as 'the surface of the proximal half of the horn was smoother' were used. It would be very difficult to reproduce this method.

Following subjective visual assessment of the hoof, clippings from the equine hoof capsule were investigated using electron microscopy by Kempson, (1987). Structural features such as tubule shape and cell integrity were found to differ although the horses were visually assessed as having the same gross level of quality. The lack of relationship between gross appearance and underlying histology might be explained because of:-

- variations in the original subjective assessment
- anomalies of sampling as sampling sites under the microscope were not tightly defined and therefore comparisons were inappropriate
- variability in samples which were taken at random, so that it would have been impossible to take comparable samples over time
- the small sample population, (two horses) differences may have been due to individual variation

This illustrates that it is important to define and grade visual parameters first and then to investigate whether there is a link between these and the underlying histological and material properties of the horn. Results will be difficult to interpret unless more attention is paid to initial grading and characterisation of quality. If gross appearance is quantified it will then be possible to characterise the underlying histology of the correctly graded gross appearance and

consequently to define and establish if there are links between the gross expression of quality and the underlying quantified histology.

The aims of chapter three were to discuss the usefulness of qualitative and quantitative methods of measurement and to develop a method to quantify the gross appearance of the equine hoof wall epidermis by measuring cracks. The relationship between cracks and the material property of fracture toughness was also investigated.

1.6 The relationship between shape, changes in stress concentration and cracks

Some researchers attribute the changes in mechanical properties and visual appearance of epidermal tissues with changes in the epidermal material due to differences in nutrient supply at keratinisation. However the mechanical properties of a material should not be studied in isolation, especially if the properties are being related to the visual appearance of the tissue in terms of cracks. This is particularly relevant when specialised weight bearing integuments are being studied, because the structure and shape of the material contribute to the functionality of the epidermal tissue. Changes in shape, as reviewed below, have been shown to alter strain distribution in the hoof wall. Changes in strain distribution may affect stress concentration. Cracks occur in areas of stress concentration thus changes in shape may have an affect on crack distribution.

1.6.1 Relationships between claw shape and lameness in domestic ungulates

The functionality of weight bearing epidermis will depend upon its shape, size and the material from which it is made, (Biewener 1992; Gordon 1987). The epidermal material can be isolated and its material properties and trace element content analysed for relationships, (chapters 3 and 4) enabling predictions as to its propensity to cracking. This technique can be likened to the testing of one brick. A brick is of little use in isolation and can only function when it is part of a structure, (wall) which is then built to a specific shape to withstand the appropriate loads, (curved arch, single brick wall). However in order to know how well the brick might work in

the structure, it is necessary to know its composition and mechanical properties. It is hypothesised that both trace element concentration and the shape of the equine hoof wall will contribute to its integrity.

There is evidence in the literature that there is a relationship between aspects of shape and the incidence of lesions. Andersson and Lundstrom, (1981) measured aspects of shape as well as position and severity of cracks and lesions in cattle claws. Toe length to heel height was expressed as a ratio and the relationship between this ratio and severity of lesions was investigated. Severity of lesions increased as the difference between the two measured parameters increased. There was also a relationship between increased severity of lesions and increasing toe length and sole depth. If cracks occur when the stress concentration of the material is exceeded, it is hypothesised that these changes in shape of the claw alter the load bearing sufficiently to exceed the strength of the material. Smitt *et al.* (1986) noted a relationship between claw measurements and lesion scores where cows with longer toes and deeper heels had higher incidences of lesions. They noted that high claw angles in cattle reduced the incidence of lesions but long toe lengths increased the risk of lesions. A similar relationship has been noted in the field with horses, Moyer, (2003) and O'Grady, (2002) associated heel and quarter cracks with under-run heels and long toes.

It is possible that the relationship between hoof shape and disease, (which in the above examples is related to the incidence of cracks or lesions) exists in certain shapes of hooves and claws because the shape predisposes specific areas of the epidermis to cracks due to changes in stress[g] and strain[g] distribution. A review of the literature below illustrates that changing linear or angular measurements of the equine hoof wall is associated with redistribution of strain.

1.6.2 Relationship between hoof shape and load bearing in the horse

Changes in toe angle and its measured effect on distribution of load

Changing the shape of the hoof wall by altering the angle of the toe will affect the way in which the hoof wall functions as a load bearer. Trimming the hoof to different angles has been recorded for well over a century. Glade and Salzman, (1985) reported that Winter, (1852) recommended trimming hooves to give a hoof angle of 32^0 in the fore and 35^0 in the hind, because he believed that steeper angles resulted in increased heel and frog damage. In addition, they noted that Fleming (1872) estimated the natural hoof angle to be 56^0 and stated that angles

of less than 45^0 were dangerous and damaging to the foot and lower leg. Whilst there was obviously disagreement in the older literature as to which was the most suitable toe angle, there was agreement that a change in the angle caused damage to the foot or leg due to a change in the load displacement. Glade and Salzman, (1985) changed the shape of hooves by trimming hooves to achieve a toe angle of 40^0 with a long toe and short heels. After a period of time this resulted in reducing the distances between what the authors described as the angles of the hoof wall by 7%. The distance to which the authors referred was the distance between the heel bulbs, measured by dividing the sole into an equal number of radii with their origins at the tip of the frog. They measured the change in angles between theses radii over a period of time and indicated that the change in shape was as a response to more caudal weight bearing, because of the original change in the toe angle. Other workers have also confirmed the effect of low toe angle on the change in load distribution through the hoof wall. Barrey, (1990) used instrumented horse boots and measured vertical forces at four positions on the hoof. A low toe angle of 37^0 increased the loading at the heels moving 75% of the vertical force caudally[g]. In the same experiment an increase in toe angle to 55^0 resulted in 57% of the total vertical force distributed cranially[g] towards the toe. Balch *et al.* (1991) altered toe angles to 56°, using hoof wedges and after exercising horses on a treadmill measured increased impulse at the medial quarters away from the heels with a three point transducer shoe which confirmed the results of Barry, (1990).

Although there is agreement that altering the shape of the hoof by changing toe angle does change the way the hoof functions as a load bearer, researchers still do not agree on the specifics. Thompson *et al.* (1993) using strain gauges *in vitro,* increased toe angle from 58^0 to 78^0 with wedges and noted an increase in strain at the quarters and a decrease in strain at the toe. However, Thomason, (1998) using strain gauges *in vivo* found that steeper toe angles 57^0 increased magnitude of strain at all sites. Twelve horses of mixed breed, age and size were studied, the author acknowledged the heterogeneity of the groups explaining that he was looking to study strain patterns and variations which are common to all hooves not a particular breed. The differences in results between the two researchers might be confounded by the fact that *in vivo* more than one geometric variable is likely to change; in addition factors such as ground surface, bodyweights, individual horse variation and the initial shape of the equine hoof capsule will affect the results.

Changes in lengths of the medial and lateral hoof wall and measured effects on weight distribution

Balch *et al.* (1991) used a wedge to increase the medial and lateral sides, (quarters), of the hoof wall by 95mm. They recorded a resultant increase in the peak vertical force[g] on the medial quarter, however increasing the lateral wall length had no effect on the weight bearing of the lateral quarter. This was in contrast to Chang *et al.* (1993), who raised the medial or lateral sides by 12mm with a resultant increased side angle of 8^0. They recorded increased tension at both the lateral and medial wedges measured on all the strain gauges[g] specifically at the proximal and distal toe. It was hypothesised that if the hooves were different sizes or shapes and asymmetrical before the wedges were used or if the horses were different bodyweights, the load distribution would differ before the experimental changes. Changes in strain gauge measurements could have been a result of both the experimental investigation and individual variation and the individual variation may have been bigger than the effects being studied. Balch *et al.* (1991) studied two horses only and reported that there was a between horse difference in the way the hooves landed before any wedges were used due to a difference in hoof conformation. This inter-individual difference was consistent throughout the experiment. Chang *et al.* (1993) studied six horses of the same breed and bodyweight but made no reference to any base line data being taken before wedges were used.

Thomason, (1998) in a study looking at the variation in surface strain measured a difference in the lateral and medial angles and recorded that all the hooves were more upright medially by between 1°and 7°, however he did not record a difference in the strain gauge as a result of the angle differences. Rooney (1978), Barrey (1990) and Balch *et al.* (1991) suggested that the side of the hoof that interacts with the ground first (e.g. medial side) is under compression whereas the opposite side (e.g. lateral) will expand in tension. In this example over a period of time, the medial hoof wall will become straighter and the lateral side will become more flared, however this has not been validated experimentally. This difference in natural weight bearing may be one of the factors which over rides any experimental changes *in vivo*. Chang *et al.* (1993) recorded large individual differences between horses in the changes of strain orientation due to the wedges.

Differences in heel angles and lengths and the effect on load bearing

Medial lateral balance is measured at the heels, both in terms of length and angle differences. Turner, (1992) has defined a difference in length between the heels of more than 0.5cm as sheared heels. Under-run heels have been defined as heels, which are 5° less than the toe angle, as measured from radiographs, (Turner 1992). It is possible that differences in heel angles alter

the way in which the hoof carries load in a similar manner to changes in angles at the quarters. Balch *et al.* (1991) recorded an increase in force at the medial quarter and an increase in toe angle when they used a wedge to increase the length of the heel. Some researchers relate this change in load bearing to foot related lameness; under run heels were associated with 77% (Turner *et al.* 1988) of lameness cases.

Differences in medial and lateral wall heights and angles at both the quarters and heels have been shown to change strain distribution and force on the hoof wall. As strain is proportional to stress, it is possible that the hoof may suffer a stress concentration which exceeds the material properties, causes failure of the material and results in cracks.

Changes in the shape of the capsular base and its affect on load bearing

Stress in terms of the ground reaction force[g] can be qualitatively recorded using pressure sensitive film (Colahan *et al.* 1993). Photo-elastic film, (Colahan *et al.* 1991; Dejardin *et al.* 1999) showed that the surface area at the bearing border available for load bearing varies between the hoof wall and the sole depending on the shape of the hoof, independent of ground surface.

1.6.3 Distribution of cracks in the equine hoof wall

Hoof wall cracks are ubiquitous with regard to breed, athletic pursuit and work load; they are universally and historically encountered in the horse world. A great deal of descriptive literature is available in both veterinary and farriery publications; however there is little information regarding analytical and comparative studies. Much of the information available is based on personal prejudice and opinion which is based on the individual's own experiences, (Moyer 2003).

Pardoe and Wilson, (1999) reported that equine hoof wall quarter cracks are relatively common in horses performing exercise, but did not give details on the incidence or severity. However they did note that the injuries often penetrate to the stratum internum resulting in severe lameness. Numerous terms are used as descriptors of cracks and often different terminology is used to describe the same type of crack, *(table 1.6.3.i)*, in addition subjective terminology continues to be used.

Table 1.6.3.i Summary of definitions used to describe cracks

Name of crack type	Definition	Author
Sand crack	Toe and sides of hoof that they are seen to invade more than other parts. The nearer the crack is to the front of the hoof, the more direct and perpendicular its position	Bracey Clarke (1834)
Sandcrack	The chronic form consists of an extensive split on the antero-abaxial aspect of the hoof from a point about 1 inch below the coronet down to the bearing surface The acute form may be described as an infected vertical fissure extending downward from the coronet (cattle)	Greenough (1962)
Sandcrack	Is a fissure in the wall of the hoof , parallel to the horn tubules, commencing at the coronet and extending a variable distance down the wall Common sites are the toe of the hind foot and inner quarter of the fore foot	O' Connor (1956)
Sandcrack	9 types:- Complete extending downwards from the coronet to the plantar border of the wall; incomplete, extending downwards through part of the height of the wall; superficial, only going through a part of the thickness of the wall; deep extending into the sensitive tissues; straight or sinuous in direction; Recent; Old; Simple; Complicated	O'Connor (1956)
Sandcrack	is a fissure , rent or separation of the horny, fibrous tubes of the hoof to a greater or lesser extent When slight it causes little or no inconvenience ; but when the fissure extends into the sensitive parts and any dirt gets in , inflammation is set up, and , if infected, pus is formed *(first attempt of severity scoring)*	Duncan (1935)
Quarter cracks	Destabilisation of the hoof structure is caused by a crack forming along the axis of the horn tubules, from the coronary band to the sole	Pardoe and Wilson, (1999)
Quarter crack	Typically originate at the coronary band and continue distally, They are full thickness and extend into the dermis of the hoof, leading to inflammation, instability and infection	O'Grady (2001)
Quarter crack	Free movement is often apparent demonstrating that a breakdown in the intrinsic laminar support mechanism has occurred	Curtis (2001)
Quarter crack	Shear forces acting on the horn matrix are considered to be the major factor in the breakdown of the hoof structure Shape of foot may play an important part in altering the shear forces	Rooney (1978)
Quarter cracks	Occur over the point of load impact. This is the point where the coronary band is deviated proximally and usually contains the most shear within the hoof wall. By evaluating the hoof balance parameters and making the necessary corrections , shear is relieved , support is corrected and the crack generally heals	Snow and Birdsall, (1991)
Wall crack	If the coronary corium is severely damaged, the coronary papillae are replaced with scar tissue, a permanent wall defect (wall crack) results which will always be refractory to treatment	Pollitt (1992)
False quarter	A thin layer of modified brittle horn on the hoof, mainly on the sides and where it is connected to ordinary horny hoof, forms rifts and furrows (resembling sandcracks) on each side	Duncan (1935)
Seedy toe	Separation of the wall from the subcorneal tissue and the formation in the interspace of crumbly pumice –stone like horn secreted by the sensitive laminae	O'Connor (1956)
Chronic Coronitis	No apparent cause but it may result from repeated injury or irritant of the coronet. The disease affects mostly the toes and the quarters. The periople band appears to be more active secreting a horny material , disposed in irregular masses separated by narrow fissures (like the bark of an old tree)	O'Connor (1956)

	The coronary cushion in the affected part produces thickened abnormal horn similar to that produced by the periople, if the condition has been present for a time then there maybe separation of the horn at the coronet	
Name of crack type	**Definition**	**Author**
Toe wall separations	Associated with excessively long toes, wall flares away and splits and cracks in the wall at the toe	Moyer (2003)
Quarter and heel cracks	Associated with long toe and underrun heel	Moyer (2003)
Linear cracks	Form parallel to the coronary band and represent pre-existing damage to the coronary dermis (trauma or infection) and interrupt normal wall growth	Moyer (2003)
Defects	Thin walled, flaky, crumbly, split or cracked	Slater and Hood (1997)
Wall defects	Sandcracks, grass cracks, quarter cracks, splitting of hoof wall; all used without definition	Ellis (1998)

Descriptive terms may be used, as discussed further in chapters three and four because clinicians and farriers feel they cannot capture the appearance by one scientific term or test, particularly because hoof horn is an isotropic[g], non homogenous material. It is likely that the use of descriptive terminology restricts comparative work and that it would be difficult to justify the differences in definition, where they exist, between a sandcrack, a quarter crack and a grass crack.

The significance of a crack should be related to its affect on function as it is possible that cracks prevent total wall failure and their presence ensures a fully functional hoof. Therefore cracks should be defined not just in terms of their anatomical position but also by a measure of the affect that they have on the function of the hoof. For example a crack at the bearing border will reduce the area of wall available for weight bearing and concentrate load onto other parts of the wall. In chapter three, a method to count and measure cracks within predefined anatomical positions and a new scoring system which objectively measures the effect a crack might have on the function of the hoof wall is reported. The score is adjusted mathematically into a severity crack score system adapted from Greenough and Vermunt, (1991).

1.7 Programme of experimental work

Some of the problems in testing hoof are comparable to those encountered in skin research described by Marks (1991).

- mechanical properties are anisotropic
- changes are time dependant
- environmental conditions vary
- loading history varies
- there is a lack of standardisation amongst researchers in sampling as discussed in each experimental chapter
- different units of measurement are used as discussed in each experimental chapter.

In order to ascertain and investigate associations between functional integrity and factors which may affect function, there needs to be an attempt to agree on standardised practises for sampling and recording and an agreement on uniformity of nomenclature.

This thesis resolves some of these issues by providing researchers with

1. methods to quantify the visual appearance of the equine hoof epidermis
    i. by measuring cracks
    ii. by developing methods to quantify the severity of cracks
    iii. by measuring incidence of cracks by anatomical position (chapter three)
2. protocols for sampling and measuring fracture toughness in hoof wall blocks, (chapter three)
3. protocols and standardisation of sampling and analytical methods for analysing trace elements from hoof wall blocks(chapter four)
4. Measurements taken directly from the capsule or photographs for the development of ratios to define the shape of the hoof capsule,(chapter five)

The following questions were then investigated:-

- Equine hoof wall defined as 'poor quality' in the literature has been shown to have lower concentrations of certain trace elements compared to hoof defined as being of 'better quality'. However, cracks will occur in keratinised material when the fracture toughness of the material has been exceeded. Is the term 'quality' related to mechanical properties or trace element concentrations and can a measurement of cracks be related to either of these characteristics? Is there a relationship between trace element concentrations and the crack score of a hoof?
- If cracks occur in specific places on the equine hoof capsule, is it because the fracture toughness was exceeded because of a defect in keratinisation, due to a change in trace element concentration? Alternatively was it due to localisation of the stress concentration because of the shape of the hoof?

Chapter six aimed to answer whether;

1. there was a relationship between the fracture toughness of the hoof wall and its trace element concentration
2. cracked hooves had different trace element concentrations to uncracked hooves
3. there was a relationship between fracture toughness and the crack score of a hoof, (chapter three)
4. there was a relationship between hoof shape and the incidence of cracks
5. grouping hooves according to their shape changed any of the above relationships

2 Methods for obtaining samples from the hoof wall

In order to carry out comparisons across a number of hooves it is important to develop methods that are repeatable and reproducible. The lack of clear relationships between visual assessment and other parameters may be due to the large biological variation in the material or more equally, as discussed in the introduction, due to a lack of standardisation in methodology.

In order to ensure sampling of material is consistent throughout this thesis, anatomical points and areas were defined and samples taken from the defined areas. The standardisations of methods which share commonality across the chapters are described in this chapter.

1. Designating anatomical reference points and areas
    a) For shape categorisation; chapter 5, page 194.
    b) For counting crack areas and numbers; chapter 3, page 77.
    c) For harvesting blocks for fracture toughness tests; chapter 3, page 82 and for trace element analysis; chapter 4 page 133.

2. Cutting blocks
    a) For fracture toughness tests; chapter 3 page 82.
    b) For trace element analysis; chapter 4 page 134.

Hooves

One hundred and two random hooves were obtained from veterinary schools, abattoirs and hunt stables in order to obtain a selection of hooves from disparate geographic areas; having different dietary and farriery management. The feet were removed at the fetlock and frozen at -24^{0}C immediately after slaughter. The hooves were kept frozen for all gross measurements, unless stated otherwise in the specific chapters.

On collection and before measurement, each hoof was cleaned, shoes were removed where necessary, and the hoof was bagged and allocated a unique identification. In addition a separate set of left fores (28) were also used to reduce some of the variation due to differences between hind and fore feet and to check reproducibility of all the techniques.

2.1. Location of anatomical sites for sampling and measurement

2.1.1 Designating anatomical reference points on the capsular base

The hoof was inverted and the following measurements taken from the underside of the hoof, described as the capsular base in this thesis. The capsular base, (sole) was used as the template from which all other measurements and points were related.

Capsular base, Midline Dead Centre, (CBmdc)

The bulbs of the heel were measured across its width, X/Y in diagram B *figure 2.1.1.i.* and a point in the middle of the centre of the bulbs of the heel was marked with Tippex. A ruler was placed on this point and a line drawn from the base in the yz direction through the centre of the tip of the frog, (central succulus if present) and continued until it reached the most forward dorsum point of the hoof wall. This line is the sagittal axis as described in Thomason *et al.* (2001). A point was marked with correction fluid at the distal margin of the hoof capsule at the intercept with the dorsal wall (*plate 2.1.1.iii*). Reilly *et al.* (1996) defined the MDC from the dorsal aspect of the hoof wall, where the MDC was the centre of the dorsal wall in the x plane measured medially/laterally. Unless the hoof capsule is very asymmetrical, the two methods should result in the same point on the toe.

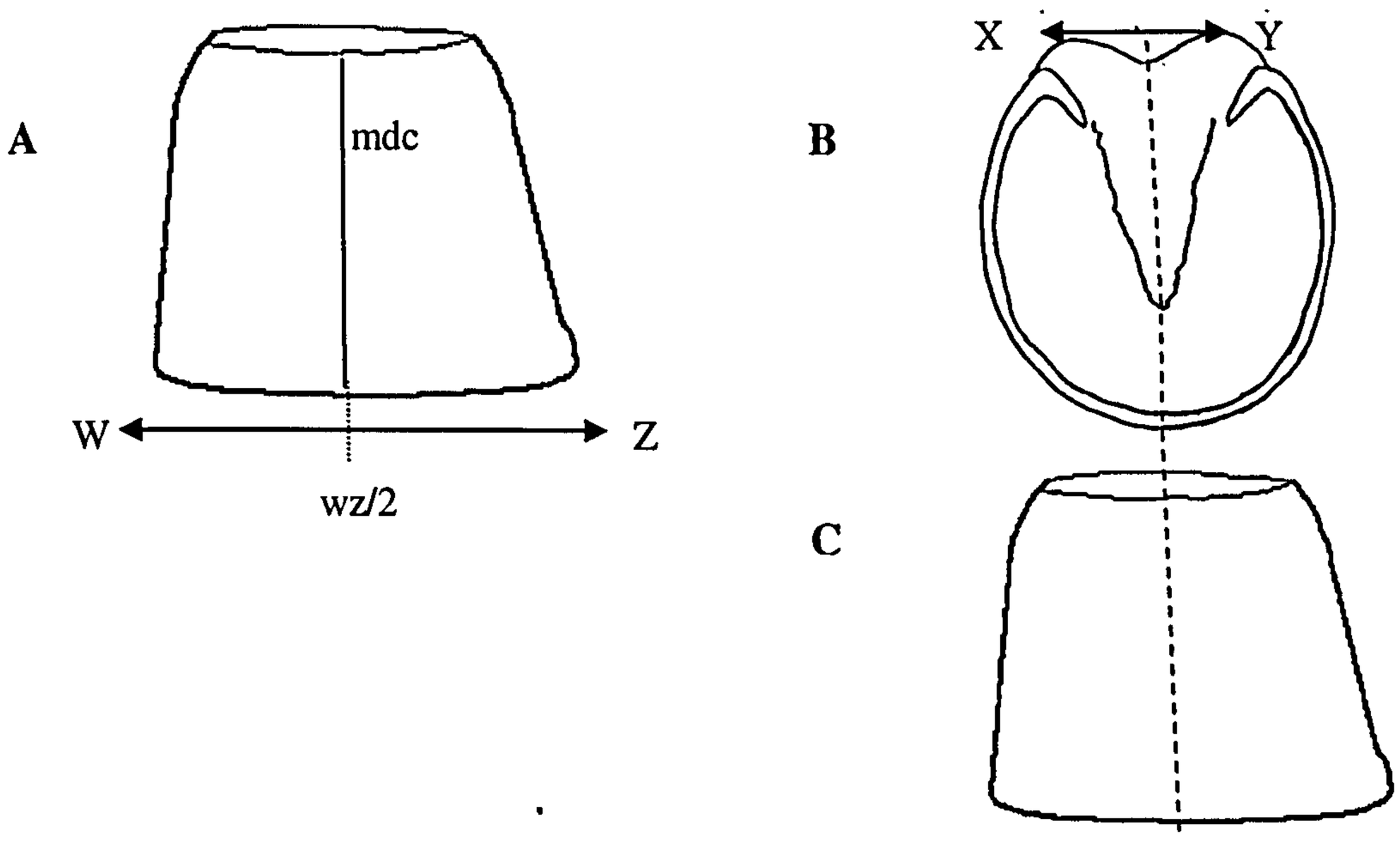
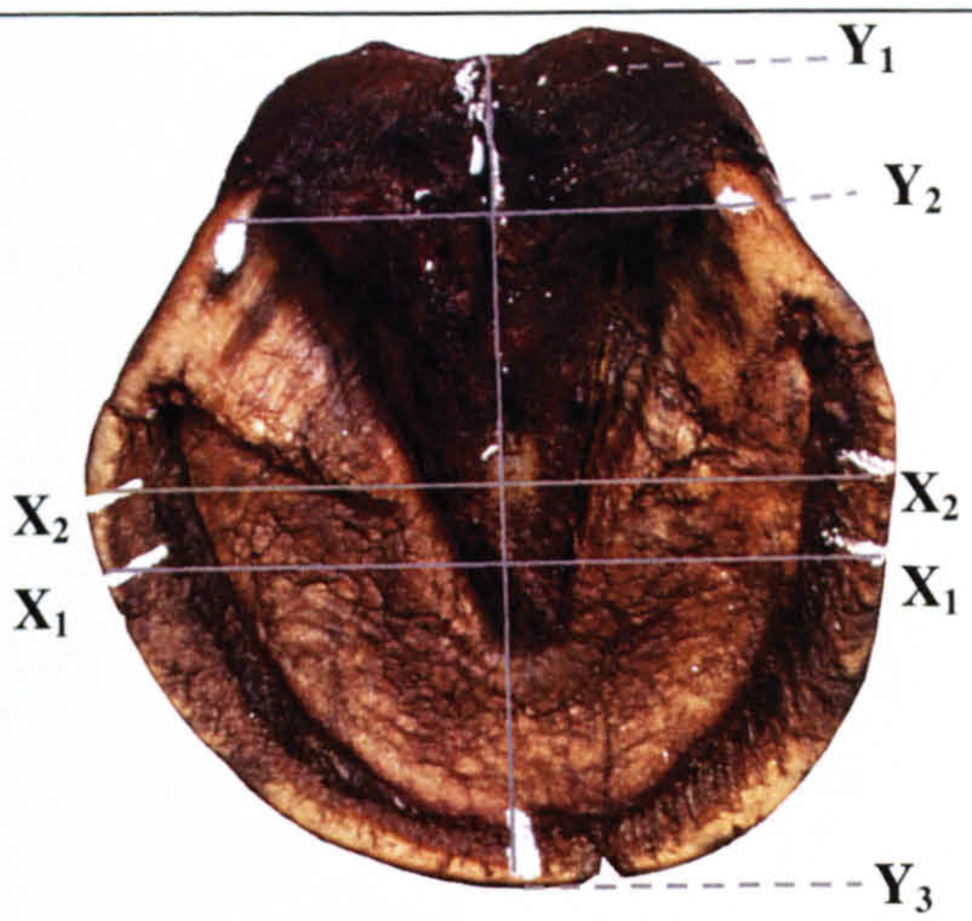
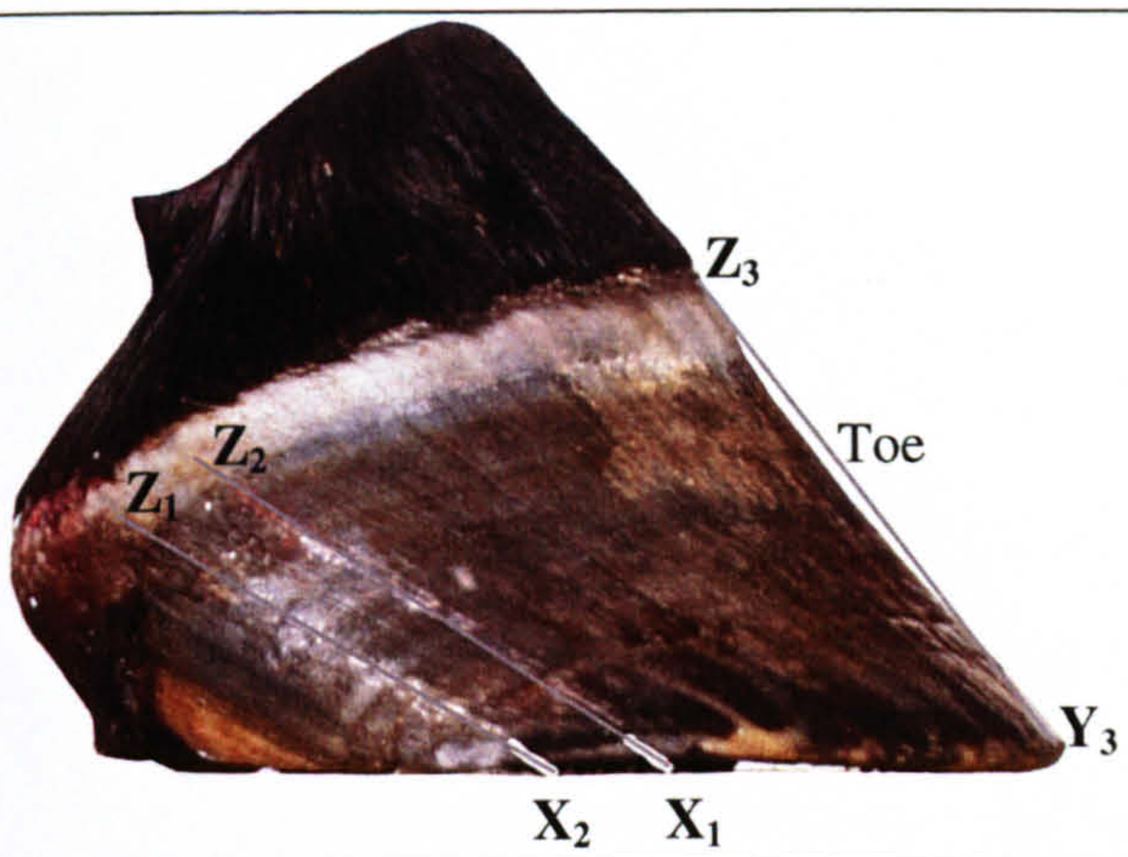


Figure 2.1.1.i The midline dead centre (MDC) from the solar and dorsal aspect of the hoof capsule



Y_1-Y_3	Capsular base midline dead centre (CBmdc)
Y_2-Y_3	Capsular depth (CD)
X_1-X_1	Capsular width 50% (CW50%)
X_2-X_2	Capsular width, widest point (CWWP)
Y_3-X_1	Capsular depth 50% (CD50%)
Y_3-X_2	Capsular depth widest point (CDWP 50%)



Y_3-Z_3	Toe midline dead centre (mdc)
X_1-Z_1	Point of quarter
X_2-Z_2	Widest point of quarter

Plate 2.1.1.ii Summary of the anatomical areas from which samples were taken

Artwork © Dave Gibson

Disal perimeter points.

The most caudal points of the weight bearing distal perimeter were established by putting the hoof onto graph paper ensuring that the point of toe and the succulus of the frog were perpendicular. The point of toe and the midpoint of the frog succulus were marked on the graph paper. An outline was then drawn around the distal bearing surface. Because of asymmetry, there was often a discrepancy between where the hoof wall ended at the caudal surface and where the hoof wall was in contact with the ground. A needle was used to mark the end of the weight-bearing surface at the heels on the graph paper, by making a mark at either side of the heels, where the wall last touched the surface of the graph paper. Correction fluid was used to mark the hoof wall capsule at these two points.

Capsular depth and width points

The hoof was removed from the graph paper. A line was drawn medially/laterally between the two caudal points previously marked on the capsular base template, on the graph paper, to designate the most caudal weight bearing points of the capsular base. A second line was drawn from the previously marked point of the toe to the caudal line. This line was designated the capsular depth and represented the depth of the weight bearing surface, in some instances this would be the same depth as the capsular base mdc, *(figure 2.1.1.iii).*

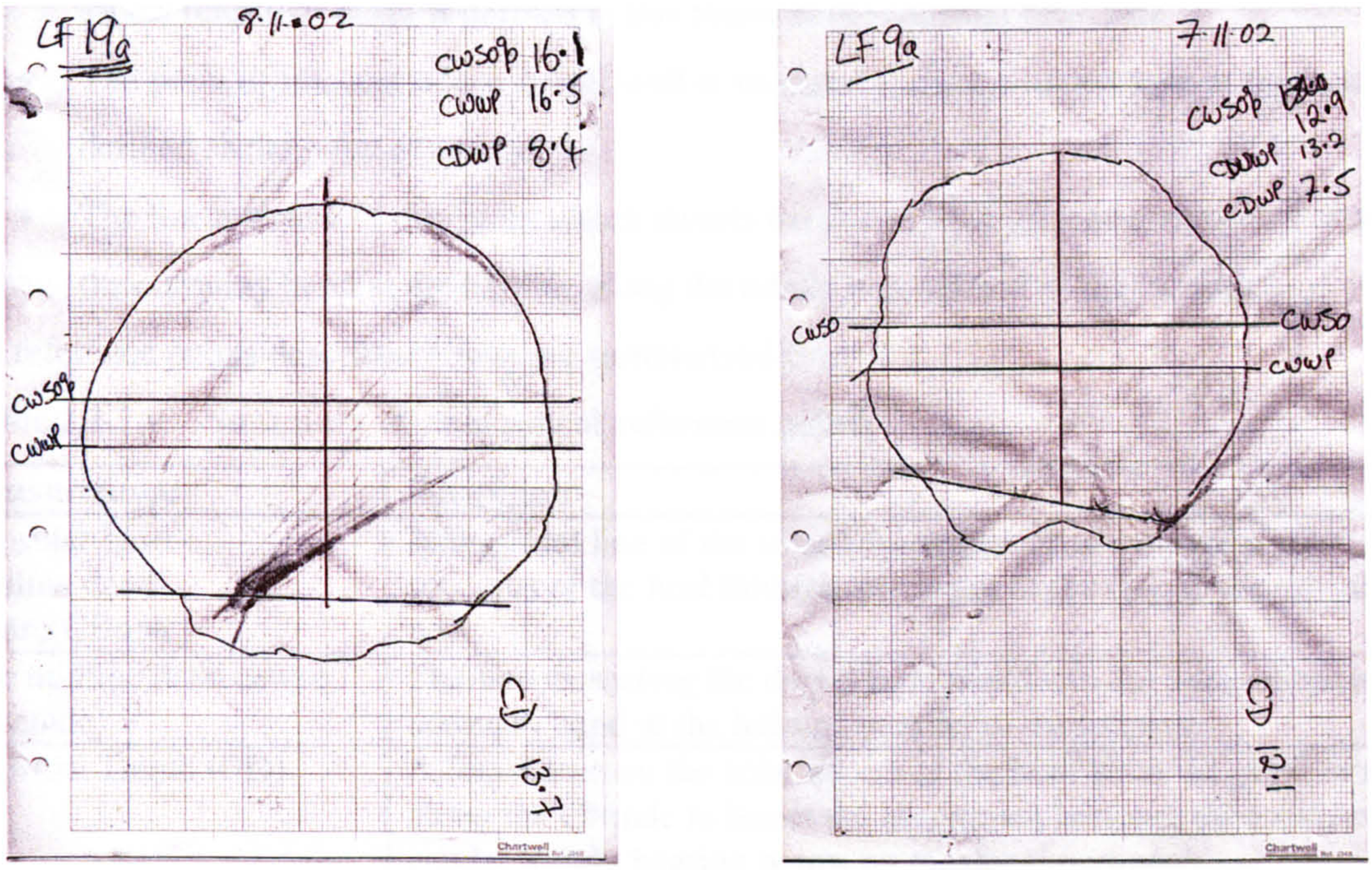


Figure 2.1.1.iii Templates of capsular bases to show measurements and annotations used to measure the shape of the capsular base

Point of quarters

The length of the capsular depth was measured and a mark was then made on the line at 50% of its length and a line drawn medially/laterally to bisect the capsular depth in equal halves. Where the lines met the hoof wall was defined the point of the quarter on either side.

Widest point of quarters

In addition the capsular width at the widest point was measured with a ruler and points marked on the graph paper where the line disected the bearing border on each side, these points were designated the widest point of the quarters.

Reference Points on the hoof capsule

The hoof was replaced on the outline of its perimeter on the graph paper and the designated points described above were marked on the hoof capsule with correction fluid. At each designated point, a score was made from the point on the distal border to the hair roots of the hairline at the coronary band along the line of the tubule at that point, *plate 2.1.1.ii.*

The toe mdc, the point of quarters and the widest point of quarters could all be replicated for sample collection from these areas. To avoid confusion in nomenclature: -

- the midsagittal line drawn from the mid point of the heels to the point of toe on the solar aspect (capsular base) is defined in this thesis as the capsular base mdc
- The point of intecept of the dorsal wall at its distal surface with the sole at the mdc was defined as the point of the toe
- The linear aspect of this point which disects the dorsal hoof wall from the point of toe to the coronary band at the hairline along the tubule was defined as the toe mdc

All reference points described above are summarised in *table 2.1.1.v.*

Table 2.1.1.v Summary of anatomical reference points

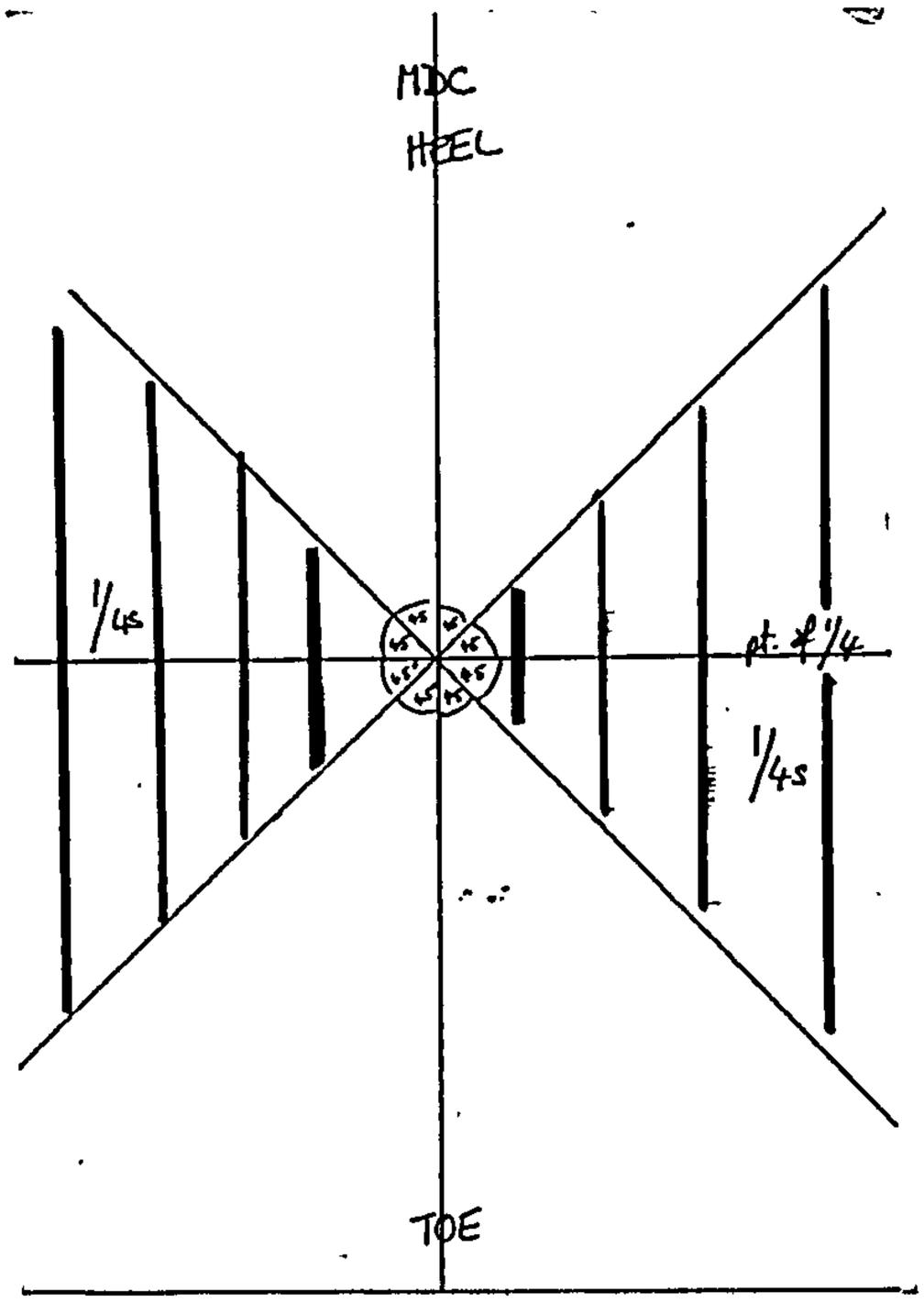
Measurement	Definition
Capsular base, midline dead centre(CBmdc)	Midsagittal line of the solar base drawn from the caudal mid succulus of the heel through the centre of the frog to the dorsal wall.
Toe midline dead centre (Toemdc)	The line dissecting the dorsal hoof wall from the point of toe to the coronary band at the hairline parallel to the tubules.
Capsular Depth (CD)	A line drawn on the solar aspect of the hoof from the point of toe along the CBmdc to bisect the line drawn between the two most caudal weight bearing points on the distal perimeter
Capsular width 50% (CW50%) Point of quarter	The width of the capsular base at CD50% measured along a line, which bisects the CBmdc at 90^0. The points at which the above line intercepted the hoof wall at the distal surface were designated the point of the quarters
Point of toe (Pt toe)	Intercept of the capsular base mdc with the dorsal wall at its distal surface

Measurement	Definition
Capsular width widest point (CWWP) Widest point of quarter	The width of the capsular base at its widest point, measured along a line which bisects the CBmdc at 90°. The points at which the line intercepted the hoof capsule were designated the widest point of the quarters
Proximal limit of the hoof wall	All measurements were taken from the junction of the hairline with the proximal border of the hoof wall at the coronary band. The hairline was defined as the distal hair roots, which are independent of hair length.

2.1.2 Designating anatomical areas for counting crack areas and numbers

The hoof was divided into anatomical regions, so that crack scores could be investigated for the toe, quarters and heels by using a template which had been drawn onto tracing paper so that it could be used on all hooves irrespective of their size. A horizontal and vertical line was drawn to bisect each other at ninety degrees in the centre of the tracing paper. Using these two lines as alignment, two diagonal lines were drawn to bisect the previous two at 45^0. This template was then positioned onto the crack map, so that the vertical line lay on the CD and the horizontal line on the CW50%. This effectively divided the 'circular' base of the hoof into quarters. The area defined as the quarter was delimited by two lines which formed 90° to each other, with the point of quarter, (50%CW) dissecting the area at 45°. Thus the quarters covered a quarter of the circle on each side; the toe area was also similarly delimited as was the heel. The toe mdc dissected the toe area at 45°. This area allocation is shown in *figure 2.1.2.i.*

Each quarter is delimited by 90° , so that each occupies ¼ of the surface area of the circle



Each line dissects the mdc and pt of ¼ by 45°

Figure 2.1.2.i Tracing paper map used to designate anatomical regions ensuring consistency of position irrespective of hoof size

2.2 The preparation of samples for chemical and mechanical tests

2.2.1 Cutting blocks from the hoof

Each hoof was cleaned and washed in Millipore water to remove any contamination from soil and other exogenous debris. Shoes were removed. The feet were then put back into the freezer and removed individually for marking and cutting.

Each hoof was scored 2.0 cm either side of the anatomically marked mdc and the point of quarters as defined in 2.1.1 along the lines of the tubules in parallel to the original mark using a ruler and a permanent marker pen. These guide lines were used to cut the blocks out from the hoof.

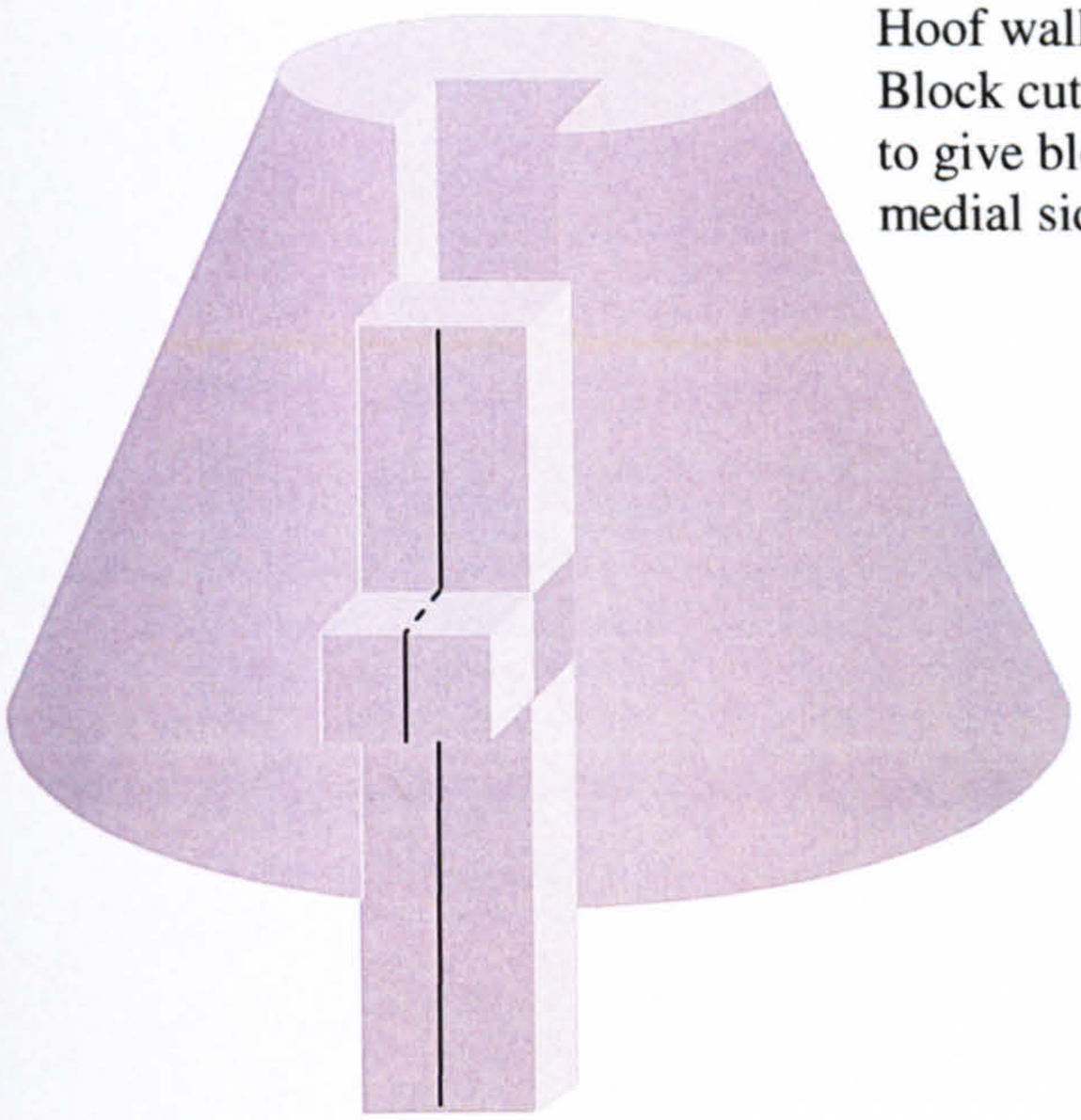
The hoof was measured along the mdc from the roots of the hairline to the bearing border as described in chapter 5; pages 183 and 195, and 50% hoof wall height was marked. The same distance was then measured from the hair roots of the quarters and also marked using a permanent marker pen, as shown in *plate 2.2.1.i.*

The blocks were cut out from the hoof using a Bandsaw* following the previously marked cutting guidelines. The blade of the Bandsaw was cleaned three times per cut using a wire brush and the hooves were cut from frozen in order to keep the blade as cool as possible. Between the cutting of each block the hoof was put back into the freezer to minimise over heating of the blade. The Bandsaw platform and blade and any instrument that came into contact with the hoof blocks was vacuumed and cleaned with Millipore water between every block and every hoof to prevent any cross contamination. Medical gloves were worn throughout and regularly changed for the same reason. The Bandsaw blade had to be replaced frequently due to the wear on one side of the blade, which meant that straight lines could not be cut. A wide tooth blade having three teeth to the inch proved to be the most efficient in cutting the hooves.

Removing the dermis and bones from the blocks

The block was secured in a vice, so that the coronary band was proximal and the dermis was furthest away from the person cutting the block. A scalpel was used to loosen the dermis and the bone from the hoof wall by cutting in a proximal/distal direction. A hacksaw was then used to saw down the epidermal and dermal junction to remove as much of the dermis as possible. The hacksaw was also used to remove the sole from the epidermis. The block was then removed from the vice and repositioned in a medial /lateral direction so that the dermal laminae could be removed using the scalpel. Each block was wrapped in parafilm and bagged in the appropriately marked bag and put back into the freezer.

Dorsal view of hoof wall

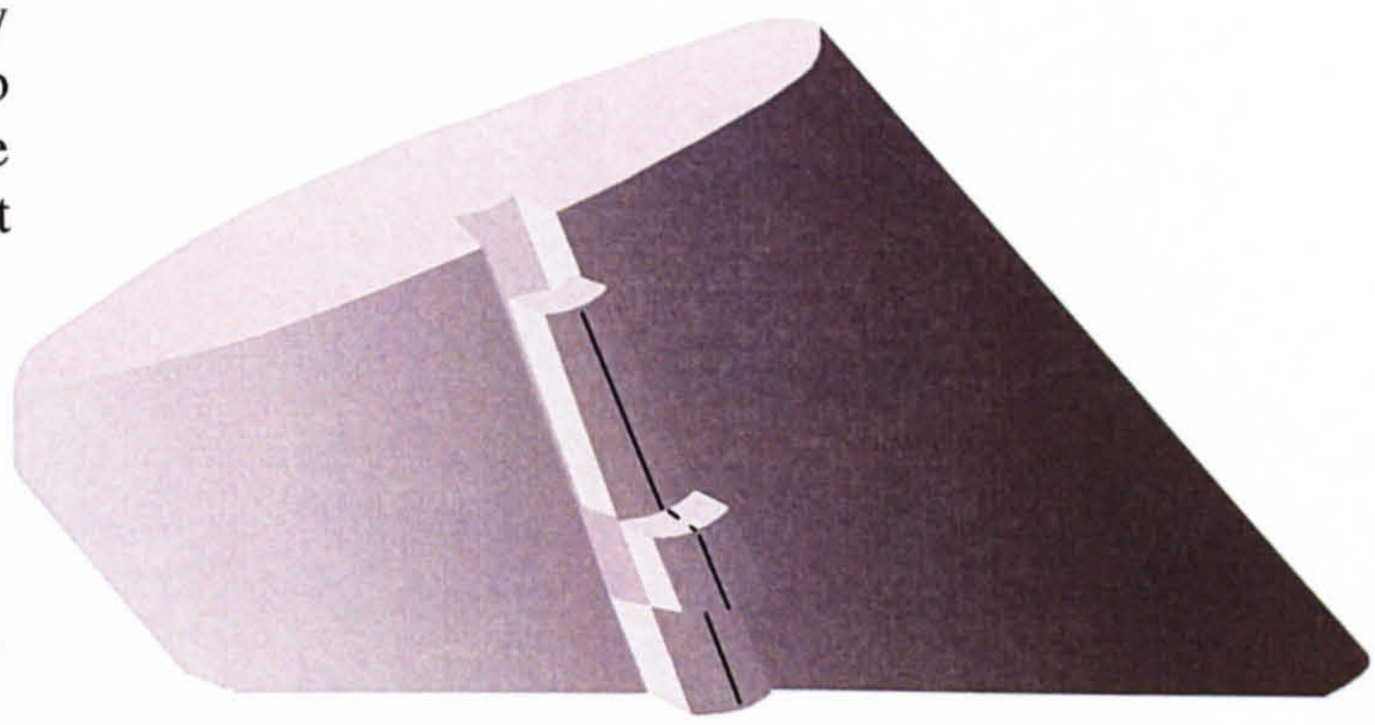


Hoof wall marked 2.0cm either side of score mdc Block cut out from hoof. Block then cut down mdc to give block A on lateral side and block B on medial side.

50% hoof wall height, 0.1g of sample shaved from the top of a 1.5cm block

Lateral view of hoof wall

Block marked at the same distance from the coronary band as 50% HWH at mdc, to match material of the same age. 1.5cm block was cut distally from this point.



Hoof wall marked 2.0cm either side of point of quarters and block cut down point of quarters; the dorsal block was marked A and the palmer block B

Plate 2.2.1.i Splitting of total wall hoof block into 50% HWH blocks to obtain material of common anatomical character, for investigation into the effect of washing and repeatability of techniques.

Note 1 For fracture toughness the whole hoof wall height was used.

Note 2 For the main experiments when material was being tested for trace elements and fracture toughness from the same anatomical position, the blocks were removed in the same way but not cut in two down the common anatomical position

Note 3 for details of cutting specific to either investigation, please refer to the appropriate chapter

2.2.2 Evaluation of repeatability

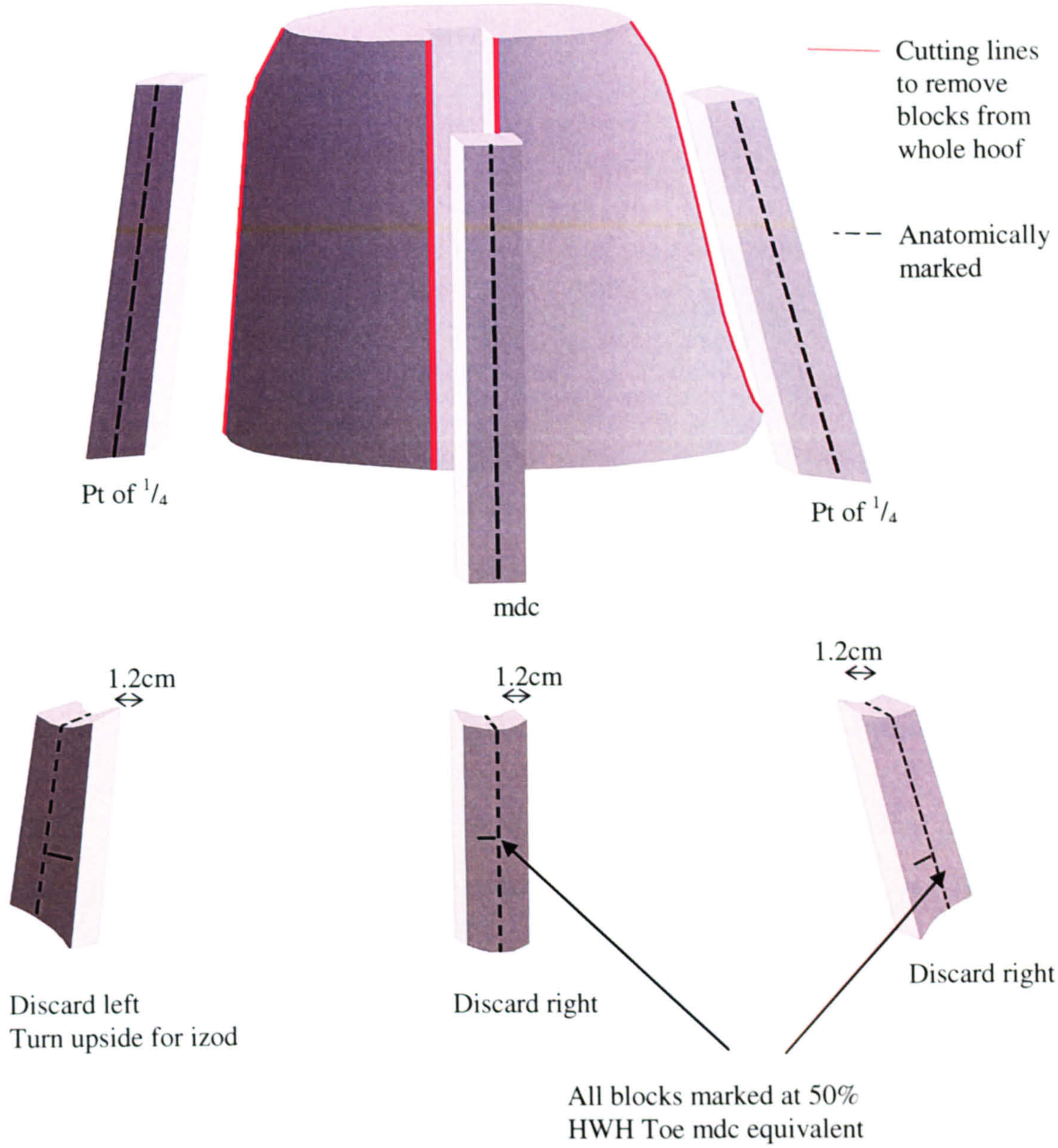
A subset of six hooves was used to test the repeatability of the techniques developed to prepare blocks and measure fracture toughness. An additional six hooves were used to investigate the differences in trace element concentration between different washing techniques. Using a permanent marker pen the blocks were marked A on the lateral side of the mdc and the dorsal side of the quarter blocks, and B on the medial side of the mdc and the palmer side of the quarters. Each block was cut into two of equal size down the anatomical marked line, *plate 2.2.1.i.* The blocks were relabelled A and B with hoof number and anatomical position, then wrapped in parafilm, bagged and frozen. The methods used for the specific tests are described in the appropriate chapters.

2.2.3 Refinements of method

Removing blocks for trace element and fracture toughness analysis from the same anatomical position

In order to compare fracture toughness and trace elements both from the same anatomical positions on the hoof and between hooves, it was crucial that the block being sampled had commonality of material, width and tubular pattern. Toe mdc, and point of quarters were marked and 50% hoof wall height was measured at the toe mdc and marked. The same distance from the hairline at the point of quarters was measured and marked; as described above.

The toe mdc and the point of quarters were used as the common line to ensure commonality of material, width and similar tubule patterns, *plate 2.2.3.i.* The hoof was then cut down its full length along lines parallel to anatomically marked lines to produce a hoof block. The block was then cut once down the anatomical line. The mark which had been made on this anatomical line at 50% hoof wall height or equivalent on the quarter blocks for cutting of the notch for fracture toughness was re-applied. The other edge was then trimmed until the block was exactly 1.2cm wide.



Artwork © Dave Gibson

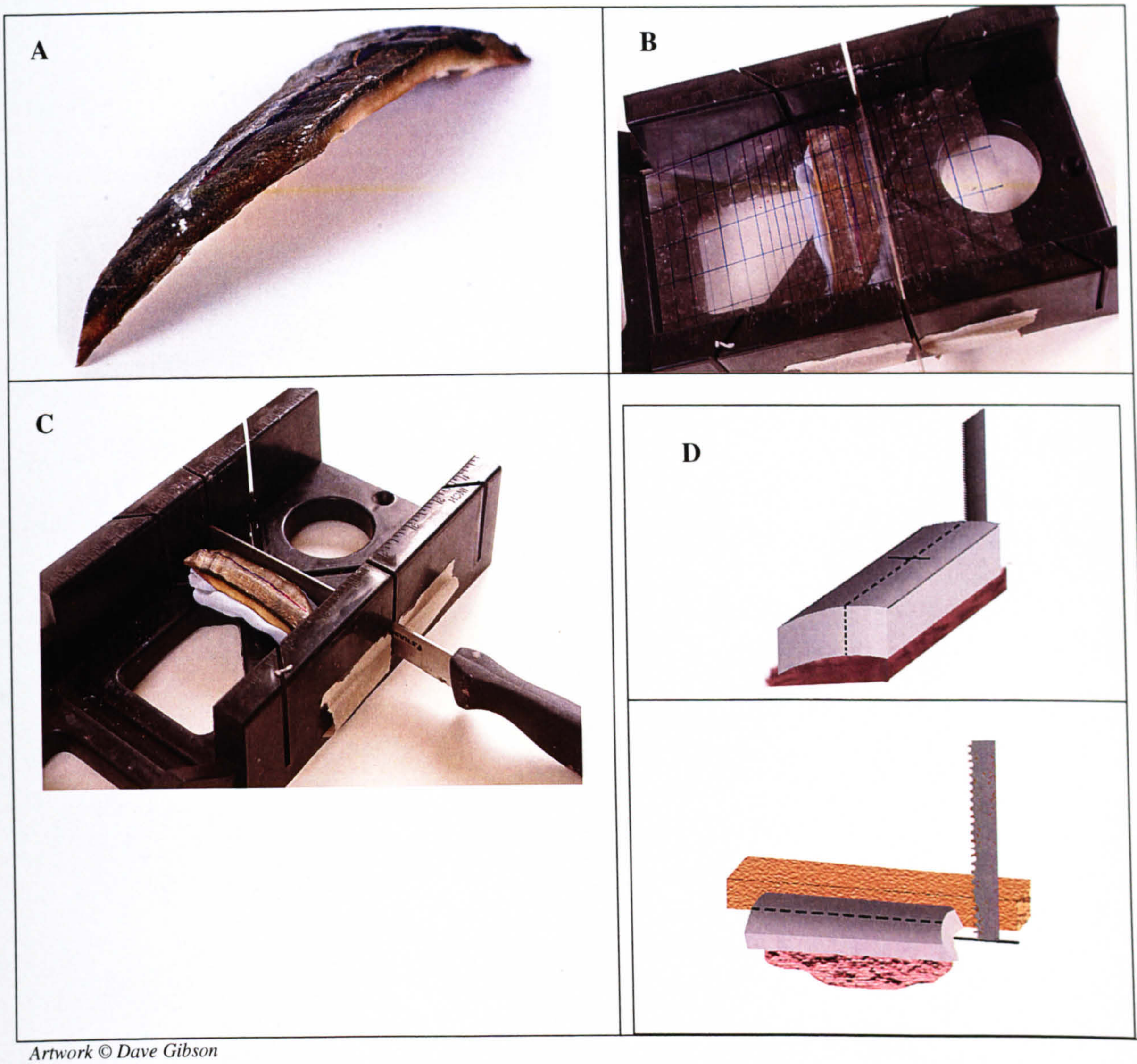
Plate 2.2.3.i To illustrate preparation of blocks for impact resistance tests

Modifying quarter blocks

The following method was developed to ensure accuracy of cutting material, predominantly from the quarters, which was curved in 2 planes as shown in *plate 2.2.3.ii*. Each block was mounted in plasticine, (which had been previously frozen so that it maintained its shape), to fill the underside of the block, so that the cutting surface on the outside of the hoof wall was parallel with the table surface and flat. The mounted block was placed within a mitre board. Clear acetate paper, onto which graph paper had been photocopied, was used to align the edge of the plasticine block so that it was parallel to the anatomically marked line on the hoof block. The plasticine block was cut with a kitchen knife using the mitre block as a guide; the resultant block edge was exactly parallel to the anatomically marked line. The plasticine bloc with the embedded hoof was placed in the freezer overnight, to maintain the hardness of the plasticine bloc, *plate 2.2.3.ii*.

Ensuring consistency of block width

The bloc was cut along the anatomically marked line with the bandsaw and the plasticine bloc used as a parallel cutting guide. A metal template which was machined to be exactly 1.2mm wide was used to position the bandsaw blade the precise distance from the guide. The bloc was then cut with the anatomical cut against the bandsaw guide. The resultant hoof block was exactly 1.2mm wide. Any modification specific to either fracture toughness or trace elements is described in the appropriate chapter.



Artwork © Dave Gibson

Plate 2.2.3.ii Steps to prepare blocks for fracture toughness tests

Note 1 Picture A quarter block with typical curvature

Note 2 Picture B block mounted on frozen plasticine mould and acetate of graph paper used to align the anatomical line parallel to cutting line on the miter

Note 3 Picture C cutting the plasticine block to provide a parallel line for to the anatomical line in order to guide the bandsaw

Note 4 Picture D diagram of plasticine mount and hoof block to provide a cutting surface perpendicular to the table and parallel to the bandsaw and the block in situ ready to cut

3 The relationship between the appearance and mechanical properties of the equine hoof.

"in buying a horse, the first things you ought to look at are his feet....high ones keep the frog off the ground and their hollow ringing sound is a proof of good feet". Xenophon, (380BC)

3.1 Introduction

The protective function of the epidermis relies on its structure, (defined in chapter 5), being integral or intact. The protective function of the hoof wall will be reduced if the hoof wall becomes sufficiently damaged by cracking. Cracking might occur because of the presence of material which is unable to maintain its proper function, (Leach 1996). Alternatively a structure will break or crack at its most stressed point, (Gordon 1976). Equine hoof wall cracks represent a wall failure, (Moyer 2003) and can occur anywhere on the hoof wall, they are mostly orientated in the direction of the tubules. The close interdependence of the dermis and epidermis means that if the hoof wall loses its protective function then the dermis is vulnerable to infection or injury.

An infected skin dermis due to cracks in the skin epidermis will cause pain but is unlikely to prevent an animal or person from fulfilling their economic role. However ungulates rely on a functional hoof wall epidermis for load bearing. If the presence of cracks prevents this function, then the consequence of poor quality hoof wall will be of economical significance. Jeffcott *et al.* (1982) reported that 68% of lost days in racing were due to lameness, with the foot being identified as the most common site of lameness. Although the authors did not specify which aspect of the foot was responsible for the lameness, others have recorded a high incidence of cracking in the equine hoof wall. Slater and Hood, (1997) surveyed horses in the USA and

reported that 27 % had hoof wall problems, Josseck *et al.* (1995) found that 90% of Lipizzaner horses had hoof defects. In both surveys the defects and problems were due to different types of cracks and Josseck *et al.* (1995) associated poor quality hoof horn and the presence of cracks to incidences of lameness and restricted use. The association with lesions, (cracks on the sole), lameness and economics has been more closely studied in other ungulates. Russell *et al.* (1982) recorded 82% of lesions as occurring in the claw, (foot), of the cow; Murray *et al.* (1991) in an epidemiological survey associated lesions with lameness in nearly five thousand dairy cows. In 1990, lameness in dairy cattle in the UK was estimated to cost £90 million a year (Esselmont 1990) due to reduction in milk yield and weight, (Singh 1993). In order to determine whether the presence of cracks on the equine hoof wall because of their effect on function are of economical significance, it is necessary to be able to record the cracks.

Currently the one of the ways that the function of the hoof in the clinical situation is assessed is by the visual appearance of the wall usually in terms of cracks. Vets and farriers describe hoof wall cracks by their location, (toe, quarter, heel), by their length, (full or partial) and by their depth, (deep or superficial), (Moyer 2003), and these descriptive terms have not been anatomically delineated. An overview of the affect of these cracks is then used to describe the hoof as either good or poor quality. This method is still descriptive and is not adhered to by all workers; indeed no single group of researchers has defined the gross appearance of equine hoof horn quantitatively, so both the researcher and the clinician continue to make subjective judgements, (Kempson and Rob 2004).

Reilly, (1995) indicated that there is a need for objective measurements of characteristics which represent normality, so that the term quality can be more closely defined. Reilly, (1998) discussed the usefulness of quantitative measurements such as hardness (Buffa *et al.* 1992), tubule density (Reilly *et al.* 1998), and moisture content (Leach and Zoerb, 1983) as parameters to provide researchers with objective methods of material and physiological characteristics of the equine hoof horn.

All the measurements discussed by Reilly, (1995) are post clinical judgement and sampling and therefore it is not possible to relate them to the gross, visual quality of the equine hoof wall. Subjective terms such as quality (Reilly 1995) and adjectives such as cracking around the nail holes should be replaced or described by measurements which are reproducible across workers thus removing the error of the individual's interpretation.

A system to record the severity of cracks needs developing before any relationship with mechanical properties of the epidermis can be studied because if the clinical judgement is not objective then it cannot be correlated with other objective measurements such as shape, trace

element content, moisture and fracture toughness. A method of recording the gross visual appearance will be valuable for studying the structural changes at gross level, which appear to influence or affect the functional capability of equine hoof horn. If the measurements can be characterised and defined and it is possible to link underlying histology with gross appearance then it may be possible to decrease the incidence of poor quality horn, commonly described as sand and grass cracks, brittle horn, seedy toe, thin walls, thin soles as defined in *table 1.6.3.i*, by taking steps to correct the problem. In order to achieve this, the appearance of the hoof capsule needs to be based on objective measurement of defined hoof characteristics, so that the measurements can be repeated and correlations with other aspects of pathology investigated.

If cracks occur in material when the fracture toughness[g], which is a measure of the material's resistance to crack propagation, has been exceeded, then the relationship between fracture toughness and severity of cracks should be investigated especially in view of the welfare implications of cracks on the equine hoof wall.

3.1.1 Qualitative versus quantitative assessments of epidermis

Reilly (1995) indicated that objective measurements of the normal hoof are required, so that subjective terms such as quality can be dismissed. Quality is defined in the dictionary as either a distinct attribute or a degree of excellence and is not necessarily a subjective term, if that attribute can be measured. Perhaps the grading of the hoof epidermis in terms of quality should not be dismissed. Whilst the word quality is often used colloquially, the terms good and poor quality could be continued to be used, if they were defined properly. Quality is a term which is recognised in every day language; however the interpretation of the word is often based on an individual's views and is thus subjective. In order to use the term scientifically it is important that the characteristics which distinguish between good and bad quality are defined and measurable. Horse owners believe black hooves to be of better quality than white hooves. Researchers, (Landeau *et al.* 1983), have shown that there is no difference in the ultimate strength or modulus of elasticity between coloured and non coloured hooves. Colour is an objective (factual) measurement but owners have associated the objective characteristic of colour with a subjective (personal) interpretation of quality. This illustrates that whilst objective measurements can be made, it is important that attributes are not associated to that measurement unless they too are objectively measured.

Subjective measurements are routinely used in 'equine science' and there may be difficulties in moving from a subjective view to a more measurement based assessment of the hoof, (Hood and Jacobson, (1977). All measurements by nature of their definition are quantifiable and therefore objective. Hood and Jacobson, (1977) expressed a concern that the measurements would be used to define an 'ideal', which in reality is unlikely to exist. They noted that there would be large individual variation in, for example the ideal shaped hoof capsule, dependant upon breed and work requirements of the horse. Many measurements such as angles and lengths are already objective and quantifiable. The concern expressed by Hood and Jacobson (1997) is related more to the association of the objective measurements with the term quality in situations where quality is being subjectively defined or subjectively related to the attribute of function.

The requirement to develop a method to define the visual appearance to allow further investigation with other parameters is not unique to the equine hoof capsule. Researchers of skin epidermis have appreciated the need to develop a reference chart which relates visual signs with mechanical properties. They highlighted (Batisse *et al.* 2002) that comparisons of clinical assessment with measurements of physical parameters are rare; not dissimilar to the situation in equine hoof epidermis. They acknowledged that the complexity of the terms used in clinical assessment such as 'looseness, tone and firmness cannot be reduced simply to the measurement of one or two physical parameters'. The terms used are actually subjective in the same way that the terms brittle, dull, soft are used to describe the appearance of the hoof. In addition it is unlikely that the various measurements can be contained in a simple equation to describe quality or gross appearance.

It is difficult to evaluate some of the attributes that the horse owner or vet considers important or characteristic of quality. Buffa *et al.* (1992) described poor quality in hooves as being characterised as 'soft, shelly horn which tends to develop cracks and flake away; Kempson, (1987) described poor quality hooves as crumbling around the nail hole, dull and soft, *table 3.1.1.i.* These evocative and imaginary adjectives are several conceptual and practical steps away from mechanical tests. It may prove difficult to define an adjective by one property only, (Prall 1973). For example whilst dullness might be easily and consistently measured in the laboratory, light conditions and the colour of the hoof may affect the result in field conditions; in addition it is unlikely that dullness could be related to any meaningful functional property.

Table 3.1.1.i A summary of descriptive terminology used to capture the term quality by researchers describing the visual appearance of the hoof wall and other epidermal tissues

tissue	summary of descriptions of quality	author
cattle claw	claw quality is the product of horn characteristics, claw shape and anatomy and physiology of the inner structures	Vermunt and Greenough, (1995)
cattle claw	poor horn quality in lame cows has been associated with a disruption of the synthesis and deposition of keratins	Hendry *et al.* (1997)
equine hoof wall	poor quality horn is characterised by soft shelly horn which tends to develop chips and cracks and flake away at the lower part making it difficult for the horse to retain shoes	Buffa *et al.* (1992)
equine hoof wall	thin, friable horn thin shelly horn with large areas of the lower bits crumbling away, particularly around the nail holes and are frequently tender on their feet	Kempson (1987)
equine hoof wall	poor hoof horn quality is a well recognised problem in horses frequently leading to restricted use and lameness. Signs of poor quality include chipped hooves, cracks and crumbling horn in the stratum medium of the horn	Josseck *et al.* (1995)
equine hoof wall	sand cracks and crumbling horn around the nail holes ; flat feet and collapsed heels. Good quality described as good shape and appearance, being able to withstand prolonged drought or mud. Poor quality horn appears to lose its natural waterproofing properties	Kempson (1990)
equine hoof wall	hoof wall diseases described on the basis of the gross appearance of the lesion. Superficial or deep cracks, thin flaky walls, crumbly fragile hooves	Slater and Hood (1997)
human skin	aging skin is of poorer quality tendency to looseness, reduced firmness and tone, reduced softness	Batisse *et al.* (2002)
human skin	perfect skin ; lack of blemishes, evenness of colour, smoothness and lack of flakiness	Prall (1973)

Unlike dullness, hardness could be easily measured using specific tests both in field and laboratory conditions and the characteristic of hardness might be related to attributes which ensure integrity of the wall.

Whether quantifiable or qualitative methods are used to assess the visual appearance of the epidermis it is important that method is both repeatable and reproducible. If a researcher cannot go back and repeat their methods and obtain results which are not significantly different to their first set of results, it will be impossible to tell if the differences in future work are due to method differences or true differences in the measured parameters. In addition it is important that other groups of researchers will be able to reproduce the method, so that they can compare results from other data sets using the same technique.

In order to develop a method of scoring or describing cracks on the equine hoof wall, quantification might be the best approach. Quantification can be done on several levels. It is possible to simply count the number of cracks, (nominal scale). A scale could be allocated to the

cracks so that they are ranked from the highest to the lowest, (ordinal scale). The cracks could be accurately mapped so that information on their distance from a pre-specified anatomical position is recordable, (interval scale). Alternatively the cracks could be measured by their length or area, which would mean that a hoof without cracks was given a value of zero, (ratio scale).

The measurement of hoof colour could be considered both repeatable and reproducible but is unlikely to be related to function. In addition the measurement of hoof colour is both qualitative and quantitative. Colour is a distinct attribute (qualitative measure), the number of black hooves as opposed to white hooves can be counted; colour is therefore quantifiable. Thus the relevance of the objective quantification of a characteristic requires consideration and needs to be related to an attribute of function which in turn might allow quality to be quantified. It is unlikely that the various measurements can be contained in a simple equation to describe quality or gross appearance, because of the complexity of the hoof's function. In addition what is considered ideal in appearance versus that which is ideal for function precludes the development of a simple measurement system. However the number of cracks could be mapped and scored for their severity which would give an indication as to the ability of the wall to protect the inner structures.

Any method of measurement and recording might need to be lexicon, but it must also be adaptable so it can be acceptable universally by researchers, vets, farriers and horse owners. An epidemiological study of hoof wall problems in equines illustrates the need for quantitative measurements of gross quality and standardisation of terminology so that the measurements are reproducible. Slater and Hood, (1997) carried out a survey to establish the presence and type of 'owner hoof wall problems'. The classification of the hoof wall diseases was based on previously used systems and described on the basis of the gross appearance of the lesion - for example superficial or deep cracks, shelly walls, brittle or thin and flaky, (Kempson 1987; 1990; Buffa *et al.* 1992; Comben *et al.* 1984). Six gross classification choices, (all subjective), for the survey were used: - i. thin walled, ii. flaky, iii. crumbly, iv. split, v. cracked or vi. other.

Over half the respondents reported more than one problem. This indicates that either the descriptions were too subjective, (the difference between flaky and crumbly would be difficult to quantify), for the owners to distinguish between the types or that the terminology was inappropriate. Alternatively as discussed above, it is possible that poor quality hoof cannot be described in singular terms, as there may always be more than one defect present. 28% of the horses surveyed had hoof wall problems compared to 90% in a comparable survey (Josseck *et al.* 1995). Survey methods varied, Slater and Hood, (1997) relied on owner response whereas

Josseck *et al.* (1995) used the same assessors for all the horses. The difference in methods emphasises that any classification of gross appearance needs to be checked for reproducibility. Josseck *et al.* (1995) also used a type of quantitative scoring which included a note of the type of crack and its severity, which will have contributed to the reproducibility. If a survey is to be meaningful, gross appearance must be quantifiable. Several systems of grading were considered as part of the method development in this thesis and results compared. In addition it was considered necessary to compare the scoring methods with a material property to see if there were any correlations with the visual appearance.

Batisse *et al.* (2002) attempted to provide physical and therefore more objective measures that could be used in clinical procedures. Clinicians generally compress the skin horizontally between their fingers to assess its firmness, elasticity and density, in the same way as vets and indeed researchers, (Josseck *et al.* 1995) indent the hoof horn with a hoof pick to superficially test for firmness, hardness and elasticity. Batisse *et al.* (2002) developed a small mechanical device that allowed reproducible skin wrinkling and sequentially made measurements of mechanical properties at the same site, in order to obtain some physical explanations to the different wrinkling characteristics. Extensibility of the skin, changes in elasticity and thickness were measured although it is unclear what methods were used. The Shore D test has been used as a method to measure hardness in cattle, pig and horse horn *in vivo* and used as an indication of quality by relating to the number of lesions present on the sole of the claw. Hardness measurements were taken in this thesis and correlations with crack scores as well as with other mechanical tests being carried out on the same material *in vitro* were investigated.

The subjective versa objective dilemma has also been encountered by those working in the epidermal field of hair, (de Berker and Sinclair, 2002); how does a clinician define a subjective diagnosis through the microscopic appearance of the hair? Concentrating on sample collection, the authors emphasised that both affected and unaffected samples should be collected, the method of collection should be carefully controlled to avoid damage to the hair shaft and the method of microscopic assessment must be detailed. In conclusion they stated that findings should only be used when strictly defined in statistical and morphological terms, emphasising the prevailing view that subjective assessment is limited in its usage.

If quantifiable methods are to be developed to provide objective evaluations of the hoof, then variables such as differences in sampling procedures which would affect the observations and measurements should be standardised. Methods should be refined so future workers can follow reproducible standardised procedures. The defining of quality in the hoof should follow the systems developed for quality control in manufacturing industries. Quality control systems

continuously evolve as they are the systematic study of a subject following an accurate and exact method for performing the observations. The soundness of the measurement is then tested against a standard, for the hoof the standard needs to be related to function *in vivo*. Any system used to describe the gross appearance of the hoof needs to be objective, repeatable and quantifiable. In order for the measurement to have any relevance it needs to relate to function. A quantifiable method to measure cracks will therefore be developed in this chapter, however the definition of quality relative to the measurement system will need postponing until the method has been investigated as to its reproducibility and also investigated as to its relationship with a function, which will need to be quantifiable.

3.1.2 Evaluation of current methods used to assess the visual appearance of the epidermal structure

Most of the techniques used in research to evaluate the gross appearance of the hoof or claw epidermis have concentrated on the subjective visual appearance of either the equine hoof wall in terms of cracks or the objective lesion mapping and scoring of lesions on the sole of the ruminant or pig claw. The techniques were developed so that researchers could investigate the cause of lameness, (Penny *et al.* 1963) or the effect of biotin, (Penny *et al.* 1980; Brooks *et al.* 1977), in relation to the lesions of the claw.

Clinical lameness in cattle is reported to be caused by digital lesions in 90% of cases, (Andersson and Lundstrom 1981). Because of the economic effect of lameness in cattle, many authors have investigated lesions and attempted to correlate them to a variety of parameters mainly as an indication of diseases such as laminitis. The welfare significance of lameness in pigs, (Mouttotou *et al.* 1997; Mouttotou and Green 1999) has also meant that the objective classification of gross lesions which are considered to be one of the main causes of lameness has been further progressed than in equines.

Using maps of the bovine and porcine claws to show the presence of lesions has been used extensively with the porcine industry adopting the original work by (Penny *et al.* 1963; 1965). Lesions are described by the position of their incidence by using the same diagrammatic map of the claw, to differentiate between for example heel crack and median horn crack (Brooks *et al.* 1977; Bryant *et al.* 1985a,b and c; Misir and Blair 1986; Mouttotou *et al.* 1997; Mouttotou and Green 1999). The bovine industry also maps the claw but various workers used different

divisions (Philpot *et al.* 1990; Boosman 1990; Murray *et al.* 1996; Logue *et al.* 1994) until more recently when workers adopted the recommendations of the VIth Symposium on Diseases of the Ruminant Digit, 1990 to use an agreed mapping of the claw, (Greenough and Vermunt 1991; Leach *et al.*1997; 1998). As well as describing their position, the lesions on the bovine claw are described by type for example haemorrhage, sole ulcer, (Booseman 1990; Murray *et al.* 1996; Greenough and Vermunt 1991), with some researchers identifying lesions only by type, (Philpot *et al.* 1990).

The development of lesion maps and scoring follows a logical strategy which may be appropriate to develop for equines, although it needs to be appreciated that lesions occur on the sole of a claw, whereas in horses, the crack is a hoof wall phenomenon. It is probable that methods developed to map lesions from the flat surface of the sole may need adapting.

Any method developed to measure cracks on the surface of the equine hoof wall should be objective; it must not rely on common terminology or purely descriptive adjectives. Current opinion indicates that quarter cracks occur most frequently, (Pardoe and Wilson, 1999; O'Grady 2002) thus there is a need to quantify the cracks on the hoof wall by anatomical position to quantify this perception. In order to monitor changes, over time, in the incidence of cracks in response to nutrition or changes in shape or as a consequence of a disease process, the crack would be defined ideally by its location and its size and probably its severity so that any associations with function could be assessed. A mean hoof crack score is likely to be most useful, in the clinical situation together with a count of the anatomical distribution of the cracks; unless the vet or farrier wishes to follow the repair process of one particular crack or the study of individual cracks is required in research. The system needs to reproducible and repeatable; otherwise its usefulness is limited. Whilst the systems used to categorise cracks on the equine hoof wall are very subjective, the systems developed for porcine and ruminant claws are objective and it is plausible that one of the systems could be adopted for the equine.

3.1.3 The use of maps to record the position and incidence of cracks

Brooks *et al.* (1977) used a map to note position and type of lesion on the porcine sole in order to compare the effect of a dietary supplement on the incidence of lesions. No differences were found between the control and supplemented groups after three months but differences in toe lesions, ($p<0.01$) between the groups were recorded after six months. This may have been

because new tissue had not appeared on the sole after three months and recording was presumptuous. Alternatively it could have been because the severity of the lesions was not recorded and simply noting presence or absence of lesions was not sensitive enough to record changes due to dietary influences. Murray *et al.* (1996) used a claw map for an epidemiological investigation into the incidence of lesions in relation to lameness in cows using vets, stockman and students to record the data on nearly five thousand cows. In addition to the map, the recorders were asked to note the site of the most important clinical lesion. Although over thirty seven farms were being used, all recorders measured a similar distribution of lesions within the hind and fore claws as well as a significant difference in the number of lesions within the hind claw with the outer claw being more affected compared to the inner claw,($p<0.001$).

The use of a map to record lesions was a reproducible method for recording both lesion position and number, (Murray *et al.*1996; Brooks *et al.* 1977; Bryant *et al.* 1985a; Logue *et al.* 1994; Mouttotou *et al* 1997). All researchers were able to measure the incidence of lesions by their type and by their distribution within claws using maps and descriptions of the cracks and all groups measured significant differences, ($p<0.01$) in lesions dependant upon anatomical position and type.

A method to record cracks by position needs developing to provide a repeatable method for identifying the location of cracks on the equine hoof wall, which is sufficiently robust to establish differences in incidence according to anatomical position. The use of maps in other species meets this requirement and will be investigated as a technique.

3.1.4 The use of severity scores to evaluate significance of cracks

In the survey described above, Murray *et al.* (1996) found a significant difference between the way recorders ranked the lesions, ($p<0.001$) which was dependant upon their perception of the clinical significance of the lesion. Whilst the map gave the recorders an objective method of recording position, relying on personal judgement to score the clinical significance appeared to introduce an element of subjectivity and decrease the repeatability of the method. Severity of lesions needs to be objectively recorded, particularly if workers seek to investigate associations with other factors rather than simply record incidence.

For example, Philipot *et al.* (1990) co–ordinated a large scale survey to investigate the associations between lesions in dairy cows, the incidence of laminitis and lameness by grouping the lesions into eleven groups using a detailed descriptive system and noting their presence or

absence. The relationship between the lesions and lameness was inconclusive. Ninety four percent of cows with two or more lesions were recorded not lame. However when the authors explored the data further using multivariante analysis, they found that the percentage of lame cows with lesions increased to 50% when correlated with lesions that they would have clinically defined as serious. This result highlighted further the need to objectively score the severity of the lesion and the authors recommended that the recording of the area and extent of the severity of the lesion should be collected in further work.

Researchers have described severity using different techniques. Goonewardene and Hand (1995), mapped lesions and addressed the severity of cracks by allocating a score from 1-3 based on the depth of the crack (where 1 =hairline and 3 = visible separation >3mm); in addition they noted the presence or absence of cracks and the number of cracks. They recorded that cows with cracks were heavier and older than cows without cracks but did not find any correlations with severity of cracks. Despite using a different method of scoring severity, Bryant *et al.* (1985)a and Bryant *et al.* (1985)b were unable to find a correlation between lesion severity and lameness scores or structural soundness in either young or mature pigs. They mapped the lesions and in addition scored them between 1-5 according to their size with 1 equalling a small lesion and 5 a very large lesion. The severity scores were adequate to record a reduction in severity, (size), of all lesions in response to a dietary treatment over time and thus were repeatable. The authors tried to claim a correlation between the number of heel cracks and soundness scores in the mature pigs, they failed to inform whether Spearman's Rank or Pearson's product moment coefficient was used and the correlation coefficient was 0.12. The square of the correlation coefficient can be calculated which represents the proportion of the total variance in one variable which can be explained by its relationship with the other variable, (Petrie and Watson 1999). Thus only 1% (0.014 X 100) of the change in soundness score can be attributed to a change in heel cracks. There was no correlation between soundness scores and toe lesions in the developing gilts. Size of lesions may not always be correlated to its severity and the lack of association might have been due to the fact that the wrong characteristic of the lesion was being associated with severity.

It is highly likely that there are correlations between an aspect of lesions and lameness. Lesions occur on the sole of the claw and if the dermis is exposed, then the animal will be in pain and lame. Even if the lesion does not expose the dermis, it is likely to become full of grit or stones resulting in an uncomfortable sole surface. It is possible that Bryant *et al.* (1985) were recording lesions which were not normally associated with the incidence of lameness. Philpott *et al.* (1990) only found lameness correlated with sole ulcers or detachment of heel horn, whereas Bryant *et al.* (1985) recorded toe lesions. Murray *et al.* (1996) recorded greater incidences of

lameness with heel horn erosions, sole ulcers or penetration of the sole with a foreign body. Lesions are more significant with respect to lameness if animals are kept on concrete floors, (Penny *et al.* 1967), thus a standardised method of recording lameness should also be adopted and scoring systems evolved to stay relevant to function. There may be a species difference in pain tolerance, Bryant *et al.* (1985) were working with pigs, Philpott *et al.* (1990) and Murray *et al.* (1996) were working with cows.

Further consideration needs to be given to the development of a method for severity scoring cracks on the equine hoof wall. Cracks can be described in terms of their position, which may or may not have an effect on an aspect of hoof function; they can be counted and the number of cracks may or may not be related to function, their area can be measured which may or may not have an effect on function. All of these methods are objective and quantifiable but none are actually related to the severity of the crack and severity is the parameter most likely to affect function, possibly in relation to either number or area.

Since 1990, the description of haemorrhages within each zone of the sole map of cattle claws has been scored between 1 and 4 in relation to their possible effect on function after agreement at 6th Symposium on Diseases of the Ruminant Digit by allocating 1 to slight discolouration and 4 to exposed dermis. Greenough and Vermunt, (1991) argued that a cumulative score for three slight haemorrhages may give the same score as one severe lesion and whilst the severe lesion might be debilitating, the slight lesions may only be cosmetic in significance; thus numerical lesion scores would be meaningless if trying to correlate them with function. In order to recognise the greater clinical importance of the higher scores, they adjusted each observation geometrically to produce a severity score which gave a greater emphasis on the more severe lesions as summarised in *table 3.1.4.i*. They used the scores to investigate factors which might influence the occurrence of lesions. No correlations were carried out between scores and any other factors despite noting that cows with the lowest bodyweights had the lowest severity scores; nor was the relationship between the arithmetic score and any other factors investigated, so no comparison could be made between the two scoring systems.

Table 3.1.4.i Geometric adjustment of numerical scores used to weight clinically severe cracks

Arithmetic/ numerical score based on severity of haemorrhage	**Geometric adjusted score to increase significance of severe haemorrhage ($2^{\text{numeric score -1}}$)**
1	1 (2^{1-1})
2	2 (2^{2-1})
3	4 (2^{3-1})
4	8 (2^{4-1})

Note 1 the four point scale was adjusted by $2^{\text{numerical score -1}}$
Note 2 after Greenough and Vermunt, (1991)

The lack of evaluation of the adjustment may have been one of the reasons that Logue *et al.* (1994) chose not to use the weighted severity scoring when investigating the relationship between locomotion score and lesions of the sole and white line in dairy cows. The authors extended the score type from 1-4 to 1-10 to cover ulcers but omitted to describe the appearance relative to the score, so the system immediately became unrepeatable. Although no correlation coefficients were given, the authors recorded a correlation between the mean lesion score of all four feet and locomotion score. Further analysis between the lesion score of the hind feet only and locomotion score increased the correlation and when analysis was done between the lesion scores of the zones of each foot, there was only a correlation with one of the zones, (zone b) and locomotion scores. The authors had reported that lesions were most severe in the hind feet and zone b. This indicates that if a weighted severity score had been used, the mean score of all four feet would have reflected the effect of the severe lesions on function more closely. Indeed the authors concluded that recognition of clinical severity of lesion should be adopted for future work.

Whilst the presence of cracks is generally considered to be a reflection of poor quality horn in the equine hoof capsule, (Slater and Hood 1997; Kempson 1987; 1990) it needs to be appreciated that a cracked material does not infer loss of function; bricks, cast iron, plaster and masonry are stronger in compression than they are in tension because they are full of cracks, (Gordon, 1976). It is hypothesised that the equine hoof is likely to have numerous small cracks similar to other biological materials like bone, (Currey 1999) wood, (ASCE 2002) and dentine, (Marshall *et al.* 2003). In fact the equine hoof wall is similar to other tough biological materials and appears to have a sophisticated mechanism for dissipating energy through numerous small cracks, (Kasapi and Gosline 1998; 1999). It was therefore considered important to develop a method to measure both the number and the severity of cracks on the equine hoof wall especially as a relationship with a mechanical property was to be investigated.

Leach (1996); Leach *et al.* (1998) in response to the work by Greenough and Vermunt, (1991) and Logue *et al.* (1994) discussed a further development of severity scoring of lesions in cattle and used five different methods of scoring: - i. number of lesions, ii. arithmetic severity, iii. adjusted severity, (geometric score), iv. size, measured from photographs and v. size multiplied by adjusted severity. The same footmap as Greenough and Vermunt, (1991) was used which increased the usefulness of the data for comparisons between published work. Repeatability and reproducibility of the severity scoring system and the area measurements from the photographs were tested statistically and scores were collected for individual lesions, per claw and per cow. The systems were then used to follow the development of lesions in dairy cows during lactation, (Leach *et al.* 1997).

Relationships between the size and severity of the individual lesions were recorded, (Leach *et al.* 1998). Less severe lesions were more numerous compared to severe lesions, this was reflected in the size of the lesions; 94% of the lesions accounted for less than 34% of the total sole area. In addition, Leach *et al.* (1998) adjusted the score for individual lesions geometrically and then multiplied the score by the area in order to plot distribution graphs. This highlighted that individual lesions with a high severity affected only a small proportion of the claw. However when they calculated the mean scores for a claw, they reported that there were only a small number of low severity lesions, this actually contradicts their previous result. In their 1997 paper the scores were used to follow development of lesions, the same contradiction is reported and displayed graphically i.e. as the number of lesions increase over time, so does their area and severity. On the basis of that result they concluded that as the area of claw affected follows the same pattern as severity, then there is no need to measure area as 'the simpler, quicker assessment is therefore considered to be an adequate indicator of the degree of damage'. Although in the same paper, they reported that there were no correlations between the different types of scores. This is in contrast to their 1998 paper, when they recorded a slight but significant correlation in arithmetic score, geometric severity score and combined lesion score, (geometric score times area).

The equine hoof capsule is exposed to a variety of environments including sand, mud (dry and wet), the farrier's rasp, ammonia in bedding, all of which can be considered environmental insults and it is hypothesised that these will cause numerous, small, mild cracks. This is in contrast to the cow's claw, which is likely to develop large mild lesions because the sole is exposed to friction, whereas the hoof wall is under compression which closes cracks rather than opens them up. This might be one of the explanations for the different results recorded by Leach *et al.* (1997; 1998). If cows had just been bought in from the field, they may have numerous small lesions on their sole, on a concrete floor due to friction they may increase in size and if the floor is dirty, infection would occur and severity increase. A severe crack on the equine hoof wall is likely to occur due to a physical insult and is therefore more localised and possibly singular in its existence; however because it represents a wall failure, the area may be quite large. Because of these possible associations, it is hypothesised that there will be an inverse correlation between the number and severity of cracks in the equine hoof wall but a positive correlation between severity and area.

To test this hypothesis and in the light of the apparent contradiction in results of Leach, *et al.* (1997;1998) and because severe cracks will have a greater effect on function compared to mild

cracks on the equine hoof wall; a scoring system which distinguishes between severity and allows weighting by number and area needed to be developed.

3.1.5 The choice of an appropriate mechanical test to evaluate susceptibility to crack formation

Boosman, (1990) investigated the relationship between the gross objectively scored appearance of the cow's claw and its histological appearance. Soles of calving cows and a control group were mapped for the presence of haemorrhages which were scored according to clinical terminology. The rest of the claw was not mapped but descriptors of severity were used to score its appearance. A total laminitis score was then recorded which, was a summation of all the parameters measured. A sagittal slice was taken from the claw to include sole and wall, six areas were sampled for histology, although the sampling sites were not clearly defined. Twelve parameters the authors considered indicative of laminitis, for example, haemorrhages, formation of new vascular capillaries and fibrosis were looked at under the microscope and scored semi–quantitatively according to their severity. The authors were investigating the hypothesis that calving cows were severely affected by laminitis, however there was no difference in histological scores between the non calving and calving cows; in fact many of the histological scores were higher, (more severe) in the control group. More relevant to this thesis was the fact that the histological scores were related to the gross appearance as measured by the macroscopic scores. This highlights the need to quantify microscopic as well as macroscopic scoring systems in order to investigate common perceptions. Had the authors simply compared the microscopic appearance of samples from the controls and the calving group, they would have found no differences in the histological scores as they reported; however they also found that there was no difference in the macroscopic laminitis score between the two groups. Thus the important relationship was not inter- group but inter–laminitis and histological scores.

If mechanical or histological properties are being compared to the gross appearance of the hoof or claw, it is important that the appropriate tests are chosen. As gross appearance of the equine hoof wall is described in terms of cracks, a mechanical test which characterises the material's resistance or propensity to crack needed to be chosen. Most of the correlations described in the literature compare lesion scores and cracks with the material property, hardness. A material test should be appropriate to the physiological characteristic being measured, in this instance cracks.

Mechanics of cracks

All structures deflect or change shape when a force or weight is applied to them. One of the common characteristics of biological materials is their toughness and resistance to rupture, (Vincent 1990). The deformation of the hoof in response to the horse's bodyweight (e.g. in the standing horse) is not visible to the naked eye, but the wall is distorted so that some parts of the wall become shorter due to compression and other parts are stretched due to tension. The hoof wall should deflect just far enough to counter or resist the load from the bodyweight of the horse and at the same time recover from these deflections without damage to the structure of the hoof wall.

A structure will break or crack at its most stressed point, (Gordon 1976). An average horse weighing 600kg may carry 180kg on one front foot (60% of the bodyweight is carried by the front feet). The magnitude of the force is likely to be greater when the horse is moving as the force is being taken by either one or two feet rather than equally shared between four feet. At moderate trot, the peak force on each forelimb is considered to be nearly equal to the horse's bodyweight, at gallop, the force increases to over one and half times the bodyweight. These differences would affect the size of the stress concentration and if the foot landed on unequal ground surface, then this would further concentrate the stress on what maybe only a small proportion of the hoof wall.

The quantity of energy required to break a given cross-section of a material is known as its fracture toughness. When a structure breaks or cracks, two surfaces are formed. In order to form these new surfaces, the chemical bonds, which held the material together, have to be broken. Generally a material will crack in areas where stress is concentrated. In terms of hoof mechanics, fracture toughness can be considered as the hoof wall's resistance to the formation and spreading of cracks, (Bertram and Gosline 1986). In the hoof, fracture toughness is very dependant on the intertubular material and hydration of the hoof wall, (Bertram and Gosline 1987).

The hoof wall needs to be tough, a property it shares with bone, (Currey 1999); it needs to be able to absorb energy without fracturing and it needs to be relatively insensitive to the presence of cracks and other imperfections. The hoof appears to be relatively insensitive to cracks as any shod hoof will have a minimum of seven potential stress concentrators where the nail holes exit the hoof wall. Various parameters will affect the stress concentrations on the hoof and the hoof's ability to resist this stress is complex. In order to remove some of these variables, the material of the hoof was tested for its toughness rather than the structure itself.

Hardness

Various researchers have measured hardness as a mechanical property and related it to either the visual appearance or the structure of the epidermis. Many correlations have been attributed to hardness and some of the relationships could be considered tenuous, because the mechanical property being measured is actually not relevant in terms of the physical characteristics of either the material or its underlying morphology. Hardness is considered to be an indication of the material's resistance to wear, because the material has a resistance to flow and plastic deformation, (Robson and El-Tahawi, 1971) this is tested by measuring the resistance of a material to an indentation and is a physical characteristic that can be easily measured, (Robson and El-Tahawi 1971) using an indentation test known as Shore D. The Shore D test measures the resistance of a material to penetration. The degree of indentation of a hand held spring loaded probe is read from a dial which is calibrated to the Shore industrial scale of hardness. The probe used in Shore D is sharp and will make a hole in hoof wall material.

Vincent, (1990) expressed reservations regarding the use of hardness tests for evaluating the mechanical properties of materials. He pointed out that hardness is not related to stiffness and should not be confused with it. 'The idea of using an indenter on a piece of material and taking the force required to do this as a material parameter is an attractive one; however it really is impossible to ascribe the numbers to any particular deformation. Robson and El-Tahawi, (1971) measured the hardness of fingernails as an index of nutritional status of a group of healthy and malnourished people. They found that the fingernails of the well nourished group were softer than the malnourished group and the hardness of the malnourished group decreased over a three week period. It is unlikely that the structure of nail could change in such a short time period in response to nutritional changes. Forslind *et al.* (1980) pointed out that 'hardness is not a feasible definition for the property of withstanding bending and buckling in a composite organic material like that of the nail.' He highlighted that a complex structure like a nail is likely to exhibit complex viscoelastic behaviour under load. Forslind *et al.* (1980) tested nail at different water contents under three point bending to find its elastic modulus having measured its thickness and width. They showed that the modulus was correlated with water content.

Hardness testing in the field is very dependant on the water content of the hoof material, (Vermunt and Greenough 1995) and hardness will decrease with increasing water content. It would be difficult to control for water content in field conditions. A working party, (Brizzi, *et al.* 1998) concluded that methods to measure hardness 'applied to the claw wall of the living animal are both unreliable and inconsistent. The hardness of the sole can be measured but consideration needs to be given to the variation in hardness caused by different levels of water content.' It is unlikely that measuring hardness of the equine hoof wall will be very meaningful

unless the moisture content of the wall could be measured at the same time as hardness is being tested.

Webb *et al.* (1984) used both Shore D and A, (Shore A uses a blunt tipped probe and is therefore less invasive), tests to measure hardness and in addition measured the compressive strength of pig hoof horn from the same predefined anatomical sites on the claw wall. Increases in compressive strength and hardness after dietary treatments at the same sites were recorded but correlations between results of the two tests were not investigated. However strength and hardness are not related.

Work on bone, (Hodgskinson *et al.* 1989) indicates that Vickers hardness can be correlated with Young's modulus and be used as a predictor for both yield stress in tension and for Young's modulus. Bonser, (1995) says that shear failure is responsible for the formation of the indentation in Vickers hardness test and that the physical properties responsible for shear failure, (i.e. mineralisation in bone) are the same for stiffness and tensile failure.

Penny *et al.* (1965) measured the friction of concrete surface and related the increased friction of concrete compared to other surfaces to the increased wear of the pig's claw and the associated increase of lesions in pigs kept on concrete floors. If hardness is related to wear, then the measurement of sole hardness may give an indication of the sole's resistance to both wear and lesion incidence. The Shore D test has been adopted in the equine literature, (Coenan and Spitzlei 1997) however one has to question the suitability of this material test for explaining an aspect of quality in the equine hoof capsule. The majority of cracks in the equine hoof wall capsule originate in the wall not the sole, (Kempson 1997; Josseck *et al.* 1995) and many hooves are protected to a large extent against wear by shoes. Resistance to wear is not related to cracks on the equine hoof wall.

The relevance of hardness in the formation of cracks is dubious; a material's toughness is a more pertinent relationship. However if hardness tests are carried out on material followed by sequential toughness tests, it would be interesting to see if the results correlated. As hardness is not related to any other mechanical property and is limited in its physiological application, it is important not to over interpret a cause and effect relationship where none exists. More work needs doing on whether a simple, fairly meaningless test has any correlations with other mechanical tests. If this is the case then the hardness test could be used as an arbitrary scale simply to correlate with other more meaningful parameters which cannot be measured in the field as they are invasive. It was therefore considered appropriate to measure hardness on the piece of hoof which would later be tested for its impact resistance, even though Budras *et al.*

(1998) cautioned that the Shore durometer could be problematical as even very small inhomogenities in the horn can lead to false results.

Using Shore D on the equine hoof capsule

The measurement of hardness using a Shore D durometer proved problematical on the entire hoof capsule. It was not possible to find an area flat enough for the durometer to sit horizontally due to the curvature of the hoof in two planes; in addition the results were not reproducible. Vermunt and Greenough, (1995) noted that the instrument needed to be placed flat on the material being tested and that a constant pressure needed to be applied. The application of pressure in order to achieve an indent would vary amongst operatives. Therefore should this test be adapted for field use, there would need to be some form of standardisation required by way of including a test block, or maybe automating the machine so that a set amount of pressure was applied independently of operator. Baggott *et al.* (1988) also had concerns regarding the use of a durometer and noted that a flat surface of 30mm X 15mm with a depth of 6mm was required. Despite the shortcomings of the method, they were able to obtain a standard deviation within 4% of the mean and concluded that whilst the technique might not have been ideal, they were able to obtain repeatable measurements. All their measurements except for one were taken from the sole of the foot, which is flatter than the wall. This was in contrast to Buffa *et al.* (1992) who was able to measure Shore D on the equine hoof wall but was unable to obtain accurate readings from the sole. Webb *et al.* (1984) noted that pig horn exhibited post compaction stress release in response to the Shore D tester and therefore took measurements at 0 and 5 seconds after application of the tester. It was only possible to take one reading using the equipment available as the tester was hand held and the reading was taken when the probe had been pushed into the wall as far as possible. In addition, Webb *et al.* (1984) noted that Shore D probe made a small hole in the hoof wall, which would influence the integrity of the hoof wall for further tests. Two other hardness tests were considered and they are summarised in the appendix, *section 3.1.5A*.

Fracture toughness

Impact resistance is defined as the kinetic energy needed to initiate fracture and continue the fracture until the specimen is broken. The energy required to break the specimen is considered a measure of the material's toughness. Energy is needed to create a crack and energy is needed to enlarge the crack to failure. Fracture toughness was considered a more appropriate material parameter to measure as fracture toughness is actually related to the physiological mechanism of cracking. Impact resistance is tested by an Izod machine, which provides a quick and easy method to compare materials for their general toughness. A pendulum impact machine consists of a base, a single arm pendulum and a striking head. The mass and the drop height determine the potential energy of the hammer. A pointer is moved by the swinging pendulum and the

pointer stays at the highest point reached by the pendulum after striking the sample. Izod pendulum tests do not provide information on the mechanism of failure nor the cause of a fracture but they do provide an estimation of the toughness of a specimen. It is assumed that the material fails in shear due to crack propagation. Two values for the fracture toughness of the hoof wall can be obtained from this test. The first is called impact resistance and is a measure of how much energy is absorbed by the width of the hoof block and is calculated by dividing the amount of energy absorbed by the width of the block, expressed as J/m. The second, impact strength, takes into account the full geometry, the energy absorbed is divided by the width and depth of the block, this takes into account the variation in depth across the blocks and is expressed in kJ/m^2.

3.1.6 Experimental aims

The following points have been highlighted from the literature:-

- Cracks have been measured in the equine hoof wall, but the methods used have been subjective and therefore limited in their use as they cannot be reproduced nor repeated by other researchers
- Cracks are used as an indication of the failure in the functional integrity of the hoof wall and nutrition has been used to reduce the appearance of cracks but due to the subjectivity of measurement, results cannot be condoned
- In other species, severity of cracks has been correlated to anatomical position
- Severity weighting of lesion scores has reduced the significance of minor cracks which have little or no effect on functionality
- Using maps of the claw and agreeing on specified zones has allowed changes and differences in lesions to be recorded and provided a reproducible and repeatable method of recording the location of a lesion
- The relationship between function in terms of lameness and lesions was dependant upon the severity of the lesions
- Objective measurement of lesions allowed comparison with underlying histology

The aims of this chapter were therefore three fold a) to make an observational study of the number, area, type and position of cracks and to make comparisons between the incidence of cracks at the toe and quarter; b) to compare two methods of describing the severity of cracks on

the hoof capsule to see if sufficient emphasis was put on cracks which are of clinical significance; c) to investigate whether there was a relationship between crack scores and the impact resistance of the epidermis. Having established the above, it was then important to ascertain if using different methods of crack scores imparted the same information and relationships between scores and material properties of the hooves.

The following were therefore developed

- An objective method to record the position of a crack
- An objective method to measure the area of a crack
- A scoring system to type cracks dependant upon their potential effect on function, thus giving the crack a severity score
- A system to adjust the severity number geometrically, thereby weighting the significance of the more severe cracks
- A protocol for sampling hoof wall material for fracture toughness testing

Thus cracks will be defined as shown in *figure 3.1.6.i.*

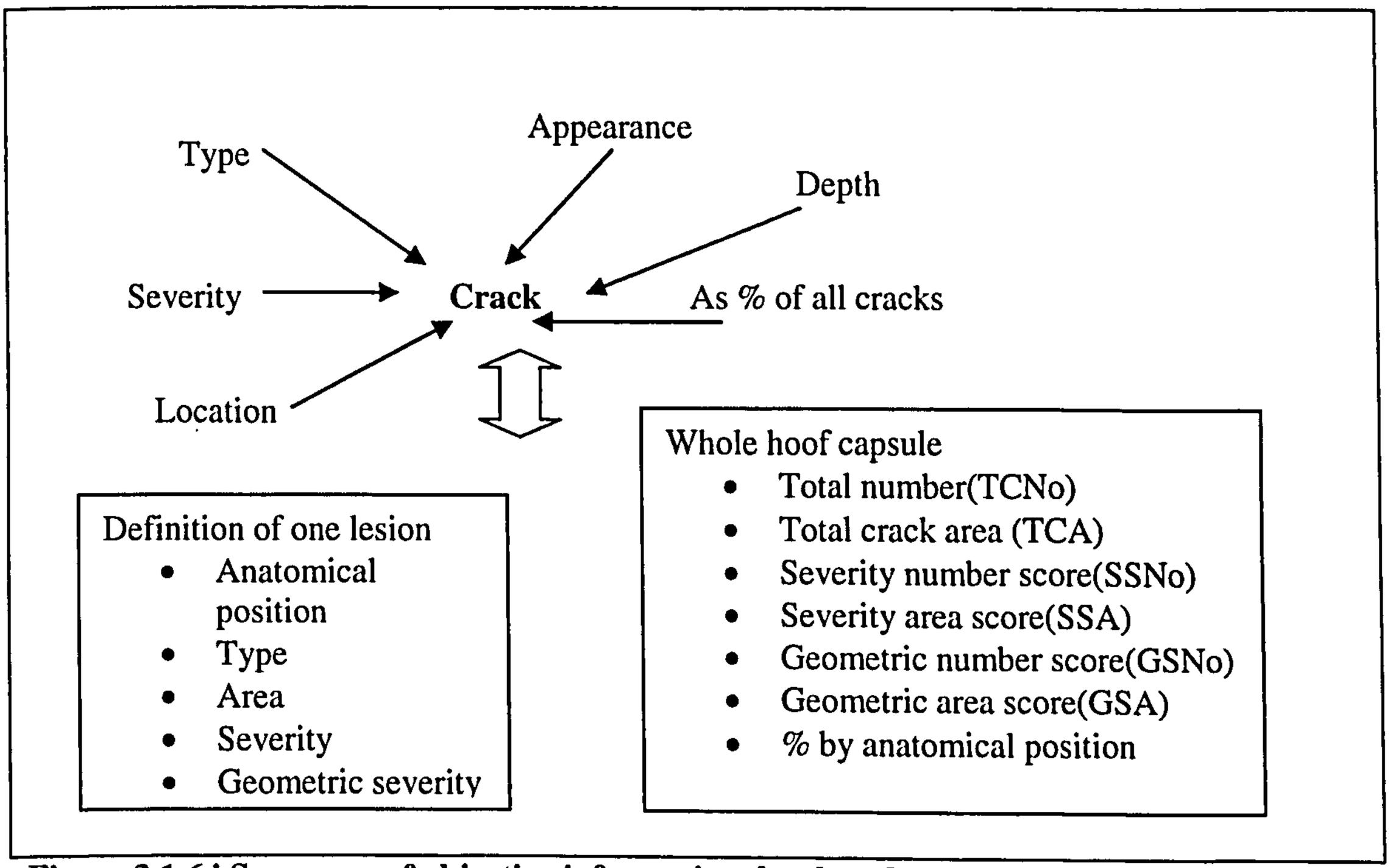


Figure 3.1.6.i Summary of objective information developed to categorise cracks

The following hypothesise were then investigated:-

- That there was no difference in incidence of cracks between anatomical positions
- That the total number of cracks per hoof is not related to the severity of the cracks as measured by type (SSNo) or geometrically weighted (GSNo)
- That there is no relationship between the area and number of cracks

- That there is no difference in the fracture toughness between anatomical positions
- That there is no relationship between any crack score and fracture toughness

3.2 Methods

It was not possible to detect cracks in sufficient detail from photographs; therefore their position could not be mapped from a digitised photograph. In addition the parallax and curvature of the hoof wall meant that the geometry of the wall could not be captured in two - dimensional photography; thus it was not possible to measure surface area of either the crack or the wall of the capsule from photographs. The experience of other researchers, discussed in the introduction, highlights very clearly that it is important to develop a method which is objective and reproducible. In order to measure both the position and the surface area of the cracks, a crack map was developed, using the solar base as a template.

3.2.1 Mapping the location of cracks

The hoof was placed onto graph paper which had been divided into quarters. Each hoof was positioned so that the point of quarter lay on the horizontal line and the point of toe on the vertical line. This ensured that all anatomical comparisons would be repeatable and comparable across different sizes of hooves, a variation not taken into consideration by the use of claw maps.

Mapping the position of the crack

An outline of the solar base was obtained by drawing around the hoof. The graph paper was marked with the hoof number, the orientation of the toe and medial and lateral sides. A pair of compasses were used to mark the position of any cracks on the wall on the outline of circumference of the solar base plate drawn on the graph paper. This was done by positioning the compass on the distal end of the crack and marking where this lay perpendicularly at the solar base, *plate 3.2.1.i.*

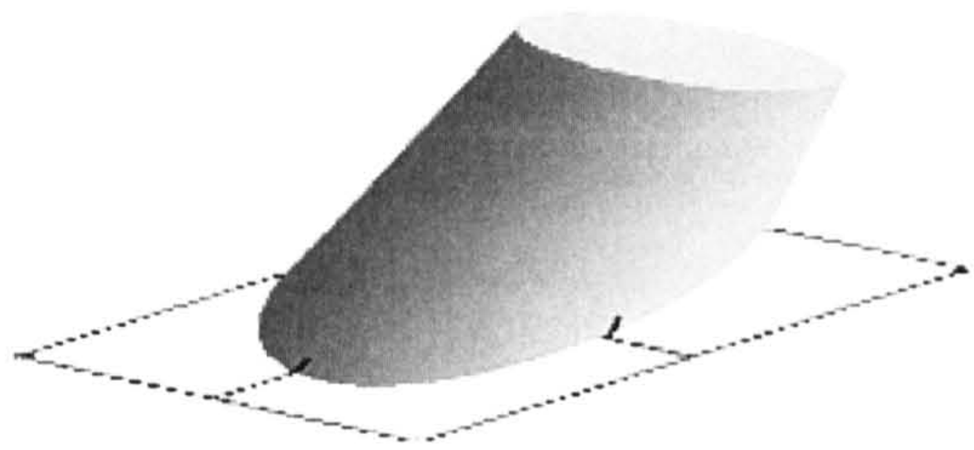
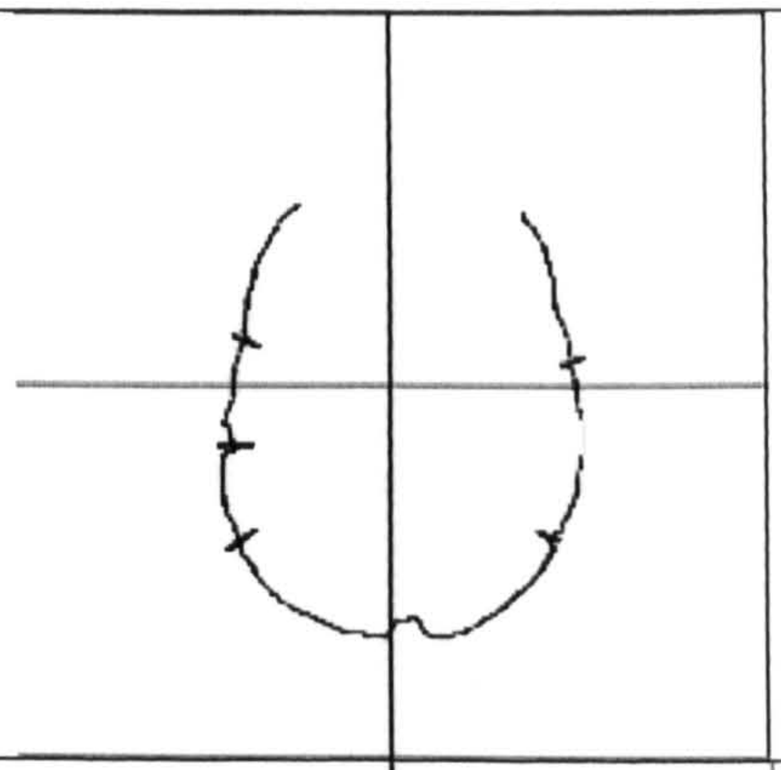
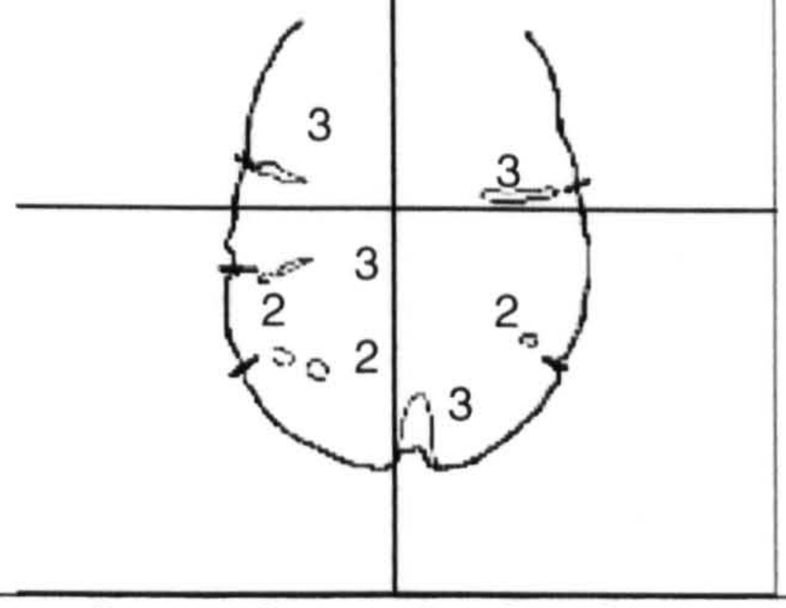
Graph Paper marked with a cross	
Hoof placed on graph paper and aligned so mdc CD is positioned on the perpendicular and CD50% / CW50% is positioned on horizontal	
Draw around sole and mark positions of cracks	
Cracks then measured, mapped and recorded. Sole divided anatomically, by placing tracing paper over the graph and cracks allocated by position. Scanned with NIH image, measured and recorded in excel.	

Plate 3.2.1.i Summary of the steps taken to map the cracks on the equine hoof capsule

Artwork © Dave Gibson

Mapping the size

The xy co-ordinates of crack length were measured and mapped accurately onto the base map; first the distance of the distal end of the crack, (x) was measured from the distal bearing surface and marked on the map. The length of the crack was then measured and the proximal distance (y) marked on the map. The outline of the crack was measured by measuring a series of points along the xy spine in the z direction (90° to xy) so that the width of the crack was measured. If the crack was circular, then the distance to the middle of the circle was measured from the distal base, the diameter measured and the compass used to draw the outline. If the crack or chip was an irregular shape, then the shape was broken down and each area accurately measured and located on the map. The number of measurements taken was determined by the irregularity of the shape. The outline of the crack was then drawn on the crack map.

3.2.2 Classification of cracks by score type

Visual assessment

Once the cracks had been mapped, they were visually assessed from the hoof capsule using the system described in *table 3.2.2.i* developed specifically for this thesis and given a score type according to the perceived effect that they would have on the function of the hoof wall capsule in terms of protection and load bearing. The type number was allocated to each crack on this basis.

Table 3.2.2.i Description of crack score system developed for this thesis

Crack type	Effect of crack
1	Cosmetic, surface effects only, e.g. toe clip; less than 0.25cm in depth. No effect on weight bearing and no reduction in protective properties
2	Scratches and surface abrasions less than 0.5cm deep; no effect on weight bearing capability
3	Nail holes and other chips or cracks deeper than 0.5cm on wall which do not affect the ground bearing surface. Sensitive structures not exposed.
4	Small areas of missing wall at ground surface; surface delamination, chipped weight bearing areas; e.g. old nail holes at ground surface. Deeper than 0.5cm but not exposing sensitive structures. Affects ground bearing surface but unlikely to have catastrophic effect on weight bearing
5	Distal surface missing; reducing weight bearing surface but not exposing sensitive structures
6	Deep cracks which expose sensitive structures; delamination at weight bearing surface, reducing weight bearing and reduction of protection.

3.2.3 Counting cracks and measuring their areas

Each hoof crack map was scanned, saved and labelled by its hoof number as a TIFF file, for use with NIH image. The scale was set by measuring the graph paper on which the maps had been scanned. The squares were measured horizontally and vertically three times over a series of different lengths, the difference between the mean of the horizontal and vertical measurements over all the lengths was only 0.001mm. The measurements were averaged using Excel and the pixel count adjusted to the correct length. Areas and numbers of the different type cracks were recorded. Their position was determined by using an overlay which delimited the toe, the quarters and heels, as described in chapter two, *section 2.1.2 and figure 2.1.2.i*, so that distribution of cracks between hooves could be compared.

3.2.4 Calculation of weighting scores

Three types of scores were used, table *3.2.4.i*. The total crack number, (TCNo) or total crack area, (TCA) which is a simple count of either the total number of cracks on the hoof or the measure of the total area of cracks on the hoof. The severity score (SS*) takes into account the type, (severity) of the crack by multiplying the crack type by its incidence, either by the total number of cracks (SSNo) of each type or by the total area of each crack type (SSA) to give a total severity score by type, SSNotype or SSAtype. All severity scores can be added together to give a total severity score of all the cracks counted, (SSNo) on the hoof or the total area of all cracks measured, (SSA) it is hypothesised that this may give too greater a weight to cracks which are not clinically significant. The geometric score, (GS*) emphasises the more clinically significant types of cracks by using the geometric weighting system, *section 3.1.4 and table 3.1.4.i* , to weight the higher crack types.

Table 3.2.4.i Summary of crack information taken from each hoof

by number	by area
1. The total number of cracks,(TCNo) counted on the hoof	1. Total surface area of the hoof covered by cracks in mm^2 (TCA)
2. The total number of each crack type (TCNo1 – TCNo6)	2. The total surface area of each type of crack per hoof (TCA1-TCA6)
3. The percentage that each crack type contributed to the total number of cracks	3. The percentage surface area that each crack type contributed to the total crack surface area
4. The severity score number per hoof (SSNo) and per crack type (SSNo type 1-6) (type x total number of cracks designated that type)	4. The severity score area per hoof (SSA) and per crack type, (SSA type1-6) (type x total area of cracks designated that type)
5. The geometric score number per hoof, (GSNo) and per crack type (GSNo type 1-6)	5. The geometric score area per hoof, (GSA) and per crack type, (GSA type 1-6)
6. The above were calculated for the medial quarter(TCNoM/4, , SSNoM/4, GSNoM/4); the lateral quarter and the toe,(TCNoT, TCAT, SSNoT, GSNoT)	6. The above were calculated for the medial quarter (TCAM/4 SSAM/4, GSAM/4), the lateral quarter and the toe,(TCAT,SSAT, GSAT)

Note 1 cracks were counted , no account taken of type; areas of cracks were measured and totalled
Note 2 The number of each type of crack was counted and recorded. The area of each type of crack was measured by tracing around the outline of the crack and recorded. The absolute area for each type of crack was totalled.
Note 4. Using Excel a whole hoof numerical severity score (SSNo) for each crack type was calculated by multiplying the occurrence of each type of crack by its numerical type and a SSNo was summed for the whole hoof; A severity score area (SSAtype) for each crack type was calculated by multiplying the area by each numerical type and summed to give a total SSA for the hoof
Note 5. A geometric score for number, (GSNo) and area (GSA) was calculated. This gave a greater weighting to the more severe cracks.
Note 6. The numerical occurrence of each type according to its anatomical position was recorded; the area of each type of crack according to anatomical position was recorded and the total types for each area summed. The geometric and severity scores for each type of crack at each anatomical area was calculated as were the total geometric and severity scores for each position

In order to illustrate the effect of weighting the scores, a hypothetical hoof has been scored and tabulated, *table 3.2.4.ii.*

Table 3.2.4.ii The effect of severity and geometric adjustment on the number and area of cracks on a hypothetical hoof

crack type	1	2	3	4	5	6	total
number of cracks TCNo	5	3	2	1	2	1	14
severity number score SSNo *number x crack type*	5	6	6	4	10	6	37
geometric number score GSNo *number x* $2^{Crack\ type-1}$	5	6	8	8	32	32	91
area of cracks mm^2 TCA	100	30	10	25	27	30	222
severity area score SSA *area x crack type*	500	60	20	25	54	30	691
geometric area score GSA *area x* $2^{Crack\ type-1}$	500	60	40	200	432	960	2192

3.2.5 Block preparation for fracture toughness testing

Blocks were prepared as described in *chapter 2 sections 2.2.1 page 49 and 2.2.3 pages 52*

Notching

It was necessary to notch all blocks to prevent deformation of the samples when they were hit by the pendulum, otherwise some of the energy would have been absorbed by the specimen bending thus reducing the accuracy of the test. It is important that the notch geometry is repeatable for each specimen as it will affect the degree of stress concentration. One of the main causes of error in the pendulum impact tests is incorrect or inconsistent notching of samples. It is possible to buy specialised notchers; however these were not available, so steps were taken to standardise the notching of the hoof blocks.

A template was used to cut the notches to ensure that the notch size was consistent across all the blocks. A large metal sheet which had been cut in the middle from its back edge towards its front edge, exactly parallel to its free edge, so that the groove acted as a runner for the blade, was placed on to the cutting area of the bandsaw. A second, smaller piece of metal which also had a parallel groove was positioned from the back of the bandsaw blade, on top of the first piece of metal. A third piece of metal had a 2mm groove cut into it; and this was used to position the back plate, so that the blade could only cut 2mm in length, *figure 3.2.5.i.* All the 'positioners' were held secure with clamps. Each block was then notched 2mm at the previously marked 50% mdcHWH.

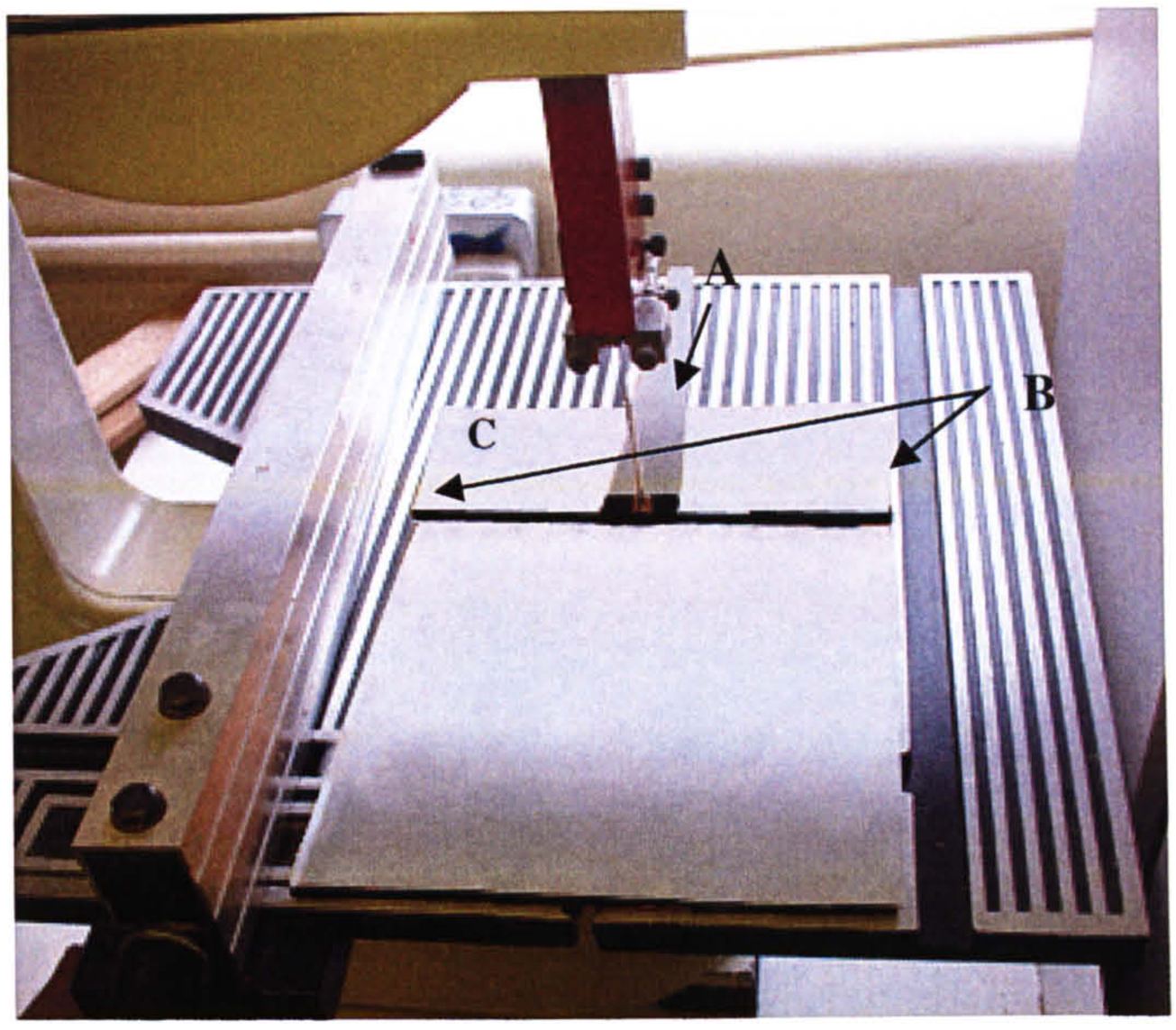


Figure 3.2.5 .i Notch template used for preparing blocks for Izod testing

Note 1 B shows where clamps were positioned to hold back plates secure. Due to changes in the lab, the clamps were not available for photography
Note 2 top piece of metal was machine notched to 2mm, hoof block positioned perpendicular to the blade at 50% hoof wall height
Note 3 Metal template A has notch of 2mm, which is used to align C by placing piece A in front of the blade and clamping C so that the hoof block can only move 2mm towards the blade.

The main crack resistance in hoof horn material is perpendicular to the tubules (Kasapi and Gosline 1998; Baillie *et al.* 2000). The notch was placed circum-radially as defined by Kasapi and Gosline (1998) so that the fracture toughness was measured perpendicular to the tubules. The cutting of the notch in the quarter blocks was difficult due to the curvature in two planes, making it both hard to cut straight parallel lines to make the block in the first instance and secondly to present a perpendicular surface to the Bandsaw to cut the notch. The method used for the mdc blocks was refined as described in section 2.2.3 to overcome these difficulties. The hoof block was then removed from the plasticine.

Hydration status

In order to minimise the variability in fracture toughness due to moisture content, all the blocks were placed in double distilled Millipore water for two weeks until their weight had equalised and they were fully hydrated. The alternative would have been to either test fully dehydrated blocks, but this would have put the material past its glass transition[g]; or to have tested the blocks as received and then to have measured their water content and expressed all values on a dry matter basis for comparison. However because the blocks were also used for trace element analysis from the same position, taking samples for dry weight determination at this stage would have interfered with the standardised methodology. The conditions under which the samples were kept were identical and therefore any error introduced would have been consistent across

all samples. Landeau *et al.* (1983) re-tested hoof block samples which had been repeatedly dried and re-soaked and found that this had no effect on the material properties specifically the stress strain response. This finding was confirmed by Kasapi and Gosline, (1999) who tested samples which had been hydrated and subsequently dehydrated as well as samples which had been frozen and subsequently defrosted and found no significant differences in the modulus of stiffness. However Kasapi and Gosline, (1999) quoted work by Leach, (1980) who found that freezing did have an effect by lowering modulus. Leach, (1980) froze his samples for a longer period and at a lower temperature compared to Kasapi and Gosline, (1999). The protocol for keeping samples in this thesis was more comparable with the time scale of Leach, (1980).

Sample size

It is important that samples to be tested on the IZOD are the same size in order for comparisons to be made. With hoof wall material it is only possible to control the width of the block, as depth and height will vary according to the original size and shape of the hoof from which it was taken. The width of the block had to be 1.2 cm in order to fit the clamp of the IZOD machine. A special metal template was made which was exactly 1.2 mm wide. This was put between the blade and the positional guide of the bandsaw and the positional guide moved so it fitted exactly perpendicular to the template and secured. The template was removed and the hydrated blocks were cut with their anatomically marked line and notch against the positional guide and thus any excess material was trimmed to exactly 1.2 mm. The positional guide was repositioned every ten blocks to make sure that the blade had not warped.

3.2.6 Procedure for fracture toughness testing

During pilot studies, none of the blocks fractured so the weight was doubled from 3lbs to 6 lbs and a similar test rerun on the IZOD machine, *figure 3.2.6.i*. All the blocks fractured and the readings stayed within the range of the dial. Before the test can be carried out, the hoof block has to be placed in the vice. It should be noted that the force used to clamp the block can vary and add a significant stress to the block. In order to minimise this, two small pieces of plywood were used either side of the blocks to try and standardise the force applied to the blocks.

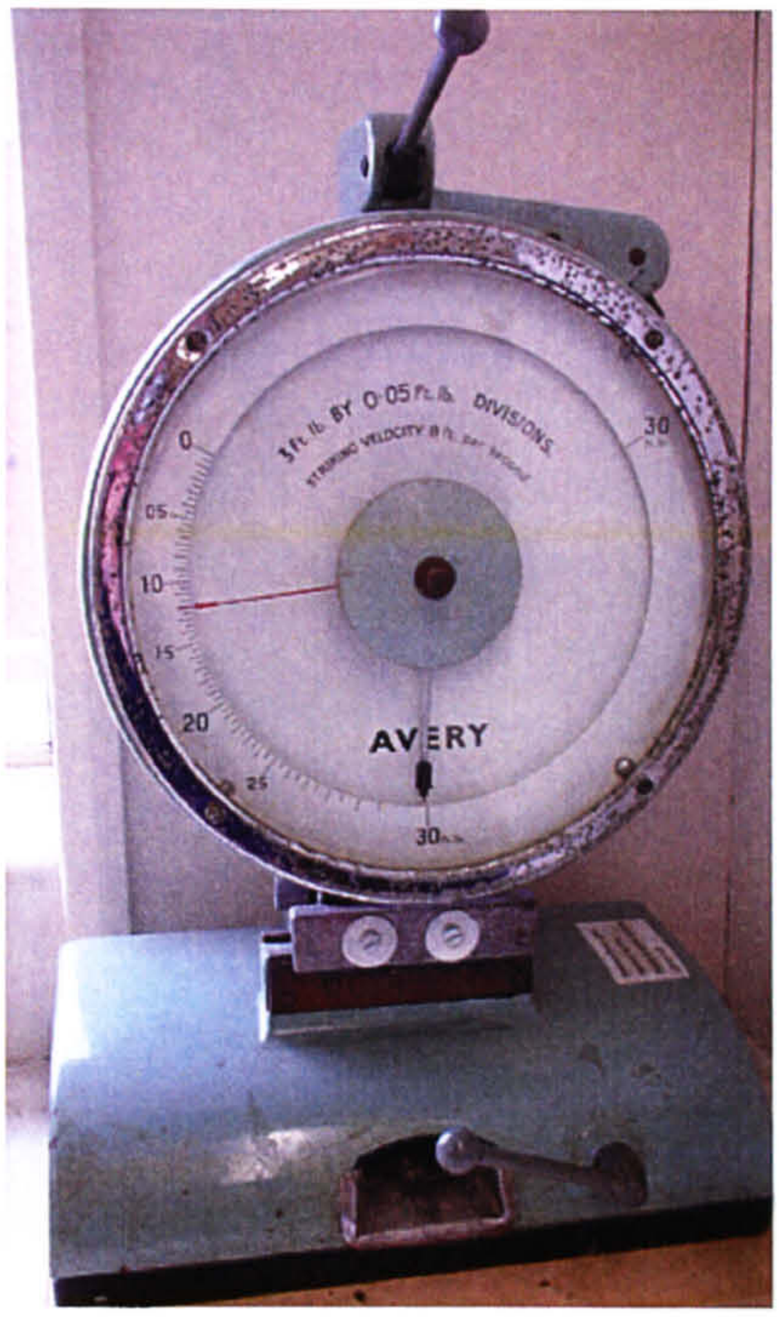


Figure 3.2.6.i Izod Pendulum used to test the fracture toughness of hoof wall blocks

Impact results are dependant upon moisture content, impact velocity, geometry, sample notching and sample mounting, thus all these were standardised as described above, accepting the limitations of trying to standardise biological materials.

Calculating the impact energy absorbed

The impact energy absorbed in breaking a notched block of hoof wall takes into account the original cross sectional area of the block at the notch and the pendulum striking the block at the notch edge; it is expressed in kJ/m^2. The block was placed so that the blow was edgeways parallel as recommended by the British Standards Institute (BSI 1997). The energy absorbed W was noted from the gauge after the specimen broke. The thickness h and the remaining width b_N, in millimetres at the notch base, *diagram 3.2.6.ii* were measured. These were then entered into the following equations.

$$A_{iN} = \frac{W}{h.b_N} \times 10^3 \qquad kJ/m^2 \text{ impact strength} \quad \text{equation 1.}$$

$$\frac{W \times 10^3}{b_N} \qquad J/m \text{ impact resistance} \quad \text{equation 2.}$$

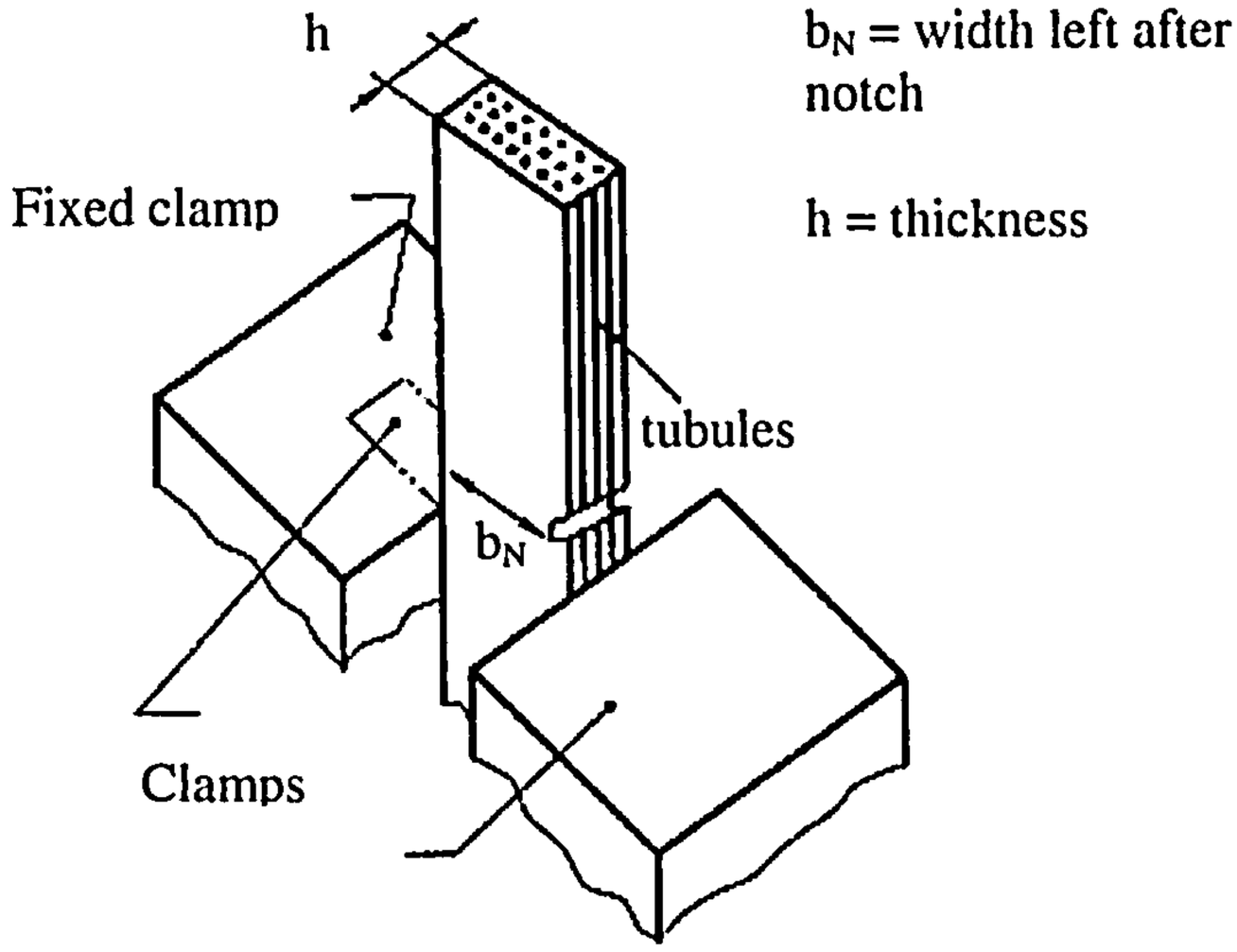


Figure 3.2.6.ii Hoof block in vice for fracture toughness testing
Note 1 annotated to show from where the measurements were taken
Note 2 b_N and h entered into equation 1; b_N entered into equation 2

Four types of break were recorded:-

FB	full break; a break in which the block separates into 2 or more pieces
HB	hinge break ; an incomplete break such that both pieces of the block are held together only by a thin peripheral layer in the form of a hinge having no residual stiffness
PB	Partial break; an incomplete break that does not meet the definition for a hinge break
NB	non break ; in the case where there has been no break, the specimen is only bent , possibly combined with stress whitening

As the Izod measured in ft.lbf, W was multiplied by a correction factor of 1.356 to convert to Joules. It was noted that this test should be carried out on blocks free of twist, with mutually parallel surfaces and the surfaces and edges should be free from scratches. Whilst it was not possible to fulfil all these requisites, methodology was standardised as described above and both impact strength and resistance were calculated in order to compensate for differences in the geometry of the blocks. Repeatability of the technique was tested on six hooves which were split along a common anatomical line as described in chapter two, section 2.2.1.

3.2.7 Statistical Methods

All statistical analysis were performed using Miintab[13]. Data from the set of 48 mixed hooves were analysed separately to the subset of 28 left fores feet as the subset was being used i) to test the assumptions reached from the first set of data and ii) to test repeatability of the different methods.

Summary data including the mean, standard deviation and range of fracture toughness for each anatomical position are presented in section 3.3.3 .Where appropriate the summary data from the blocks which had been split down a common anatomical line into blocks A and B are collated and are presented in section 3.3.6Aiii in the appendix.

Severity and geometric scores were calculated for the whole hoof, for each type of crack and for each anatomical position. Summary data including the mean, standard deviation and range for the number, area, geometric and severity scores of each crack type of the whole hoof and of the toe, medial and lateral quarters are collated and are presented in the appendix, pages VIII - XIV

The distributions of crack number, area and score data and fracture toughness data were tested for normality by plotting histograms and confirmed using the Anderson Darling Test of normality. All sets of data were subjected to the following tests and analysis.

Comparative tests

Data were tested for differences in variances using Levene's F test before any parametric comparative analysis was undertaken.

Paired t tests were used to compare the fracture toughness of full hoof wall blocks which had been split into two along a common anatomical line and marked A and B to test the hypothesis that there was no difference in fracture toughness between material sampled from common anatomical positions; thereby testing the repeatability of the method.

The one way analysis of variance test, (ANOVA) was used to investigate differences in fracture toughness between the toes and the quarters (28 fore feet only); testing the hypothesis that there was no difference in the fracture toughness of the quarters compared to the toe. Multiple comparisons were carried out using the Tukey test at a significance level of $p < 0.05$ to reduce the likelihood of type1^{f} errors.

The ANOVA test was used to test the differences in the total number of crack numbers and areas at the toe and the quarters and the Kruskal Wallis test with the Wilcoxon Rank Sum test was used for any multiple comparisons at a $p < 0.05$, using a Bonferroni correction to investigate the differences in their calculated severity scores to test the hypothesis that the incidence of cracks is not dependant upon their location.

The two way analysis of variance test (2ANOVA) was used to investigate differences in the number and area and severity of the individual crack types, (types 1-6) between and within the quarters and toe; to test the hypothesise that there is no difference in the incidence of individual crack types between anatomical positions. It was hypothesised that weighting the scores would increase the differences between the more severe types of cracks compared to the less severe

types leading to a truer reflection of the clinical significance of the cracks. It was hypothesised that this difference would be more apparent for geometric area scores compared to the other types of scores and that the differences would be greater for the higher scores. A general linear model was used with hooves blocked for anatomical position; post hoc multiple comparisons were carried out using Tukey at a significance of $p<0.05$.

Finally the 2ANOVA test was used to investigate the differences between weighted number and area scores; to test the hypothesis that there is no difference in the types of scores. Therefore weighted number scores might be used instead of weighted area scores to give the same information about the clinical significance of cracks as found in cattle claws.

In addition, hooves were grouped using the inter-quartile ranges, appendix, *table 3.2.7A.i*, to divide them according to whether they had a high, medium or low crack score. This reduced the degrees of freedom and increased the clinical relevance of the system, similar to the grading concept used for lameness scores. The ANOVA test was used to investigate differences in toe mdc fracture toughness between the three different crack score groups in the group of 48 feet; the 2ANOVA test was used to investigate differences in fracture toughness between the scores groups and within anatomical positions in the group of 28 feet; to test the hypothesis that there is no difference in fracture toughness between hooves from high, medium and low crack score groups.

Correlations

Multiple scatter plots were used to investigate relationships between the different types of crack scores and between crack scores and fracture toughness. Data which showed linear association were further investigated using Pearson's Correlation, (fracture toughness, absolute number and area scores) or Spearman's Rank Correlations, (weighted crack scores) in order to establish if :- i) number scores were inversely related to area scores, ii) the absolute number or area score were inversely related to geometric or severity scores, iii) severity scores were related to geometric scores or if iv) fracture toughness of the material was related to a crack score from the same anatomical positions. The limitations of multiple *a posteriori* tests, were accepted and correlations of marginal significance($r<0.45$) were ignored.

Due to the fact that when hooves were filtered according to their GSNo score to see how well GSA was predicted, the predictions were strongest in the high and low values; hooves were grouped into high, medium and low crack scores. *Post hoc* Spearman's rank correlations were carried out, within the groups to investigate whether the same correlations existed between number and crack score methods.

3.3 Results

Summary data of the mean number, area, severity and geometric scores are presented in the appendix, *tables 3.3.A.i-vii*

Overview

1. There was no difference in the mean number, area or median severity of cracks between the toe and the quarters on the whole hoof
2. All hooves had significantly more type 3 and 4 cracks compared to less severe (types1, 2) and severe (types5, 6) crack types
3. Geometric area scores, (GSA) were not significantly different to geometric number scores (GSNo), the scoring systems were strongly correlated and GSNo ranked hooves in the same order as GSA. The use of either GSA, GSNo or SSA gave the same information about the fracture toughness of the toe
4. The fracture toughness of the quarters, (41.9 ± 11.5 kJ/m^2) was significantly higher than at the toes, (28.6± 11.9 kJ/m^2)
5. Low crack groups had lower fracture toughness, (FT) at the toe but higher at the quarters compared to high crack score groups
6. Within each crack group , the FT at the quarters (40kJ/m^2) was greater than the toe, (29kJ/m^2), except the high crack group
7. There were no correlations between fracture toughness and crack scores
8. Grouping hooves into low, medium or high crack score groups increased the correlations between the different scoring methods and between crack scores and fracture toughness

3.3.1 Distribution of cracks and differences in number, areas and types between anatomical positions

Differences in crack differentiation between anatomical regions

Cracks were evenly distributed between all the anatomical positions in both sets of hooves. The differences in the mean number of cracks, the total mean area of cracks and their severity between the quarters and the toe were not significant ($p>0.05$), *figures 3.3.1.i - .iv graphs A*.

Differences in crack distribution within anatomical positions

There were significantly more type 3 cracks compared to the other crack types. All differences are at a significance of $p<0.05$ unless stated in the text.

- **Mixed Feet**

Differences in numbers of crack types

The medial quarters had significantly more type 3 cracks compared to type 1 and the toe had significantly more type 2 cracks compared to type 5 cracks. The distribution is summarised graphically in *figure 3.3.1.i graph B*. A comparison of the mean number of crack types on the whole hoof indicated that there were significantly more type 3 cracks compared to types 1,($p<0.00001$) 4, 5 and 6 ,($p<0.00001$) cracks and significantly more type 2 cracks compared to type 5,($p<0.0001$) and type 1,($p<0.00001$) cracks.

Differences in areas of crack types

The area of type 6 cracks was significantly greater than the areas of all the other crack types within the medial quarter and within the toe. Within the medial quarter, the difference between the most severe crack and the other cracks was highly significant; between 6 and 1, ($p<0.00001$), between 6 and 2, ($p<0.001$) and between 6 and 3 ($p<0.01$); *figure 3.1.1.iii graph B*. A comparison of the areas of the different crack types on the whole hoof showed that the area of type 6 was significantly greater than the areas of types 1 and 3.

- **28 left fores**

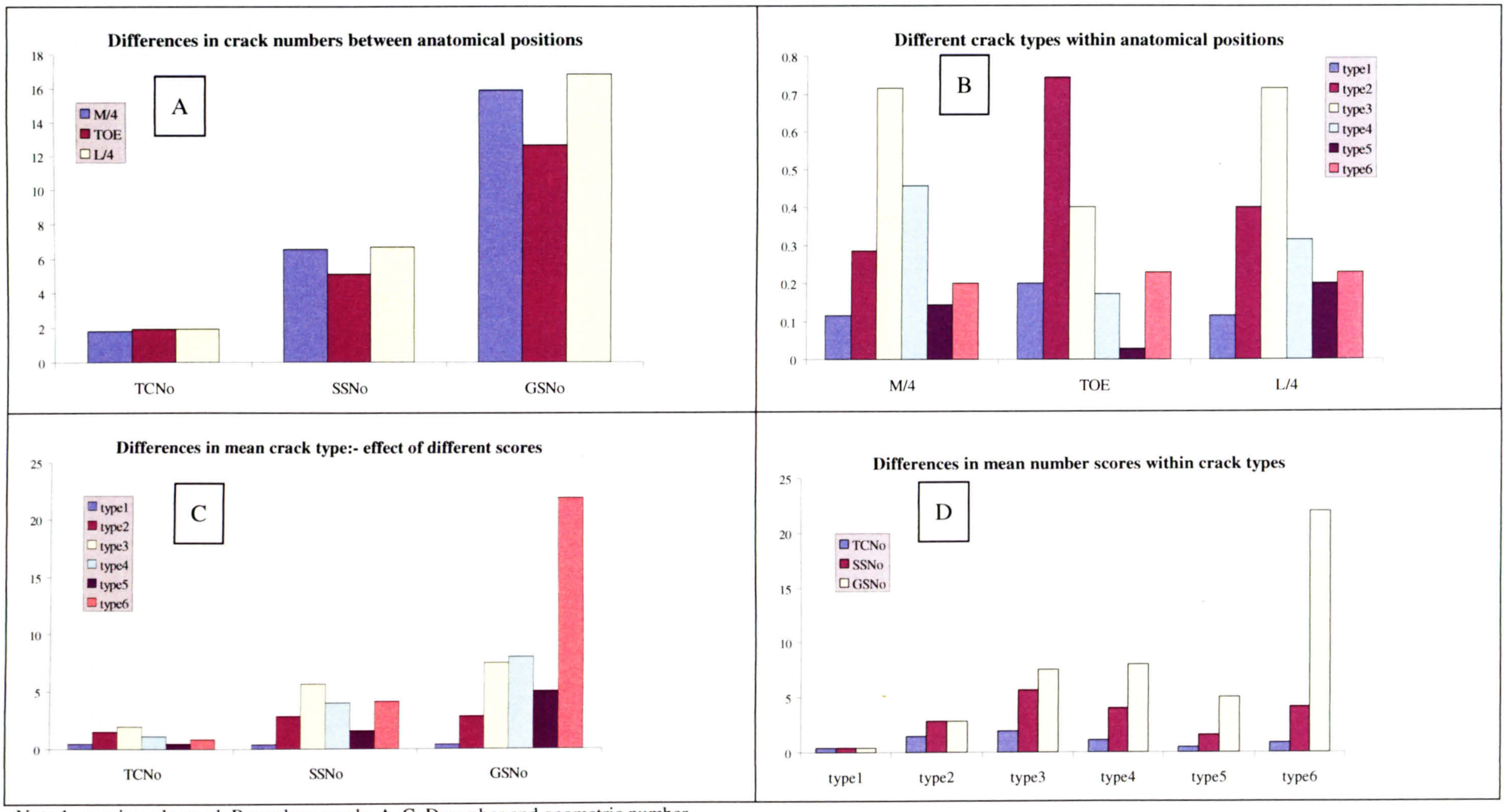
Differences in numbers of crack types

The lateral quarter had significantly more type 3, ($p<0.01$) and 4 cracks compared to type 1 cracks and significantly more type 3 cracks compared to type 5, ($p<0.01$) and 6, ($p<0.01$) cracks. The medial quarter had significantly more type 3 cracks compared to type 1, 5 and 6 cracks and significantly more type 4 cracks compared to type 1 cracks. The toe had significantly more type 4 cracks, ($p<0.00001$) compared to type 1, 5 and 6 cracks, *figure 3.3.1.ii graph A*. A comparison of the number of crack types on the whole hoof showed that there were significantly less type 1 cracks compared to types 3, 4, ($p<0.0001$) and 5. There were significantly less type 2 cracks compared to type 3 and 4 and significantly more type 3 and 4 cracks compared to types 5 and 6, ($p<0.00001$).

Differences in areas of crack types

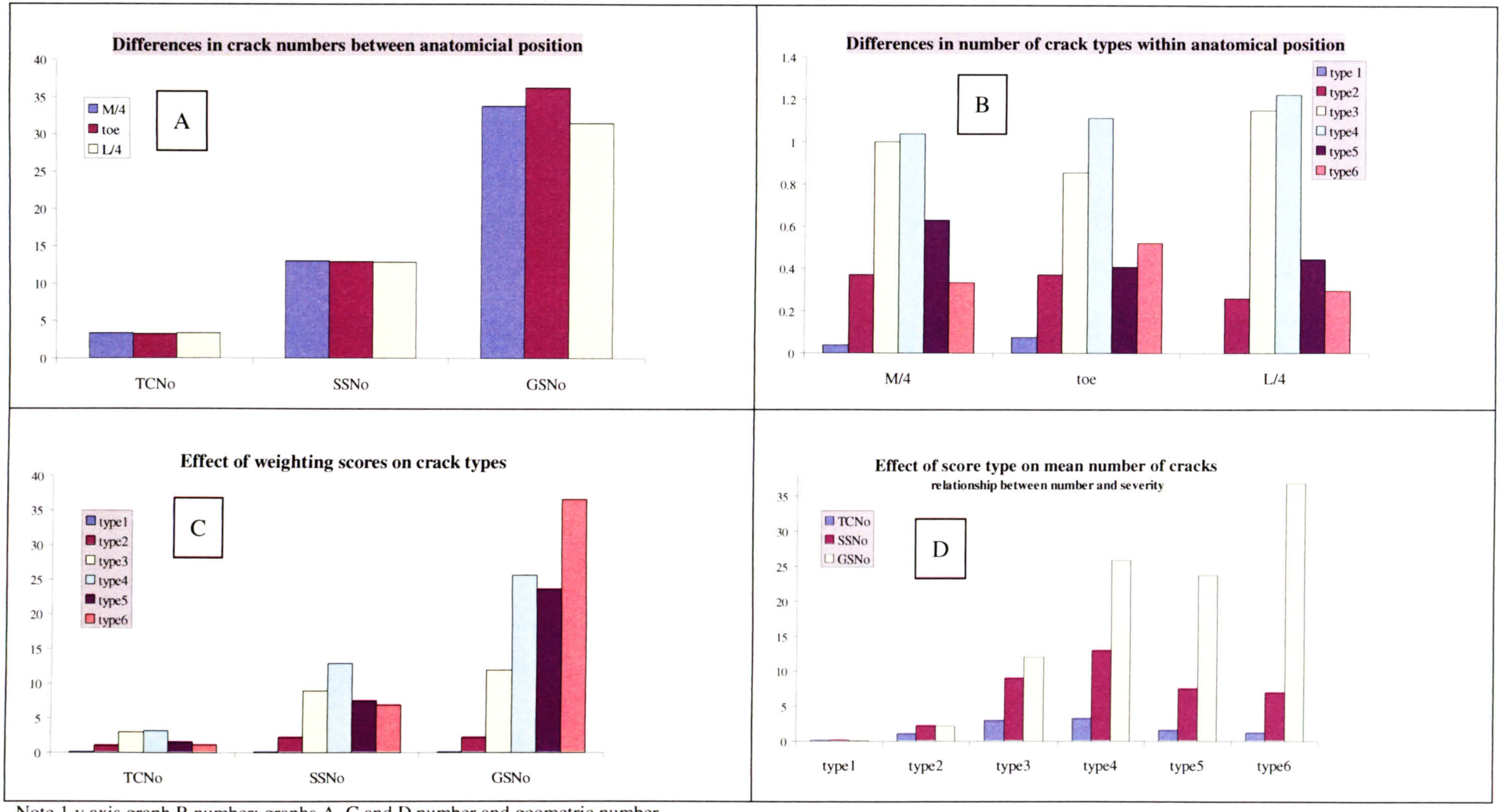
In the group of 28 left fores, type 4 cracks covered a significantly higher area than the area of crack types 1 and 2 at the medial quarter and in the toe region the area of type 4 cracks was also significantly greater than the area of the type 1 cracks, *plate 3.3.1.iv graph B*. A comparison of

Figure 3.3.1.i Distribution of crack numbers by type, severity and anatomical position (mixed feet)



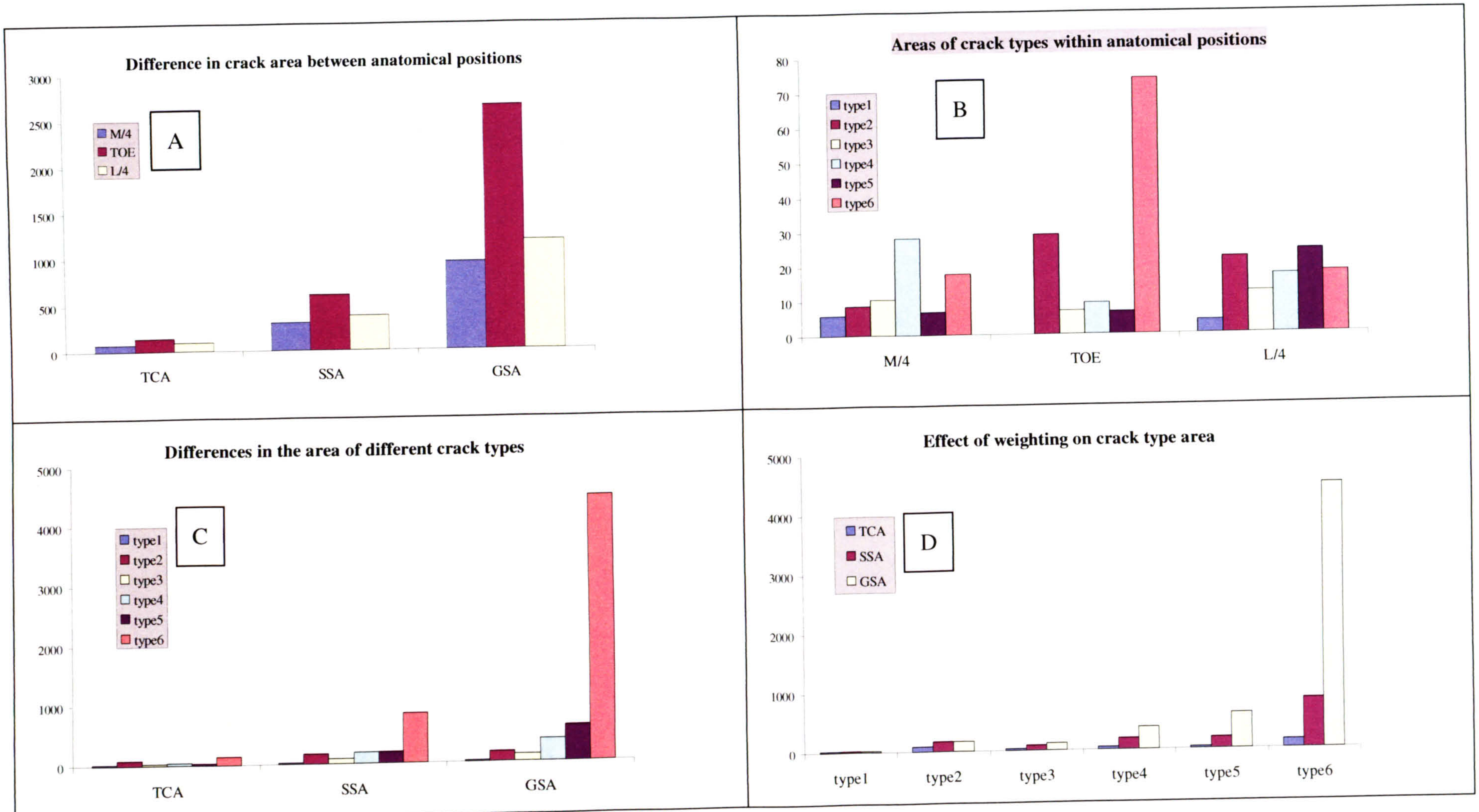
Note 1 y axis scale graph B number; graphs A, C, D number and geometric number
Note 2 L/4 lateral quarter,M/4 medial quarter, TCNo total crack number, SSNo severity crack number, GSNo geometric crack number

Figure 3.3.1.ii Distribution of crack numbers by type, severity and anatomical position (28 left fores)



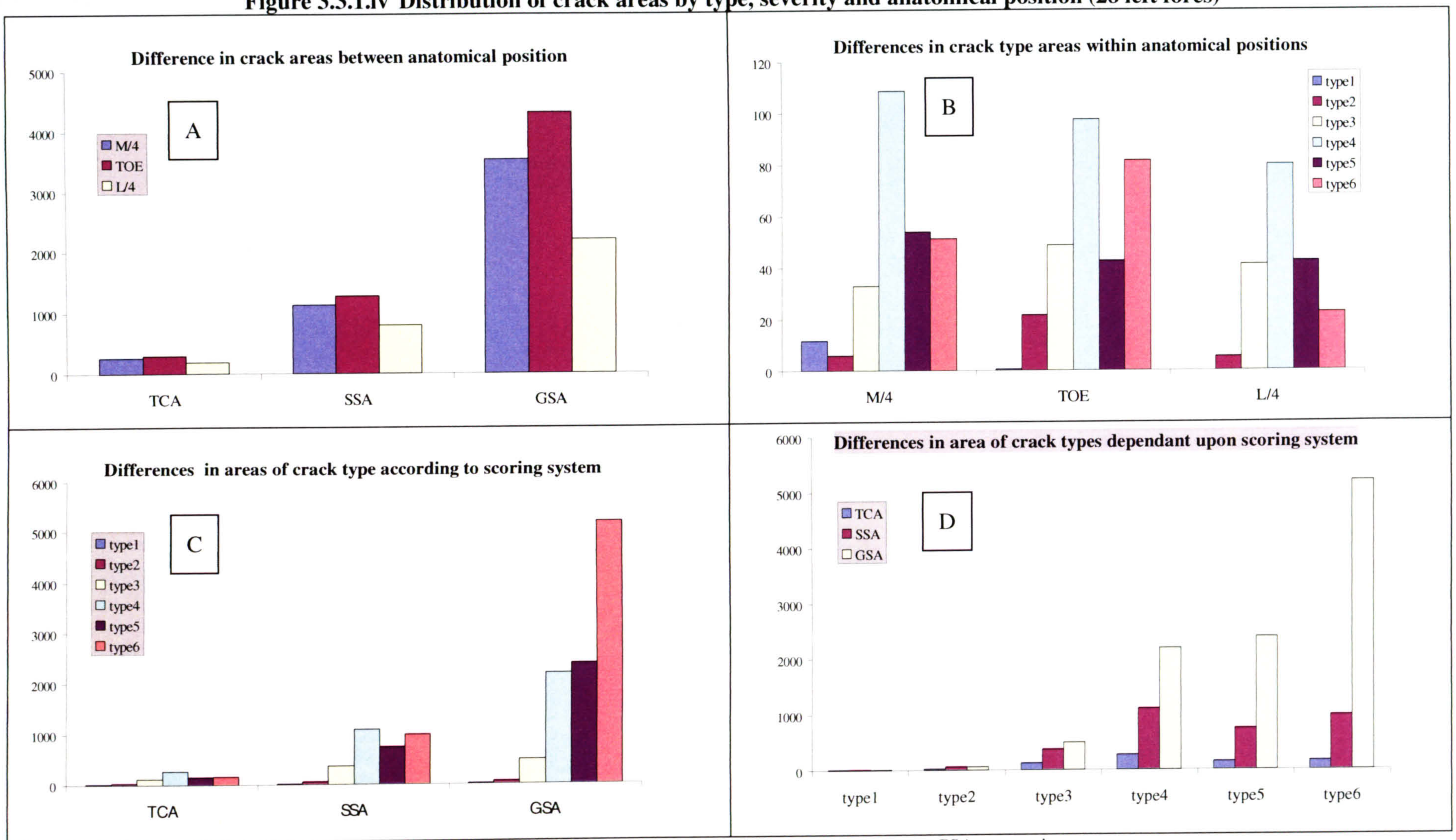
Note 1 y axis graph B number; graphs A, C and D number and geometric number
Note 2 L/4 lateral quarter, M/4 medial quarter, TCNo total crack number, SSNo severity crack number, GSNo geometric crack number

Figure 3.3.1.iii Distribution of crack areas by type, severity and anatomical position (mixed feet)



Note 1 y axis graph B mm^2 graphs A, C ,D geometric area score
Note 2 TCA total crack area, SSA severity score area, GSA geometric score area

Figure 3.3.1.iv Distribution of crack areas by type, severity and anatomical position (28 left fores)



Note 1 y axis graph B mm^2 graph A, C, D geometric area score; Note 2 TCA total crack area, SSA severity score area, GSA geometric score area

the areas of the different crack types on the whole hoof indicated that the area of type 6 was significantly greater than the area of types 1 and 4. The area of crack type 4 was significantly greater than the area of crack types 1, ($p<0.00001$) 2, 3, ($p<0.001$) and 5.

3.3.2 The effect of geometric weighting scores on crack types

In both sets of feet, the effect of using the weighted geometric scores was to increase significantly the difference between the most severe cracks and all other crack types. Thus crack type 6 was significantly greater compared to the other scores, whether the score being compared was an area score or a number score.

- **48 feet**

Differences in weighted number scores of crack type

GSNo6 was significantly greater than GSNo1, GSNo2, GSNo3, GSNo4 ($p<0.00001$) and GSNo5 ($p<0.0001$). However the differences between TCNo6 and the total number of other crack types and the differences between SSNo6 and SSNo1-SSNo5 were not significant, *figure 3.3.1.i graph C.*

- **28 feet**

Differences in weighted number scores of crack type

The differences between TCNo6 and the total number of other crack types and the difference between SSNo6 and SSNo1-SSNo5 were not significant, *figure 3.3.1.ii graph C.* Using the geometric weighting scores increased the differences between the more severe and the less severe crack types. GSNo6 was significantly greater than GSNo1, ($p<0.00001$), GSNo2 and GSNo3; GSNo5 was significantly greater than GSNo3, GSNo2 and GSNo1, ($p<0.00001$); GSNo4 was significantly greater than GSNo2 and GSNo1, ($p<0.001$) andGSNo3 was significantly different to GSNo2 and GSNo, ($p<0.001$).

- **Both data sets**

Differences in crack type weighted area scores

The effect of weighting area scores on crack types had no affect in either group of hooves except for the most severe cracks. There was no difference in the area crack scores when TC*, SS* or GS* were used except for crack type 6. The median geometric score area of type 6

cracks was significantly different and larger than the GSA of types 1, 2, 3, 4 and 5 ($p<0.00001$), *figures 3.3.1.iii and 3.3.1.iv graphs C.*

Comparison of number and area scores

a. to measure different crack types

There were no significant differences between number, area and geometric and severity scores for crack types 1 to 3 in both sets of hooves. However there were significant differences in both sets of hooves between GSA4 and TCA4; GSNo4, SSNo4, TCNo4, ($p<0.001$) and between SSA4 and all other type 4 scores. GSA5 was significantly different from TCA5, ($p<0.001$) and all the other scores for type 5 cracks, ($p<0.0001$). GSA6 was significantly different from SSA6, ($p<0.001$) and all other type 6 scores, ($p<0.0001$), *figure 3.3.1.v, graph A in the group of mixed feet and graph B 28 left fores.*

b. to give a whole hoof mean crack score

Weighting the scores increased the difference between the crack score methods; the differences varied according to the data set, *figures 3.3.1.vi* mixed feet *and 3.3.1.vii* 28 left fores. In both sets of data GSA was significantly different to all other scores. Graph A in both plates shows the number and area of the different crack types, graph B and C the affect of using a severity and geometric score respectively and Graph D the affect of geometric and severity score on the whole hoof score.

3.3.3 Fracture toughness

Fracture toughness ranged between 9.9 and 48.2 kJ/m^2 at the mdc in the set of 48 feet, *table 3.3.3.i.*

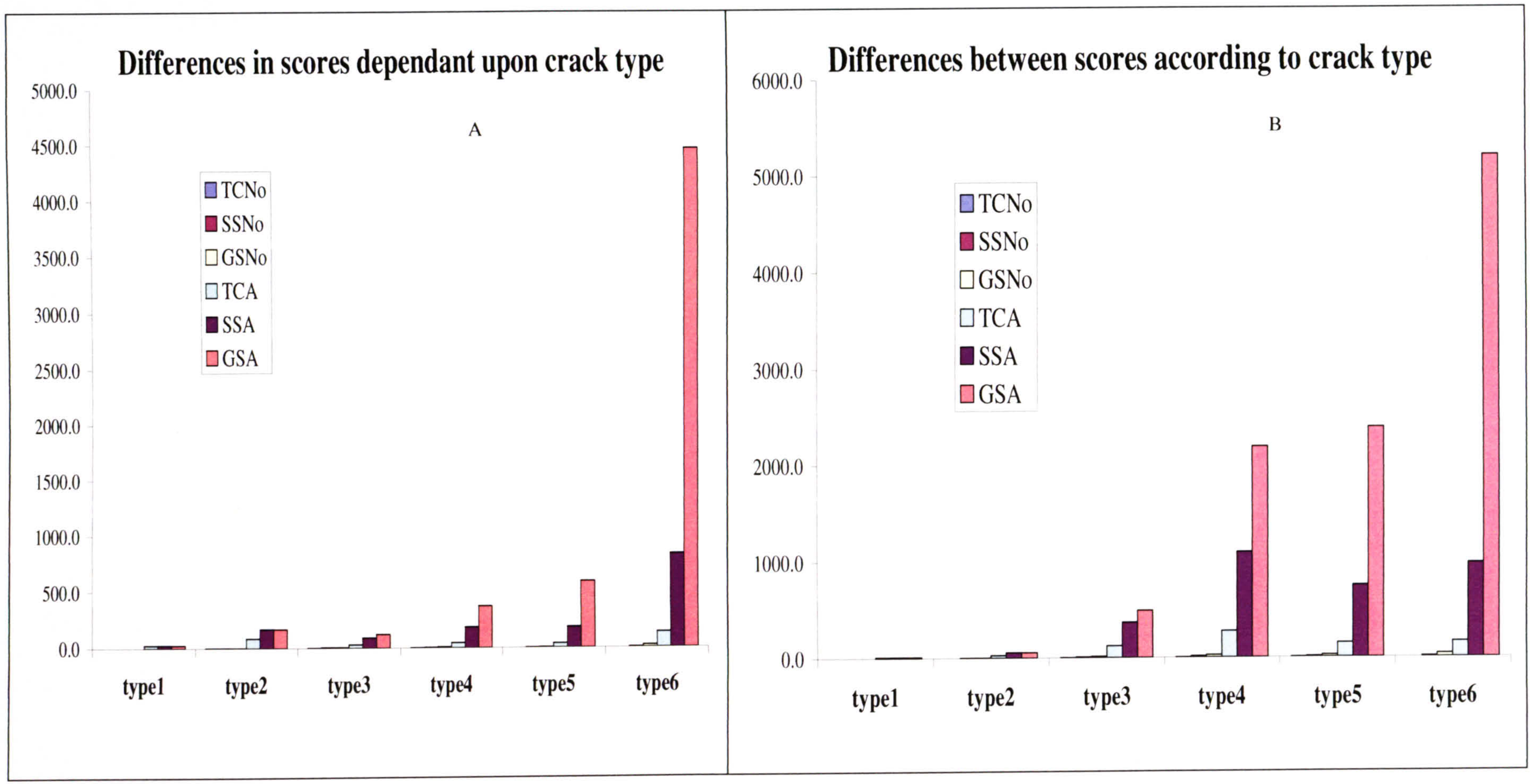
Table 3.3.3.i Fracture toughness of 48 mdc blocks from 48 mixed hoof capsules

mdc	mean ± SD *min-max*
impact resistance	280.4 ± 100.5 *81.4 – 569.5*
impact strength	28.8 ± 8.0 *9.9 –48.2*

Note 1 IR measured in J/m
Note 2 IS measured in kJ/m^2

In the group of 28 left fore, the mean impact resistance and strength was significantly greater at the quarters compared to the toe, ($p<0.05$) *table 3.3.3.ii*. The fracture toughness at the toes was similar in both groups of hooves.

Figure 3.3.1.v The effect of crack type on the use of different scoring systems



Note 1 left hand graph, data set mixed feet; right hand graph data set 28lfs
Note 2 y axis geometric crack score
Note 3 TCNo total crack number, SSNo severity crack number, GSNo geometric crack number; TCA total crack area, SSA geometric crack area, GSA geometric crack area

Figure 3.3.1.vi The effect of different scoring systems and their effect on the relationship between severity, area and number of cracks:-the clinical relevance of using weighted scores, (mixed feet)

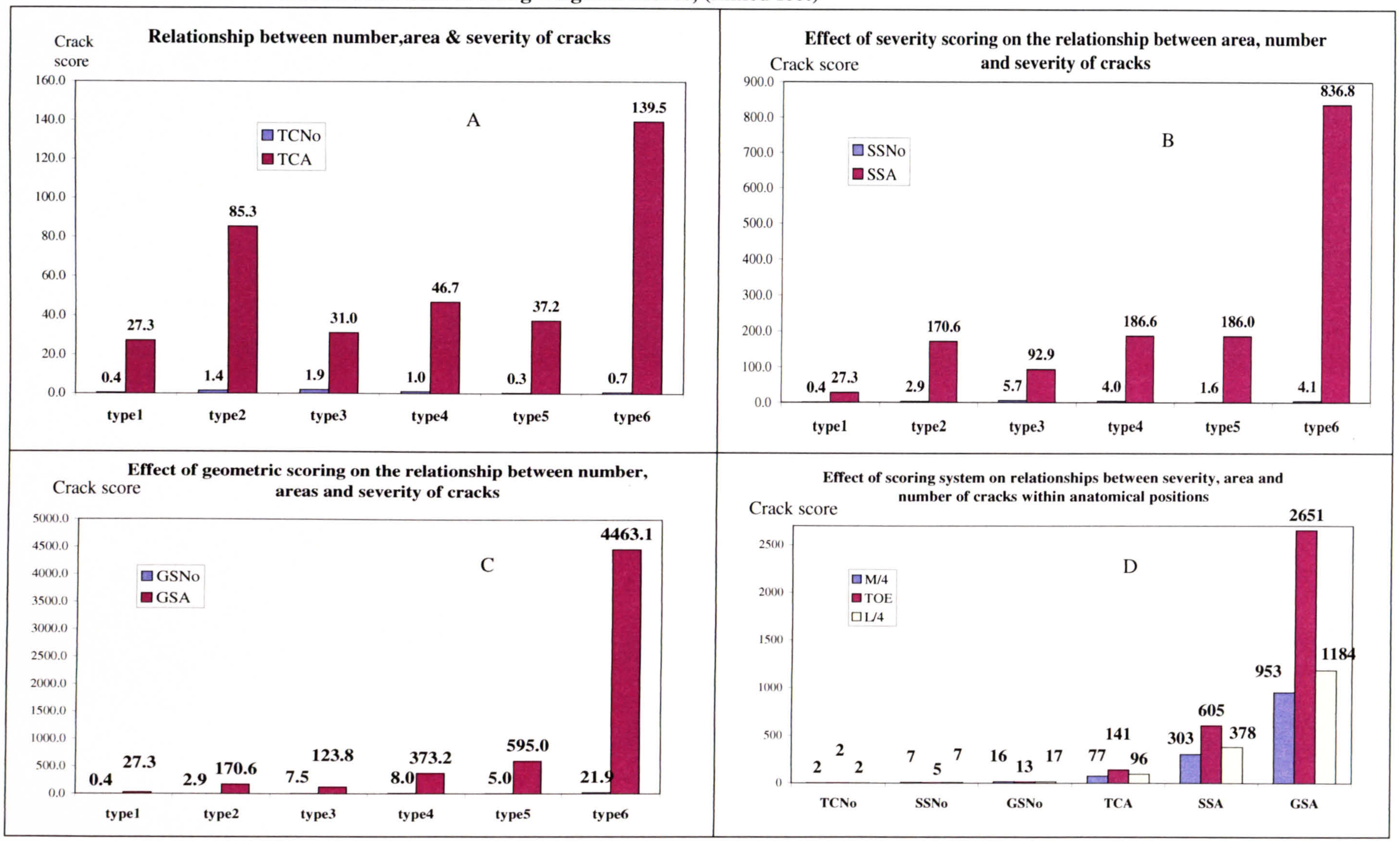


Figure 3.3.1.vii The effect of different scoring systems and their effect on the relationship between severity, area and number of cracks:-the clinical relevance of using weighted scores, (28lfs)

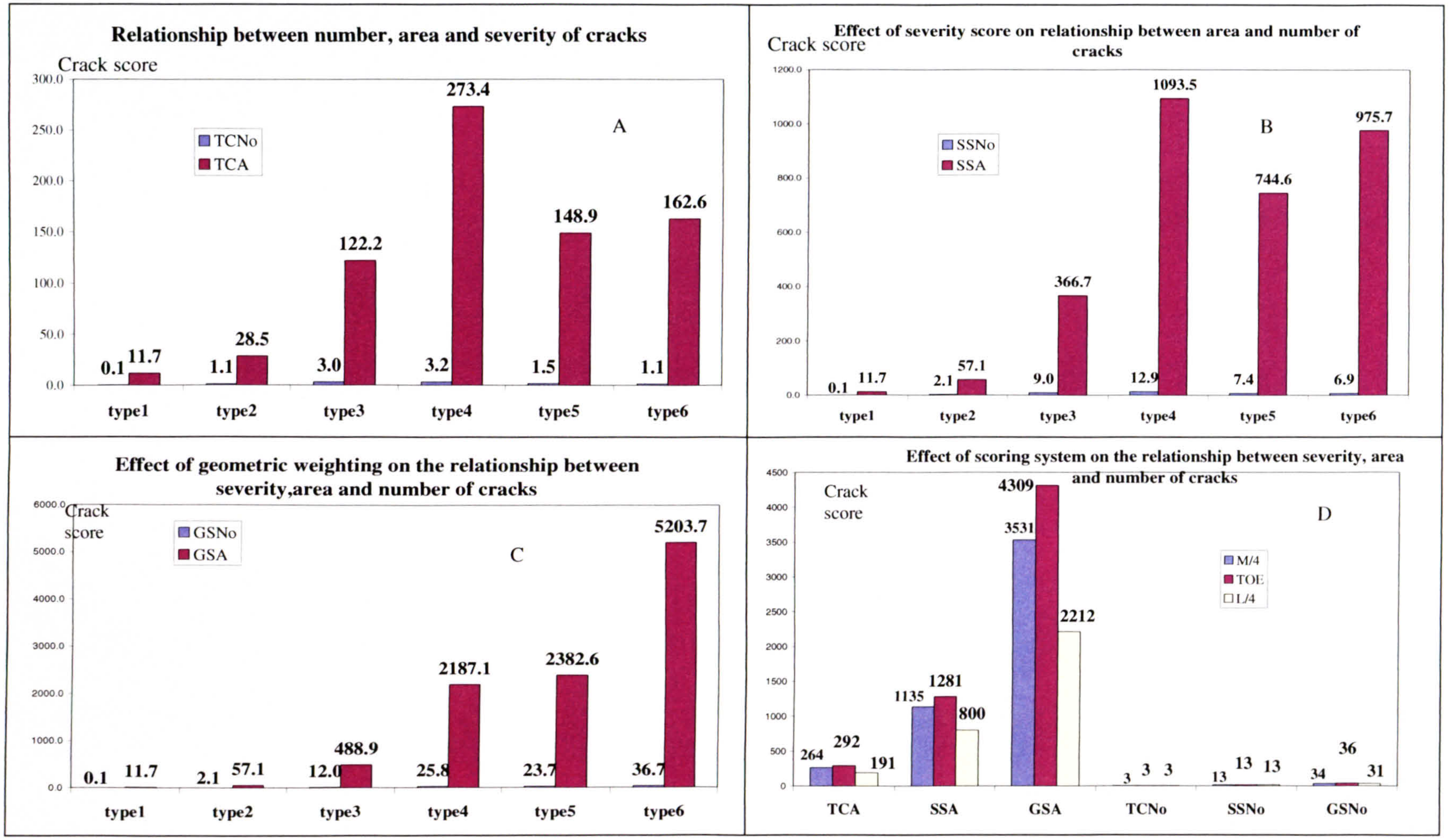


Table 3.3.3.ii Fracture toughness of 28 toe, lateral and medial quarter blocks from 28 left fore hoof capsules.

	Whole hoof	M/4	toe	L/4
	mean ± SD			
impact resistance	327.1 ± 97.8	[a b]344.2 ± 111.2	[a]285.5 ± 69.2	[b]351.6 ± 97.9
impact strength	37.6 ± 11.9	[b]41.9 ± 11.5	[a]28.6 ± 11.9	[b]42.3 ± 11.7

Note 1 different superscripts indicate significant differences in fracture toughness between different anatomical areas at a significance $p<0.001$ not including the whole hoof
Note 2 impact resistance is measured in J/m
Note 3 impact strength is measured in kJ/m^2

3.3.4 Relationship between crack score and fracture toughness

The effect of using different methods of crack scoring on fracture toughness results

There were no significant differences in the mean fracture toughness at the toe of both sets of hooves within high, medium or low crack score groups regardless of whether geometric or severity scores were used to group the hooves, *table 3.3.4.i*.

Table 3.3.4.i Comparisons of toe fracture toughness within high, medium and low crack sore groups using either geometric or severity area or geometric number to group the hooves

Crack score group	GSA^{48}	SSA^{48}	$GSNo^{48}$	GSA^{28}	SSA^{28}	$GSNo^{28}$
	mean impact resistance scores		J/m of hooves with either high, low or medium crack			
high	[a]352.0	[a]342 .0	[a]307.7	[b]301.3	[b]321.0	[b]269 .0
medium	[a]279.6	[a]277.1	[a]276.3	[b]287.0	[b]273.5	[b]289.1
low	[a]190.9	[a]191.9	[a]197.0	[b]276.6	[b]288.8	[b]308.5

Note 1 superscript a or b The differences between fracture toughness are not significant regardless of which crack scoring system is used within high, medium or low crack score groups, at a significance of $p> 0.05$
Note 2 superscript 48 or 28 shows which data set is being compared

Comparisons between hooves with low, medium or high crack scores

- **Differences in fracture toughness at the toe of hooves with low, medium or high crack scores**

The fracture toughness of the toes of the hooves in the low crack score was less than the fracture toughness of hooves with either a high or medium crack score in both sets of hooves; the difference was significant ($p<0.05$) in the 48 hooves (*table 3.3.4.ii*) but the difference was not significant in the 28 left fores (*table 3.3.4.iii*).

- **Differences in fracture toughness at the quarters of hooves with low, medium or high crack score**

In the group of 28 left fores, the quarters of the low crack groups had higher mean fracture toughness compared to the quarters of the hooves from the higher crack score groups, although the differences were not significant, (*table 3.3.4.iii*).

Table 3.3.4.ii Comparisons of mean fracture toughness of the toe of 48 hooves between hooves with low, medium or high crack scores.

medians	low	medium	high
GSA	*132.6*	*1279.0*	*19730.0*
impact resistance	$^{a}190.9 \pm 74.8$	$^{b}279.6 \pm 85.3$	$^{b}352.0 \pm 108.6$
impact strength	$^{a}23.4 \pm 8.3$	$^{b}28.5 \pm 7.7$	$^{b}35.2 \pm 7.0$
SSA	*104.4*	*707.1*	*4324.0*
impact resistance	$^{a}191.9 \pm 75.1$	$^{b}277.1 \pm 85.7$	$^{b}342.1 \pm 107.6$
impact strength	$^{a}23.9 \pm 8.4$	$^{b}28.3 \pm 8.0$	$^{b}33.2 \pm 7.0$
GSNo	*3.6*	*33.7*	*114.9*
impact resistance	$^{a}197.0 \pm 93.1$	$^{b}276.3 \pm 49.1$	$^{b}307.7 \pm 108.4$
impact strength	$^{a}21.9 \pm 7.7$	$^{b}30.9 \pm 8.4$	$^{b}32.5 \pm 5.5$

Note 1 subscripts indicate significant differences in the fracture toughness between hooves with low and high or medium crack score at a significance level of $p<0.05$ unless otherwise stated
Note 2 low SSA and high SSA; low GSA and high GSA $p<0.001$

Differences in fracture toughness at different anatomical positions within high, medium and low crack score groups

There were fracture toughness differences within the score groups between anatomical positions in the 28 left fore hooves, *table 3.3.4.iii*. The fracture toughness of the quarters within all the low and medium area crack score groups was higher than the toe. The impact resistance of the lateral quarter was significantly ($p<0.01$) greater than the toe in the medium SSA crack score group. The impact strength of both quarters were significantly greater ($p<0.0001$) than the impact strength of the toe in both the medium SSA ($p < 0.0001$) and medium GSA crack score groups. There were no significant differences between impact scores of the quarters and the toes in any of the high crack score groups. However when hooves were grouped into high, medium and low crack scores using GSNo, the only significant difference in impact scores was between the toe and the two quarters in the medium score group, ($p<0.0001$).

Table 3.3.4.iii Fracture toughness of the toe, lateral and medial quarters of 28 hooves divided into groups according to whether they have a high, medium or low crack scores

crack score gp	Low			Medium			High		
Score	M/4	toe	L/4	M/4	toe	L/4	M/4	toe	L/4
	score median								
SSA	*202.2*	*76.5*	*112.0*	*837.6*	*931.0*	*595.2*	*2620.0*	*3362.0*	*2001.0*
	J/m mean ± SD								
IR	412.6 ± 182.0	288.8 ± 16.6	361.6 ± 102.4	[ab]320.6 ±70.8	[a]273.5 ± 69.2	[b]367.0 ±106.8	314.6 ± 66.4	321.0 ± 97.7	312.0 ± 76.2
	kJ/m^2 mean ± SD								
IS	[a]47.0 ±13.9	[b]29.0 ±5.2	[ab]39.0 ±10.4	[b]42.2 ±12.0	[a]26.0 ± 6.0	[b] 46.1 ± 12.2	35.3 ±5.0	35.1 ±7.5	39.4 ±9.0
GSA	*270.7*	*116.6*	*197.0*	*1937.0*	*2343.0*	*1553.0*	*10776.0*	*13785.0*	*6103.0*
IR	427.1 ± 195.3	276.6 ± 35.7	397.1 ±113.4	318.6 ±66.0	287.0 ± 80.3	344.8.0 ± 99.5	319.6 ± 71.9	301.3 ± 80.9	323.2 ± 76.4
IS	[a]47.0 ± 15.2	[b]28.5 ± 4.9	[ab]42.8 ±12.3	[a]42.0 ±11.3	[b]27.2 ± 7.8	[a]43.5 ± 12.2	35.3 ± 5.4	32.7 ± 6.4	42.3 ±10.0
GSNo	*7.8*	*3.0*	*7.0*	*25.2*	*21.7*	*27.4*	*81.3*	*99.3*	*66.0*
IR	427.1 ± 195.3	308.5 ± 32.0	422.6 ± 107.9	323.6 ± 63.0	289.1 ± 69.0	346.2 ± 94.3	306.9 ± 77.6	269.0 ± 92.8	302.0 ± 73.6
IS	49.2 ± 14.4	26.6 ± 7.9	48.3 ± 18.9	[a] 41.2 ±11.0	[b] 28.8 ±6.2	[a] 41.4 ±7.9	35.17 ± 5.37	29.8 ± 9.5	41.8 ± 9.6

Note 1 different superscripts indicate differences at a significance $p<0.05$ between fracture toughness measured at different anatomical positions

3.3.5 Correlations between the different methods of quantifying cracks

Correlations between fracture toughness and crack scores

There were no correlations between crack scores and fracture toughness in either set of hooves.

Correlations between different methods of crack scoring

The results from correlations between crack scores of mixed feet are summarised in *table 3.3.5.ii* and in 28 fore feet in table *3.3.5.iii*. Linear regression was investigated between correlations with an $r > 0.8$, to see if number scores could be used to predict area scores, *table 3.3.5.i. and table 3.3.5.iv.*

Table 3.3.5.i Predictive equations using easier to collect scores to predict weighted scores in 48 feet

Linear or polynomial regression between crack score methods	p and R^2 values
GSA = - 1966.73 + 5.16570SSA	R^2 = 97.3% P = 0.0001
SSNo = 1.42 + 3.1TCNo	R^2 = 74.5% P = 0.0001
SSA = -556 +5.65 TCA mm^2	R^2 = 93.5% P = 0.0001
GSA = 3.27342 + 0.818144GSNo	R^2 = 66.9% P = 0.0001

Table 3.3.5.ii Correlations between different methods of crack scoring in 48 feet

Correlations between different crack scores				
	r Pearsons/Spearmans correlation P value			
a) numbers	**whole**	**M/4**	**toe**	**L/4**
TCNo SSNo	0.863 0.0001	0.869 0.0001	0.775 0.0001	0.905 0.0001
SSNo GSNo	0.748 0.0001	0.781 0.0001	0.715 0.0001	0.802 0.0001
TCNo GSNo	----	----	----	0.554 0.001
b) areas	**whole**	**R/4**	**toe**	**L/4**
TCA SSA	0.967 0.0001	0.959 0.0001	0.970 0.0001	0.939 0.0001
SSA GSA	0.986 0.0001	0.911 0.0001	0.994 0.0001	0.913 0.0001
TCA GSA	0.927 0.0001	0.773 0.0001	0.945 0.0001	0.751 0.0001
c) areas & numbers	**whole**	**R/4**	**toe**	**L/4**
GSA GSNo	0.509 0.002	0.629 0.0001	0.541 0.001	0.638 0.0001
SSA GSNo	0.525 0.001	0.605 0.0001	0.555 0.001	0.490 0.003
SSA SSNo	---	---	---	0.471 0.006
TCA GSNo	0.5 0.003	---	---	0.490 0.003

GSA and GSNo were correlated in both sets of hooves, (*tables 3.3.5.ii and 3.3.5.ii*), *figure 3.3.1.v.*

Table 3.3.5.iii Correlations between different methods of crack scoring in 28 left fore feet.

Correlations between different crack scores				
	r Pearsons/ Spearmans Correlation P value			
a) numbers	**whole**	**R/4**	**toe**	**L/4**
TCNo SSNo	0.835 0.0001	0.9 0.0001	0.916 0.0001	0.855 0.0001
SSNo GSNo	0.817 0.0001	0.863 0.0001	0.923 0.0001	0.778 0.0001
TCNo GSNo	---	0.601 0.001	0.739 0.0001	---

b) areas	whole	R/4	toe	L/4
SSA	0.951	0.908	0.951	0.94
GSA	0.0001	0.0001	0.0001	0.0001
TCA	0.967	0.67	0.764	0.61
SSA	0.0001	0.0001	0.0001	0.001
TCA	0.856	0.651	0.713	0.513
GSA	0.0001	0.0001	0.0001	0.006
c) areas & numbers	**whole**	**R/4**	**toe**	**L/4**
GSA	0.800	0.606	0.824	0.763
GSNo	0.0001	0.001	0.0001	0.0001
SSA	0.714	0.572	0.767	0.94
GSNo	0.0001	0.002	0.0001	0.0001
SSA	---	---	0.611	0.573
SSNo			0.001	0.002
TCA	0.598	0.465	0.654	0.568
GSNo	0.001	0.015	0.0001	0.002
GSA	---	---	0.623	0.537
SSNo			0.001	0.004

Table 3.3.5.iv Predictive equations using easier to collect scores to predict weighted scores in 28 left fore feet

Linear or polynomial regression between crack score methods	p and R^2 values
GSA = -4032 +4.42SSA	p= 0.002 r^2 = 90.1%
GSA = -3813 +141GSNo	p= 0.0001 r^2 = 60.8%
GSA = -6072 + 21.9TCA	p = 0.0001 r^2 = 73.2%
SSA = -741 + 5.34TCA	p = 0.0001 r^2 =93.6%
SSNo = 4.01 + 3.43TCNo	p = 0.0001 r^2 = 69.7%
SSNo = 19.3 + 0.191GSNo	p = 0.0001 r^2 = 66.8%

Hooves were ranked in descending order of their scores to establish if the hooves ranked in the same order regardless of the crack score used, *tables 3.3.4A.i- 3.3.4A.ii*. The hooves were more closely ranked at either end of the scale.

3.3.6 Correlations within groups of hooves ranked according to high, medium and low crack scores

Hooves were grouped as described in the statistical methods and in *table 3.2.7A..i*.

Correlations between methods of crack scoring

Ranking hooves according to their crack scores increased the correlations between the different methods of crack scores in the group of 28 feet and 48 feet. This is illustrated in the appendix, where hooves are filtered according to GSNo high, medium and low scores to see how well

GSA was predicted, *tables 3.3.6A.i and 3.3.6A.ii. Table3.3.6.i and 3.3.6.ii* summarise the Spearman's Rank correlation coefficients within the groups.

.

.Table 3.3.6.i Correlations between geometric numerical and area scores within mixed hooves ranked according to high, medium or low crack scores

GSNo score group	**high**	**medium**	**low**
	spearman's coefficient p value		
GSAT/GSNoT	--	----	0.888 0.003

Correlations between the other anatomical positions in the mixed feet were not calculated as fracture toughness results were only available for the toe.

Table 3.3.6.ii Correlations between geometric numerical and area scores within 28 left fore hooves ranked according to high, medium or low crack scores

GSNo score group	**high**	**medium**	**low**
	spearman's coefficient p value		
GSAM/4 GSNo M/4	0.03 p = 0.954	0.625 p= 0.013	-0.042 p= 0.938
GSAT GSNoT	**0.907** **p= 0.013**	0.497 p= 0.07	**0.937** **p= 0.002**
GSAL/4 GSNo L/4	**0.920** **p= 0.009**	**0.667** **p= 0.009**	**0.857** **p= 0.014**
Anatomical position	**Medial ¼**	**Toe**	**lateral ¼**
GSNo h,m,l predicting GSA, h,m,l	**0.822,** **p=0.0001**	**0.806** **p=0.0001**	**0.775** **0.0001**

Note 1 figures in bold indicate significant correlations

When the data set of 28 left fores were filtered according to whether they had a high, medium or low GSNo score, the equivalent GSA score was the same in over 80% of the hooves, as shown in the last row of *table 3.3.6.i and table 3.3.6A.ii.*

Correlations between crack scores and fracture toughness

Grouping hooves according to their crack scores resulted in some correlations between fracture toughness and crack score within the crack groups. There were correlations between crack area scores and fracture toughness in all crack score groups in the data set of 48 feet. However there were no correlations between fracture toughness and crack scores in the data set of 28 left fores.

Table 3.3.6.iii Correlations between fracture toughness and crack scores in 48 feet ranked according to high, medium or low crack scores

Correlations between	correlation p value regression equation R^2
low GSA GSAT IR	0.719; p = 0.045 IR = 161.71 + 1.21812GSAT; R^2 = 51.6%

Correlations between	correlation p value regression equation R^2
GSAT IS	0.647; p= 0.05 ; IS = 20.9490 + 0.122505GSAT; R^2 = 41.8%
medium GSA GSAT IR GSAT IS	 0.622 0.018 0.647 0.012
high GSA IR GSAT	 0.584; 0.05

Table 3.3.6.iv The differences between the number and area of cracks between hooves grouped according to whether they had a high, low or medium crack score as measured by GSNo or GSA

Hooves grouped by	**GSNo high** **> 63**	**GSNo medium** **≥ 9.5 ≤ 63**	**GSNo low** **< 9.5**
	mean number or area ± SD		
TCNo	[a]6.1 ± 2.3	[a]6.7 ± 3.1	[b]1.6 ± 1.0
TCA mm²	[a]636.8 ± 650.0	362.0 ± 326.5	[b]112.0 ± 131.2
	GSA high **> 5242**	**GSA medium** **≥ 376 ≤ 5242**	**GSA low** **< 376**
TCNo	5.4 ± 2.6	6.2 ± 3.3	3.4 ± 3.5
TCA mm²	[a]860.8 ± 617.3	[b]285.5 ± 165.3	[b]60.0 ± 71.5

Note 1 superscripts indicate differences at a significance of $p<0.05$
Note 2 Data set of mixed feet

3.4 Discussion

One of the main aims of this chapter was to develop an objective method of measuring the cracks of the equine hoof wall epidermis, which was repeatable and which took into account the severity of the cracks so that in future, correlations between the integrity of the hoof wall and function can be studied.

Six numerical representations were studied; number, area, severity number and area and geometric number and area, to investigate which crack score of the equine hoof wall best described the apparent functional integrity of the epidermal tissue and which score was related to the mechanical test of fracture toughness.

3.4.1 Methods of assessing cracks

Quantitative: Numerical

Counting cracks is easy and objective. A number count has been used to demonstrate a difference between laminitic and non laminitic cattle, (Maclean 1971), but Philipot *et al.* (1990) were unable to find a relationship between the number of lesions and laminitis in diary cows. Neither Bryant *et al.* (1985)a nor Bryant *et al.* (1985)b found any relationship between the number of lesions in gilts and their soundness score, nor between the number of lesions, histological appearance or soundness scores in sows. A simple number count in this thesis gave no indication as to the effect of cracks on the functional integrity of the hoof wall as illustrated in *figure 3.4.1.i.* When hooves were grouped (*figure 3.3.1.v*) according to their severity scores, which will be discussed later, it is very clear that a total numerical count cannot be used as an objective indication of the gross appearance of the hoof wall, particularly if a relationship to function needs to be investigated.

Greenough and Vermunt, (1991) argued that numerical counts of cracks was misleading as it gave a higher score to claws which had a number of 'cosmetic' cracks which are often more frequent compared to claws which may have only one lesion of greater clinical significance.

This hypothesis was partially disproved in this thesis as the cosmetic cracks were similar in number to the cracks which were assumed to have the most clinical significance. However there were more of the cracks which fell between both the extremes. The affect of these cracks on the functional integrity of the hoof wall can only be surmised as no correlations with lameness or lack of protection was carried out due to the morbidity of the specimens. Certainly a simple count of cracks provided little useful information in this thesis.

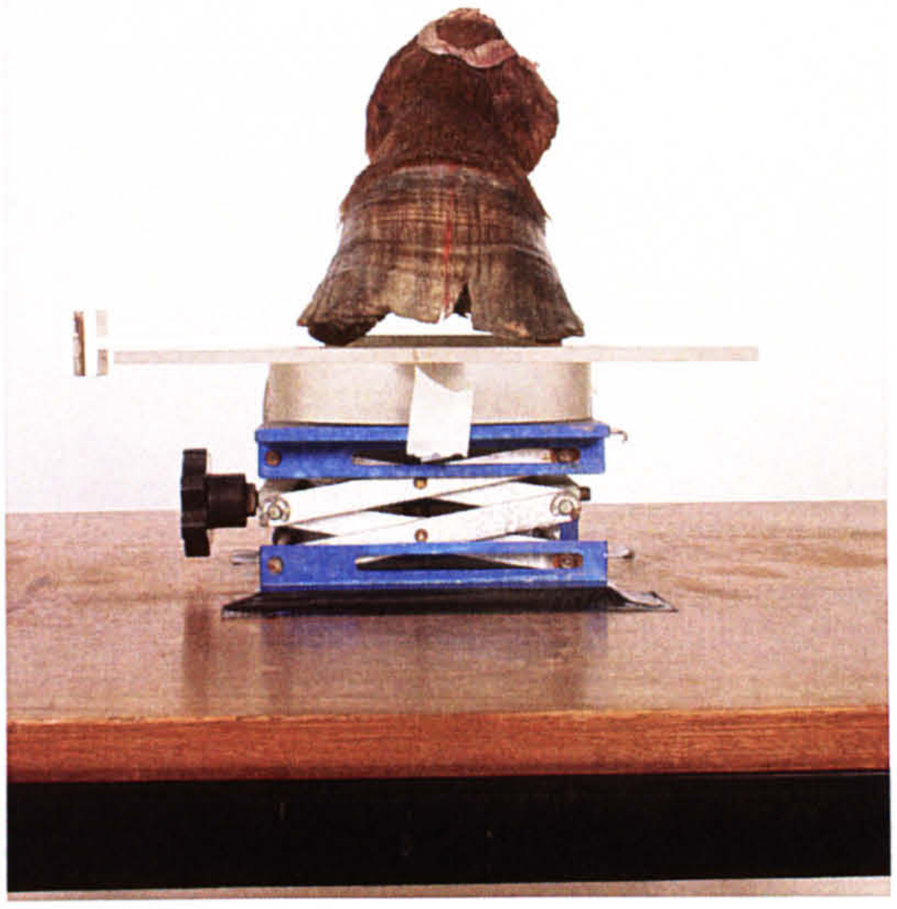


Figure 3.4.1.i Visual illustration of why a simple numerical count of cracks was inadequate to quantify the gross appearance of the hoof wall epidermis

Quantitative: Area

The area of the cracks was measured because it provided an objective measurement of the size and position of the cracks. Objective measurement of the size of the cracks was considered important as it would give a repeatable measurement of the extent of damage due to cracks and would also allow a chronological record in the changes in size of the cracks over time. Whilst measurement over time was not possible as the hooves used were cadavers; a subset of ten hooves was crack scored and measured on four occasions over twelve months without reference to the original measurements. These four data sets were compared using separate ANOVA for the areas and positions of the cracks; there were no significant differences in the scores between the measuring dates, ($p > 0.05$).

Bryant *et al.* (1985) also used area as a method of characterising lesions on pigs' claws but equated size with severity, so a small lesion was given a score of 1 and a large lesion a score of 5. There was no correlation between the final score and soundness score. Leach *et al.* (1998) argued that measuring area may be a way of providing a severity adjustment score and would

give sufficient emphasis to the severe lesions. However a severe lesion/crack which only covers a small surface area is likely to be of greater clinical significance compared to less severe cracks which in total may still cover a greater surface area. If area is to be meaningful, then it needs relating to some form of severity score. The depth of the lesion was the only measurement that Goonewardene and Hand, (1996) used when assessing lesions, but rather than noting the actual depth, a number was allocated with the deepest cracks having the largest number. This is actually a form of severity allocation but several less deep lesions would still result in the claw having a greater score than one with a single deep lesion.

Categorical: Arithmetic Severity

Counts and area measurements are objective but in order to relate them to functionality, a severity adjustment must be included. Researchers (Logue *et al.* 1994; Goonewardene and Hand, 1996; Leach *et al.* 1997) who recorded an association with function and lesion assessment had all used a method of defining severity by either allocating a number based on the description of severity, (Logue *et al.* 1994; Leach *et al.* 1997; 1998), or on the perception of depth in mm of the lesion, (Goonewardene and Hand 1996).

To an extent severity categorisation will always be subjective and dependant on the recorder. Due to the preconceptions that might have been associated with the common names, the more general descriptive terms for cracks were avoided in this thesis. A system was developed that described the crack in visual terms relative to the perceived effect that the crack would have on the integrity of the epidermis and by the depth of the crack. The cracks were numerically typed as described in the *table 3.2.2.i, (p79)* where 1 had no clinical significance and was cosmetic and 6 was likely to compromise all aspects of the epidermal integrity. Counting or measuring the area of each crack type and multiplying the total by the crack type provided the arithmetic severity score described in this thesis (SSNo or SSA). Some researchers would argue that this type of adjustment only provides ordered and categorical data, (Leach 1996). This is the correct definition as we have assigned a number to a variable and have created an appearance of a numerical scale. But this does not mean we should dismiss this type of data, ordinal scores are used successfully in measuring both body condition and lameness in horses. The success of an ordinal scale appears to lie in the description of the category being comprehensive, with not too many choices of scale. The use of two ordinal systems for body condition 1-10 and 1-5 has shown that the 1-5 is significantly more reproducible and repeatable than the 1-10, (Ellis and Hollands 2000; unpublished). It might be possible to make the severity score a more quantifiable variable by developing a well defined scale, using a much larger epidemiological study and a measure of function would need to be taken at the same time, otherwise the crack

type allocation could not be related to anything meaningful. Allocating types to the cracks does not confer a continuous quantitative relationship to them, crack type 4 is not necessarily four times as bad as crack type 1, it is just worse.

Arithmetical weighting may put too much emphasise on some less severe crack or lesions as shown in *figures 3.3.1.vi* and *3.3.1.vii, graphs B*. Certainly this bias was a concern expressed by Greenough and Vermunt, (1991) and Leach, (1996) but the bias is unlikely to occur if area, number and severity are correlated with each other.

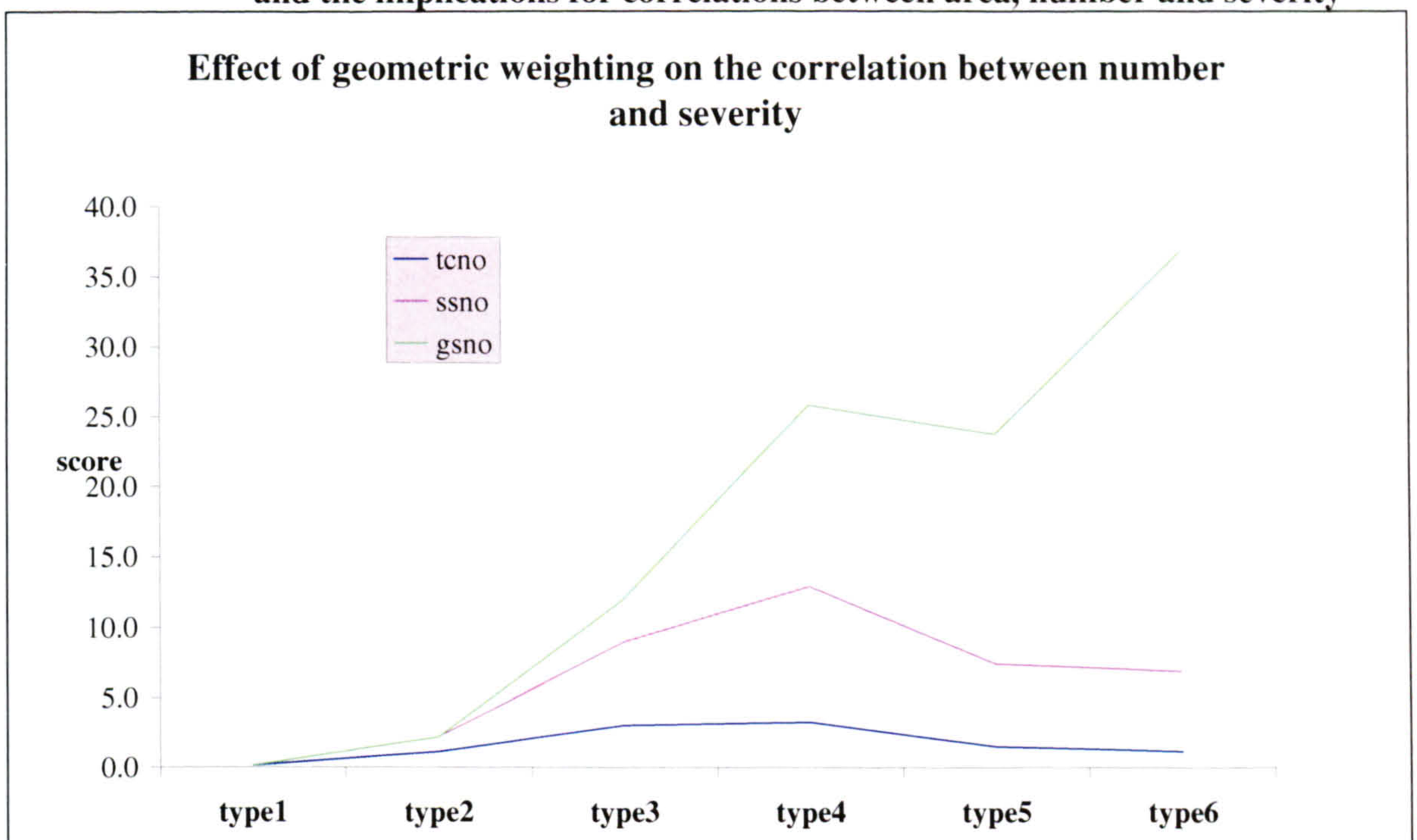
Categorical: geometric severity

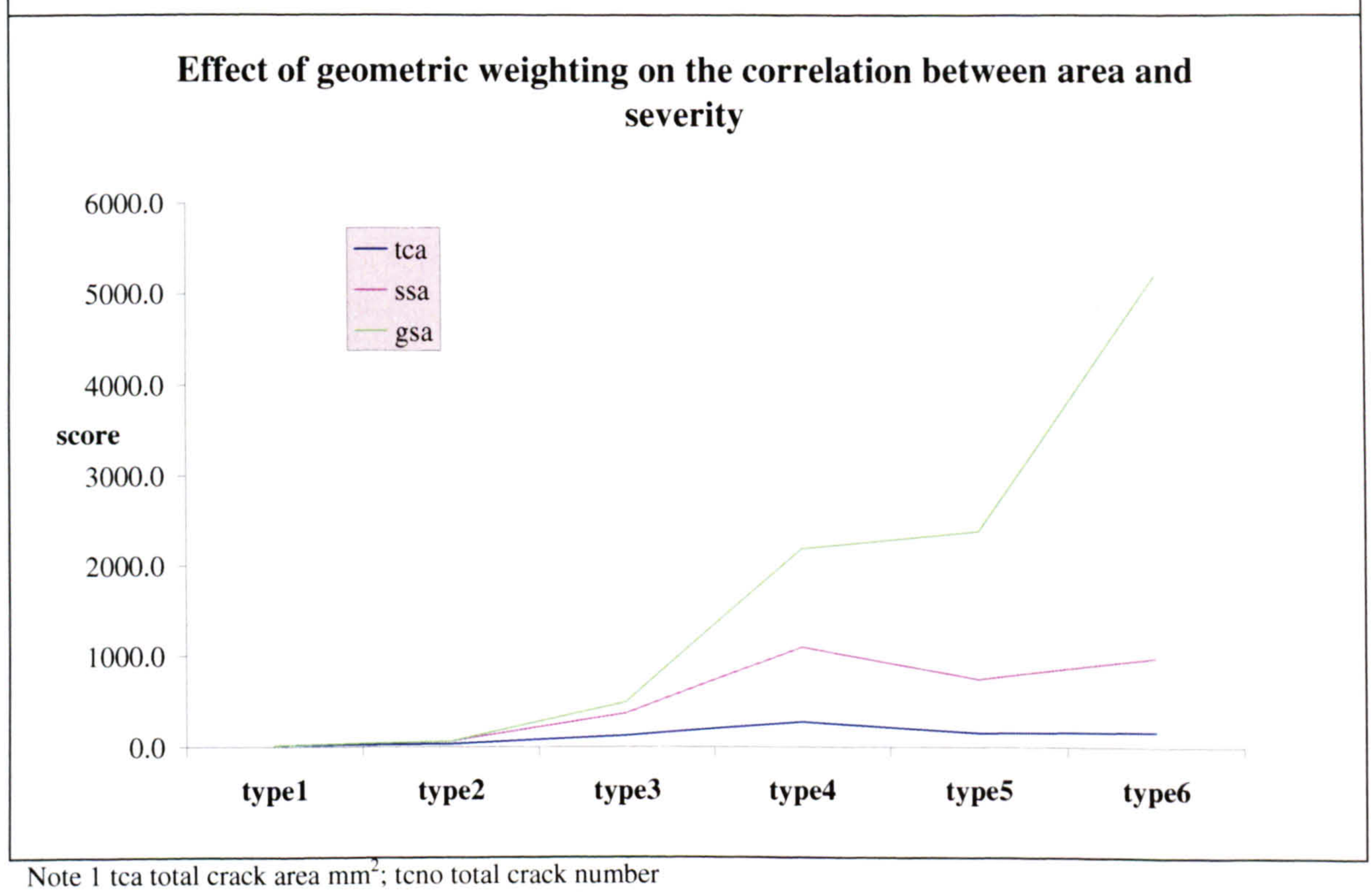
In order to increase the emphasise on factors which have a greater influence on outcome whether in terms of cracks or other risk factors, weighted scores are commonly used, for example in patient survival, school grades, suitability of job applicants. Geometric weighting increased the relationship between the x and y axis exponentially as shown in *figure 3.4.1.ii*.

The geometric weighting score (GSNo and GSA) used in this thesis followed the same weighting as Greenough and Vermunt, (1991); Leach, (1996); Leach *et al* (1997; 1998), *tables 3.1.4.i and 3.2.4.ii*. The development of a weighted score is used to describe a risk, in this instance the effect of a severe crack. However as with any risk assessment there will be a measure of uncertainty, the measured attribute might affect function or it might lead to a complete loss of function. The score should try and take into account the likelihood of both happening and therefore the clinical relevance. The weighting needs careful consideration, it should be quantifiable, an assessment should be taken and then a conclusion reached. In this thesis, not all these criteria were met. The weighting of the score should be based on criteria which have been defined; until the system proposed in this thesis is used and the scores related to clinical significance, the weighting cannot be adjusted to reflect the significance of the crack to functional integrity. But this has in part been addressed by the initial type allocation.

Ideally geometric adjustment should be made according to outcomes measured and a regression model is used. The only outcome measured in this thesis was fracture toughness and the correlations were not robust enough to develop regression equations. This system should not stay static, it should be developed, a performance index such as lameness should be tested and the observed outcome of the crack scores be tested against expected outcomes, the weighting may then need adjusting. It is possible that crack type 6 is of great functional significance regardless of its area or number and therefore any other crack score is irrelevant, thus the weighting to this type should be increased or the other score disregarded. When weighting candidates for job applications, the top scores are often the only ones considered. Alternatively when the system is tested, it may be that the combination of types 4 and 5 are more clinically

Figure 3.4.1.ii The exponential effect of weighting scores on area and number of cracks and the implications for correlations between area, number and severity





Note 1 tca total crack area mm^2; tcno total crack number
Note 2 ssa severity area score; ssno severity number score
Note 3 gsa geometric area score; gsno geometric number score
Note 4 data set 28 left fores

significant than type 6. Certainly the cumulative number of both these crack types in the equine hoof wall is greater than crack type 6, *(figures 3.4.1.i-ii; graphs B)*, although only the cumulative area is greater in one data set, *(figures 3.4.1.iv-v.; graphs B)*.

3.4.2 Characteristics of cracks in equine hoof wall

Distribution

The effect of anatomical position on the number and type of cracks was not significant and there was an even distribution of cracks between the quarters and the toe in both groups of hooves. This is in contrast to lesions recorded in dairy cattle claw, (Leach 1996) where the effect of claw position on severity of sole lesions was highly significant with the outer hind claws always having the highest scores.

Cows bear more weight on their lateral hind claw which is generally larger in size than the other claws, (Leach 1996), and most researchers have recorded this greater concentration of lesions in the outer hind claws of cows, (Greenough and Vermunt 1991; Bergsten 1993). The sole of the claw is frequently in contact with the ground, and unlike horses, few cows are shod; thus friction wear and lesions are more likely to be associated with weight bearing in the cow compared to the horse. Friction lesions due to weight bearing were not expected on the equine hoof capsule, but a difference in cracks between the anatomical areas may have been predicted due to the way that the hoof capsule deals with load. The literature indicates that quarter cracks are more frequent in the horse compared to other types of cracks, but this perception was not supported by the results in this thesis. Although a distinction was not made in this thesis between shod and unshod hooves, it should be taken into account in future work. Shod hooves might have a propensity to quarter cracks because farriers try and balance the hoof in a mediolateral aspect despite a lack of scientific evidence to support the concept of medio-lateral balance. If the farrier changes the balance of the foot, then weight will be redistributed and this may over time result in cracks.

Relationship between size, number and severity of cracks

Leach, (1996) stated that severe lesions on the cow's claw tended to be smaller than less severe lesions. This was the main argument used to support the use of geometric scores in order to weight the severe lesion so that its clinical significance was recognised, (Greenhough and Vermunt 1991; Logue *et al.* 1994; Leach *et al.*1997; 1998). Leach, (1996) also noted that the

less severe lesions were large. However biological and mechanical principles would indicate that a shape or structure under compression is more likely to have multiple small cosmetic type cracks as a way of dissipating energy and preventing failure. In fact the hoof is a complex composite at ultra-structural and microscopic levels where the keratin filaments and the tubules are designed to divert cracks and the matrix and the intertubular material to absorb the energy which might otherwise cause a failure, (Kasapi and Gosline 1997; 1999). Leach, (1996) measured lesions over a period of time and despite indicating that small cracks were the most severe, her results showed that with time both the number and the GSA of sole lesions increased as did the proportion of the sole affected by lesions and the differences over the time period were significant. This finding was further confirmed, (Leach *et al.* 1997) by the change in lesions on the white line which also showed an associated increase in the number, severity and area of lesions. Concluding their results, (Leach *et al.* 1997) stated that as the changes in the extent of lesions, i.e. the percentage of claw affected followed the same pattern as severity, there was no need to measure area and the simpler severity assessment was an adequate indicator of damage, which in this thesis is described as GSNo.

In this thesis, the relationship between the number, area and severity varied according to crack type. There were not many cosmetic crack type 1s, (*figures 3.3.1i and 3.3.1.ii graphs B*), and their area was small. This may be due to the fact that most horses receive regular hoof care and the farrier would remove many of the insignificant cracks through rasping. The numbers of crack type 6s were similar to crack type 1 but their areas were large. A crack of this significance in the equine is probably due to some catastrophic damage such as injury to the coronary band which results in material unable to support load; alternatively it could be due to inappropriate hoof shape which when exposed to excess loading such as work on hard or uneven surfaces simply fails. If this is the causal mechanism, then it would be surprising if crack 6s were numerous, and in addition their effect would be catastrophic from a functional perspective and immediate remedial action would be taken. Crack types 3, 4 and 5 were the most numerous and had the greatest area, the association with area, number and severity for these crack types would be similar to that recorded in cattle claw.

Bertram and Gosline, (1986) recorded a much lower fracture toughness in samples taken from close to the bearing border of equine hoof wall compared to younger material and suggested that hoof wall accumulates fatigue damage with long term loading which weakens the wall due to the formation of micro cracks. It would be interesting to relate crack incidence with the time the horse was last seen by the farrier. In addition, it must be appreciated that in this work, the crack scores were a static measure and ideally changes in time should be recorded especially if

attempting to correlate with other attributes such as trace elements and fracture toughness which also change with time.

Relationships between methods of severity scoring

Adjusting the area of cracks geometrically in this thesis resulted in a significant difference between GSA type 6 cracks and GSA1 – 5 crack types and therefore this scoring method is likely to be more meaningful from a clinical perspective. As already noted this thesis was unable to record changes in crack development. However when comparing how the hoof score differed according to which scoring method was used, GSNo methods showed significant differences between type 6 cracks and crack types 1, 2 and 3. In fact GSNo scores further differentiated between type 6 and the types 4 and 5, *figures 3.3.1.i-ii graphs C*. This indicates that in a routine examination, GSNo may be adequate to classify the hoof in terms of its cracks. The relevance of which scoring system is used to describe crack types 1-3 is less significant as differences between the scores were not significant. However as the severity of the cracks increased geometric scores were significantly different to the other scores; showing that the weighting system was effective, *figures 3.31.i - iv, graphs D* assuming that the increase in severity of cracks would be related to a decrease in hoof function.

Choosing which score to use

Although there were significant differences between GSA and GSNo scores, they were correlated in both sets of data, *figure 3.4.1.ii*, with over 60% of the differences in GSA being explained by GSNo differences. This thesis recommends that GSNo is a sufficiently robust method to describe the cracks objectively on the equine hoof wall, not only because as Leach, (1996) indicated it is less time consuming and therefore more applicable in the clinical situation, but also because it is likely to be more precise than GSA. In cattle claws the area of the lesions is measured from photographs, this is not currently possible in the equine hoof simply because the measurement of three dimensional cracks from a two dimensional photograph is neither accurate nor precise, particularly for cracks 1-3, which cannot be easily seen on the photograph. The precision or repeatability of the area measurements using the method developed in this thesis was tested. The reproducibility or accuracy of measurement could not be tested and it is hypothesised that the use of area measurement would result in a greater variation compared to using GSNo.

The use of weighted scores to categorise hooves into low, medium and high crack score groups

It is unlikely that correlations between crack scores would be greater than 0.9, because of the inherent accuracy problems encountered during the area measurement of the cracks, particularly

when the cracks were an unusual shape. Consideration also needs to be given to the detail required to asses the gross appearance of the wall, so that it is appropriate for any future correlations. Grouping hooves into low, medium and high crack groups should provide sufficient detail for the significance of the cracks to remain meaningful. Using crack score groups in this thesis was meaningful; it increased the correlation between the two methods of scoring and between crack scores and fracture toughness, there were differences in fracture toughness between the groups and there were differences in the total number and area of cracks between the groups, *tables 3.3.4ii-iii, tables 3.3.6i-iii and table 3.2.6.iv*. The inter-quartile ranges were used, *table 3.2.7A*, to divide the hooves into the groups in this work, but this is an arbitrary division as the inter-quartile range is specific to the data set being analysed. The recommendation for future work would be to categorise a low score group as hooves with > 70% of their GSNo from crack types 1 and 2; medium as hooves with > 30% of their GSNo being accounted for by types 3-4 and the high crack group as hooves with any type 5 or 6 cracks. This would remove the likelihood of two hooves getting the same score from an accumulated score of several types 4 and 5 or from a crack 6. This also illustrates that the refinement of the weighted score is likely to be of greater significance in creating a meaningful relationship between a crack score and function. The geometric weighting will need adjusting if it is found that an accumulated score of crack types 4 and 5 are actually more clinically significant that a high crack score due to type 6. It is appropriate to note that mean scores or groups are used in large population studies to remove individual factors.

3.4.3 Interpretation of fracture toughness in context of other measured parameters and previous published studies

The fracture toughness of the quarters (41.9kJ/m^2 ±11.5) was significantly higher than that of the toe, (28.6 kJ/m^2 ±11.9); the fracture toughness values at the toe were similar in both sets of hooves. This is in comparison to values between 5.2-13.5 kJ/m^2 (Bertram and Gosline, 1986), mean of 12±3kJm^2, (Kasapi and Gosline, 1996) and 5.5-7.8kJ/m^2, (Bertram and Gosline, 1997) for hoof wall. There are numerous reasons why there is a difference in the values obtained from this work and others and none are likely to be related to the material. Impact results are sensitive to moisture content, impact velocity, impact geometry, the shape and dimensions of the sample, the direction and angle of impact, how the sample is notched and how it is mounted in the tester (Sherman 2003). In addition, Bertram and Gosline, (1986) were using a different technique to measure fracture toughness, where pins are loaded either side of a pre-cut notch and the sample

pulled apart to create a stress concentration near the crack tip. They measured the J-integral analysis which is a measure of the instantaneous change in energy with a change in crack length at the critical point of failure.

There are also several possible explanations as to why differences in fracture toughness were recorded between the quarters and the toe in this work. Bertram and Gosline, (1986) recorded a significant difference in fracture toughness between the most distal part of the hoof, (5.2 kJ/m^2) compared to all the other sections they measured down the hoof wall, (mean 11.9 kJ/m^2) except the most proximal sample. The difference was hypothesised to be due to the fact that the distal sample is the oldest tissue and therefore it would have accumulated fatigue damage with long term impact loading. This would not explain the difference in fracture toughness recorded between the quarters and toe in this thesis as sampling was from material of the same chronological age nor why Bertram and Gosline,(1986) did not find a difference between their proximal, (youngest) and distal, (oldest) samples.

It is possible that the difference found by Bertram and Gosline, (1986) was due to differences in water content of the tissue, the blocks were not maintained at full hydration, which would possibly explain why the proximal block was not significantly different to the distal block. The distal portion might absorb more moisture from the ground surface and the proximal portion is closer to the dermal tissues and might absorb water from the dermis. Leach, (1980) and Bertram and Gosline, (1987) in separate experiments manipulated moisture content and found that horn with high moisture content had a lower modulus of elasticity which they described as stiffness compared to horn that was not hydrated. Moisture content is also directly correlated with hardness, (Forslind 1980), thus it is likely that the effect of moisture will influence the results of mechanical tests.

Douglas *et al.* (1996) examined the differences in the modulus of elasticity[g] between the dorsal, (toe) wall and the wall at the quarters. They noted, but did not measure, that the width of the quarter blocks were half that of the toe blocks. They recorded that the moisture content of the outer dorsal wall was less than the inner dorsal wall, thus they surmised that the full wall blocks from the quarters would have a higher moisture content compared to the outer dorsal wall because they were closer to the fully hydrated dermis and were more comparable to the inner dorsal wall. In this thesis, the mean depth of toe blocks was 9.4±2.0mm, the lateral blocks 8.6±1.4 mm and at the medial quarter 8.9±1.9mm, the difference in the widths were not significant. Douglas *et al.* (1996) recorded that the toe outer wall samples were stiffer[g], as measured by modulus of compression and tension, than the whole hoof wall samples at the quarters and equated the difference to moisture content which they suggested was the

consequence of the different wall thickness; no value was given for full dorsal wall blocks. During testing, they standardised the size of the blocks taken from either the toe or the quarters to 30mm long and 5mm square but did not equilibrate for water content so the blocks were representational of the *in vivo* situation. They reported the moisture content of the quarter blocks as 35.5 ±2.5% compared to 32.5±1.2% of the inner dorsal blocks no value was given for the full dorsal wall blocks. Whilst they reported correlations between moisture and stiffness, it is difficult to compare the results with the work in this thesis as they did not look at full dorsal wall blocks and they were comparing full quarter blocks with outer dorsal wall samples. As moisture decreases in bone, (Currey 1999) the material becomes stiffer and its fracture toughness decreases. It would not be possible to deduce if this relationship exists in hoof due to the difference in sampling. However Bertram and Gosline, (1987) showed that fully hydrated horn, (which was similar to the moisture content of the inner dorsal wall measured by Douglas *et al*, (1990) has a lower work of fracture compared to normally hydrated horn. It appears that moisture content, stiffness and fracture toughness may all be correlated in the hoof. The fracture toughness of the toe measured in this thesis was significantly less than the quarters. Interestingly a note of the fact that the 'horn at the quarters is more elastic and flexible than at the toe' was made in 1834 by Bracey Clark. Differences in moisture content should not have been a confounding factor in this thesis as blocks were fully hydrated. There might have been a difference in bound water, which was not measured in this thesis.

The differences in results might be explained by the fact that the quarters might have been presented at a different angle to the pendulum compared to the toe, due to the curvature because of the difference in anatomical position. The notches in both blocks were cut at 90° to the tubules, but if the tubules were presented at a slightly different angle then the crack could have propagated differently. Bertram and Gosline, (1986) showed significant differences in the fracture toughness of blocks whose notches were cut with different orientations to the tubules. The difference in fracture toughness between a notch cut at 180° to the tubules and one cut at an angle to the tubules was 38.6% (Bertram and Gosline 1986); the difference in fracture toughness between the quarters and the toe in this thesis was 68%, so it is unlikely that slight differences in tubular orientation was the only explanation for the difference.

The thickness of the quarter blocks was generally less, but not significantly, than that of the toe. The effect of this geometry was accounted for by measuring impact strength but it may also mean that the notch had been cut through a different tubular pattern. Tubular density differs across the hoof wall, (Reilly *et al.* 1996). Tubular density was not measured in this thesis. Kasapi and Gosline, (1997; 1999) have shown that the fracture control of the hoof wall is

complex but is dependant upon both the tubules and the intertubular material, thus differences in their number, orientation and density may be reflected in different fracture toughness values. Douglas *et al.* (1990) by comparing full hoof wall blocks with split inner and outer blocks may have been measuring an effect of tubules as well as moisture content. The stratum medium of the hoof wall is very resistant to cracks, (Kasapi and Gosline 1997) and the mechanism is by necessity complex. There was no correlation between any impact score or crack score in this survey except within low, medium and high crack score groups.

Is fracture toughness related to severity of cracks?

When both data sets of feet were divided into low, medium and high crack score groups, the fracture toughness, (FT) of the quarters was significantly higher than the toe in the low and medium crack score groups, *table 3.3.4.ii*, but there was no difference between anatomical positions in the high crack score groups. In addition there was a difference in the FT between groups. The fracture toughness (FT) of the quarters in the low crack group was higher than the FT of the quarters in the higher crack score groups. It is possible that there is a minimum lower fracture toughness value to which the material of the hoof wall conforms, a biological safety net that ensures the FT is physiologically capable. A material with a low FT is more likely to crack than one with a higher FT, so the mechanical test of the hoof wall material appears to be related to the gross appearance of the wall at the quarters.

However there were also surprising differences (*table 3.3.4.ii, - 3.3.4.iii*) between the fracture toughness of the toe which increased from its lowest value in the low crack score group to its highest value in the high crack sore group, the difference between the low crack group and the other groups was significant. At the toe, it appears that there was no correlation between the objective measurement of the visual appearance of the equine hoof capsule by crack scores and the material property of impact resistance. One of the reasons a material cracks is because the stress concentration that the structure can cope with is exceeded. The load bearing of a structure is dependant not just on its material properties but also on its shape. It is highly likely that differences in shape maybe confounding the relationship between crack and impact scores.

Hoof cracking is influenced by numerous factors; environment, genetic and conformation disposition, nutrition and hoof care. Recently a system to predict predilection to cracking has been developed using artificial neural networks, (Suchorski-Tremblay *et al.* 2001). Over one hundred different factors were considered to be pertinent to crack development but the only information taken on the cracks were their length and position. It would therefore be very difficult to distinguish between cracks which were purely cosmetic and those which were of clinical relevance. The authors concluded that the 'precise quantification of hoof cracking

damage is difficult at best and one would normally expect answers in terms of fuzzy, natural language descriptors such as " hardly damaged at all", " severely damaged". Meaningful data can be collected on cracks if the system developed in this thesis is adapted.

Why this method needed developing

The quantification of the visual assessment of any epidermal structure has proved problematic to researchers working in the field, whether they are studying hoof capsule, (Moyer 2003) skin, (Carli *et al.* 2002; Rubegni *et al.* 2002; Miyamoto *et al.* 2002) or hair. Visual judgement is used in the diagnosis of lesions, (malignant or otherwise) in human epidermis but no standard procedure of measurement or reporting has been established, (Carli *et al.* 2002; Miyamoto *et al.* 2002). Researchers working with human epidermis wished to establish a standard procedure so that a remote observer could diagnose a three dimensional lesion from a two dimensional photographic or microscopic image. It is particularly pertinent that this diagnosis is accurate due to the possible medico-legal implications and therefore the method needed to present the highest degree of information possible. Whilst this degree of accuracy is not needed in the measuring of crack on the equine hoof wall, the same requirements for standardisation of measurement and reporting is required. It may be possible to develop a similar system for evaluation of hoof wall cracks, so that farrier and vet could communicate remotely. The development of a photographic method that enabled capture of the cracks and a way of correlating a two dimensional image with a three dimensional area, may improve repeatability and reproducibility, currently however GSNo is considered robust enough to describe the problem.

This thesis has developed a system which will allow both clinicians and researchers to report and compare the incidence of cracks on the equine hoof wall. The fact that this system was long overdue was highlighted by Moyer, (2003) who commented that the structural damage caused by cracks varies from being insignificant to catastrophical; however 'there is little retrievable published evidence of the actual incidence of such problems'. A great deal of descriptive literature is available both in farriery and veterinary publications; but there is little information regarding analytical and comparative studies. Farriery is an ancient practise which has accumulated a number of theories, and management schemes; but few studies are available to provide accurate incident rates of cracks, understandings of the cause or comparative information on management or prevention. The first step will be to use this first standardised method developed to report the anatomical position and the mapping and severity of cracks on the wall of the equine hoof capsule.

3.4.4 Future work

1. Lab tests which can be correlated with field work:-
    - Akazaki *et al.* (2002) has developed a method by which the morphology of the skin surface can be evaluated directly in three dimensions. Using a white light of halogen origin which is non invasive it is possible to measure wrinkle depth and width. Whilst this technique could not be widely used in clinical practise for horses' hooves, because of the lighting constraints, it maybe practical to use within a hospital or research environment where more detailed information on the cracks incidence and severity is required. In order to measure the wrinkles depth and width, they used a roughness specimen with known projection and depression sizes which conformed to Japanese industrial standards. This device could be adapted and its use in the field for measuring depths of cracks on the equine hoof capsule should be investigated to improve the objectivity of crack type allocation.
    - The development of a simple field test such as Shore D, which can be standardised regardless of operator and which is not affected by the curvature of the hoof capsule. Laboratory work could then be carried out on morbid specimens to correlate the field test with a more meaningful mechanical test such as fracture toughness.
    - The correlation of crack scores with material properties of hooves both in the clinical situation and the research environment.
2. Testing the developed method against general clinical judgement as well as testing for reproducibility.
    - This is now needed in order to refine the use of the geometric score.
    - Use the method to carry out a larger epidemiological survey of cracks to investigate relationships between crack scores and factors considered to affect crack formation.
    - The objective quantification of the cracks will mean that techniques such as neural network analysis could be more meaningful and move away from adjectives described by the authors of such papers as 'fuzzy'.

4 An evaluation of two methods for measuring trace element concentration

'this organic matter,(sample of horse's hoof) is extremely difficult to destroy by fire, it takes several hours of intense heat to get rid of it; the horn swells and liquefies, bubbles of inflammable gas break through the surface..... boiled with sulphuric acid it appears for a minute or so unaffected, when suddenly a hissing sound is heard and dense white fumes of gases are given off in torrents' (Smith, 1887).

4.1 Introduction

Altering trace element concentrations either in the diet (Sugg *et al.* 1996) or in the cell culture medium (Hennings *et al.* 1980) can potentially alter the integrity of the epidermal structure which may affect the visual appearance of the epidermal tissue and influence the maturation of the keratinocyte. Work with epidermal tissue indicates that there is a relationship between the trace element content of the keratinised tissue, its mechanical properties, (Marston 1946) or its visual appearance, (Kapp and Simon, 1980; Weismann *et al.* 1980) and that this relationship might be affected by changes in dietary intake of the trace elements, (Suttle 1983). Whilst some researchers have established relationships between trace element intake or concentration in the tissue and epidermal integrity of the ungulate hoof wall, (Baggott *et al.* 1988; Coenen and Spitzlei, 1997); others have been unable to establish similar relationships, (Ley *et al.* 1998). It is not clear if there is a link between trace element concentration within the tissue and its integrity which might be in part due to the large variation in the methods used to collect and prepare the samples. Several reviews, (Strain *et al.*1971; Chyla and Zyrnicki 2000; Chittleborough 1980; Bass *et al.* 2001) highlighted the concern that researchers have about the effect of non standardised techniques on trace element analysis in human hair. The effect of biological variation on trace element metabolism has already been discussed in *section 1.4*. However other sources of variation such as external contamination, lack of standardisation in sample collection

and preparation and analytical accuracy, (Bass *et al.* 2001) must be considered before the relationship between the concentration of trace elements in the equine hoof wall and other properties can be investigated. It may be necessary to develop or adapt current methods to establish standardised protocols of sample collection, preparation and analysis.

4.1.1 Review of methods for sample collection

Obtaining material of standardised chronological age within a hoof

Dietary intakes have been shown to alter trace element composition of the equine hoof wall, (Coenen and Spitzlei 1997). As dietary intakes will vary, it was considered important that material being analysed should be collected from sites of standardised chronological age within the hoof wall in order to minimise the inherent variability in trace element concentration due to changes in diet. This would allow comparison between anatomical positions within a hoof.

There are several factors which must be considered when sampling from the hoof wall, sampling must be:-

a) from pre- defined anatomical sites, *section 2.1.1, plate 2.1.1.iv,* to ensure repeatability and consistency of sampling areas both between feet and within feet, if the effect of time is to be studied.

b) at an anatomical position other than the distal bearing surface. The distance from the coronary band to the ground is less at the heels than at the toes (*figure 4.1.1.i.*). Heel horn at ground surface will be younger than the toe horn and in addition, contamination from shoes and or the ground is likely to confound results.

d) restricted to the proximal surface of the block taken, so that laser and solution comparisons can be carried out on the same age material within a hoof when sampling from different anatomical positions.

e) from the full hoof wall depth in order to minimise variability within the hoof, because hoof material will vary in chronological age across the hoof wall depth.

f) from the same distance as 50% HWH at the mdc measured from the hairline, as described in chapter 5, to sample from the same growth line in order to compare anatomical positions.

Information on the differences in the rate of growth between heels, toe and quarters is limited and inconclusive and varies by over 100% between authors. The rate of growth of the equine hoof wall has been reported to be between 4-7mm/month (Ryan 1999), 4.9mm/month, (Reilly *et*

al. 1998) 7.5mm/month, (Pollitt 1990), 2-3.5mm/month (Sheta 2002) at the toe and between 3.5-6.6mm/month (Ryan 1999) and 2-3mm at the heels, (Sheta 2002). It appears that the growth rate of different anatomical positions is similar.

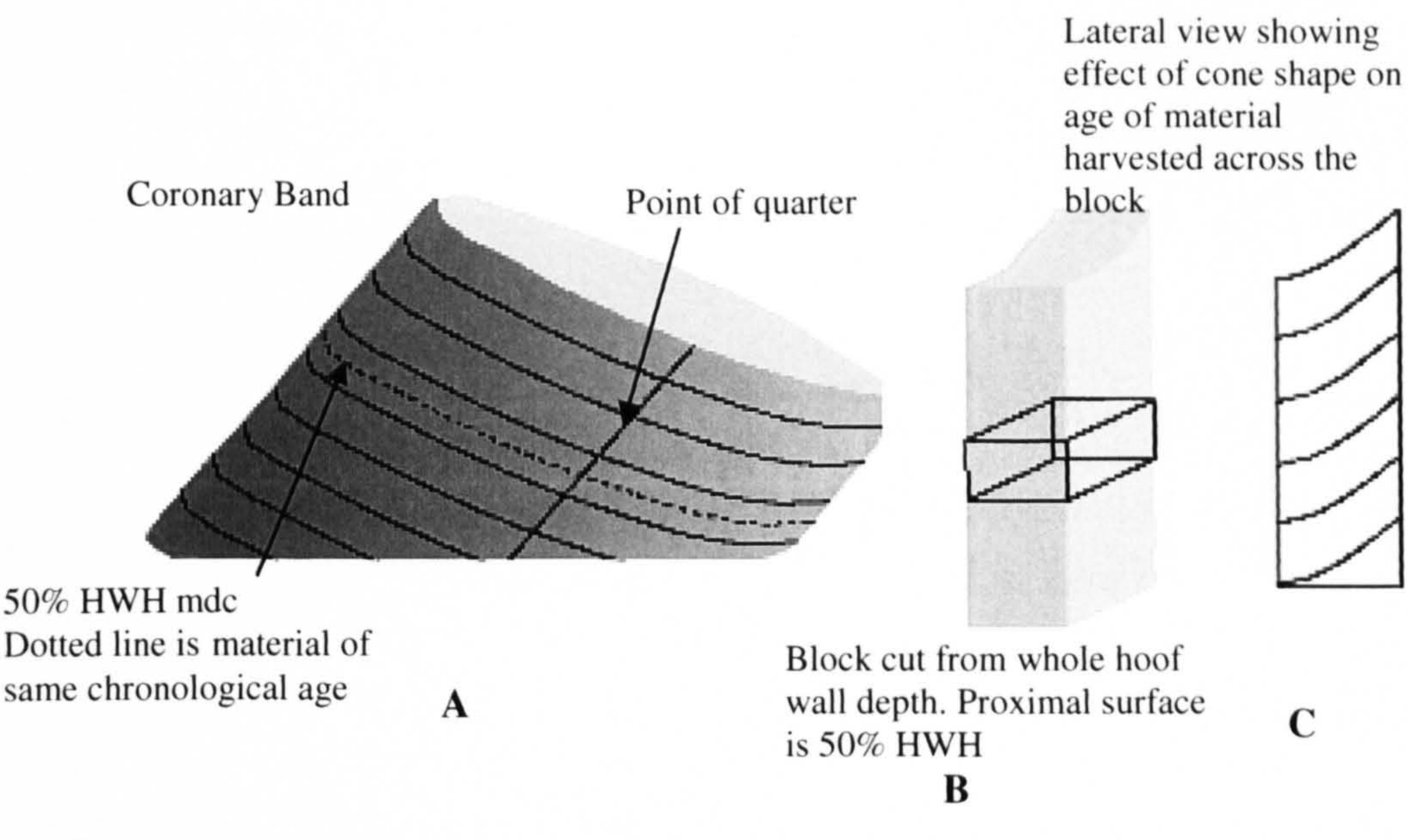


Figure 4.1.1.i Growth pattern of hoof wall and the effect on material sampling to ensure chronological consistency

artwork © Dave Gibson

Note 1 growth lines used to determine position to harvest block
Note 2 full depth sample will contain material of different chronological age.
A whole hoof; **B** block taken from full hoof wall depth and 1.5cm sample from 50% Hoof Wall Height (HWH); **C** effect of the hoof shape on material taken from full hoof wall block

Minimising variability of material tested for fracture toughness and trace element concentrations

Existence of a link between trace element concentrations of the equine hoof wall and mechanical properties is inconclusive as discussed in chapter one, *section 1.4.4*. Ley *et al.*(1998) did not find any association between mechanical properties and trace element concentrations, whereas others report an association, (Coenen and Spitzlei 1997; Hennings *et al.* 1980). Cattle research, (Baggott *et al.* 1988b) reported an association with hardness and zinc concentration, but compared sole with wall which are different in their composition as did Coenen and Spitzlei (1997) in horses. It is possible that the differences in material properties were due more to the hierarchical structure of the corneocytes and the lipid cement or to the variation in the ratio of keratin intermediate filaments to intermediate filament associated proteins. Ley *et al.* (1998) sampled only from the hoof wall, but the sampling was from the bearing surface, a sample was taken from the mid toe region for mechanical tests and the rest of the clipping was used for trace element analysis. This meant that as well as material of different chronological age being compared, there was an increased risk of external contamination from the ground. In addition diet varied through the trial, (Ley *et al.* 1998) therefore the material

tested for trace elements may have had a different composition to the material tested for tensile strength. To minimise the effect that this might contribute to the variability, a protocol will be developed so that material for trace element analysis can be sampled at the crack plane from blocks which had been tested for fracture toughness.

4.1.2 Review of methods for sample preparation

Washing procedures

Epidermal tissues such as hair and nail are exposed to exogenous contamination of trace elements, (Harrison and Tyree 1971; Bass *et al.* 2001; Kruse-Jarres 2000; Chittleborough 1980; Salmela *et al.* 1981). Due to the considerable variation that this may introduce, a large body of the literature reports that trace element analysis of hair and nail is inappropriate for any type of monitoring except forensic, (Bencze 1990; Chyla and Zyrnicki 2000; Combs 1987). Others argue that the problem with hair analysis is not reliability of measurement but in the preparation of the samples, (Bass *et al.* 2001; Chittleborough 1980; Salmela, *et al.* 1981). Human hair and nails are subjected to environmental contamination, as is the hoof, and to manufactured contaminants such as cosmetic, medical and hygienic treatments. Shampoos containing selenium can result in scalp hair having twenty to thirty times normal values, (Chittleborough 1980); dyes containing zinc directly affect the concentration in the hair, (Bencze 1990).

In order to minimise this variability, various washing techniques have been researched to investigate the difference in trace element concentration between washed and unwashed samples. Results from washing pooled homogenised dog hair, (Chyla and Zyrnicki 2000), fingernails, (Harrison and Tyree 1971) and human hair, (Salmela *et al.* 1981) with non ionic detergents (Triton X-100%), a complexing agent (EDTA), de-ionised water, an organic solvent (acetone) and alcohol (methanol) indicated that all washes reduced the amount of trace element present compared to the unwashed sample but to differing degrees which were similar across the different epidermal structures.

Different elements have different binding activities; therefore the wash which removes the most trace element varies according to the element. There continues to be debate as to whether the washings are removing endogenous as well as exogenous trace elements. Harrison *et al.* (1969) and Chyla and Zyrnicki, (2000) found that a non ionic detergent removed substantially more iron and manganese and only small quantities (approximately 14% and 1% respectively) of

copper and zinc compared to alcohol or organic washes. Whilst Harrison *et al.* (1969) thought that either the detergent was leaching the endogenous trace elements, (Fe and Mn) or the organic was failing to remove the exogenous trace elements, they were unable to clarify which. Even by the present century, Chyla and Zyrnicki, (2000) were unable to explain which procedure was affecting the different results; they too thought that either the detergent was failing to remove the exogenous trace elements, (copper and zinc) or the organic solvent was leaching the endogenous trace elements.

Despite the debate, it has been accepted that using a non ionic detergent removes exogenous zinc due to external contamination. Researchers recorded different effects of washing with a non ionic detergent, Triton-X removed more than forty-five percent more zinc compared to unwashed hair, (Salmela *et al.* 1981). Compared to distilled water, Triton-X removed over 20% more calcium, 35% less copper and 20% more zinc from hair and this affect was the same for dog and human hair, (Chyla and Zyrnicki 2000). However others recorded only 3-7% difference in zinc, calcium and copper levels when comparing Triton-X and distilled water, (Borella *et al.* 1996). This probably reflects the method of washing, Salmela *et al.* (1981) and Chyla and Zyrnicki, (2000) carried out twelve successive washes, Borella *et al.* (1996) only carried out one wash.

Some researchers (Chittleborough 1980) advocate reducing the pre-analysis of hair to a minimum if not zero due to the fact that the trace elements may easily be leached from the disulphide bonds of the IFAPs in the cuticle. There is some support for this argument from the treatment of hair with perming lotion. Perming lotion and setting gel leached calcium from the hair by up to 99% (Hilderbrand and White 1974) and zinc concentrations increased by a factor of three. Perms alter the structure of hair resulting in a change in visual appearance which may resemble the change in bonding of sulphur- sulphur bonds induced by dietary deficiency of copper or excess zinc at cornification.

All authors agree that whilst some washing procedures cannot distinguish between exogenous and endogenous trace elements; it is of vital importance that wash procedure and times must be standardised; continuous washing will affect the amount of trace element removed (Harrison and Tyree 1971; Salmela *et al.* 1981) and therefore results cannot be comparable.

Whilst it is unlikely that hooves will be exposed to the same number of endogenous treatments as human hair and nail, it was felt that non ionic detergent may be required to remove any grease or oil, both of which are common hoof preparations. Therefore it was proposed that a non ionic detergent should be compared to millipore water and that washing times and rinses should be standardised. Because using different rinsing times and repetitive washings with non ionic

detergent removed different quantities of the trace elements, (Chyla and Zyrnicki, 2000; Salmela *et al.* 1981; Borella *et al.* 1996), and because others recommended no washes, (Chittleborough 1980), the procedure was modified with a reduced rinsing time compared to Borella *et al.* 1996 but increased number of washes in a non ionic detergent which was then compared to millipore water.

Digestion procedures

The aims of digestion were to dissolve totally the hoof sample, to minimise any losses due to volatisation and to avoid contamination from external sources. Original digestion techniques followed those reported in the literature (Harrison, 1971; 1972) and dissolving 10mg of hoof wall with 6mls of 70% nitric acid, 2ml perchloric acid and 1g of lanthanum chloride was successful; initial results fell within published literature ranges. Due to changes in H&S policy a different technique had to be found which was a) quicker and b) did not use perchloric acid. Finding alternatives initially proved problematical due to the difficulty in dissolving the hoof. The Kjeldahl[g] technique was investigated as an alternative and digestion was successful within an hour. However despite using Buchner and micropore filters, to ensure a 'clean' solution with no dissolved solids; the dissolved solutions continuously blocked the ICP-MS. After contact with the manufacturer of the machine, it became clear that the sodium selenite used as a catalyst resulted in too many dissolved solids for the plasma to ionise and a matrix dependency problem had occurred. This happens when there are excess salts in the ICP, (Dean, 1997). Using sodium selenite as a catalyst meant that the nebuliser which was not a high solids type became blocked, in addition there was probably a build up of solids on the sample cone at the ICP-MS interface. If it had only been the second problem, then the solution could have been diluted further, but this would have resulted in decreased sensitivity of the trace elements being measured specifically copper. The first problem was the dominant problem as the plasma kept going out, it was not feasible to buy a new nebuliser. An alternative method of dissolving the hoof was required. Microwave digestion was therefore investigated as a technique.

Microwave digestion has several advantages:-

a) It does not require the sample to be dry ashed nor ground into fine powder. Grinding increases the risk of contamination from the mortar and pestle especially if either have surface marks. Hoof wall material is very resistant to grinding even after being cooled by liquid nitrogen to try and make the material more brittle. Dry ashing is considered a suitable procedure to oxidise hoof wall material as it is high in organic carbon, but because it is an open system, there is some risk of losing volatile analytes which may include some of the trace elements.

b) It is carried out in a closed system which uses a bomb, *figure 4.1.2.i.* This isolates the digestion process in a closed container which can withstand the increase in pressure and excludes any external contamination. In addition, it minimises loss of volatile analytes.

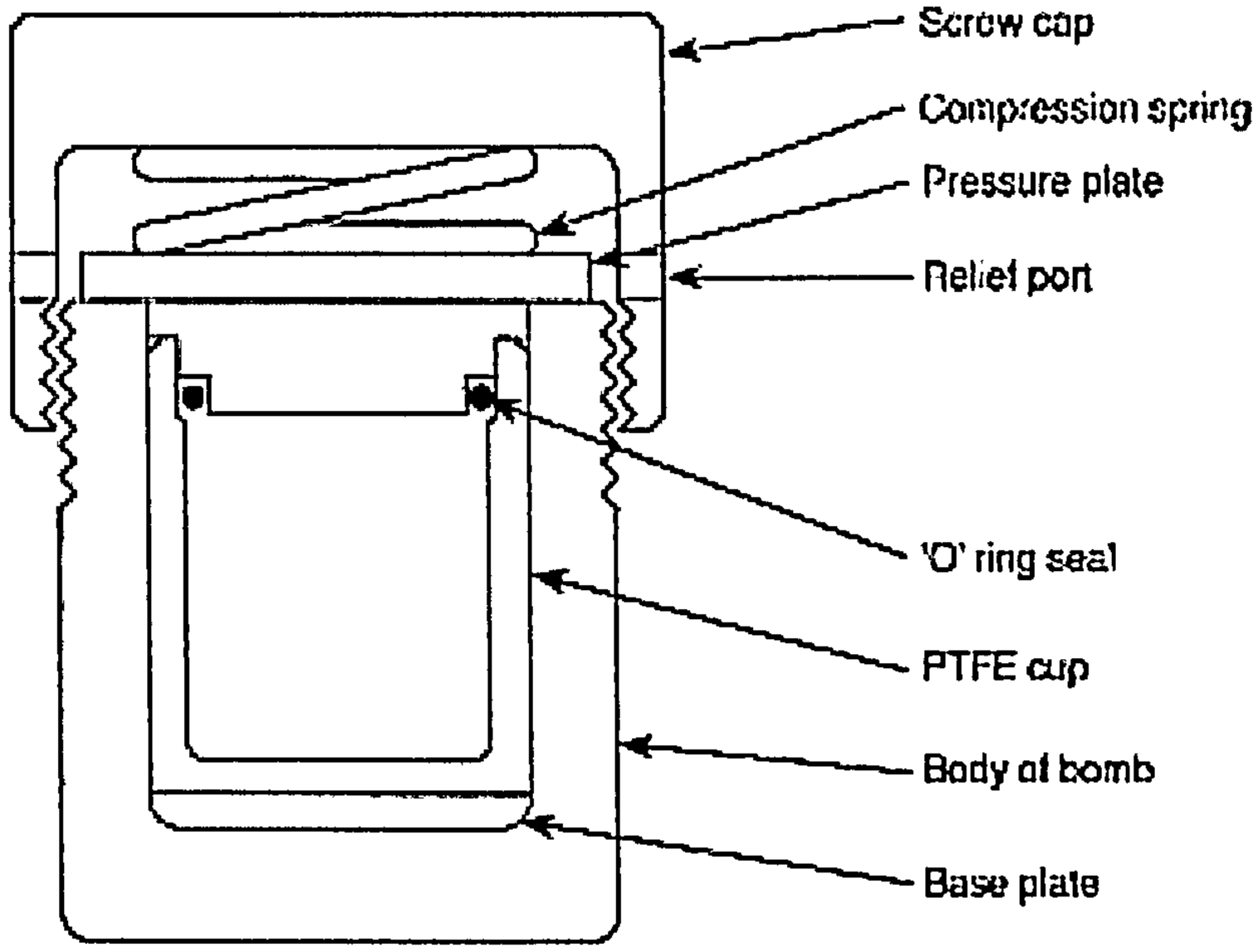


Figure 4.1.2.i Bomb used for closed digestion of hoof wall material in a microwave
(adapted from Howard and Statham,1993)

c) Only, ultra pure nitric acid[2] is required, the process takes less than 30 seconds to dissolve the material and the closed system precludes any external contamination.

Minimising sources of interference

When analysing trace elements steps must be taken to minimise contamination, and the stringent conditions recommended for trace element analysis was considered for sample preparation, (Howard and Statham 1993). In order to minimise matrix[g] effects, standards must have the same composition as the analytical sample i.e. the dissolved hoof in terms of acid and millipore water, this was achieved as described in the methods, section 4.2.2. In addition a series of procedural blanks were made up; these do not contain any hoof or analyte and can be used for checking for contamination.

Nalgene[g] bottles made from PTFE rather than glassware were used. Scratched glassware can be a source of trace elements, in addition, the low pH of nitric acid may favour dissolution and desorption of trace elements from the glass (Howard and Statham 1993).

[2]*(70% weight by volume. i.e. 69-71% (w/v). specific gravity of 1.42, approximately 16M), ROMIL-SpA Super Purity acid, Zn, Cu, Ca <1ppb*

Blanks were run through the ICP to check for losses or contaminants due to storage for the length of time that the solutions were kept.

Non powdered surgical gloves were used to handle the hoof samples and were changed between each hoof. All surfaces were cleaned with millipore water between samples as it had been noted that cleaning materials may be a source of trace elements, *table 4.1.2.ii.*

Table 4.1.2.ii Some potential sources of contamination in the laboratory

Material	Zinc µg/g	Copper µg/g
dust leached by conc nitric acid	2400	406
paper towels	49	
rubber tubing	40000	0.6

Adapted from Howard, and Statham (1993)

Water supply

Originally double distilled water was used for making up the samples, stocks and standard solutions during the initial stage of gaining familiarity with analytical techniques. Water is a possible source of trace elements. Calcium content in domestic water can vary between 7mg/kg to 104mg/kg, (Howard and Statham 1993); even treated water can contain different quantities of trace elements, demineralised and distilled water contain 1-1.5 µg/l of copper; water doubly distilled in quartz contains 0.3-0.5 µg/l and distilled and millipore purified contains only 0.1-0.3 µg/l copper, (Vandecasteele and Block 1993). Thus for all experiments millipore water from the cell signalling laboratory was used and collected on the day, to minimise storage in Nalgene bottles. In addition, pure millipore water was analysed through the ICP-MS to check for levels of calcium, zinc and copper.

4.1.3 Choice of analytical techniques

Inductively Coupled Plasma-Mass Spectrometry

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was chosen as it is considered a very powerful tool for *trace* (ppb-ppm) and *ultra-trace* (< ppb) elemental analysis. In ICP-MS, a plasma or gas consisting of ions[g], electrons and neutral particles is formed from Argon gas. The plasma is used to atomize and ionize the elements under analysis from a sample which has been pre-digested in solution. The resulting ions are passed through a series of apertures (cones) into the high vacuum mass analyzer. The isotopes of the elements are identified by their mass-to-

charge ratio[g] (*m/e*) and the intensity of a specific peak in the mass spectrum is proportional to the amount of that isotope (element) in the original sample.

The system is calibrated using standard solutions of differing concentrations and a best fit calibration line is plotted using Minitab. Samples are entered into the mass spectrometer under similar conditions to return a count rate at the detector that was converted directly to the concentration for each element. The response of the mass spectrometer in counts per second is directly proportional to the concentration of a given element in a sample.

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP- MS)

Since the development of inductively coupled plasma most applications have required digestion of solid samples with heat and/or strong acids, which is time consuming and could be hazardous. An alternative sample-introduction technique for ICP-MS, laser-ablation, was developed by Gray, (1985) and became commercially available in the mid-1990s. LA-ICP-MS is an extension of the well-established technique of plasma torch mass spectrometry. Laser energy is used for *in-situ* sample excavation of solid sample material, which greatly reduces sample preparation required and therefore minimises contamination. Samples are located in an enclosed sample cell that is continuously purged with argon gas and are mounted as irregularly shaped samples or as unpolished thin sections. The physical form of the sample is irrelevant as long as the sample surface remains in laser focus. Samples are positioned under the laser beam and viewed using a high-resolution colour video camera. A small volume of material of interest is ablated from the sample surface. Ablated material (probably in the form of condensed droplets) is transported in an argon carrier gas directly to the inductively coupled plasma and mass spectrometer. A glass standard (NIST 612) is used to calibrate the machine and an internal standard carbon is used to standardise the ppm as unknown quantities of solid material are ablated.

It was considered appropriate to see if this method could be developed to measure trace elements in hoof, because of all the inherent difficulties in solution analysis including contamination associated with the preparation of solutions, the time involved and the increasing pressure from Health and Safety to minimise the use of reactive acids. It was unlikely, however, that the two methods would give directly comparable results for the following reasons:-

1. Laser ablation measures a very small quantity of ablated solid material and is representational of that area alone. Solution work measures a full hoof wall depth and gives the average of that piece of hoof.
2. A known quantity of hoof is dissolved in solution and a dilution factor allows calculation back to mg/kgDM of the original hoof. Laser ablates an unknown quantity

of material. A hard piece of hoof might result in less sample being ablated during the specified time and therefore a lower ppm would be recorded, which was indicative of the quantity of the material ablated not the true concentration. It is therefore standard procedure to divide the ppm of the trace element recorded at the detector by the ppm of an atom which is in sufficient abundance in the material that it can be considered as relatively constant in its uptake. In this instant, it was advised to use carbon ppm to represent the uptake of the material. Thus the figures are purely qualitative and can be used for comparative purposes only.

3. When using solutions the counts can be calibrated against known standards which are made to ensure that any matrix effects are common across the unknowns and the standards. For example the same supply of 2ml of ultra pure nitric acid and millipore water used to make up the standards, was also used to dissolve the unknowns. Any calibration on the laser is purely qualitative as a glass containing known quantities of only of few trace elements is used; carbon is not included as one of the standards. In addition glass is a completely different substrate to the hoof and thus matrix effects cannot be taken into account.
4. Solution work is accurate to ppb whereas laser is only accurate to ppm.
5. Various factors can affect the precision and accuracy of an ICP-MS machine.

The main advantage of the laser technique is that comparative analysis can be done along the hoof wall depth and down the hoof wall length, provided that various steps (as described above and in the methods), to control variability were taken. In addition, previously lasered blocks were used as an internal standard by calibrating their solution results against a second ablation to 'train' the machine.

4.1.4 Choice of trace elements

The reasons for choosing to analyse copper, calcium and zinc were discussed in chapter 1. Because the dietary history of the horses from which the hooves were collected was an unknown, it was possible that there would be a large individual variation in the trace elements within the epidermal wall; although this may also exist in individuals fed the same diet. Due to the large metabolic differences, the ratio of trace elements to each other might be more meaningful and removes the variability due to absolute dietary intakes. Kovacs *et al* (1973) and Hidiroglou and Williams (1986) analysed calcium, magnesium, copper, zinc, and sulphur in

healthy claw of three different breeds (cattle, sheep and pigs). Hidiroglou and Williams, (1986) sampled at different times of the year and during differing dietary regimes showing affects of both. They found that hoof concentrations of calcium and magnesium decreased, whereas the inverse trend was observed for copper and zinc. There were strong correlations between calcium and the other minerals and between zinc and copper in the horn and they suggested that an excess of one mineral can cause a deficiency of another mineral. Hintz, (1996) indicated that an animal with zinc deficiency may be induced by a high intake of calcium, mainly because calcium and zinc are absorbed from the small intestine; therefore it is likely that calcium could interfere with zinc in horses. Hintz, (1996) also showed that high intakes of phosphorous can decrease the efficiency of magnesium, calcium and potassium absorption in horses. It was therefore considered appropriate to investigate correlations between trace elements and to calculate ratios of trace elements so that correlations with other parameters could be investigated in further chapters.

4.1.5 Experimental Aims

The literature review has highlighted that the following are likely to be contributing both systematic and random errors which might be either hiding or contributing to variations in trace element measurements.

Sampling from tissues which differ in anatomical position or age

Sampling from different sites in order to measure relationships between trace element concentration and a mechanical property

Differences in washing techniques

Using equipment for sampling and storage which itself might be a source of trace elements

Inconsistency in reporting the concentration of the trace element in the original sample

The aims of this chapter were i) to measure trace elements in the hoof from predefined anatomical positions, ii) to see if trace element concentration was affected by age of the material or by anatomical position so that iii) in chapter 6, the relationship between fracture toughness at the same anatomical position and trace elements can be investigated, iv) to investigate the effect of washing techniques on trace element concentration, v) to investigate the application of LA-ICP-MS for the analysis of trace elements in whole hoof blocks, vi) to compare the results from

ICP-MS solution analyse with those obtained for LA-ICP-MS, vii) to investigate if the ratio of trace elements was consistent and to ascertain if any of the trace elements showed correlations.

The following were therefore developed

- An objective method for defining anatomical position
- A protocol for sampling hoof wall to minimise external contamination
- A method to compare two washing techniques
- A method to compare two analytical techniques
- A method for measuring trace elements down (proximally/distally)the hoof wall height and across (cranially/caudally) the hoof depth

In order to test the hypothesis

- That there is no difference in trace element concentration regardless of the washing technique used
- That the trace element concentration of the hoof wall is not affected by anatomical position if sampling from material of the same chronological age within a hoof
- That there is no difference between the results obtained from ICP-MS solution and LA-ICP-MS when material of the same age and the same anatomical position from the hoof wall is analysed for trace elements
- That there is no difference in trace element concentration in the hoof wall when sampling from material of different chronological age

4.2 Methods

A subset of six hooves was used to sample material from three different analytical positions, mdc and both quarters to establish differences in trace elements between anatomical positions. In addition the block from each analytical position was spilt into two and labelled A & B to test the effect of different washing techniques on trace element concentration. Analysis was carried out using both ICP techniques. A further forty eight hooves were sampled just from the toemdc in order to investigate the relationships between the ICP-MS solution and LA-ICP-MS. Twenty-eight left fores were then analysed using LA-ICP-MS to investigate differences in trace elements between anatomical positions by analysing quarter and toe blocks. In addition within anatomical differences were investigated by analysing within entire blocks of hoof wall.

4.2.1 Preparing blocks for trace element analysis

Blocks were prepared as described in chapter two, *section 2.2.1*. Sampling to compare washes was restricted to 1.5cm distal to the first cut to remove approximately 3 months or one season's growth to ensure consistency of material sampled within a hoof, *(plates 2.2.1.i).* For washing technique comparisons six hooves were used and each block was cut into the A and B sections along the anatomical line to ensure consistency of material, the distal surfaces were marked with a pen. Blocks A were allocated to washes in millipore water and blocks B to washes in a non ionic detergent and bactericide solution, (Lance) and wrapped in parafilm until ready for testing.

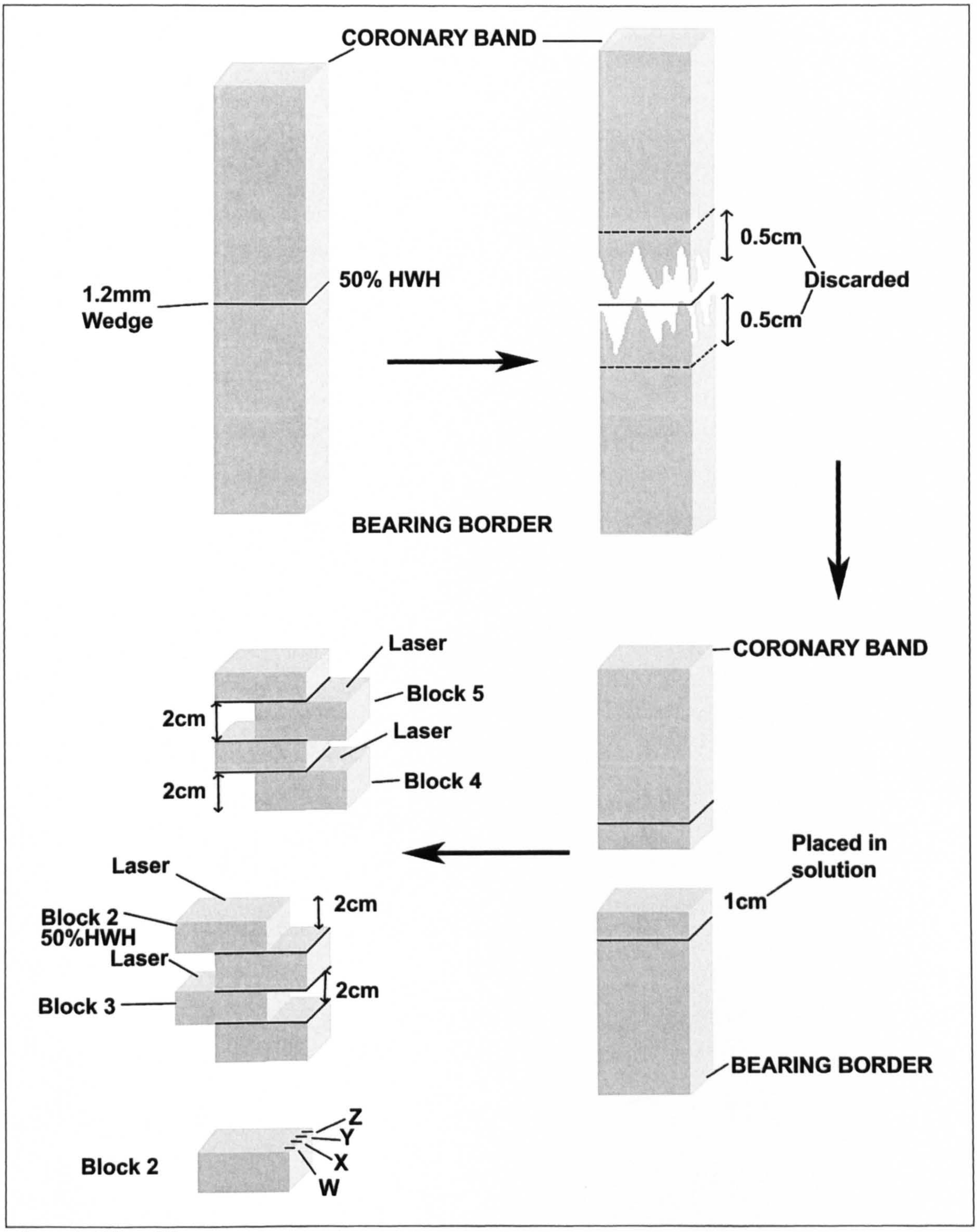
Washing Procedure

a) Five Nalgene bottles were used for successive washings in Millipore water. Using stainless steel forceps and medical gloves to transfer the block, the block was rinsed in each bottle for 5 minutes by agitating the bottle. New bottles or bottles cleaned in 2%ultra pure nitric acid were used for each new block. After the final wash, the block was put in a desiccator until shaved.
b) Similarly 5 bottles of 15 % Lance were used for successive washing of the other allocated blocks. Each block was rinsed in the bottle for 6 minutes and then rinsed in millipore water for 5 minutes. After the final wash, the block was placed in a desiccator.

The above procedure was modified from the technique described by Borella *et al.* (1996).

Block preparation for laser and solution comparisons

The blocks were prepared for fracture toughness testing, *section 2.2.3, and plate 2.2.3.i.* After testing with the IZOD, the hoof wall block split in two at the mdc at 50% hoof wall height, (HWH) and mdc blocks from 48 hooves were prepared so that they could be used to compare analysis by solution and laser. After the full hoof wall height blocks had been tested on the IZOD, each block was cut at 90° to the tubules with a Bandsaw** medially/laterally at 50% mdc hoof wall height, as shown in *plate 4.2.1.iv*. A second cut was made 1.5cm below the first cut. The blocks were wrapped in parafilm. The procedures below were followed for all blocks being prepared for solution and laser work during establishment of washing technique and for all subsequent ICP-MS solution analysis.



Artwork ©Dave Gibson

Plate 4.2.1.iv Block preparation for laser ablation across the hoof wall depth and down the hoof wall length at the toe mdc

Taking shavings for solution analysis

Each block was mounted into a microtone. The proximal surface was rinsed with Millipore water to improve cutting and shaved. A clean environment was maintained by dust extraction and the machine was vacuumed between each block. The shavings were put into plastic specimen tubes, each tube was appropriately marked and the contents weighed. The tubes were then put back into a desiccator containing anhydrous phosphorus pentoxide to dehydrate them, (Hopegood, 2002) so that analysis could be compared on a dry matter basis. The remaining block was put in an appropriately marked specimen tube and left in a desiccator until it reached equilibrium in weight for using for laser ablation.

Once the shavings had reached equilibrium in terms of weight, 0.100g dry weight of shavings were weighed from each sample and kept in the desiccator for making into solutions the following day.

Preparing blocks for laser ablation

mdc blocks

The distal portions of the blocks from 48 mixed hooves were used for trace element analysis and 0.5cm was removed, using a Bandsaw, from the split edge of every block before microtoming to ensure comparisons at similar anatomical positions. Following any cutting procedure, the blocks were rinsed rapidly in millipore water to remove any surface material. Material for solution work was collected as described above and the microtomed blocks were put into a desiccator and kept for laser ablation studies.

Cutting blocks for HWH and HWD comparisons

Eleven toe mdc from left fores were cut to allow laser investigation of trace element variability, firstly proximo-distally down the hoof wall height and secondly across the hoof wall depth from the outer wall to the inner wall. The decision on how to divide the blocks was based on an average growth rate of 1.5cm/season, to try and ensure that the samples were taken from growth which occurred throughout the whole growth cycle.

0.5cm was removed from both fracture surfaces, to standardise the sampling as the crack surface varied from block to block. Shavings were taken from the proximal surface of block 2. The remaining hoof wall heights were measured and a mark made at 50% of their length. The distal and proximal blocks were cut along this line (*plate 4.2.1.iv*); a permanent mark was made on the 'bottom' of all four blocks and the surface for laser ablation was cleaned using very fine sandpaper.

The blocks were marked and placed in a desiccator until used for laser ablation. Lateral and mdc blocks were ablated at 12.5% hoof wall depth on their right side and the medial blocks were ablated on their left side to ensure commonality of anatomical position.

Measurement of HWD

Eleven mdc blocks were measured at 12.5%, 37.5%, 62.5% and 87.5% *(w, x, y and z on plate 4.2.1.iv)* of their hoof wall depths, using the scale within the laser chamber during focusing.

4.2.2 Preparing sample and stock solutions and standards

Microwave digestion

Microwave digestion was used to prepare the hoof samples for trace element analysis. The protocol used fell within the guidelines issued for the use of a 4781-23ml microwave digestion bomb[2], i.e. 0.100g of sample dissolved in 2.0ml of nitric acid.

Procedural blanks

Procedural blanks were made by decanting ultra pure nitric acid into a small 100ml beaker in the fume cupboard. 2mls of the nitric acid were autopipetted into the teflon sample cup and the lid put on. The sample cup was placed into the outer body and the casing top was screwed on firmly. The bomb was placed in the microwave, (400watt, 14litre) and 'cooked' on the highest setting for 30 seconds. The bomb was left for 25minutes to cool to prevent loss of material by vapourisation, and the outer casing was unscrewed; the lid of the bomb was carefully opened and any droplets accumulated on the lid or sides of the cup were washed back into the cup with a pipette containing millipore water. The contents of the cup were transferred into a clean volumetric flask. The cup was rinsed again with millipore water and transferred into the flask; the flask was then made up to 100ml with millipore water. The contents of the flask were transferred to PTFE bottle and labelled procedural blank.

Sample dissolving

The preweighed shavings (between 0.100g and 0.200g) were placed into the cup and 2ml nitric acid was autopipetted. The above microwave procedure was followed. The final dissolved solution was made up to 100ml and transferred into the PTFE bottle marked with the sample number.

[2]*Parr Instrument Co., 211 Fifty Third Street, Moline, Illinois, 61265, USA, patent no 4882128*

Washing procedure for auto pipetting for stock and standard solution preparation

Auto-pipettes were used and the correct sized auto-pipettes, (10ml, 1ml and 0.1 ml), and disposable tips were selected to ensure as few replicate uptakes as possible. The pipette was filled with 2% ultra pure nitric acid, (the wash solution), and the solution discarded; the correct quantity of standard was pipetted and discarded and finally the correct quantity of standard pipetted into the stock bottle. The pipette tip was discarded and a fresh tip used for every new pipetting.

Stock solution preparation

A stock of 100ppm of zinc and calcium and a stock of 10ppm of copper was prepared, *(table 4.2.2.i)* from these a further stock of 0.1ppm zinc, 0.5ppm calcium and 0.01ppm copper was prepared. New PTFE bottles previously rinsed in ultra pure nitric acid were labelled and used to store the stock solutions. The stock was made up to 100ml with pipetted millipore water, following the above washing procedure.

Table 4.2.2.i Quantities of absolute standards and millipore water used to make stock solutions.

	Zn stock flask A	**Ca stock flask D**	**Cu stock flask H**	**O stock flask O**
	mls			
1000ppm standard	10	10	1	0.1ml flask A 0.5ml flask D 0.1ml flask H
millipore water	90	90	99	99.3
to obtain ppm of stock	100	100	10	0.1ppm Zn 0.5ppm Ca 0.01 ppm Cu

Preparing standard solutions

Seven flasks were labelled 1a through to 5 and washed with 2% ultra pure nitric acid. The amount of stock added to these bottles is shown in *table 4.2.2.ii*. The correct size pipette and tip was chosen for each measurement. The washing procedure described above was followed.

Table 4.2.2.ii Quantities of stock solutions in mls used to make up multi trace element standards

Flask number	Zn stock Flask A	Ca stock Flask D	Cu stock Flask H	stock O Flask O	Millipore water	ultra pure nitric acid
	mls					
flask 1a				1.0	97	2
flask 1b				10.0	88	2
flask 1	0.1	0.5	0.1		97.3	2
flask 2	0.2	1.0	0.2		96.6	2
flask 3	0.5	2.0	1.0		94.5	2
flask 4	1.0	3.0	5.0		88	2
flask 5	2.0	5.0	10.0		81	2

These stock solutions were used to calibrate the ICP-MS machine by providing the dilutions of trace elements which covered the detection limits of the machine, *(table 4.2.2.iii).*

Table 4.2.2.iii ppm of zinc, copper and calcium in the multi trace element standards used to calibrate the ICP-MS

flask number	zinc ppm	calcium ppm	copper ppm
flask 1a	0.001	0.005	0.0001
flask 1b	0.01	0.05	0.001
flask 1	0.1	0.5	0.01
flask 2	0.2	1.0	0.02
flask 3	0.5	2.0	0.1
flask 4	1.0	3.0	0.5
flask 5	2.0	5.0	1.0

To check the reliability of the machine and for matrix effects, a series of standard additions were also run. Five flasks containing 0.2g dry weight of hoof 14mdc dissolved in nitric acid as described above, had 0.0ml, 0.1ml, 0.2ml, 1.0ml and 5.0ml of flask H added to them, they were then made up to 100ml with millipore water.

4.2.3 Using the analytical equipment

ICP-MS

A Perkin Elmer ICP-MS[2] was used to run the standards and samples. The machine was set for a washout of 1minute, three sampling runs per sample of 1 minute each and an uptake of 1 minute. The samples were analysed in numerical order and their order was inputted into the computer before analysis was started.

The following pattern of sampling was followed:-

Blank 1, standards 1a to 5, millipore water, 7 samples, blank 2, 7 samples, standard 3, 8 samples, blank 1, wash, 8 samples, standard 3, blank 2, 12 samples, blank, standard 2, standard 3, 1 sample. After every sampling run, the tube was replaced in the procedural blank and 1 minute wash- out was run.

A main run of peak hopping was used with 50 sweeps and dwell time of 10,000 using 3 channels per mass, an acquisition duration of 27.00s and channel spacing of 0.02.

LA-ICP-MS

The blocks were removed from the desiccator as required and medicinal gloves were worn during handling of the blocks. The blocks were ablated in numerical order and the order was entered into the software spreadsheets before commencing ablation. A Perkin Elmer ICP-MS laser ablation was used, *figure 4.2.3.i.*

[2] VG PQ ExCell ICP-MS instrument (Thermo Electron, Winsford, Cheshire)
Standard nickel sample (1.0 mm diameter orifice) and skimmer (0.7 mm diameter orifice) cones Conikal (Glass Expansion, Hawthorn, Victoria, Australia) concentric quartz nebuliser, silica impact bead spray chamber, cooled to 5°C by a Peltier cooler, and standard torch (both from Thermo Electron)
Plasma parameters :- RF power/W 1350 ; argon flows/ L min-1: cool 12.6 auxiliary 0.8 nebuliser 1.10
Acquisition parameters :- 50 scans -; 3 channels per mass , 3 replicates

Figure 4.2.3.i Perkin Elmer LA-ICP-MS

Two computers controlled the LA-ICP-MS and it was essential that the computer controlling the laser was on BYPASS before the door was opened. The block was rapidly, (to ensure no air enters the system) mounted on the positioning block, which was then replaced inside the chamber and the door closed. The light was adjusted to ring light and the system switched to purge. The block was positioned and focused and the depth of the hoof wall was measured using the scale within the laser chamber .The raster was positioned at 50% of the hoof wall depth along the common anatomical edge *plate 2.2.3.i* and *figure 4.2.3.ii.*

The laser computer was put on line, the experiment button and run were activated and the continue button on the software computer was clicked. The software computer notified the user when the data had been collected and the abort button on the laser computer was then activated. The block was removed and a mounted block immediately replaced the one just ablated and the procedure was repeated. The environment was climate and dust controlled to minimise any surface contamination.

Results were reported in ppm and counts per second and were transferred to an excel spreadsheet for further analysis. Ablation variation was normalised by dividing the results of each trace element in ppm by the amount of carbon in ppm.

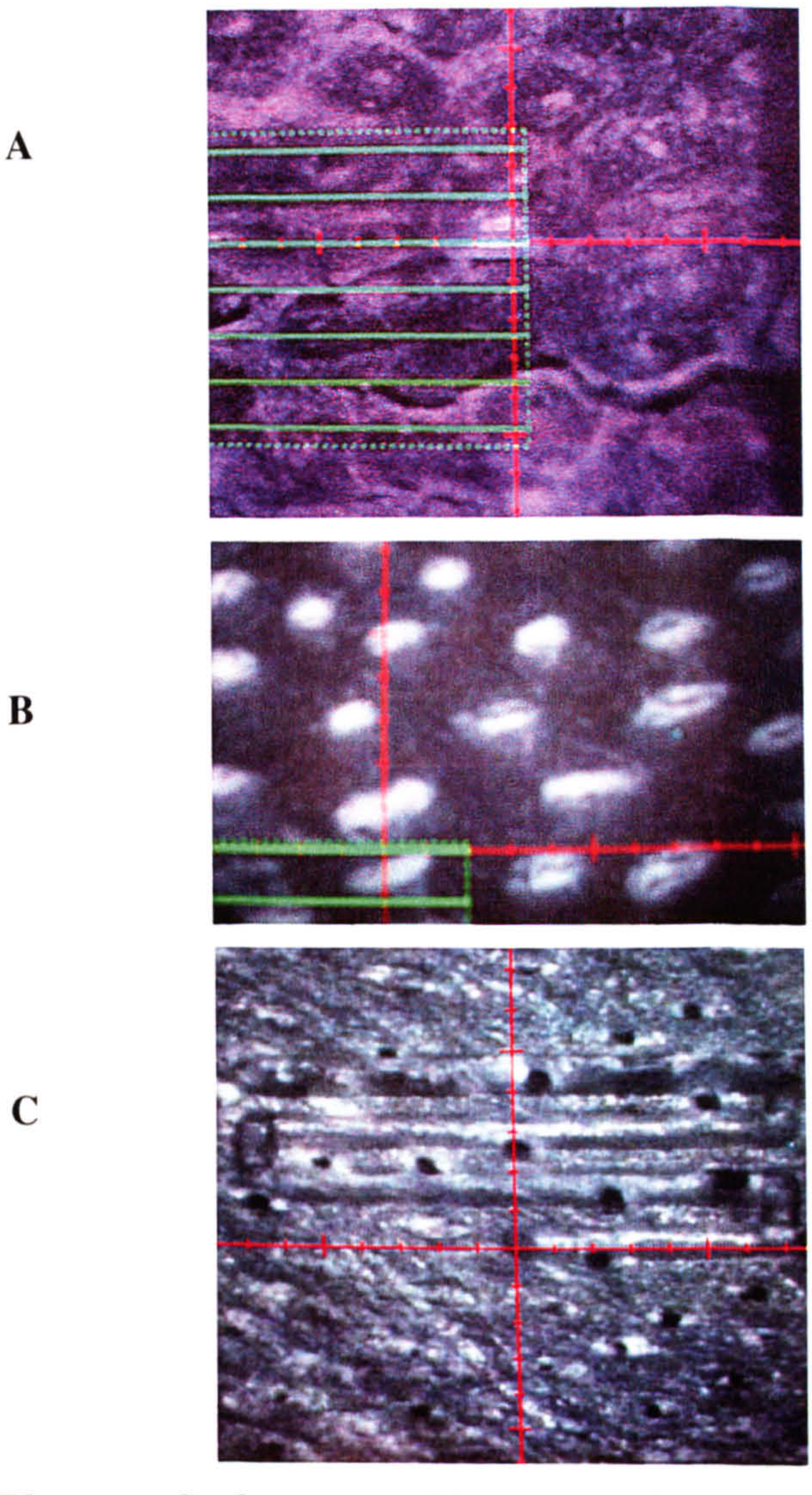


Figure 4.2.3.ii Photograph of raster positioning on a block of hoof wall
Note 1 Block set up for laser; note the raster pattern depicted by the green lines
Note 2 Rastering starting, the laser closes into the block and tubules become visible once laser is focused
Note 3 Raster pattern from laser left on hoof block surface

4.2.4 Statistical analysis

All statistical analyses were performed using Minitab[13]. Summary data including the mean, standard deviation and range of calcium, zinc and copper of the whole hoof and of the toe, medial and lateral quarters, and where appropriate, for blocks A and B are collated and are presented in appendix page XIX.

Ratios

Whilst it is difficult to equate absolute dietary intakes with absolute tissue concentrations of trace elements; an indication of the ratio in which the trace elements are found within the tissue is likely to be more meaningful. Summary data of the ratios of trace elements to each other calculated from solution work for the 48 feet at toe mdc; for laser work at the toe mdc, medial and lateral quarters and across and down the hoof wall at the toe mdc in 28 left fore feet were collated and presented.

The concentrations of the trace elements were tested for normality by plotting histograms and confirmed using the Anderson Darling Test of normality at a significance level of $p< 0.05$.

All sets of data were subjected to the following tests and analysis:-

Analysis of variance

Data were tested for differences in variances using Levene's F test before any parametric comparative analysis was undertaken.

Paired t tests, (solution results), or the non parametric equivalent, Wilcoxon Signed Rank, (laser results), for paired data were used to investigate whether there were any differences between means of the trace element concentrations of 1.5cm blocks A and B cut from 50% toe mdc, which had undergone different washing techniques measured by ICP-MS or LA-ICP-MS.

The one way analysis of variance test, (ANOVA), (solution results) or the non parametric equivalent, the Kruskal Wallis test, (laser results), were used to investigate differences in trace element concentrations between the quarters and the toe; testing the hypothesis that there was no difference in trace element concentration because sampling was being carried out on material of similar chronological age in the group of 6 hooves. Multiple comparisons were carried out using the Tukey test at a significance level of $p <0.05$ or the Wilcoxon Rank Sum test with a Bonferroni correction made for p (Petrie and Watson, 1999), to reduce the likelihood of type1[f] errors.

The Kruskal Wallis test was used to investigate differences between trace elements in blocks sampled from toe mdc, lateral and medial quarters of 28 left fores. The Kruskal Wallis test was also used to investigate differences between trace elements, (TEppm/Cppm), along the hoof wall width and down the hoof wall height in eleven blocks at a significance level of $p<0.05$, to test the hypothesis that trace element concentration does not vary according to age or anatomical position. The Wilcoxon Rank Sum test was used for any multiple comparisons at a $p <0.05$, using a Bonferroni correction.

[f] type 1 error is when the likelihood of finding a significant result is increased due to repeated testing thus increasing the odds from 1 in 20 to x in 20 depending upon the number of multiple comparisons carried out.

Correlations

Between the two analytical techniques within 6 hooves

Scatter plots were used to investigate the relationship between trace elements analysed by ICP-MS and ICP-Laser ablation. In addition, graphs were plotted of the three techniques, once the axis had been adjusted to take into account the large scalar discrepancies *table 4.2.3.i.*

Table 4.2.3.i Adjustment of scales to improve graphical representation of two methods of analysing hooves

Graph	adjustment to axis
Zn M/4A	TEppm/Cppm X E – 07
Zn mdc A	TEppm/Cppm X E + 01
Ca L/4B	TEppm/Cppm X E – 06

Between the two analytical techniques within 28 left fores

Scatter plots and the Spearman's Rank Correlation test were used to investigate the relationship between the two methods of analysis.

Between ratios of trace element concentrations in both data sets

Spearman's Rank Correlations between the ratios of trace elements measured at the toe mdc by laser and solution in 48 feet were investigated as the use of ratios removes units of measurement and may give a better indication of correlations between the two methods of analysis.

Between trace elements within both groups of hooves

Spearman's Rank Correlation coefficients between the trace elements within both groups of hooves were calculated as it is highly likely that the ratio of trace elements to each other within the epidermis is more relevant than absolute values due to the huge individual variation.

4.3 Results

4.3.1 Descriptive summaries

The mean zinc concentration in the data set of six hooves was 165.0 ±53.0 mg/kgDM with a range between 120.0-430.0 mg/kg DM and in the data set of 48 feet, 160.4 ± 54.7 mg/kgDM. Mean calcium concentration in the data set of 6 hooves was 429.4 ± 110.6 mg/kgDM with a range of 260.0-740.0mg/kgDM and in the 48 data set of hooves 674.9 ± 434.3 mg/kgDM. The mean copper concentration was 2.9 ± 6.4mg/kgDM ranging between 3.3 - 26.6mg/kgDM but in the data set of 48 hooves the mean copper was 22.8 ± 5.6mg/kgDM, a factor of ten greater than the first data set, *figure 4.3.1.i.*. The full set of descriptive statistics for the six hooves used to test the washing techniques are summarised in the appendix in *table 4.3.1A.i* – ii and the full set of descriptive results for lasered blocks and solution of the data set of 48 hooves in *table 4.3.1A.iii* page XXI.

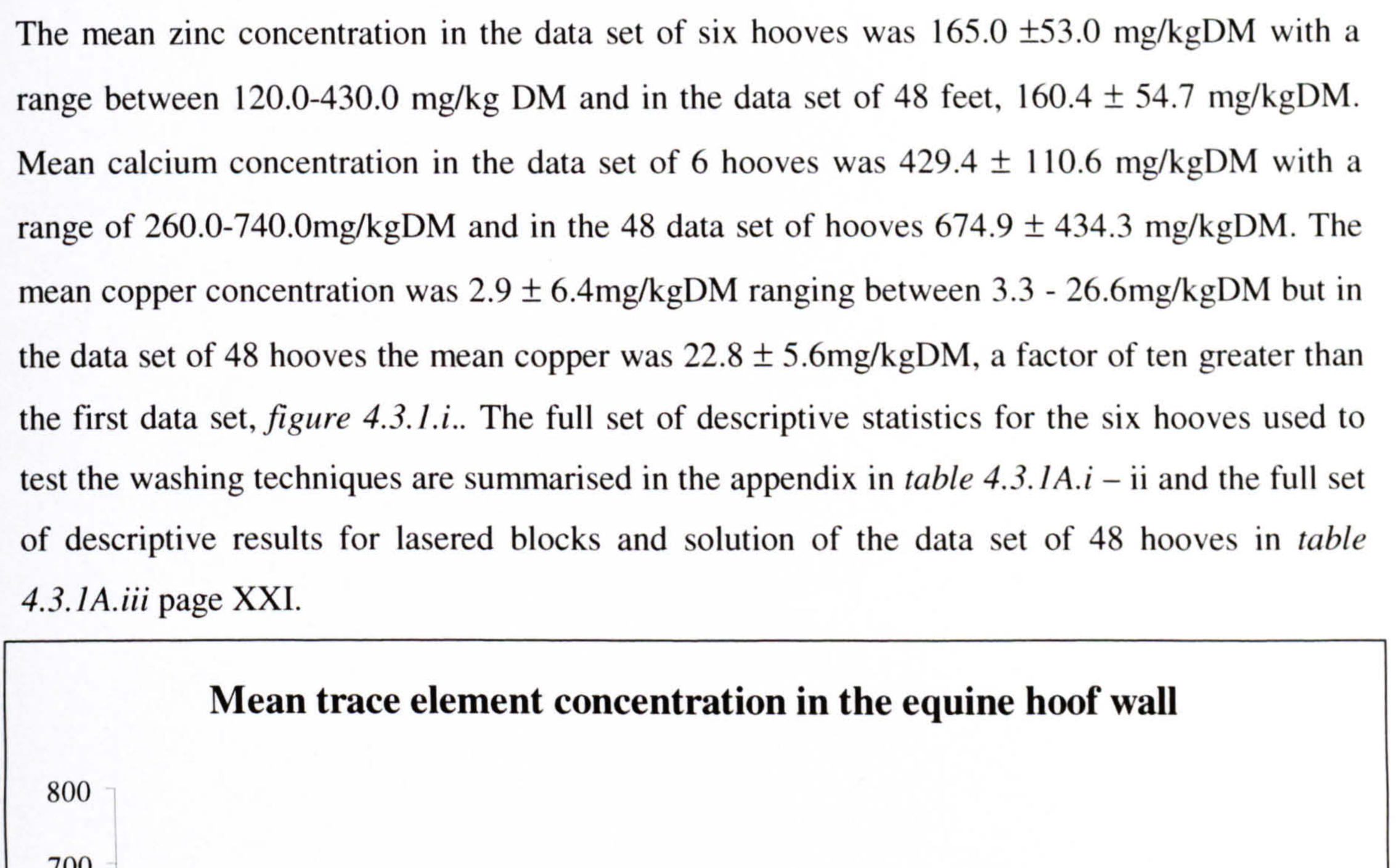


Figure 4.3.1.i Graphical summary of mean zinc, copper and calcium measured in the equine hoof wall

Laser results

The laser results from the six hooves were qualitative and the medians are reported in the appendix in *table 4.3.1.A.i,* page xix. Qualitative trace element concentration measured in the 28 left fores at the toe mdc and the medial and lateral quarters, expressed as concentration of trace element in ppm divided by carbon in ppm ([TE] ppm/C/ppm) to standardised amounts of material being ablative, are summarised in *table 4.3.1A.iv* page XX and *figure 4.3.1.ii*.

Qualitative trace elements in equine hoof wall analysed by LA-ICP-MS

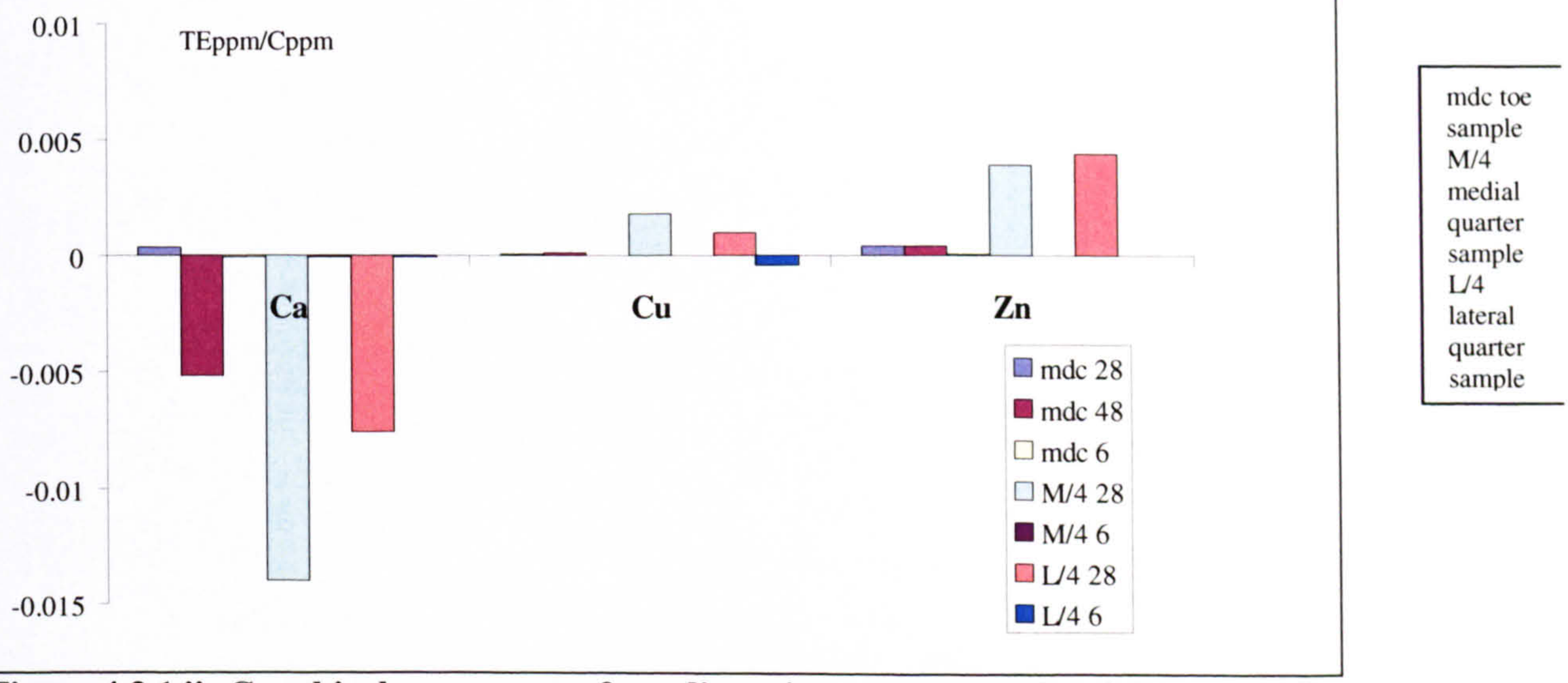


Figure 4.3.1.ii Graphical summary of median zinc, copper and calcium measured at different anatomical positions with LA-ICP-MS

Medians of [TE] ppm/Cppm ablated across the hoof wall depth and proximo-dorsally down the hoof wall are summarised in *tables 4.3.1A.v* and *4.3.1A.vi* and medians of the means of TEppm/Cppm ablated from across the hoof wall, down the hoof wall or means of the whole block are summarised in *table 4.3.1A.vii,(*page XXII). The validity of these results is raised in the discussion.

4.3.2 Comparisons between washing techniques

The differences in variances were not statistically significant, $p>0.05$. The mean zinc and mean copper (mg/kgDM hoof) did not vary significantly between the two washes at any anatomical position ($p>0.05$), *table 4.3.1Aii*.page XX Calcium did not differ significantly between the

washes on material sampled from the toe mdc and the medial quarter; however there was a significant difference between the mean calcium (mg/kgDM hoof) in the lateral quarters, ($p<0.05$) with the calcium in the block washed with millipore water having nearly 45% more calcium, *figure 4.3.2.i.*

Laser

There were no significant differences in zinc and copper, ($p>0.05$) between the two washes in material sampled from the toe mdc and the medial quarter, nor in calcium in blocks from the toe mdc and the medial quarter, ($p>0.05$); there was however a significant difference in the calcium measured in blocks A and B from the lateral quarter, ($p<0.05$), *table 4.3.1A.i.*

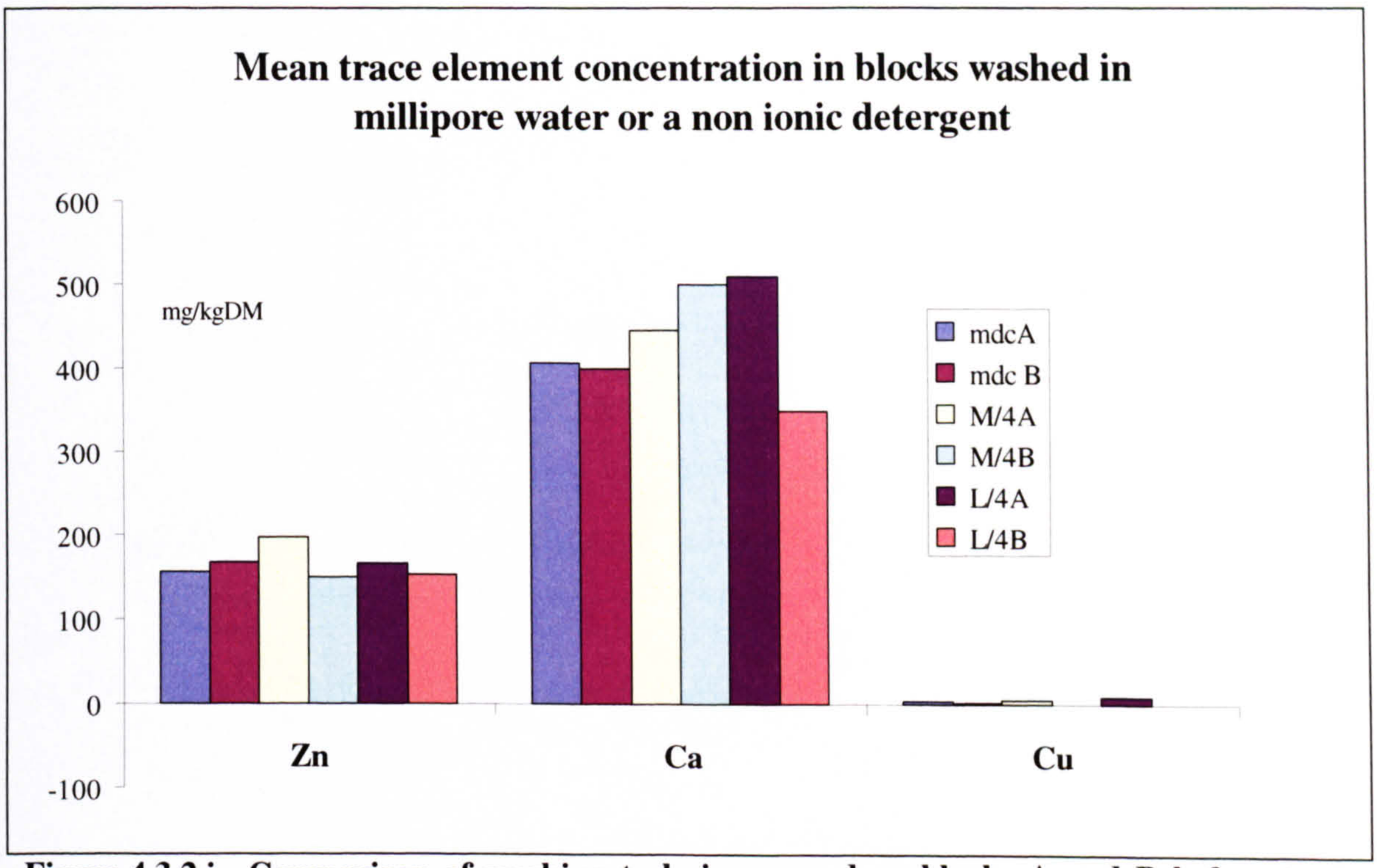


Figure 4.3.2.i Comparison of washing techniques used on blocks A and B before trace element analysis on hoof wall blocks.

Note 1 mdc toe sample, L/4 lateral quarter sample, M/4 medial quarter sample. Blocks A millipore water, blocks B detergent

4.3.3 Effect of anatomical position on trace element concentration

Trace element concentration taken from material of the same chronological age from 6 hooves

The mean concentration of zinc calcium and copper were highest at the medial quarter. The toe mdc had the lowest concentration of all the trace elements; however none of the differences

were significant, ($p>0.05$). The trace element concentration analysed by ICP-MS solution, (mg/kgDM), did not vary between anatomical sampling positions, *figure 4.3.3.i*. There were no significant differences between the anatomical positions in any median trace element recorded in ppm/C ppm by laser ablation.

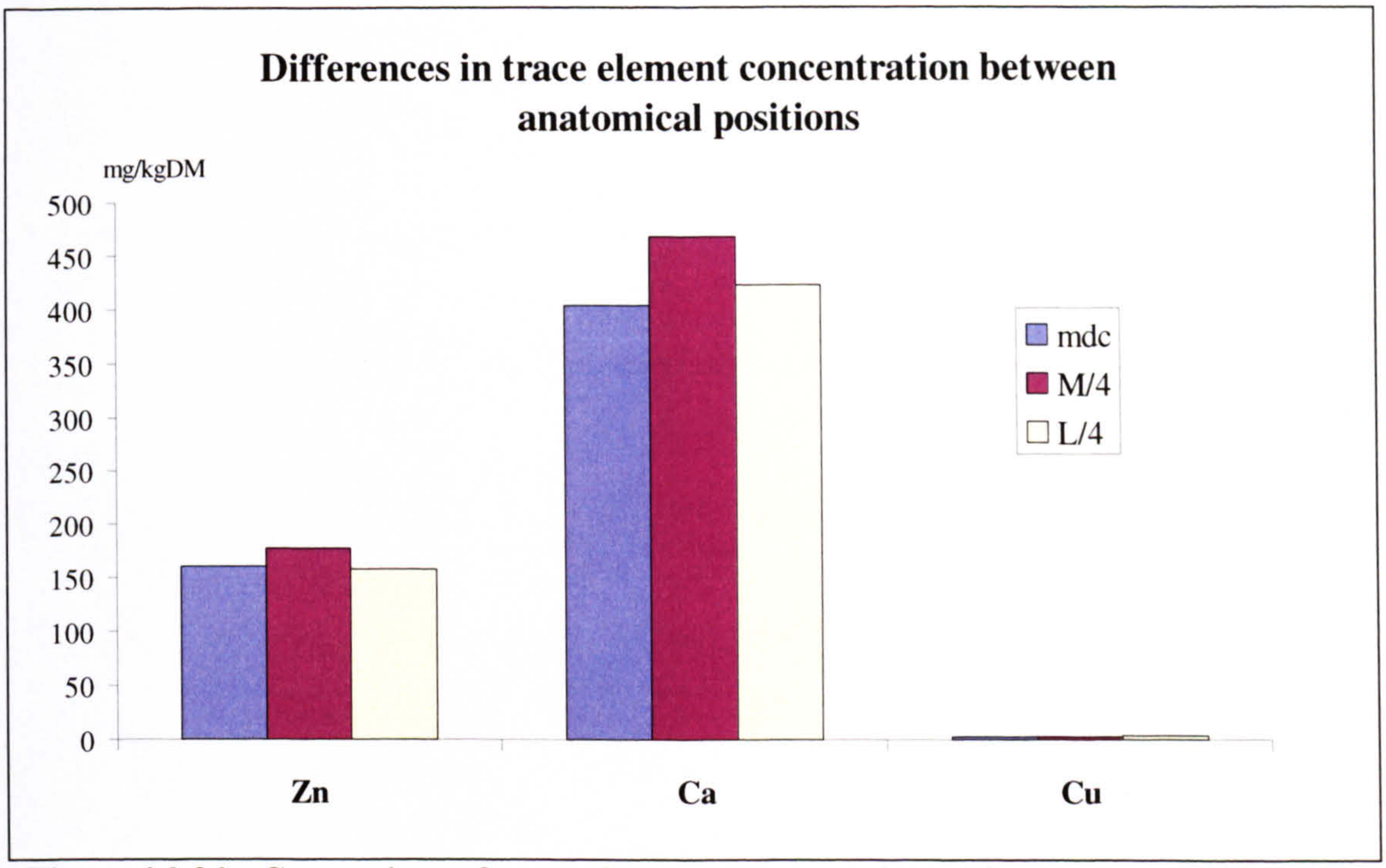


Figure 4.3.3.i Comparison of trace element concentration with anatomical position taken from material standardised for chronological age

Note 1 data set of 48 hooves, measured in mg/kgDM
Note 2 mdc toe sample, M/4 medial quarter sample, L/4 lateral quarter sample

Differences between median TEppm/Cpppm between mdc, M/4 and L/4s of 28 left fores

There were significant differences in the qualitative measurements of trace elements between anatomical positions, $p<0.05$. The lateral quarter Ca ppm/Cppm and Zn ppm/Cppm were significantly different to the medial quarter and toe mdc blocks. There were significant differences between the Cu ppm/Cppm measured at all anatomical positions $p<0.05$ *figure 4.3.3.ii, table 4.3.1A.iv*.

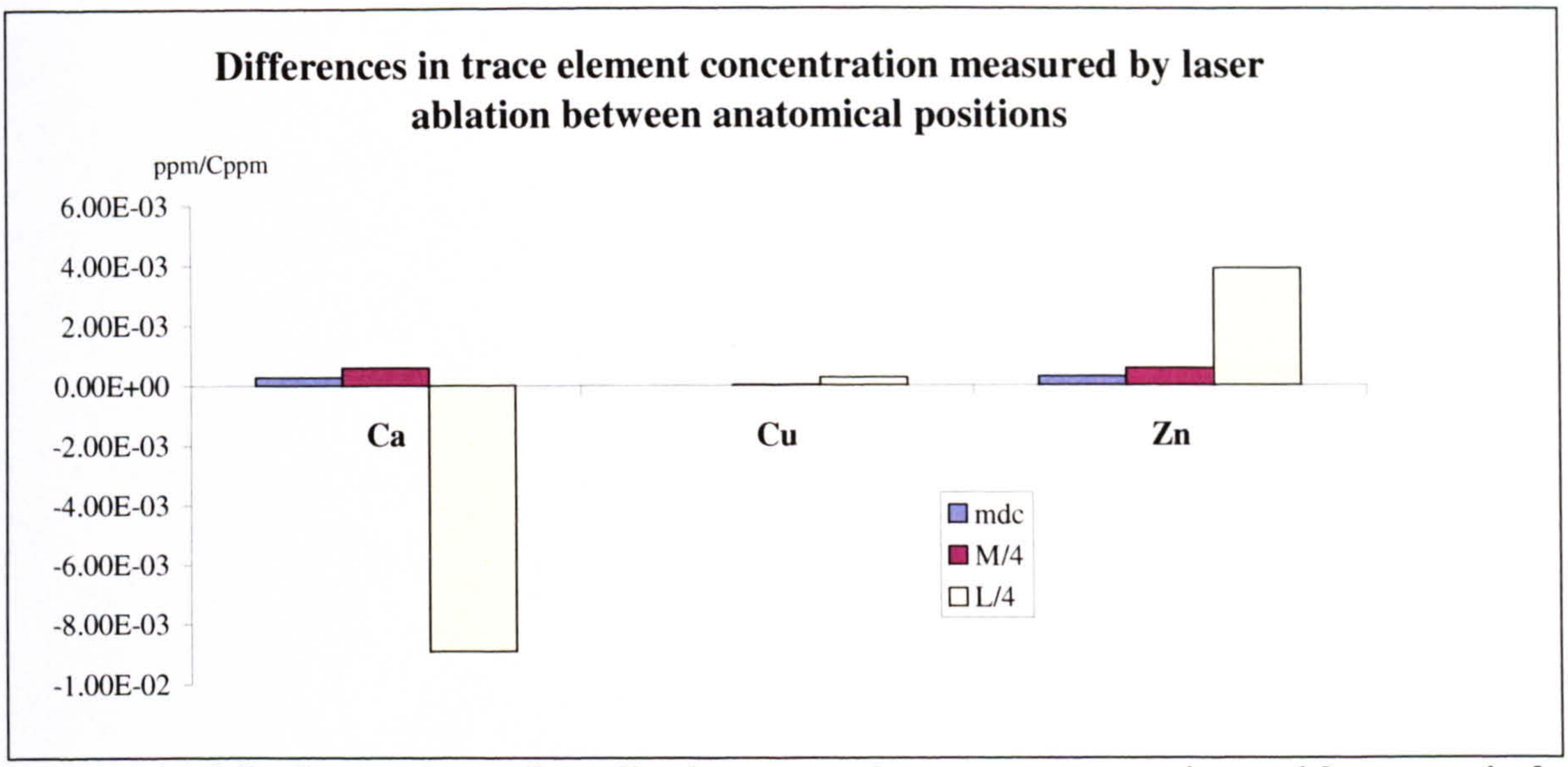


Figure 4.3.3.ii Comparison of qualitative trace element concentrations with anatomical position from material standardised for chronological age

Note 1 data set of 28left fores, analysed by LA-ICP-MS, Note 2 mdc toe sample, M/4 medial quarter sample, L/4 lateral quarter

The results obtained and shown in graphs 4.3.1.ii and 4.3.3.ii are an artefact of the analytical technique used. The reasons for the aberrant results are discussed in more detail in the discussion and these results do not detract from the validity of the results expressed in graphs 4.3.1.i, 4.3.2.i, 4.3.3.i, 4.3.4.i, 4.3.4.ii nor 4.3.5.i.

4.3.4 Effect of chronological age on trace element concentration

Differences between medians of TEppm/Cppm ablated from blocks 2-5 taken proximally/distally down the length of the toe mdc

The concentration of both calcium and zinc, measured as ppm/Cppm varied down the length of the toe mdc and the differences were significant, ($p<0.05$). Calcium was significantly greater at 50% hoof wall height compared to the block nearest the coronary band; zinc measured closest to the coronary band was significantly less to that measured anywhere else down the toe mdc. Copper measured as ppm/Cppm did not vary significantly down the length of the toe, *figure 4.3.4.i and table 4.3.1A.v*. However all trace elements were at their lowest comparative concentrations in the block closest to the coronary band.

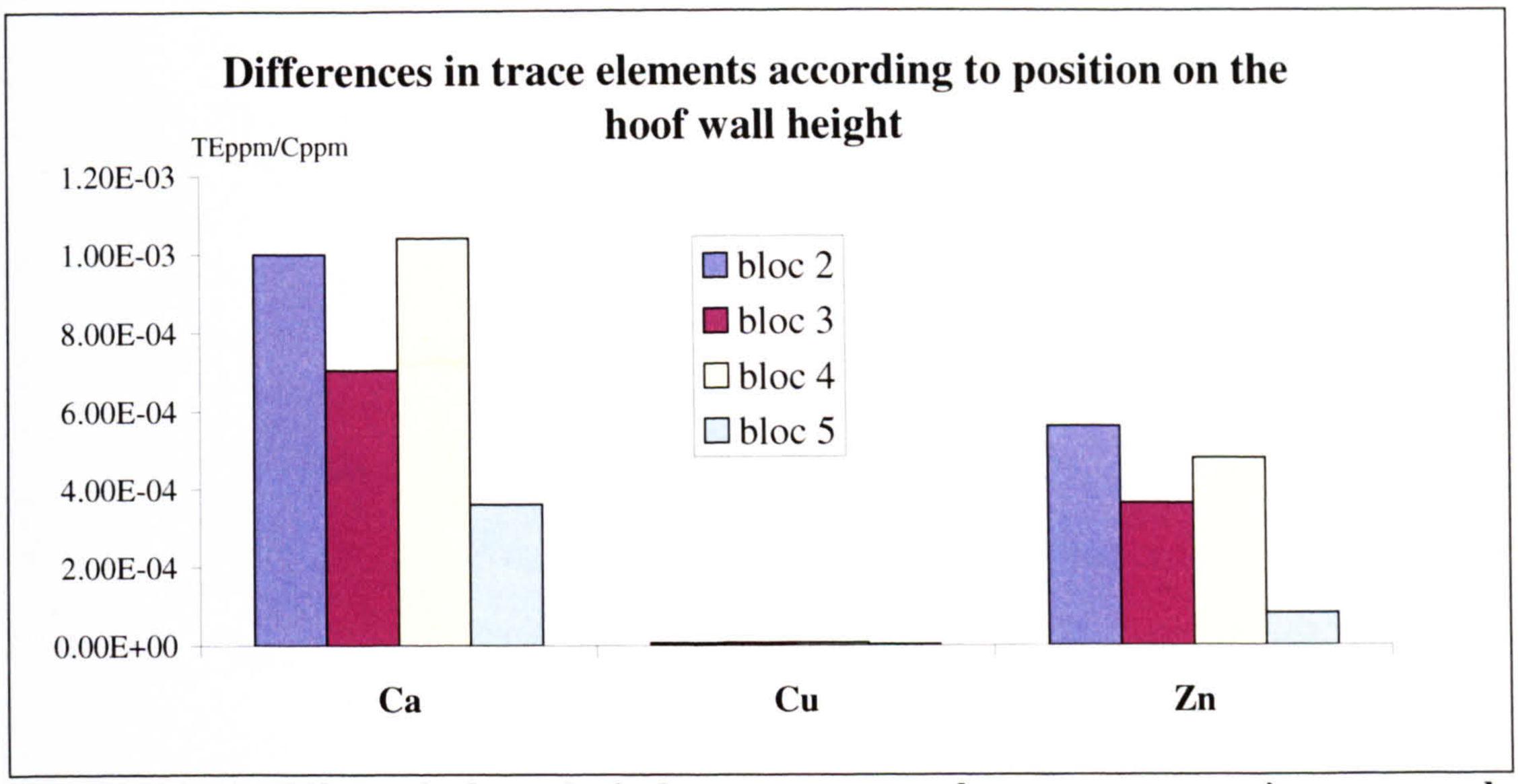


Figure 4.3.4.i Effect of chronological age on trace element concentration measured proximo-distally along the hoof wall height

Note 1 bloc 5 closest to coronary band
Note 2 bloc 3 closest to bearing border
Note 3 bloc 2 50% HWH
Note 4 bloc 4 proximal to bloc 2

Differences in median TEppm/Cppm at 12.5%, 37.5%, 62.5% and 87.5% of hoof wall depth across block 2

Trace elements varied across the hoof wall depth as measured by LA-ICP-MS. Trace element concentration decreased from 87.5% hoof wall depth to 12.5% hoof wall depth, however the significant difference in concentration between the depths varied. The differences, ($p<0.05$) between calcium ppm/Cppm measured at 87.5% HWD (inner wall, closest to the dermis) and 62.5%, 37.5% and 12.5% was significant compared to the amounts measured at the other depths. The difference between the amount of copper ppm /Cppm and zinc ppm/Cppm, between 12.5% HWD (the outer wall) and all other depths was significantly lower ($p<0.05$) compared to the differences between the other depths *figure 4.3.4.ii*.and *table 4.3.1A.vi*.

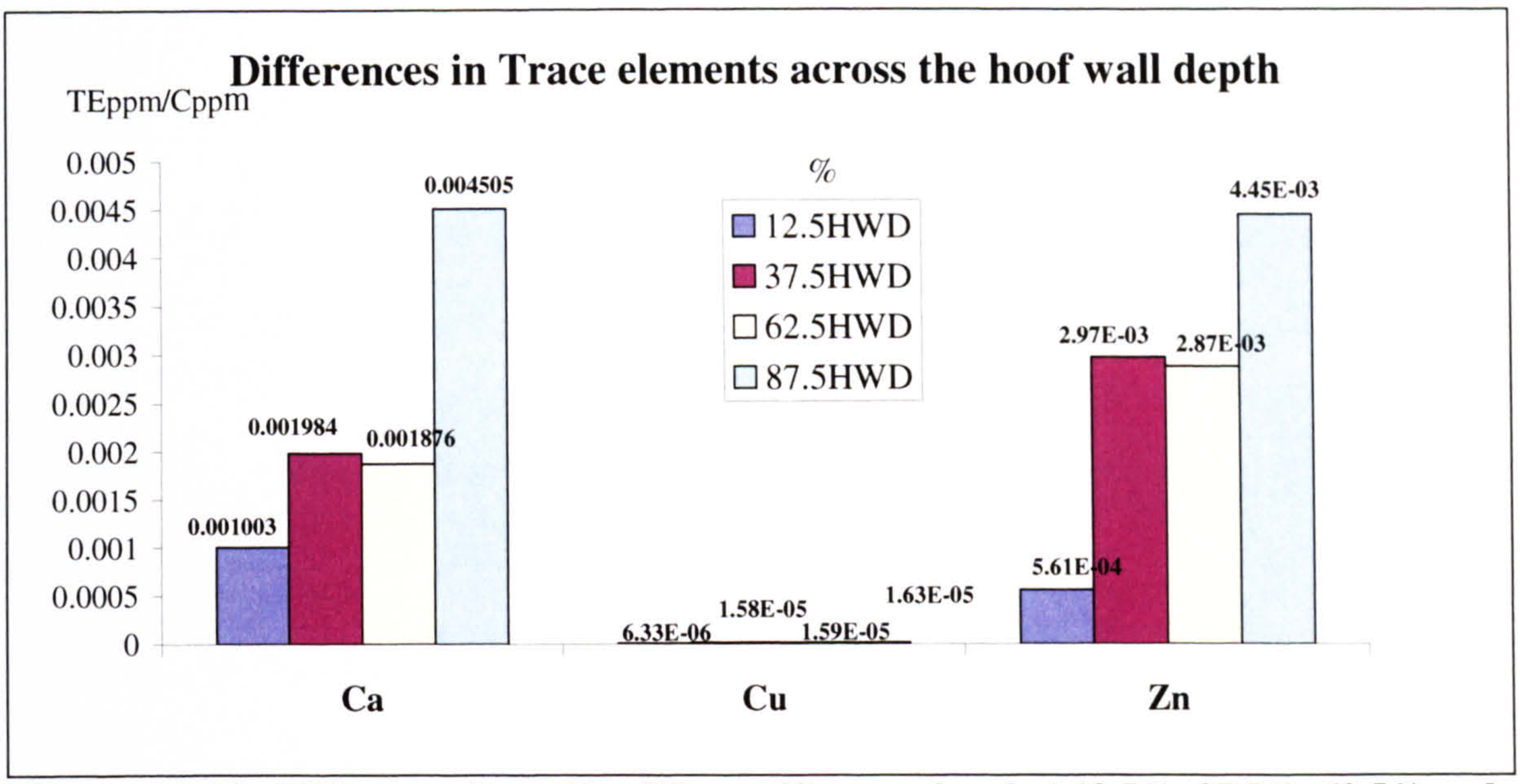


Figure 4.3.4.ii Differences in median TE ppm/Cpm analysed at 12.5%, 37.5%, 62.5% and 87.5% HWD

4.3.5 Effect of laser scanning position on trace element measurement

Comparisons between mdc median TE/C from one measurement at 12.5% HWD, mean HWD and mean HWH

There are significant differences in the median TE/C depending on whether the mdc is sampled at one position or a mean of HWH or HWD is used.

Similar qualitative results ($p>0.05$) were obtained for TE ppm/Cppm when ablation results from 12.5% HWD and the mean HWH (mean of all the ablations from the different hoof wall heights) were compared. The results from ablations across the hoof wall depth were averaged, (similar to taking a full hoof wall block sample for solution work) to obtain one value for the hoof block. This result was compared to ablation from either the average of all the ablations up the hoof wall height (HWH) or with the single value at 12.5% hoof wall depth and the values varied significantly for all the TE ppm/Cppm, ($p<0.05$), *figure 4.3.5.i.* and *table 4.3.1A.vii.*

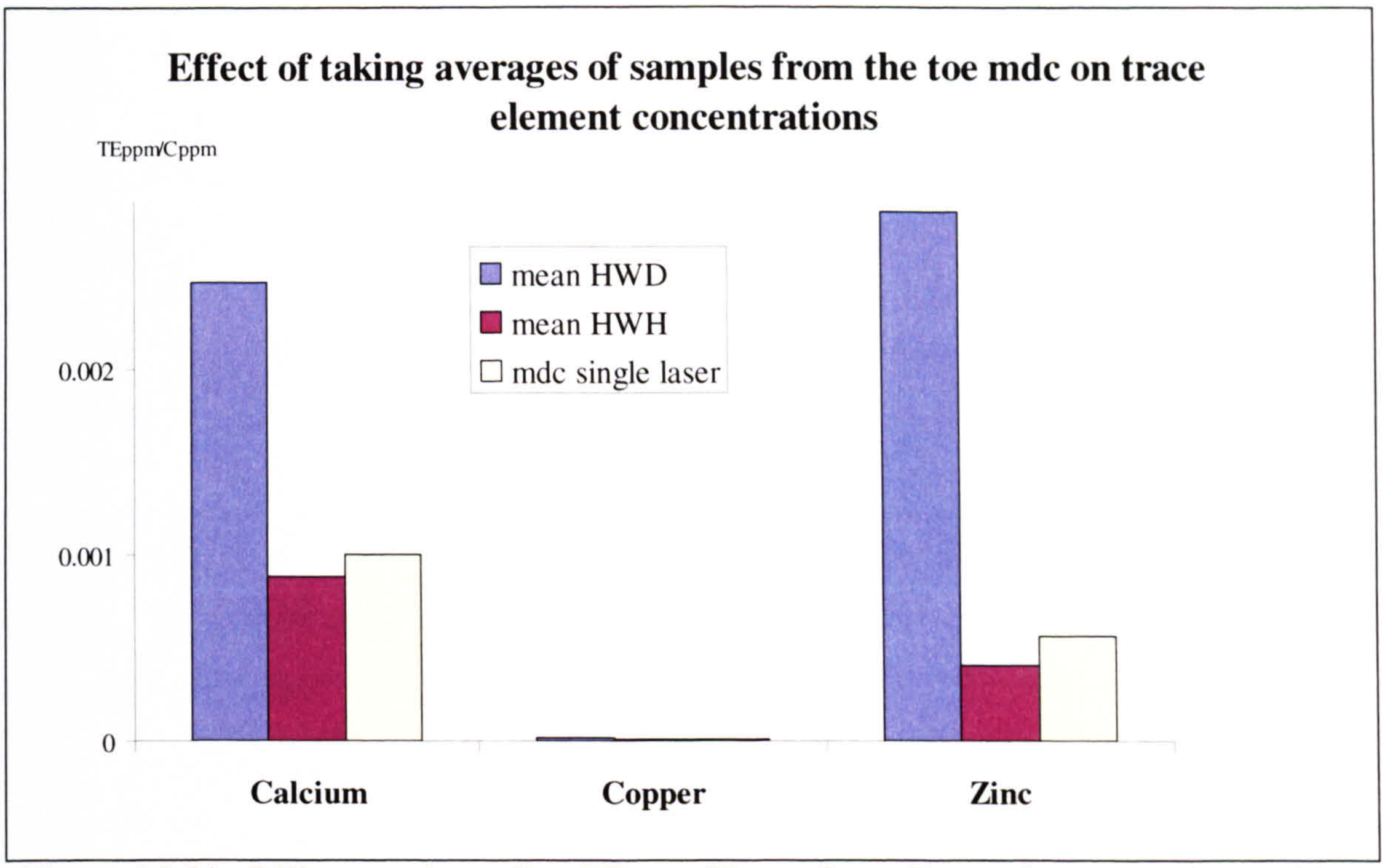
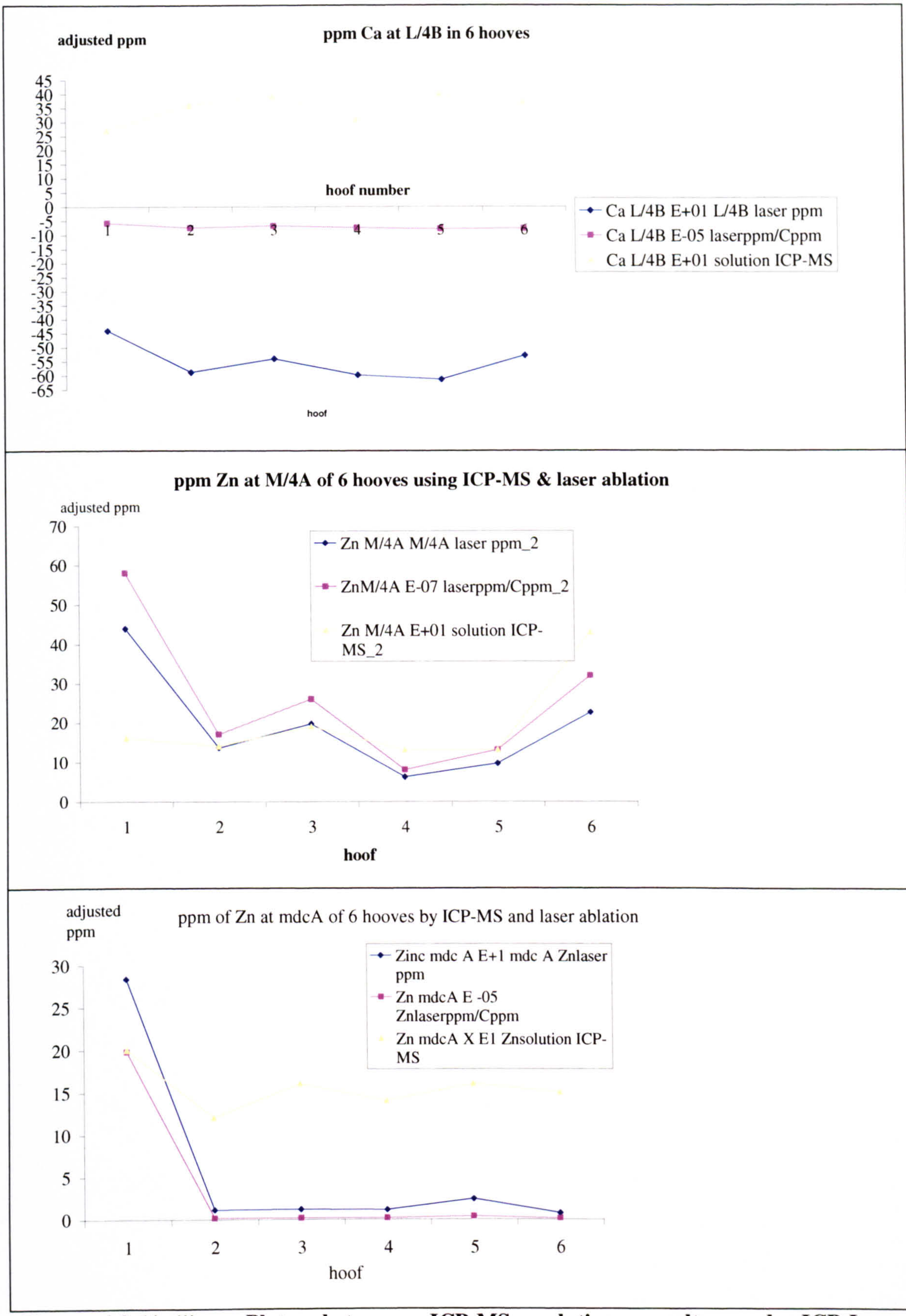


Figure 4.3.5.i Differences in median TE ppm/Cppm depending on laser scanning position
Note 1 HWD hoof wall depth , mean of the 4 readings across the block, HWH hoof wall height, mean of the 4 readings down block

4.3.6 Correlations between ICP-MS solution and ICP-MS Laser ablation

Correlations between the two techniques in 6 hooves

The scatter plots (*figure 4.3.6A.i*) indicated that there was little relationship between the two methods of analysis. However the graphs normalised by their axis, indicated that some of the results may follow the same pattern, *figures 4.3.6.i to 4.3.6.iii.* In addition the calibration between solution and laser in ppm for zinc at the mdc was significant at $p<0.05$ with a R^2 of 0.702, so the calibration was accepted as satisfactory for comparative purposes but limited for precise comparisons. None of the other correlations were significant with $p>0.05$ and R^2 as low as 0.01. Although these results indicated that there was unlikely to be a relationship between the two methods, it was felt appropriate to investigate further by using the results from the six blocks as 'check standards' to train the machine for the next set of hooves and at the same time control as many analytical variables as possible.



Figures 4.3.6.i –iii Plots between ICP-MS solution results and ICP-Laser TEppm/Cppm

Correlations between the two techniques in 48 feet

Despite controlling as many analytical variables as possible, there were no significant correlations between the two techniques, ($p>0.05$). There were no correlations between the two techniques when the ratios between the trace elements were compared, ($p>0.05$).

4.3.7 Correlations between trace elements

Zinc and calcium were correlated within both sets of hooves, *table 4.3.7.i.*

Table 4.3.7.i Correlations between zinc and calcium at different anatomical positions using different analytical techniques

correlation of zinc and calcium						
hoof group analyte	6 feet mg/kgDM	48 feet mg/kgDM	48 feet TEppm/ppm	mdc 28 feet TEppm/Cppm	M/4 28 feet TEppm/Cppm	mean 28 feet TEppm/Cppm
R Spearman's correlation coefficient p value	0.877 0.0001	0.637; 0.0001	–0.556; 0.0001	–0.454; 0.015	–0.997; 0.0001	0.977; 0.0001

Correlations of the other trace elements at the different anatomical positions are summarised in the appendix *table 4.3.7A.i.*

4.3.8 Ratios between trace element concentrations

The calculated ratios of trace elements from the toe mdc of 48 feet analysed by laser and solution and the ratios of trace elements at the toe mdc, the medial and lateral quarters as sampled by laser in 28 left fore feet are summarised in *tables 4.3.8.i-iii.*

Table 4.3.8.i Descriptive summary of trace elements ratios in material sampled from 48 mixed feet by ICP-MS

TE: TE	ratio	TE : TE	ratio
	median and *inter-quartile range*		
Cu : Ca	30.5 *26.9- 42.0*	Ca : Cu	0.03 *0.02-0.04*
Cu : Zn	7.3 *5.8-9.3*	Zn : Cu	0.14 *0.11-0.17*
Zn : Ca	4.2 *3.4-5.5*	Ca : Zn	0.24 *0.18-0.30*

Note 1 TEs were analysed in mg/kgDM hoof
Note 2 all analysis was carried out by ICP-MS solution

Table 4.3.8.ii Summary of the mean and median ratios of trace elements in material sampled from 48 mixed feet by ICP-Laser ablation

ppm/Cppm	ratio medians	ratio range	ppm/Cppm	ratio medians	ratio range
Cu/C : Ca/C	–64.3	–233.7–– 12.9	Ca/C : Cu/C	– 0.0	–0.0-0.0
Cu/C : Zn/C	10.2	4.0- 18.6	Zn/C : Cu/C	0.1	0.0-0.25
Zn/C : Ca/C	–7.5	– 24.2 - – 1.9	Ca/C : Zn/C	– 0.0	– 0.2 - – 0.0

Table 4.3.8.iii Descriptive summary of the ratios of trace elements in material sampled from 28 left fore feet by ICP-Laser ablation

	toe mdc				
ppm/Cppm	ratio median	ratio range	ppm/Cppm	ratio median	ratio range
Cu/C : Ca/C	24.0	–8.3-122.8	Ca/C : Cu/C	0.0	–0.0-0.0
Cu/C : Zn/C	27.3	18.8-55.2	Zn/C : Cu/C	6896.0	3192.0-20497.0
Zn/C : Ca/C	0.93	–0.56-2.2	Ca/C : Zn/C	0.4	–0.4-0.9
	medial quarter				
ppm/Cppm	ratio median	ratio range	ppm/Cppm	ratio median	ratio range
Cu/C : Ca/C	21.2	3.5-73.4	Ca/C : Cu/C	0.0	0.0-0.1
Cu/C : Zn/C	16.5	4.3-54.5	Zn/C : Cu/C	0.0	0.0-0.2
Zn/C : Ca/C	0.8	0.2-3.9	Ca/C : Zn/C	0.7	0.1-1.5
	lateral quarter				
ppm/Cppm	ratio median	ratio range	ppm/Cppm	ratio median	ratio range
Cu/C : Ca/C	– 11.5	– 80.6-0.0	Ca/C : Cu/C	–0.0	– 0.1-0.0
Cu/C : Zn/C	15.3	4.4-26.2	Zn/C : Cu/C	0.0	0.0-0.2
Zn/C : Ca/C	–2.19	– 3.2- – 0.0	Ca/C : Zn/C	–0.3	– 0.5-0.4

4.4 Discussion

Several techniques have been developed or adapted to improve standardisation of the protocols used for collection of hoof wall material for trace element analysis.

4.4.1 Methods for sampling material

Full hoof wall blocks were used to collect material for ICP-MS solution work to compare differences in trace element concentration between anatomical positions. Whilst the blocks had been taken from the same growth line, material of different chronological age was still included as samples were taken from the full hoof wall depth. But by including the full block it is proposed that the effect of diet would be consistent within a hoof because the block was cut perpendicular to the tubules, therefore the material closest to the inner wall at the quarter should be the same age as comparable material at the mdc.

Dietary influence is an important factor which is considered when harvesting hair for analysis. Strain *et al.* (1971) using radioactive zinc illustrated the importance of standardising collection techniques by following the uptake of zinc in hair follicles by sampling plucked and shaved hair at time intervals after the zinc had been injected. The collection was only undertaken for twenty four hours during which time the concentration in clipped hair was lower than in the plucked hair, the concentration in the hair follicle, (plucked hair) showed a surge of concentration of various injected radioactive elements and then a fall, which the researchers took to mean that the hair levels were reflecting the rise and fall in the blood of the elements after injection. If keratinisation of hoof reflects a similar pattern, then dietary intake of trace elements could vary considerably over twenty four hours, particularly if the horse is out at grass, which generally has high levels of trace elements and then is fed hay at night which is generally extremely low in trace elements. If this is the case then it is unlikely that sampling a full hoof wall depth will be sensitive enough to the changes.

Hair (Strain *et al.* 1971) and hoof are considered inert tissues as once cornified, all metabolic activity ceases. Thus it might be considered that trace element concentration stays similarly inert. In hair, however, it has been shown that whilst some trace elements such as zinc and copper stay constant throughout the length of a strand, others such as calcium significantly increase in concentration as a function of distance from the root, (Sky-Peck 1990). It was suggested that the differences were due to environmental exposure and the mild washing technique had not removed the external contamination of calcium. Sky-Peck, (1990) did not consider changes in dietary intake to be a contributing factor, yet research has shown that hair does reflect the history of copper deficiency; as copper deficiency develops, younger cells contain less copper than the older cells, (Suttle 1983). However, the copper concentration in the wool root is six times more concentrated than that analysed in the keratinised follicle; therefore only a small amount of the total copper remains in keratinised tissues, (Ward 1954). Consequently advice to standardise the collection of hair for analysis is that it should be differentiated by cutting into segments, or at the least by matching material of similar chronological age. This advice is based on using the epidermal tissue as an indication of current nutrient intakes and therefore the youngest tissue needs harvesting to ensure the relationships are relevant.

In view of the qualitative differences recorded by LA-ICP-MS across the hoof wall block in this thesis, it is recommended that a further standardisation should be investigated in future work on the effect of diet on the concentration and distribution of trace elements. Sampling should be refined so that the blocks are cut longitudinally, proximo-distally. The most appropriate place to make the divisions should be investigated but this thesis recommends dividing the wall at 12.5% hoof wall depth to reflect the change in tubular pattern, (Reilly *et al.* 1996).

Pollitt, (1990) using radioactive sulphur showed differential growth rates between the inner and outer hoof wall, with the inner wall having a 30% faster rate compared to the outer wall. It would be interesting to carry out an intra hoof wall map of trace element distribution. Initially an electron microscope map could be done across the wall. It would be important to ensure that all microscope work was carried out at the same magnification and at measured distances across the block. This is achievable and an example of some preliminary investigation is included in the appendix, *plate 4.4.1A.i* to illustrate how blocks can be marked to ensure repeatability. Full hoof wall blocks should be divided at the same distances mapped by the SEM and samples taken for ICP-MS; results could then be compared.

Alternatively, if the relationship between trace elements and mechanical properties are to be investigated, dietary intake, and the distribution of trace elements within specific areas of the

hoof might not be relevant relationships to study. The hoof material is a composite and although the majority of cracks on the wall were found in this thesis to be between 0.5cm and 0.75cm deep, it is likely that the full hoof wall depth of trace elements contributes to its integrity, not just the first 12.5% of its depth. This lends support to continue to sample from the whole of the hoof wall depth as long as the area is standardised for chronological similarity within and between hoof and within and between anatomical positions. This would facilitate the use of clippings in future work, as long as the variation due to external contamination is not considered a major issue. If clippings are to be used to investigate comparisons between trace element concentration and mechanical properties, note must be taken that the material at the quarters is not comparable in chronological age to the toe and therefore might vary in trace element concentration due to dietary influences.

4.4.2 The use of washing techniques in trace elements analysis

Earlier researchers in hoof used a defined washing technique for a standardised time period, (Kovacs and Szilagy 1973), which appeared to be subsequently ignored by most other researchers in hoof. This thesis did not find any difference in results between the washing techniques investigated and this is in agreement with others (Sky-Peck 1990) who compared the effect of washing hair with a mild detergent followed by distilled water to unwashed hair samples on trace element analysis. Sky-Peck, (1990) was using X-ray fluorescence, which is non destructive, so was able to analyse the same samples of hair before and after washing. In this thesis the techniques were destructive, so a similar comparison could not be carried out at the same time that the two washing techniques were being compared.

Complete non conformity in washing techniques may be contributing to the large range of results are reported within the equine hoof, the ruminant claw, human hair and skin, *(table 4.4.2A.i),* alternatively the differences could be valid. It needs to be appreciated that several factors influence trace element concentration in epidermal tissues. For example, age has an effect upon trace element concentrations, with significant differences in hair being recorded between people divided into 3-20yrs and 58-70yrs age groups, (Sky-Peck 1996). Various workers have established differences in trace element concentration between different colour hair, in humans, (Sky-Peck 1996; Chyla and Zyrnicki 2000) and in dogs, (Chyla and Zyrnicki 2000), as well as differences between mane and coat hair in horses, (Krusic *et al.* 1990). These

differences could be masked by different washing techniques as previously discussed, *(section 4.1.2)*.

Researchers working with the epidermal tissues of nail and hair reported a standardised washing technique as early as 1953, (Goldblum *et al*), yet standardised washing techniques still have not been adapted by researchers working in the equine field on keratinised tissue. Of two recent papers, (Dunnett and Lees, 2004; Schlupp *et al.* 2004), only the former reported the washing technique used. Because trace elements are measured in mg/kg or µg/kg the lack of standardisation in the equine and ruminant literature may be contributing to the discrepancies in results and offer some explanation as to why some researchers record relationships with other parameters and others do not.

To substantiate this hypothesis, comparisons of washed with unwashed hoof material should be carried out, to establish not only differences in washing techniques but also differences between washed and unwashed. This needs doing on material split at a common anatomical position as carried out in this thesis.

4.4.3 Comparison between ICP Laser ablation and ICP- MS solution

There was no correlation between the two methods used to measure the trace element concentrations in samples taken from 50% HWH mdc in the data set of 6 and 48 mixed hooves. Care had been taken to ensure that the material used for solution work did not vary significantly from the surface being ablated, as it was originally thought that this would minimise the effect of sampling. One of the reasons why the two techniques did not correlate might be because laser ablation was only carried out at 12.5% hoof wall depth and this was used to compare full hoof block solution results. Qualitative laser results showed significant differences in trace elements across the hoof wall depth. Unfortunately due to budget constraints the facilities to investigate the trace elements in the same material which was lasered across the hoof wall block were not available.

There are also several other reasons for this observed lack of correlation.

1. Hoof has a heterogeneous chemical and textural composition, thus there will be different sample surface and vapourisation characteristics, (Ohatas *et al.* 2002) during laser ablation. It is also recognised that a good spatial resolution for inhomogeneous samples is related to crater depth and diameter, (Liu *et al.* 2001). This study attempted

to normalise for these differences by focusing the laser on the surface of every block individually to compensate for different surface characteristics. In addition the power density was kept constant.

2. Precision and accuracy of laser are less than those from ICP-MS because of the large variation in ablated amounts due to fluctuations in laser power on a shot to shot basis, (Ohata *et al.* 2002). This study attempted to overcome this problem by increasing output energy and fixing the operating time of the laser for 30 seconds. In addition Liu, *et al.* (2002) has shown that a single pulse has poor measurement reproducibility and the amount ablated is extremely small, so a raster pattern of ablation was used, ensuring that the same amount of area was ablated for each hoof.
3. Laser ablation is recognised in the literature as being limited by lack of calibration standards, (Bove- Bigne *et al.* 1999). At present there is no universal method to match standards to sample matrix. During this research certified reference material was used; however being glass it is highly likely that matrix effects, speciation and ablation characteristics would be significantly different to organic hoof, (Stern,B personnel communication). It was hoped that using the original blocks and re-lasering them and then adjusting the machine to recognise the solution results from those blocks would improve the standardisation of the calibration curve. This assumption was based on some work by Boue-Bigne *et al.* (1999) who used aqueous standards whose absorption characteristics were modified by a chromophore. They concluded that 'the data demonstrated that aqueous standards having a modified absorption coefficient can be employed in analysis of solid material by LA-ICP-MS as long as the analytes in the liquid and solid sample behave equally regarding the internal standard'.
4. Due to the fact that it was necessary to use ICP-MS equipment from collaborative institutions, it was difficult to control the operating conditions of the machine. Possible factors that can affect the sampling conditions of the ICP-MS are: variations in plasma ionization efficiency; possible clogging or erosion of cone apertures; differing matrix concentrations in samples that could result in matrix suppression; temperature and humidity fluctuations in the laboratory environment; and formation of molecular species within the sample that may interfere with another element in an unexpected manner. Any one of these variations or conditions can render the accurate analysis of the respective sample difficult or impossible unless certain methods or procedures are employed to minimize the potential for such difficulties.

The first set of results from the main study obtained from the outsourced ICP-MS machine threw up negative results for both copper and sulphur, suggesting possible matrix effects. In

addition the results from the samples fell in the bottom 5% of the calibration curve made from the standards provided. Accuracy of results was therefore suspect. A second set of stock and standards were made up as described in the methods and the analysis repeated to obtain a better calibration line. The samples were then rerun.

To try and minimise accuracy problems, a known standard was put through the machine every 8 hoof samples. When the results were obtained from the software, it became apparent that there was serious drift on the machine as the counts had increased by 66.5% between the start of the sampling and the completion, *table 4.4A.i* p XXIV The day that the analysis was undertaken was extremely hot and the samples were run over a ten hour period in a room without air conditioning; this will have affected the accuracy of the machine. The curves were re calibrated to take this into account using Minitab. This would have undoubtedly have introduced an error as the drift will have been gradual, yet recalibration for the hoof samples is necessarily abrupt and the curve was adjusted after each standard rerun.

1. Among the steps which should have been taken, and that are utilized by chemists are the following: -
    - Incorporation of an external calibration series encompassing the elements to be analyzed. This is designed to cover a range of concentrations that will completely bracket the concentration of analyte in the sample. In the event the sample is found to fall significantly outside the bracketed range, it can then be diluted and run again so that it falls within the desired range.

During the pilot study and initially, the levels of dilution were established to ensure that the elements fell well within the detection limit of the ICP-MS, which is able to detect zinc at 0.01ppb (µg/l), copper at between 0.01 and 0.001ppb and calcium at 1.0ppb. Expected levels in hoof are copper 2-30ppm, zinc 100-200ppm and calcium 500-900ppm; approximately 0.100g of the hoof was used and diluted into 100ml of millipore water. In addition the dilution and detection limits were checked with the operators of the machine to check the appropriateness of the dilutions.

- Internal standards can be incorporated for each sample at known concentrations for the desired element(s) to compensate for any variation in the intensity of the element signal. These internal standards can then be used to correct the measured instrument response to the known concentration. By applying this same correction to other elements in the matrix solution, the correct element concentration can then readily be calculated. This procedure was incorporated into the second sampling run. 0.100g of a hoof block was

dissolved and a known quantity of a standard in increasing increments was added and the total made up to 100ml with millipore water.

- For potentially difficult matrices, the chemists can incorporate the use of spiked samples. This procedure involves the preparation of duplicate sample(s) spiked with each element of interest, which can then be utilized to measure the recovery efficiency of each element so that obvious discrepancies can be determined and investigate in more detail.

It was not possible to use spiked samples as the hoof solutions had already been made up and to introduce a spike at that stage would have decreased the accuracy as the dilutions would have had to be changed and matrix effects might have been introduced.

4.4.3.1 Comparison between ICP-Laser results

It needs to be appreciated that ICP-Laser is an ideal technique for determining the spatial analysis of trace elements over the surface of solid materials. However because of the lack of calibration standards as discussed in 4.4.3 point 3, any comparison is only qualitative. There are some inherent problems in the analysis of solid material specifically instrumental drift, (Dr B Stern, personnel communication) which would account for some of the negative results obtained as these samples were analysed over a period of days. The time period incorporated days in which temperatures were higher than 80°C and the counts of carbon obtained over the period varied considerably which resulted in the aberrant results. However the analysis within blocks both across the hoof wall depth and distally laterally down the hoof wall was carried out on the same day. Carbon counts were consistent and the variation in the material was intra-hoof rather than inter-hoof. Inter hoof variation across a surface is likely to be large because of the complex relationship between an organic matrix and the inorganic trace elements. Some trace elements may simply be adsorbed on the surface although the washing technique should have standardised the variation, other trace elements maybe absorbed into the bulk of the protein matrix and others persorbed by co-valent bonding. Depending upon which aspect of the solid sample is lasered the above may add additional variation which would not be an issue in material which is dissolved before analysis.

4.4.4 Differences in trace element concentration in the hoof wall

Despite the commonality of keratin proteins and the effect of trace elements on keratinisation and cornification, there is a large variation reported in epidermal tissues between the concentrations of copper, zinc and calcium, *table 4.4.2A.i*. However, there is a discrepancy in method of reportage. As part of the standardisation recommended in this thesis researchers in epidermal tissues should reach an agreement on how to report concentration which varies, (*table 4.4.2A.ii*) not only by the units used, but also is not consistently reported on a DM basis making comparisons across the literature onerous. All results in this thesis are reported in mg/kgDM.

There is little information available on the trace element concentration in equine hoof wall. This thesis measured a mean zinc of 165, (120-430) mg/kg DM in the data set of six hooves and 160, (31-287) mg/kgDM in the data set of 48 hooves. This is in comparison to 174-215mg/kgDM zinc measured in hoof wall, (Coenan, and Spitzlei, 1996); 195 ± 22 mg/kgDM in good quality hoof wall and 171 ± 29.5 mg/kgDM in poor quality hoof wall, (Coenan and Spitzlei, 1997). Others measured 143ppm zinc in the hooves of pasture fed horses, 133ppm zinc in hay and compound fed horses and 114ppm zinc in pasture and compound fed horses (Ley *et al.* 1998), although the authors did not make it clear whether the amounts were reported on as received or dry matter basis. There is a 69% difference in zinc concentration in the hoof wall between the mean recorded in this thesis and that by Ley *et al.* (1998). This could be the result of different dietary regimes between the UK and the USA. Coenan and Spitzlei, (1997) and Siciliano *et al.* (2003)a; (2003)b have shown that zinc in hoof horn increases in response to dietary supplementation, however Ley *et al.* (1998) recorded that changes in diet due to season had no effect on the zinc measured in the hoof horn. There was no detail on dietary intake in Ley *et al.* (1998)'s paper. Dietary intakes 47-79mg zinc/kg diet, (Coenan and Spitzlei 1997) and 110-169mg zinc/kg diet (Siciliano *et al* 2003a) are variable and it is impossible to deduce a cause and effect relationship from current data. Coenan and Spitzlei, (1997) relate zinc concentration in wall horn to hardness and quality, yet measure 174 ±17.4 mg/kgDM in 'good' quality hooves and 171± 29.5 mg/kg DM in 'poor' quality horn and the dietary intakes of zinc between the groups of horses was not different.

Whilst it was not possible to investigate any links with nutritional intakes of trace elements and the concentration found in the hoof , the use of a new technique of LS-ICP-MS on whole blocks of hoof taken from the mdc enabled a 'mapping' of trace element differences within the blocks.

A comparative analysis of copper, zinc and calcium was undertaken from the coronary band, (youngest growth), to the distal bearing surface, (oldest material) at the mdc and across the hoof wall at 50% hoof wall height to investigate if there were differences. A qualitative difference in zinc was measured between blocks sampled proximo-distally down the hoof wall height, with zinc being significantly lower in the block closest to the coronary band compared to all the other blocks. A difference in zinc concentration within epidermal tissue may not be linked to dietary intake of zinc but to protein intakes. Coenan and Spitzlei, (1997) noted that the zinc intake of horses with poor and good quality horn was similar, they also recorded a reduction in some of the amino acids in the poor quality horn and although the zinc concentration in the poor quality horn did increase after supplementation, the increase was only by 40mg/kgDM which is less than the variation in zinc in hoof wall recorded between researchers, *table 4.4.2A.i.*

It is possible that the zinc qualitatively shown to be less in the youngest horn of the horses in this thesis was due to a decrease in protein intakes. A relationship between zinc and protein was discussed in the introduction. Extracting zinc from epidermal cells with EDTA was less successful than extracting after incubation with a sulfhydryl reagent, (Calvin and Bleau 1974) lending support to the fact that the zinc is strongly associated with the –SH groups. A series of experiments feeding different levels of protein with constant zinc intake and feeding different levels of zinc with the same protein intake, (Wallwork *et al.* 1983) indicated that zinc and protein are inter-related in their effects; however the work was confounded as none of the diets were pair fed. Regardless of the actual mechanism, low protein diets do appear to impair zinc homeostasis. Horses destined for the abattoir are unlikely to be well fed and due to the time of year that the sampling occurred, it is highly feasible that the horses would be deficient in protein.

The amount of copper did not change significantly down the hoof wall but there was less copper in the coronary band block compared to the other blocks. The graph of differences in trace element concentration down the hoof wall shows a similar trend for all three trace elements, so it is possible that the analytical technique maybe masking true differences.

A comparison of differences in the trace elements across the hoof wall block shows that there is a lower concentration of all the trace elements at 12.5% hoof wall depth and the differences are significant. This may indicate that the shortened washing technique was removing exogenous trace elements from the surface of the hoof wall, but not from the inner wall. There is some evidence that prolonged soaking for ten days (Wagner and Hood 2002) removed significant quantities of the electrolytes, sodium, potassium and chloride from the hoof wall. As discussed in the method development section, longer washing times also removed more trace elements

from hair. It is recommended that future work should investigate the effect of soaking on hoof blocks by analysing the water in which they are soaked before addition of the blocks and at sequential time periods, especially as many researchers soak hoof specimens to bring them to 100% hydration.

Alternatively one of the reasons that the LA-ICP-MS might be picking up more representational differences across the hoof wall depth is because in order to measure the change down the hoof wall, four different blocks had to be ablated. Each time a block is changed, the micro environment changes and indeed the 'hardness' of the block may change. If this is the case then the differences will be hidden by the changes in carbon count. To ablate across the block, the laser is simply repositioned at the measured position along the block, the block is not removed from the chamber and the counts are done consecutively. This means that, refraction effects are kept to a minimum because the sample cell is only moved along its y axis and its x and z axes are kept constant, (Ohata *et al.* 2002). There were significant differences between both copper and zinc at 12.5% hoof wall depth and all other depths; in addition calcium varied significantly between 87.5% HWD and all other depths, this is the first account of these differences being recorded in any hoof material. Due to the lack of quantifiable information on the trace element differences measured through the hoof wall, by this technique, it would be inappropriate to try and link the differences to visual and material differences measured else where in this thesis. However, it would be interesting to study this further, especially if histological investigations could be carried out to see if there was a difference in the ratios of IFs to IFAPS. Trace elements influence the differentiation of the keratinocyte to corneocyte and may affect the ratio of IF to IFAP formation. MacCallum *et al.* (2002) took biopsies from cattle hooves after scoring them and testing them for hardness and cultured them in a medium with labelled methionine to investigate the rate of DNA and protein synthesis. They related the rate of synthesis with hoof growth but not with the original visual scores and histological samples were not investigated.

This thesis recommends that due to high individual variation, the lack of (but proposed) standardisation in sample preparation, difficulty in obtaining samples and exogenous contamination that levels of trace elements measured in the epidermis of the hoof wall should not at present be related to tissue composition nor correlated with any measured mechanical or visual characteristic unless account is taken of sample preparation and analytical techniques.

4.4.5 Trace element interactions

Some studies have concentrated on the presence of certain elements in hoof horn and their effect. In this research, calcium and zinc were correlated in all sets of hooves regardless of the method used for analysis. The ratios of trace elements *(table 4.3.8.i)* do not reflect the recommended ratios for dietary intakes and it is therefore unlikely, although suggested by Coenan and Spitzlei, (1997), that there is a simple relationship between dietary intake of trace elements and their concentration in the equine hoof wall.

The use of trace element concentration in the hoof wall should not be dismissed as long as standardised techniques are adapted. Trace element analysis of teeth is used as an indication of diet and to investigate relationships between the trace elements and pathological changes, (Szostek and Glab, 2001). Researchers acknowledged that although they were looking for variations in the level of trace elements in teeth, there was likely to be significant biological and environmental differentiation of the examined individuals. The standard deviation in trace elements in teeth between individuals was very small and Szostek and Glab, (2001) hypothesised that this was likely to indicate a close relationship between the individuals and to a homogenous habitat. The variation in this thesis was very large, *table 4.4.5.i*, in part reflecting the non homogeny of the population of hooves studied in this thesis. Future work should investigate the variation within groups of horses fed the same diets and also differences between groups of horses fed different diets.

Table 4.4.5.i Comparison of the variation in trace elements in teeth and equine hoof wall

trace element	concentration in teeth (Szostek,2001)	Standard deviation	concentration in hoof, (this thesis)	standard deviation	Coefficient of variation
calcium	136.1g/kg	13.4g/kg	0.6749g/kg	0.4343g/kg	teeth 9.8% hoof 64.3%
copper	6.31 µg/g	1.01 µg/g	22.8µg/g	5.6 µg/g	teeth 16.0% hoof 24.5%
zinc	147.25µg/g	22.73µg/g	160.4µg/g	54.7 µg/g	teeth 15.4% hoof 34.1%
Zn : Ca	10.8		4.2		

Note 1 all values on a dry matter basis

4.4.6 Future work

The structural changes in the epidermis that occur during induced cooper, calcium and zinc deficiencies dramatically illustrate the importance of adequate supply during keratinisation. However until researchers analyse the changed keratinised tissues for these trace elements as well as the proteins, it is not certain if the levels of trace elements within the final epidermal structure can be correlated to its structural changes.

Some initial work on skin has started to address this, (Forslind 2000). The author pointed out that 'the quantitative distributions of physiologically important elements and trace elements of the skin has been a neglected area of research because of a lack of tools to investigate this highly differentiated tissue'. Using proton probe analysis, (Forslind 2000) was able to delineate a complete calcium profile over the entire normal skin. He showed that calcium content was high in the spinosum and granulosum layers but was below detection limits (1ppm) in the corneum. However multivariate analysis showed that calcium correlated with the granulosum and the corneum, whereas zinc was correlated with the spinous layer. In dry atopic skin zinc was found at 300ppm in the corneum which was higher than that found the 100ppm found in normal skin indicating that there is a link between epidermal integrity and trace element concentration.

More recently calcium positions in hair shaft have been detected using sub-micometer X-ray fluorescence, (Merigoux *et al.* 2002.). Two different calcium ions were found. One ion was very variable in concentration amongst individuals and was located mostly in the cuticle but also measured in the medulla and cortex, in addition it was easily removed by HCl, (probably exogenous contamination). The other ion was not removed easily and was fairly constant from one individual to another and was located mostly in the cortex. The amount of calcium found using this technique was consistent with the concentrations quoted in other literature. The authors felt that the calcium which resisted removal by HCl was associated with protein and likely to be endogenous in origin.

The validation for any biochemical procedure for the detection of a trace element deficiency and prediction of its effect depends upon, the existence of a close association of a compositional change in tissues accessible to sampling with the development of functional defects in tissues (Mills *et al.* 1976). The change in trace element composition of epidermal tissue is remote from the original factors which may have affected its composition such as dietary intakes, metabolic

differences, interactions with other dietary nutrients. The biochemical involvement of the trace element takes place before the cornification of the tissue and it is the cornified stage which is the subject of analysis.

However the newer techniques show that trace elements at differing concentrations can be detected in the stratum corneum, (equivalent to the equine hoof wall) and that there seems to be a relationship between them and 'quality/health' of the tissue. This fact is too exciting to be ignored especially in the light that over 60% of lameness problems in the horse are associated with the hoof, (Jeffcott *et al.* 1982) at huge welfare and economic cost. The relationship needs investigating further to validate if there is a cause and effect between trace element concentration and functional properties of the hoof.

Future work therefore should include the following:-

1. Sampling from known anatomical sites as proposed in this work.
2. Washing procedures should be standardised; further work is required to investigate the effect of different washing techniques and an agreement on protocol reached.
3. Regardless of analytical technique, duplicate samples and either known additions or spiked samples should be adopted as standard protocol.
4. Any association between trace elements and other properties of the hoof wall should be tested on the same material.

Mechanical testing has been carried out on hoof sampled at a different place to that taken for trace element analysis. The work in this thesis has shown that trace element concentrations can differ across the hoof wall depth and at different anatomical positions. There is evidence which suggests that certain attributes, (quality) of equine hoof wall and rate of hoof wall growth are influenced by dietary deficiencies or excesses in levels of nutrients, (Ley *et al.* 1998, Reilly *et al.*1998; Slater and Hood 1997; Coenan and Spitzlei 1997). Still lacking, however, are baseline values relating to strength and mineral composition of equine hooves of horses.

Further work is required to establish if associations described in the literature are valid.

5. A survey of trace element composition of the hoof wall should be undertaken to obtain mean population data in different age groups and in clinically healthy and unhealthy (e.g. laminitic[g]) populations.

To date there has only been one large scale investigation into the trace element content of the equine hoof wall (Ley *et al.* 1998), however this study confounded not only the effect of season but also differing regimes without any baseline data on dietary intake. In addition the authors

stated the research was intended to investigate the effect of nutrition and management regimes but not to quantify the specific levels of trace element content of the hooves or whether they differed between individuals. Little research has been reported concerning hoof wall strength and composition in horses under conditions of adequate to good nutrition.

Further work is required to determine baseline data for different trace elements in the hoof wall and to continue to establish if there are zonal differences in trace element concentration around the hoof wall, finally to determine if diet or individual horse affects trace element composition of the hoof wall.

By investigating trace element composition of the hoof using the standardised protocol developed as part of this thesis, baseline values can be established as a reference which can be used as a standard to determine if a certain horse is lacking a certain trace element; a trace element that may be related to poor hoof quality. Poor horn quality has always been seen as a major problem within the horse industry.

5 Quantitative assessment of the shape of the equine hoof capsule

'That which is called a good, strong, sound foot has its front wall inclined at an angle from 45 to 50 degrees, and has the outside wall more rounded than the inner, which is nearly straight up and down, and has a good concave sole'. (Duncan 1935).

5.1 Introduction

Whilst the relationship between trace elements and their effects on epidermal tissue in terms of its appearance, material properties and function has been well documented in skin, wool and hair literature; for example, a reduction in zinc in skin tissue is associated with the cracking and hardening of psoriasis. The effect of dietary intake of trace elements on the integrity of the claw or hoof wall epidermis as measured by visual appearance (Baggott *et al.* 1988; Coenan and Spitzlei 1997; Josseck *et al.* 1995) is less clear.

The relationship between cracks and trace elements may be hidden because of the unique role that the epidermal hoof wall of the ungulate plays in load bearing. When external forces, due to bodyweight or locomotion, are applied to the hoof, the hoof deforms in response to these forces. If a structure is unable to resist a load then it will fail. The hoof is designed to withstand many unpredictable loading situations, (Thomason *et al.* 1992) and the tissue of the hoof wall is organised at macro and microscopic levels to absorb stress through micro-cracking at tubular level to dissipate excess energy which may result in failure of the wall, (Bertram and Gosline 1986; Kasapi and Gosline 1999).

The ability of a structure to resist the forces imposed due to load depends on its material organisation and properties, as well as on the overall organisation and shape of the structure itself, (Gordon 1987; Biewener 1992). Therefore a structure might fail under load if the fracture toughness of its material is exceeded; in terms of the hoof this might be due to inadequate keratinisation due to a trace element deficiency. The force on a specific area (stress) might be raised locally by for instance a nail hole, resulting in a stress concentration. Alternatively due to

geometric irregularities the stress in specific areas of the hoof maybe much higher than the breaking stress of the hoof wall and therefore predispose this area of the wall to cracks. This was illustrated by Cohalan *et al.* (1993) and Davis, (1996) who showed that hooves with an incomplete bearing surface concentrated the force on the remaining parts of the wall. Stress is defined as the force per unit area and a structure will respond to stress by deforming; this deformation is strain. Strain is measured on the hoof wall by recording the difference in the original length compared to the length by which the material deforms using strain gauges. Changes in shape affect the way a structure can deal with load and aspects of hoof shape have been shown to effect strain distribution in the hoof wall as measured by strain gauge[g] studies. Manipulated shape changes of the hoof wall *in vitro*, by changing toe angles, using heel wedges and changing medial -lateral balance using blocks, (Balch *et al.* 1991; Barrey 1990; ,Chang *et al.* 1993; Dejardin *et al.* 1999; Douglas 1998; Thomason *et al.* 1992; 2001; Thompson *et al.* 1993); natural differences in *vivo* (Colahan *et al.* 1991; Glade and Snow 1985; Kane *et al.* 1998; Thomason 1998) and modelling changes (Hinterhofer *et al.* 2001), all resulted in differences in strain patterns experienced by the hoof wall. If the strain on the hoof wall is changing due to shape changes, then this is as a result also of a change in stress in those areas. Changes in shape may predispose an area of the wall to cracks if the stress exceeds the fracture toughness of the wall.

Variation in horses' bodyweights is considerable and an adult horse can weigh between 50kg to 1500kg, (Ellis and Hollands, 1998; 2002). General perception, as discussed later, is that breeds of large, heavy horses have a different shaped hoof compared to the lighter breeds. This perceived difference in shape may be an actual adaptation of the hoof in response to the differences in load imposed, because of the increase in bodyweight, ensuring that the hoof can deform rather than fail in response to the extra load.

5.1.1 Why the shape of the hoof requires quantification

Hooves of specific shapes maybe more prone to cracking due to the influence of their shape on stress concentration. If there is a relationship between crack incidence and the shape of the hoof wall, then the shape of the wall needs defining and measuring, so that this relationship can be further investigated.

Summerley *at al.* (1998) investigated the effect of rider and change of gait and direction on the deformation of the front hoof wall using strain gauges and found that all the variables affected strain distribution. They noted that repeated deformation may have resulted in a change in hoof shape over a period of weeks or months and indicated that there maybe a cellular mediated response in the germinative layers, (similar to remodelling in bone). They reasoned that the results may have important implications for the nature of assumed interactions between mechanical loading of the foot and its shape and highlighted the need for further work to test for a linkage between strain distribution and change in hoof shape. However in order to investigate this link, shape needs measuring.

Thomason, (1998) investigated the effect of indices of hoof shape on surface strain by measuring toe length, toe angle, and medial and lateral wall angles from horses and ponies of different sizes. Contrary to his expectations, he recorded that hooves with upright toes experienced most strain; he also noted that his results were different to those obtained by Thompson *et al.* (1993) who altered toe angle in isolation, *in vitro.* Thomason, (1998) did not investigate correlations between toe angle and any of the other measurements, so he was unable to conclude whether a shape change had occurred in all planes of the capsule. It is unlikely that toe angle *in vivo* changes independently of the other measurements and it might be that the other measurements were affecting stress distribution on the hoof capsule. Douglas, (1998) investigated the stress/strain behaviour of the laminar junction from different horses' hooves and concluded that 'horse' had a significant relationship with some of the results, indeed there were differences between hooves of the same horse and she implied that this could be explained by different hoof shapes, but as she had only measured toe angle and toe length, she could not investigate this further.

Others have investigated the effect of ground surface, acceleration, change of direction, (Thomason 1998) and inter subject variation, (Thomason *et al.* 1992) on strain on the surface of the equine hoof capsule. Thomason *et al.* (1992) concluded that whilst the strain patterns on an individual hoof are consistent, individual variation exists, 'which probably results, in part, from the differences in shape and asymmetry of the hooves among animals'. Hooves deform in response to stress and the affect can be measured as strain. Changes in different indices of shape have been shown to change strain patterns and distribution. In order to investigate further the relationship between strain and shape of the hoof capsule a method to measure shape needs developing.

The application of shape measurements in other digits

Between 1976 and 1981 (Weaver and Andersson, 1981), a working party on the ruminant digit endeavoured to make recommendations to the veterinary profession as to the nomenclature and terminology that could be applied to the shape of the cow's foot and to make recommendations on methods of recording clinical data.

In other species, the ability to measure aspects of claw shape has become a requirement in the selection process for breeding stock. For example, breeding groups use easily obtainable parameters such as claw size measurements or visual judging of claws as part of their decision to select sires, (Peterse 1986). Obviously, it is important that the assessment of claw shape is accurate and not just subjective. Morris *et al.* (1985) looked at the variation amongst subjective foot scores of cattle and found that the correlation between two scorers marking the hind feet was only 0.39 illustrating that subjective measurements are not accurate enough to predict shape of cattle claws but a separate study using subjective scores resulted in agreements as high as 52-66% between observers, (Murray *et al.* 1994). The difference between the two techniques is likely to account for the difference in agreement; Murray *et al.* (1994) trained observers using a set of diagrams and photographs to recognise characteristics used to score the claws. Morris *et al.* (1985) gave no indication as to how the judgement of a better claw was obtained except by saying that the high scores were given to claws with a better structure. This was in contrast to Hahn *et al.* (1984), who described an objective method of scoring claws by taking measurements of claw length, angle between claw and ground and heel depth. The repeatability of measurements between and within observers was as high as 0.75-0.9, illustrating that appropriately chosen objective measurements can be developed to provide accurate records. If claw or hoof shape can be accurately measured, so that the variation between the measurements do not hide true differences in shape, then changes and differences in shape can be recorded.

5.1.2 The definition of shape

A selection of measurements used as indices of shape has been used in many papers relating to equine hoof, without an attempt to define what the author means by the word. If there is no definition, then any measurements taken may not be comparable and it is necessary to define what is meant by shape before measurements can be taken.

By combining the definitions of shape, form and geometry, (Oxford English Dictionary, 1996) and relating them to what is described colloquially as the shape of the wall, this thesis offers the following definition of shape. Shape can be defined mathematically as the spatial arrangement of an object characterised by a series of co-ordinates or major geometric elements. Therefore the external surface of the hoof wall is an outline whose area is delimited by an arrangement of points or lines, which form interrelated elements with each other. The lines and points are unambiguous and the inclination or angle of any two lines to each specific, so that the combination results in a repeatable and recognisable shape. The following distinctions have also been referred to in chapter one

- The keratinocyte is defined as the material from which the structure is 'manufactured'
- The structure is the assembly of the keratinocytes in terms of tubular and intertubular material both at macro and micro levels, (as opposed to other epidermal tissue made from keratinocytes and therefore samples of the structure can be tested independently for mechanical properties
- The shape describes the outline that the structure makes when the hoof is on the ground and therefore describes the wall and the solar base of the capsule.
- Form is defined as the shape and the size of the capsule.

This definition allows differentiation between the shape of a cow's claw and a horse's hoof, even though both have the same structure, (the arrangement of tubular and intertubular constituents) and consist of the same material, (keratinocytes). By defining structure, it is possible to differentiate between different epidermal tissues, (skin, hair, nail, hoof) which are made of the same material, (keratinocytes/corneocytes) but have different structure. Structures are made from materials and there is often no clear-cut divide between a material and a structure when describing biological materials, (Gordon, 1978). However, material is defined in the Concise Oxford Dictionary as 'the physical substance from which a thing is made.' Structures are made from materials and 'to understand structures, we have to worry about shape', Vogel, (1998).

The use of ratios to describe shape

It might be difficult to establish visually when an increase in size becomes a change in shape. For example, if the length of a toe mdc is 2cm longer on one hoof compared to another hoof, then the difference might account for a change in shape or it may be simply a bigger version of the other hoof. In order to differentiate between size and shape, it is important to control or remove size from the measurement variable. Form is defined as the shape and size of an object or organism, and the words shape and form should not be interchangeable, (Chen *et al.* 2000).

Size can be defined as a quantity that depends upon dimensional space, so when researchers measured any change in the length of the hoof wall, they might have been recording a one dimensional difference in size rather than a shape change. Chen *et al.* (2000) suggested that shape is the 'residual' which is left after controlling for size and stressed the visual aspect of shape, which in this thesis has been defined as the outline recognisable as the hoof wall.

Expressing length measurements as a ratio of each other is a method of removing the variable of size. Ratios can be used to express a unit-free shape factor or as a way to factor out size. Barlow-Irich, (2002) investigated the use of ratios as a way of measuring the shape of leaves. She found that ratios were useful in distinguishing differences in shape within a taxa of leaves particularly when the correlation between two variables was high. She also noted that proportions were useful simply because they reduced the amount of data by summarising a bivariate relationship in a single variable. In this thesis, correlation coefficients between proposed measurements to use in ratios were calculated as part of the method development to obtain an indication to their suitability. Rae, (1999) supported the use of ratios because the univariate morphometric characters such as lengths used by most practising systematists are not suitable as they are not independent due to their strong correlation with generalized body size. The use of ratios represents a practical and preferable alternative to use of the raw data and removes the dependence on body size, (Rae 1999; Adams *et al.*, 2002).

Morphometrics is the study of shape and is used to classify species, to diagnose pathologies, and to categorise plants into taxa. The science of morphometrics is becoming increasingly sophisticated and utilises finite element analysis, multivariante analysis, euclidean distance matrix analysis and elliptical Fourier functions to distinguish shape differences. However the methods are all based on initial landmarks from which measurements can be taken based on x, y z coordinates. This thesis aimed to develop a series of reproducible anatomical points from which landmark measurements could be taken. In addition, the development of ratios to describe shape changes in the hoof will be investigated to reduce the amount of data which requires processing, to remove the influence of size and allow comparison of shape differences.

5.1.3 A rationale for the choice of measurements used to determine the shape of the hoof capsule

Everyday adjectives are used to describe the hoof capsule as either steep or shallow, the sole as round or oval and the toe as upright or low which captures the external appearance of the wall in three different planes. This external appearance can be quantified numerically by a series of co-ordinates or major geometric elements. By measuring the outline and quantifying the arrangement of the points or lines, it should be possible to measure a repeatable and recognisable shape and therefore group hooves which share commonality of some of these elements. Expressing some of these measurements as ratios should also remove size as a confounding influence.

The differences in hoof capsule shape are recognised by the veterinary and farriery profession and by owners, albeit in a subjective manner. One shape is described as narrow across the base, slender with upright walls similar to a cylinder and is often associated with Thoroughbred horses; another is described as being wide and squat more like a soup plate, commonly associated with Irish draughts and heavier horses. The third shape falls between the other groups having shape characteristics from each group which are more proportional so that it is more difficult to capture the shape with adjectives, but the base plate would be round and the other parameters would fall between the two 'extremes'.

The shapes described above can be captured using linear and angular measurements which are interrelated. The aim of this part of the thesis was:-

- To investigate which aspects of geometry were being described by the visual adjectives in common use
- Consider the mathematical equations available which might capture the geometry
- Develop a series of practical measurements of these parameters which could be substituted into the geometric equations
- Use the measurements to investigate ratios
- Test the ratios and measurements to see which characterise hooves and best capture the visual appearance described by the adjectives above.
- To consider the use of photography as an alternative to taking measurements direct from the hoof capsule

Consideration of the geometry that might be used to capture the visual adjectives being used to describe the shape of the hoof capsule.

In order to objectively describe the shape of the hoof capsule, which is currently subjectively described, it is appropriate to consider the geometry underlying the visual adjectives being used. It is then necessary to consider what measurements might be needed to describe this geometry, and if any of these measurements are already in use, to critically evaluate the methods used to obtain the measurements.

Transverse plane

The transverse plane is the outline of the bearing border of the hoof when viewed from the sole or capsular base. When the terms narrow and wide or round and long are used to describe the hoof, the aspect of geometry which is being considered is the sole. For example, it has been noted that the hind foot of the horse has a different shape compared to the front foot, (Adams 1974). Rooney, (1978) described the front hoof when seen from the ground surface as having a more rounded shape while the hind hoof is rather more elliptical in shape. Bracey-Clarke, (1829), elegantly wrote, 'the weight of the animal falls with a different force upon the fore and hind feet and that the fore are more burthened with it: this is one cause of their being made larger and with more flatness'. The shape of the sole is often ignored by clinicians and researchers with measurements more commonly taken from the hoof wall. In fact as the (growth) of the hoof originates proximally at the coronary band and will influence the shape of the final hoof capsule, the coronary band might be considered the most important parameter for measurement. However an outline of the coronary band is difficult to capture but the sole, (base plate) is easier to see and measure and will reflect the 'shape' of the coronary band. The sole could be considered the foundation or base plate of the hoof capsule and is the part of the hoof to which everything is related. The root and trunk of the tree dictates the width and density of the branches it supports; the foundations of a house are always constructed first and dictate the shape of the final structure. The sole of the hoof should be given equal weighting when shape descriptions are being considered as with all foundations, it is also the driver for all the other geometric parameters. Certainly in order to construct a finite element analysis[g] (FEA) model of the hoof, the first information inputted is the measurements which delimit the base of the hoof, (Newlyn *et al.* 1998)

From a geometric perspective the sole which is equally wide as it is long might be considered representational of a circle. However some base plates are longer than they are wide and some are wider than they are long and could therefore be considered more representational of an ellipse. These differences could be captured in a number of ways, as the shape of a circle is distinguishable from an ellipse by the fact that its axes are equal in length, and by the fact that

the perimeters are related to these lengths. It might also be necessary to consider distinguishing between soles which are wider proximally or distally.

Capsular wall

There has been debate through the literature as to whether the outline of the wall is one of a cone or a cylinder. In 1820, Bracey-Clarke noted that: - 'a superficial view of the hoof has very naturally led to the apprehension that it was of conical form'. He challenged that view and stated that the hoof was 'truly that of a cylinder', *figure 5.1.3.i.* More recently, the shape of the hoof wall has been described as a two dimensional cone, (Kasapi and Gosline 1998; Leach 1980; Thomason *et al.* 1992; Hood and Jacobson 1997) and the front feet have been described as being more cone shaped than those of the hind feet, (Leach 1980). One of the reasons why there is a lack of agreement on whether the hoof is a cone or a cylinder, might be because the wall of the hoof can be described by two planes, the frontal and sagittal and the shape perception might change depending upon which plane is being considered.

The other argument is based upon the perception of the researcher. Biological principles indicate that a cone allows an increase in size, *(figure 5.1.3.ii)* without a loss of shape, (Vogel, 1999). However Bracey-Clarke, (1820) ignored this premise stating that: - 'if the hoof had been a cone, by continually growing larger downwards, it would have greatly outmoded the animal; whereas a cylinder always remains the same'. If Bracey-Clarke had considered biology, he would have appreciated that increasing the length of one of the sides of a cone also increases its base plate. Increasing the length of a cylinder, on the other hand, just increases its linearity, *figure 5.1.3.ii.*

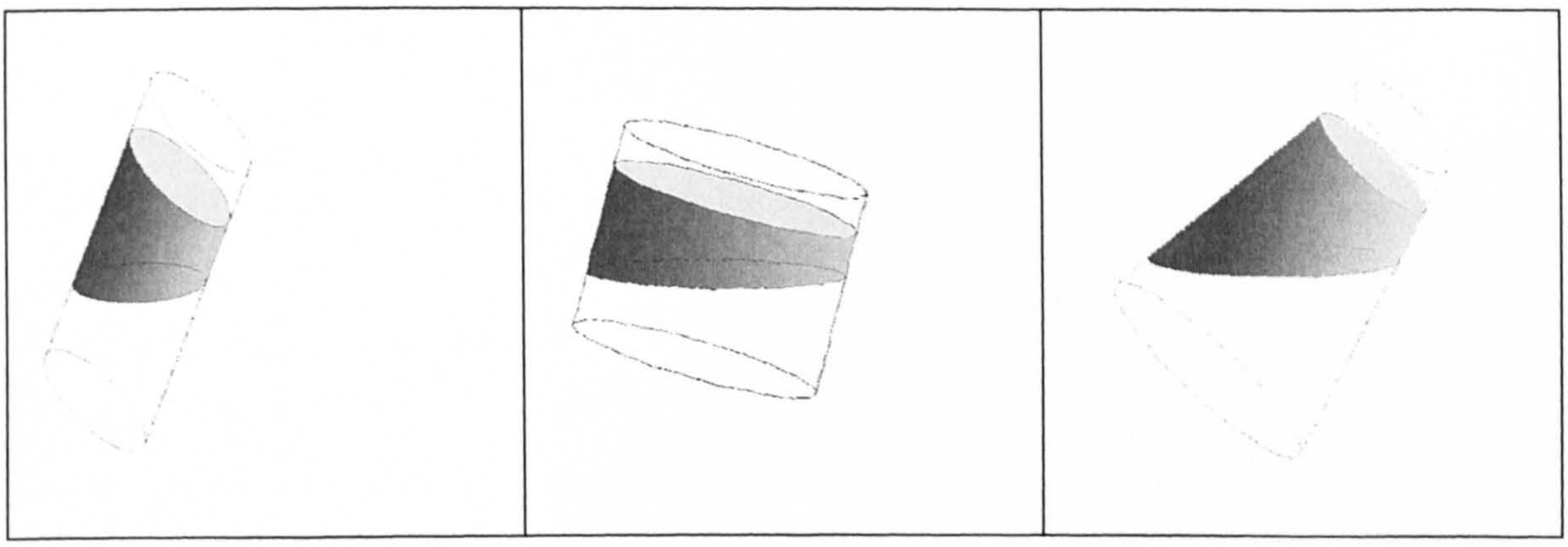


Figure 5.1.3.i The hoof as a cylinder, *after Bracey-Clarke 1820* Artwork © Dave Gibson

Note 1 Observations from Bracey-Clarke; the posterior terminations of the hoof have the same direction as the front and are parallel to them. The quarters do not project outwards as they would if the hoof was a cone. The quarters are parallel to the axis of the cylinder. If the foot was a cone, the 'contents of the foot would have slipped through on any strong exertion, as the cone is ever presenting a wider area in this direction'

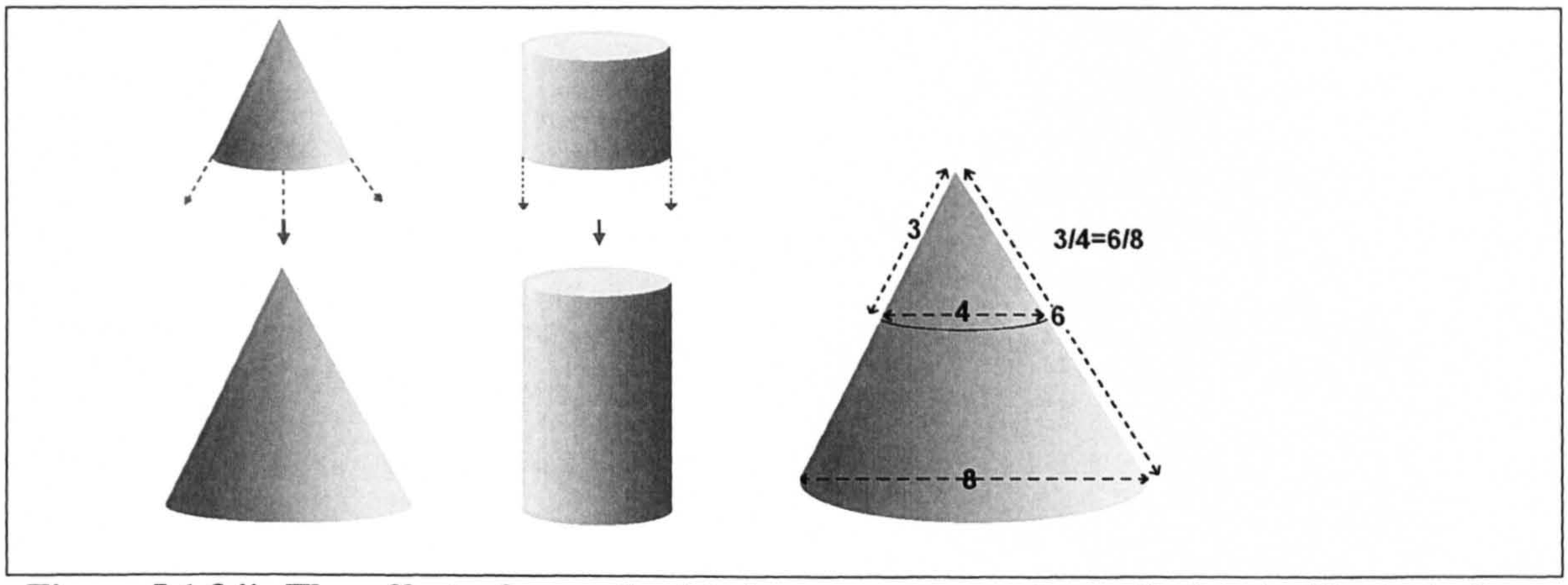


Figure 5.1.3.ii The effect of growth, (size), on a cone and a cylinder to illustrate how an increase in size can occur without a simultaneous change in shape, (*after Vogel, 1999*). Artwork © Dave Gibson

In addition, unique to the equine hoof, are changes in the basic shape as a cone. The cone is truncated at the coronary band, so that the front wall is longer than the heels, (Hood and Jacobson 1997), *figure 5.1.3.iii*. The truncation is not parallel to the ground and therefore the relationship between the circumference of the sole and the coronary band is closer to that of a frustum of a cone. The cone appears to be tilted at its tip to the rear whilst leaving the base flat on the ground. Geometrically the hoof might be considered a frustum of a cone.

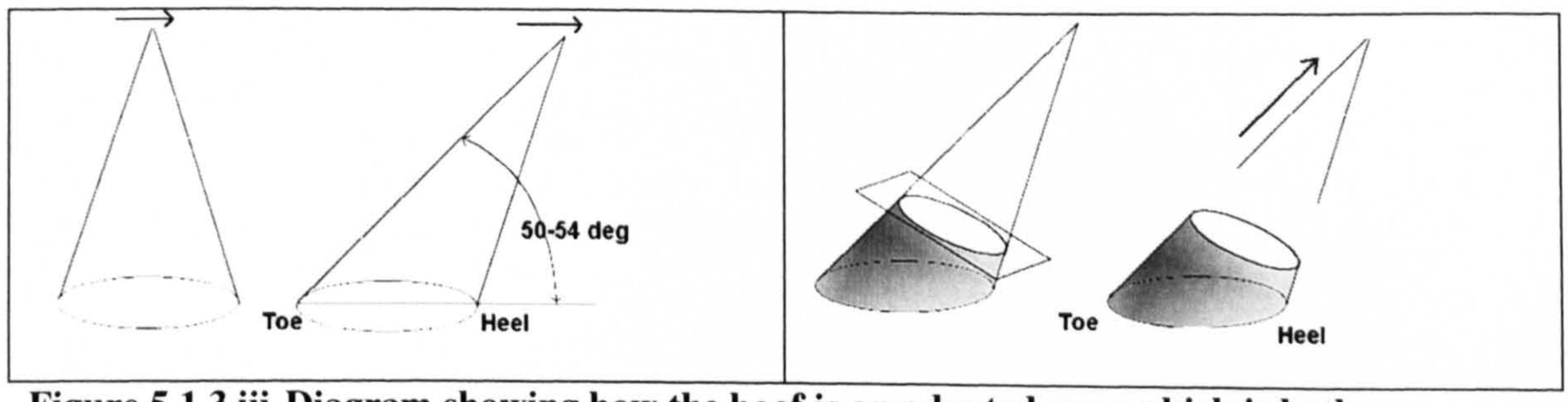


Figure 5.1.3.iii Diagram showing how the hoof is an adapted cone which is both tilted and truncated, (*after Hood and Jacobson 1997*) Artwork © Dave Gibson

Some researchers, (Helman *et al*. 2002) still considered the hoof as 'an unusual geometric shape that might be described as an oblique, expanding cylinder' and others (Arabian *et al*. 2001) considered the hoof to be cylindrical in shape. Resolution of this debate, is beyond the remit of this thesis, but if the shape of the hoof capsule is described rather than individual elements, then researchers may find that both shapes exist. Elements of the geometry of cones and cylinders were considered when deciding which measurements best captured the shapes of the hoof wall capsule and the geometry of the cone was considered both in the sagittal and frontal planes.

Frontal plane

The frontal plane in this thesis is the outline of the hoof when viewed from the front of the hoof. Researchers, (Douglas 1998; Kane *et al.* 1998; Thomason *et al.* 2001) have shown a correlation between toe angle and the medial and lateral angles at the widest point of the capsule. The visual appearance of either a cone or a cylinder may be best described by the lateral and medial angles of the capsule wall. Upright walls at the widest point of the quarter will give a cylindrical outline and their angles will be closer to 90°, compared to sloping walls which will give a more conical outline and whose angles will be proportionally less. If the angles were expressed as a percentage of 90°, then this might give a relative measure of slope. If the hoof was a true cone then the angles in the sagittal plane, (toe and heel angles) would be the same as those in the frontal plane, (medial and lateral angles). However because of the perceived rear tilt, it is possible that the angles in the sagittal plane will be more sloping. It was therefore considered that the angles of both planes should be considered independently and the angles at the widest point of the quarter were expressed as a coefficient of 90° to provide a measure of their uprightness.

Sagittal plane

The sagittal plane in this thesis is defined as the outline of the hoof when viewed from the side. The toe angle is routinely measured either accurately or by eye and together with the heel angle is likely to give an indication of the cranial/caudal tilt of the hoof wall in the sagittal plane. A normal toe angle is considered to be between 48° and 55°, (Balch *et al.* 1991; Kaneps *et al.* 1998; Turner 1992), whereas (Barrey 1990) considered angles of 38° to 50° to be more typical. There is less documentation on the hind foot but generally it is accepted that the toe angle is steeper, 55°±2°, (Turner 1992) or between 53° and 57°, (Balch *et al.* 1991). Singer, (2001) and Turner and Stork, (1988), stated that the ideal toe angle should be between 50° and 55°.

Based on the above, the use of the toe angle as an indication of the tilt of the hoof was investigated; normal 'tilt' will be between 50° and 55°, toe angles less than 50° will be categorised as having a low toe angle which would give a more cylindrical outline in this plane. Toe angles above 55° will be defined as a high toe angle, which would result in a more upright appearance in this plane and the outline would recognised as cylindrical. The relationship between the mdc toe length and heel length or the two angles may give further clarification to the geometry of the hoof in this plane. Turner and Stork, (1988) stated that if the heel angle was 5° less than the toe angle then the hoof has under run heels; Singer, (2001) indicated that the heel angle should be the same as the toe mdc angle. The heel angle as a proportion of the toe angle was investigated. Due to the difficulty of accurately measuring the heel angle

measurement, an error of ±10% was incorporated into the ratio. The toe angle is a repeatable and reproducible measurement thus any heel angle equal to or ±10% of the toe value will be considered within the recommended ratio. In addition, the ratio of heel length to toe mdc lengths may give an indication of the truncation of the hoof capsule. Singer, (2001); Turner and Stork (1988) and Turner; (1992) considered that the length of the heel should be between half and a third that of the toe mdc. The ratio of these two lengths was investigated.

Transverse, frontal and sagittal planes

The consideration of the shape of each plane individually may not provide sufficient information to be able to distinguish between the shapes of hooves. It was considered appropriate that the relationship of certain measurements across the three planes should be investigated as an alternative method of classifying the shape.

It may be possible to establish the slenderness or squatness of the capsule by considering the aspect ratio. The ratio of the capsular base to toe length and the ratio of capsular width to toe length was investigated. In addition, the ratio of the proximal perimeter, (coronary band) to the distal perimeter (bearing surface) was investigated as a possible indication of the degree of truncation.

5.1.4 Evaluation of current methods used to measure indices of shape of the hoof capsule

There is still a need for the development of a system to easily record shape of the equine hoof (Kane *et al.* 1998; McClinchey, 2001) and a requirement for the development of a three dimensional system to measure shape using easily definable parameters. This chapter proposes a method to measure the shape of the hoof wall, so that the relationship between this outline and the incidence of cracks can be investigated; no other relationships between shape and function will be considered. Before measurements can be taken, anatomical points need defining. Kane *et al.* (1998) noted that the main difficulty in establishing methods to measure shape has been the lack of easily definable reference points.

The nomenclature used to describe areas of the equine hoof capsule is a mixture of defined anatomical terms and common usage words, so that one area or point could be referred to by at least three different names. This can lead to confusion when trying to compare research findings or if conversing with either scientists or horse owners.

The area known as the toe of the hoof has long been recognised, (Lafosse 1754; White 1802; Winter 1852) and it is well-accepted term in scientific literature (Arabian *et al.* 2001; Hinterhofer *et al.* 2001; Thomason, 1998; Summerley *et al.* 1998) and general lay texts, (Your Horse; Horse and Hound; Williams and Deacon, 1999). The toe can be described in terms of its angle and its length. The first reference to the toe needing to be a specific angle is found in White, (1802), which shows a hoof superimposed on a protractor scale of 45°. However, Reilly, (1995), felt that the term toe was too general and highlighted the need to more rigoursly define anatomical points, so that all researchers could work to reproducible sites.

The adjective 'quarters' is used routinely in descriptions of the equine hoof wall but anatomically it has not been defined. Many farriers would consider the quarter to be centred halfway between the toe and the heel on both the lateral and medial sides effectively dividing the hoof into four, hence the terminology. As well as anatomical points, it is important to delineate the start and finish of the anatomical areas known as the toe, the quarters and the heels.

Weaver and Andersson, (1981) defined anatomical surface zones of the horny capsule of the ruminant digit as a result of a working party on the subject. The zones were based on an agreed nomenclature of the surface regions and a 'map' of the foot was agreed. This thesis proposes that it would be helpful if workers could come to a similar agreement on the areas and anatomical points of the equine hoof capsule.

Anatomical areas were delimited as described in chapter 2. However areas are not useful to determine shape therefore anatomical reference points from which angles and lengths were subsequently measured were required. The reference points in each anatomical area were defined first after consideration as to the best method based on reviewing other researcher's work. All linear and angular measurements currently being used were reviewed to see if they were suitable to obtain measurements from the predefined points in order to substitute measurements from the hoof capsule into formulas used to define the geometry of shape. The aim was to obtain measurements which ensured repeatability and commonality of anatomical positions regardless of the size of the hoof.

Defining the anatomical reference point for the toe

Various workers have taken measurements of the toe and have used different names for what is essentially the same anatomical point. For example, Colles (1989) and Thomason *et al.* (1992), placed strain gauges on the toe, which they defined as the 'anterior midline'; Thomason, (1998), defined the toe as 'the furthest forward point of the dorsal wall at its intersection with the

bearing surface' and Burn and Brockington, (2001) defined the toe as the dorsal midline. The hoof wall is not always symmetrical, so the anterior midline may not be the furthest point forward of the dorsal wall. Ovnicek *et al.* (1995), described how they delimited the middle of the toe to establish a repeatable point on the solar aspect by drawing a straight line which was drawn from the central succulus of the frog, anteriorly through the apex of the frog and continued across the sole to the toe. Thomason *et al.* (2001) measured the sagittal axis, which was defined as any line on a vertical plane through the midpoint of the heels palmarly and the tip of the extensor process of the third distal phalanx dorsally. Using this sagittal line would mean that the point of toe was precisely identified and could be identified by other workers; however it would rely on a radiograph of the hoof and even if a radiograph was available, it would be very difficult to ascertain where the extensor process of the pedal bone was in reality. The use of the central succulus of the frog and the apex of the frog to decide where the sagittal line is drawn on the sole till it dissects the dorsal hoof wall, may introduce a large amount of variability. The shape of the frog can be variable and is often asymmetrical, which could cause confusion as to which part of the frog should therefore 'drive' the straight line.

Kane *et al.* (1998) found differences in the frog to wall distances measured from the apex of the frog to the hoof wall. These differences may reflect the asymmetry of the hoof wall from this point or the fact that the frog itself is 'off centre', however the distance of the succulus of the frog to either heel was not measured, so it is not possible to comment upon the symmetry of the frog. Thomason *et al.* (2001) measured from the central succulus and the apex of the frog to each wall to compare the measurement between two groups of horses and they recorded that both the distance between the frog and the lateral wall and the heel widths varied significantly between the groups $p < 0.05$. In cattle, (Vermunt and Greenough 1995), the toe is defined as the apex of the claw at the dorsal border and objective measurements of both the angle and length of the claw at this anatomical site have been developed.

Point of toe
Having used all the above techniques on 10 hooves at different times, the most repeatable method of defining the point of toe was chosen as the most forward point on the dorsal wall. The midsagittal line through the sole from the middle of the heel bulbs met the wall at this point. This removed the reliability on the frog. This point was defined as the point of toe for the purpose of this thesis.

Defining toe length

The length and angle from the point of toe to the coronary band was measured. To achieve this, it was necessary to delimit the hoof wall. Balch *et al.* (1991), expressed concern that whilst the measurement of toe length is routine, confusion muddled the determination of the proximal (upper) limit. He suggested that the junction of the skin and hoof (the hairline) is too arbitrary and recommended the coronary rim, which is the most proximal point of the hoof wall and indicated that it could be palpated. The reality of being able to palpate the coronary band and then place dividers on it, maybe a challenge due to the sensitivity of the area *in vivo*. In addition this point would not be identifiable in photographs, should photography be used as a method to measure shape.

Hahn *et al.* (1984), measured toe length whilst developing objective measurements for cattle hoof shape and defined this measurement as 'from the periople line to the dorsal border'. Hahn *et al.* (1984) felt the hairline was too sensitive an area in cattle to use as the proximal limit of the toe. The periople line in the equine is not always apparent as many farriers rasp the periople (personnel observation) and it varies in length according to the individual, so this would not be an appropriate point from which to take measurements.

The junction of the hairline, (defined as the distal roots of the hairline, which is independent of hair length), with the proximal border of the hoof wall was taken as the upper limit of the hoof wall for this thesis. When photography was used to take this measurement, it was necessary to trim the hair back to the hairline before taking the photograph. This length was defined as the mdc toe length

Various workers have used different tools to measure this length; Balch *et al.* (1991a), used a pair of compasses, *(figure 5.1.4.i)* whereas, Kaneps *et al.* (1998), measured the length of the wall sagittally from the most proximal part of the cornified hoof wall to the bearing surface at the toe with a ruler.

Figure 5.1.4.i Measurement of toe length, (*after Balch, 1991*)

This would result in an approximation as a ruler is a rigid straight structure and it would be difficult to decide which measurement to read from the ruler as the hoof wall is not straight at its bearing surface nor at the coronary band. Turner, (1992) used a tape measure to determine length which is less rigid and therefore more accurate, however it maybe difficult to read from the tape measure *in vivo* unless the foot was off the ground.

Three methods were considered for measuring the length of the wall; using a pair of dividers, a piece of string which was subsequently measured or a tape measure. The string introduced too much variation as it was difficult to hold it at the hairline, follow the contours of the wall and cut the end where it bisected the point of toe. The tape measure was slightly easier to use, as there was no need to cut the end of it, simply read the number at the bottom. However it would be difficult to read the number from the tape measure if the hoof was on the ground and also it was actually quite difficult to be precise in reading the number exactly at the point of toe.

The easiest and most efficient method of measuring the length was with a pair of compasses. It was more precise in actually measuring from the hairline and to the point of toe. Therefore the length of the wall at the toe and elsewhere was measured with a pair of compasses /dividers, so that the true length of wall could be measured not the projection with the ground. The length of the toe mdc from the point of toe mdc along a line parallel to the tubules to the hairline was measured. The measurement can be made with the hoof on the ground if necessary. It is likely that this method will be more comparable with results obtained from photographs and this was tested.

Defining Toe angle

Balch *et al.* (1991) defined the toe angle as the 'dorsal to solar angulation as measured at the toe'. He recommended the use of a protractor, which remains virtually identical to the equipment designed by Russell, (1901). The hoof gauge developed for the farriery trade has been used to measure hoof angle at the toe and is both accurate and practical (Turner, 1992; Kaneps *et al.* 1988; Snow and Birdwall 1991; Glade and Salzman 1985). The main disadvantage of using a hoof protractor *(figure 5.1.4.ii)* is that it will not pick up any variability due to concavity of the wall at any point.

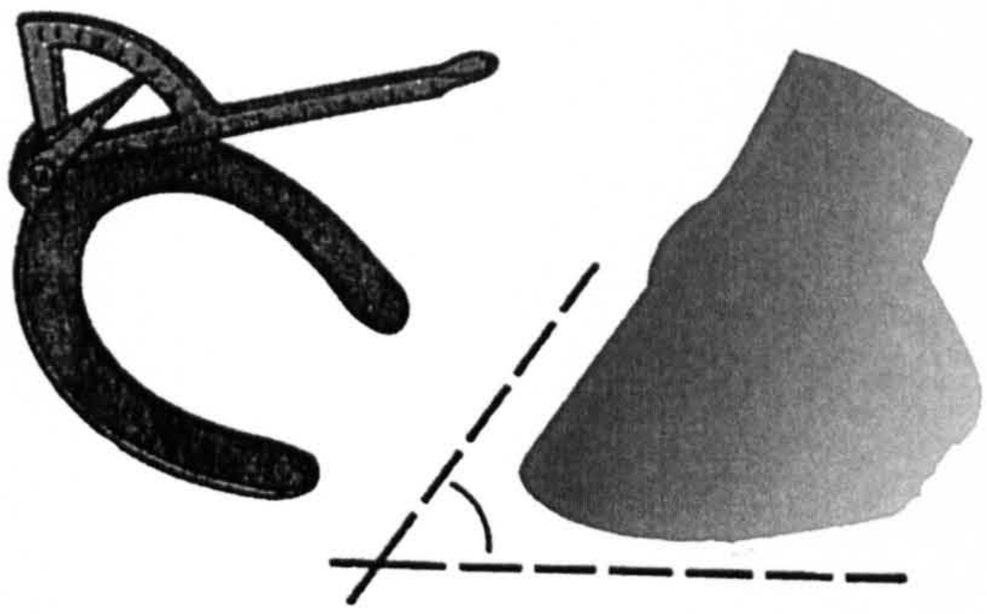


Figure .5.1.4.ii Illustration of the hoof protractor used to measure toe angle *(after Balch, 1991)*

The angle of the wall may vary along its length and therefore a specific device was developed for this thesis, which allowed the angle to be taken at 50% of the hoof wall depth on every hoof.

Defining the anatomical reference points for the quarters

Because the name quarters, is used generically, it is not always clear from the literature as to whether researchers are describing a region or a point. Thomason, (1998) used the widest point of the hoof, from a photographed dorsal view, as a reference point for measuring hoof wall angles, but did not measure the width, he described this angle as the medial quarter as did (Greenough and Vermunt 1991) when measuring the width of cattle claws. Douglas *et al.* (1998) defined the quarters as centred one- third of the way between the dorsal midline and the heel, this would mean that in a hoof with a perimeter of 30 cm, the quarters are 5cm around the wall from the toe and there is a further 10cm between the point designated as the quarter to the heel on both the medial and lateral side, If the wall was asymmetrical, as reported by many workers, (Balch and Butler 1991; Thomason *et al.* 1992; Thomason 1998), then this measurement may result in the lateral and medial point of quarter being at different anatomical points on the same hoof. Snow and Birdwall, (1991) defined the quarters by designating a point on the mid line of the frog approximately under the insertion of the deep digital flexor tendon onto the distal phalanx, a line was then made from this central point to the lateral and medial walls. Skill is required to judge the insertion of the tendon and many farriers trim the frog and therefore the mid point could become arbitrary.

Kaneps *et al.* (1998) defined the quarters by joining two points. They measured the proximal border (coronary band), and marked half way between the mid-sagittal aspect of the toe and the heel. The bearing border was then measured and a mark was made half way between the toe and the heel. The two points were joined, *figure 5.1.4.iii.*, and these were defined as the quarters. However, Kaneps *et al.* (1998) also measured the widest points of each quarter from the solar

base by drawing a line at the widest point of the capsule at right angles to one drawn mid-sagittally from the bulbs of the frog to the toe, which is likely to be a more accurate measurement and would ensure repeatability in finding this particular reference point. But if both methods of Kaneps *et al.* (1998) were to be used; then in some shaped feet, particularly those which are base wide, two points would be defined as the quarters and the author should have made a distinction between the two anatomical points.

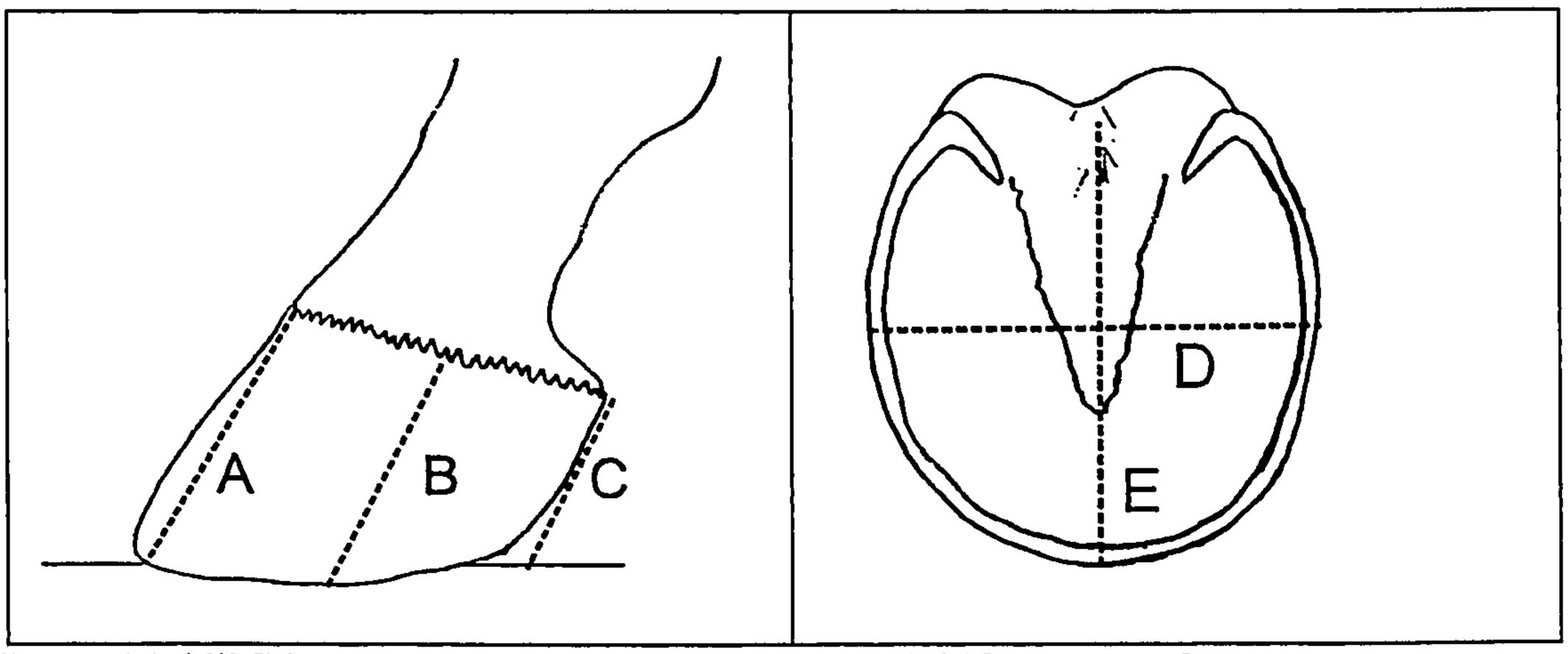


Figure 5.1.4.iii Diagram showing how Kaneps *et al.* (1998) measured quarters on a hoof capsule

Andersson and Lundstrom, (1981) measured sole length (amount of wall in contact with the ground) and sole breadth (widest point of hoof) of dairy cow claws as part measurements to describe claw shape. They compared the repeatability of all measurements and found that sole width had the lowest repeatability. They stated that the low repeatability of sole width maybe explained by the absence of reference points. In horses, the widest point of the capsule can be defined either from photographs of the sole or the frontal view.

In this thesis the widest point was measured from the sole. Where the widest part of the sole met the wall was defined as the widest point of the quarters.

It was considered important to distinguish between the widest point of the quarters and the point which, if a line was drawn perpendicular to the capsular base mdc at 50% of the capsular base, would divide the hoof capsule into quarters. Where this line met the hoof capsule was defined as the point of quarters.

Defining the quarter length

The lateral and medial walls of the hoof capsule have direction in two planes. Most researchers measured the length of the wall along the lines of the tubules in a plantar /palmer direction. Different techniques are used to take this measurement. Glade and Salzman (1985) measured lengths from photographs, Kaneps *et al.*(1998) measured lengths of the hoof capsule *in vivo*

using a ruler, Turner, (1992) measured lengths using a tape measure; the advantages and disadvantages of these methods have already been discussed. Newlyn *et al.* (1998), measured lengths of the capsule along the line of the tubules at given distances from the MDC along the sagittal axis, quarters were marked on the diagram. It was not clear which area was being defined as the quarter. Measurements were taken along the longitudinal axis of the horn tubules to the coronary band, which maybe an arbitrary point. Thought should also be given to the effect of the angle of the tubules on the perceived length of the wall. If there has been a disease process or a change in growth rate, then tubules sometimes change direction mid wall; a decision would need to be made as to which line should be followed, *figure 5.4.1.iv*.

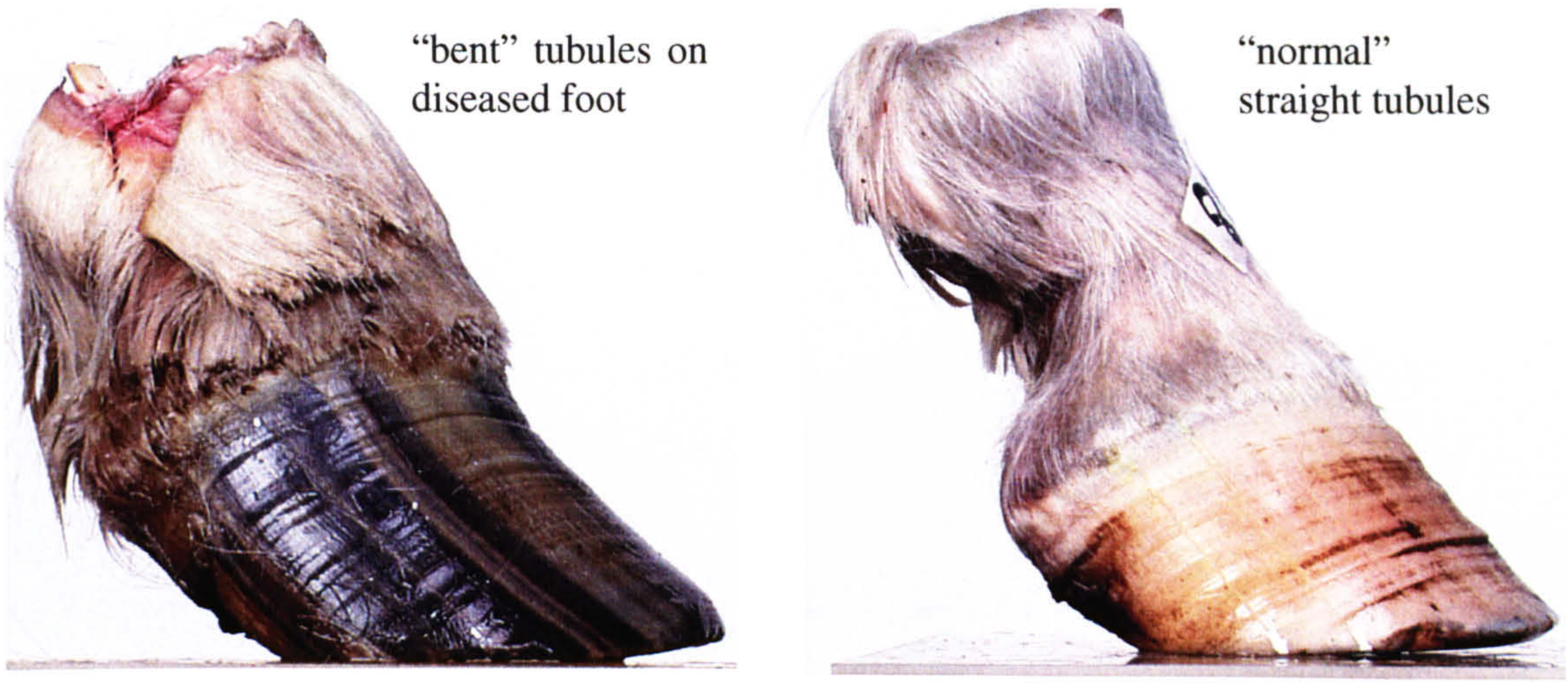


Figure 5.1.4.iv The effect of a disease process on tubular angle and subsequent effect of tubule angle on length measurement

In order to obtain the measurements needed to describe the sagittal plane, the length of the quarters in the sagittal plane were measured along the tubules both at the point of quarter and at the widest point of quarter. However, the consideration had been taken that these measurements would be unnecessary, as it is unlikely that they would give any additional information regarding the sagittal plane that could not be obtained from the toe and heel lengths.

The second length which was measured from both these points was the proximo/distal length which is the inclination of the wall in the frontal view these lengths define the length and inclination of the frontal plane of the hoof. The two measurements of length were taken from the point of quarter and the two measurements were taken from the widest point of the quarter

Defining quarter angles

Two angles can be used to describe the inclination of the hoof wall on the medial or lateral sides: -the angle of the hoof wall with the ground surface along the line defined by the tubules

or the inclination of the bearing surface towards the leg. Newlyn *et al.* (1998) measured the angles of the tubules from a lateral view in the x/z plane, where x was defined as the distal proximal plane from the bearing border to the coronary band and z the palmer/plantar plane from the toe to the heel. Five angles fell within the area described as the quarter; it would be very difficult to measure angles using this method *in vivo* on the hoof capsule of the horse. These angles give little additional information compared to the toe and heel angle for describing the outline of the hoof in the sagittal plane within the precision required at this stage of method development.

It was considered more important to measure an angle from a predefined anatomical point.
The lateral and medial angles at the widest point of the quarter in the frontal plane provide important information about the shape of the capsule in this plane and provide the information required to determine the inclination of the sides of the capsule in this view. The medial and lateral angles at the widest point of the capsule have been measured from photographs of the frontal view of the hoof wall (Thomason 1998; Thomason *et al.* 2001) but no researcher appears to have taken these measurements directly from the capsule. Consequently there has not been a comparison of angles measured from photographs with those taken *in vivo* on the capsule.
Using specially designed equipment, the medial and lateral angles at the widest point of the capsule in the frontal plane were measured

Defining the anatomical point of the heel

Much of the literature when describing medio-lateral balance fails to distinguish between the quarters or the heels, (Balch *et al.* 1991a; Snow and Birdwall 1991; Turner 1992). Very few groups have defined the two regions. Kuwano *et al*, (1999) classified the bearing border into five regions separating the heel area from the quarters. They described the division, as trisecting lines between the midline dead centre of the foot and the buttress of the heel, there is no detail as to whether a protractor or ruler was used to define these boundaries.

Because of the lack of easily definable anatomical points on the hoof capsule between the widest point of the quarter and the heel, no attempt was made to enforce one. It was considered more important that the outline of the capsule was described and therefore the point of heel in this thesis was defined as the caudal cornified aspect of the hoof wall at the distal surface

Defining heel lengths

Heel lengths have been measured from photographs of the foot, (Glade and Salzman 1985; Vermunt and Greenough 1996). Kaneps *et al.* (1998) and Turner (1992) measured lengths on

the hoof capsule following the line of the tubules. Turner, (1992) was not specific as to where the length of the heel was taken, Kaneps *et al.*(1998), described the heel length as being taken biaxially from the most proximal cornified aspect of the hoof wall to the bearing border, Newlyn *et al.* (1998) measured heel heights along the longitudinal axis of the horn tubules at sites nine and ten from the midline dead centre but these measurements measure two lines within an area described as the heel rather than one specific point. Thomason *et al.* (2001) measured the heel length from the hairline to the ground, parallel to the margin of the heel. They were specific as to where the measurement was taken at the coronet band, however from the diagram provided it was unclear where to measure the margin of the heel. If comparisons are to be made with toe length, for example, Turner, (1992) stated that heel length should be about one third of toe length, then measurements of the toe and heel must be made in the same aspect.

In this work, the heel length was measured from the hairline to the most caudal distal bearing border along the line of the tubules

Defining heel depth or height

Vermunt and Greenough, (1996) measured heel depth in cattle claw and defined it as the vertical distance from the ground surface to the skin/wall junction at the heel bulb. Arabian *et al.* (2001) measured heel height in hooves from the distal edge of the hoof wall at the heel to the coronet edge, this measurement was used in a regression equation to calculate hoof mass.

The heel height was measured in this thesis by projecting a horizontal line using a thin needle from the hairline at the heel and measuring to the ground. If it proved too difficult to measure the heel angle *in vivo*, then this measurement would be used with heel length to calculate toe angle, as sin a = opposite over hypotenuse

Defining heel angle

Measurement of heel angle is less variable between researchers. Newlyn *et al.* (1998), measured tubular angle at 100% capsular depth. Thomason *et al.* (2001) measured the same x/z angle as Newlyn *et al* (1998), at the caudal aspect of the hoof wall. Turner, (1992) measured heel angle from radiographs, which was not an option in this work.

If comparisons were to be made between toe angle and heel angle, then the angle the heel made with the ground surface needed to be measured along the line of tubules at the cornified caudal edge of the hoof wall.

Defining the perimeters

In order to measure a perimeter, reference points which show the start and finish of the perimeters needed to be defined. Measurement of perimeters varies amongst researchers; some calculate the bearing surface, (Kaneps *et al.* 1998), others take 'real' measurements, (Newlyn *et al.* 1998; Arabian *et al.* 2001). Kaneps *et al.* (1998) calculated the bearing surface of foal feet by taking measurements from the solar aspect. A line was drawn from the toe, (dorsal aspect of the hoof at the bearing surface) to the heels. A second line was drawn across the sole at the widest medial and lateral points of the hoof capsule. These measurements were then used to calculate the circumference of an ellipse[g], however not all hooves are elliptical some are circular, thus the accuracy will vary depending upon the shape of the sole.

Newlyn *et al.* (1998) accurately mapped the solar aspect, (bearing border) of the donkey foot by measuring from the mdc to the bearing border at set distances along the mdc. Arabian *et al.* (2001) measured both the base circumference at the distal edge of the wall and the coronet circumference but did not explain the reference points they used to delineate the wall at the heels. Douglas *et al.* (1996) used a flexible rule to measure the solar circumference around the outer circumference of the distal wall between the most posterior weight bearing points medially and laterally; coronary circumference was measured around the entire foot at the hairline.

Snow and Birdsell, (1991) and Turner, (1992) measured coronary band circumference and shape graphically by taking seven hoof lengths in the direction of the tubules. The first measurement was taken at the toe and three medial and lateral measurements are taken equilaterally from the toe. The measurements are then plotted starting at one heel on a graph with the distance between each point plotted on the x-axis and the length on the y-axis, *figure 5.1.4.v.*

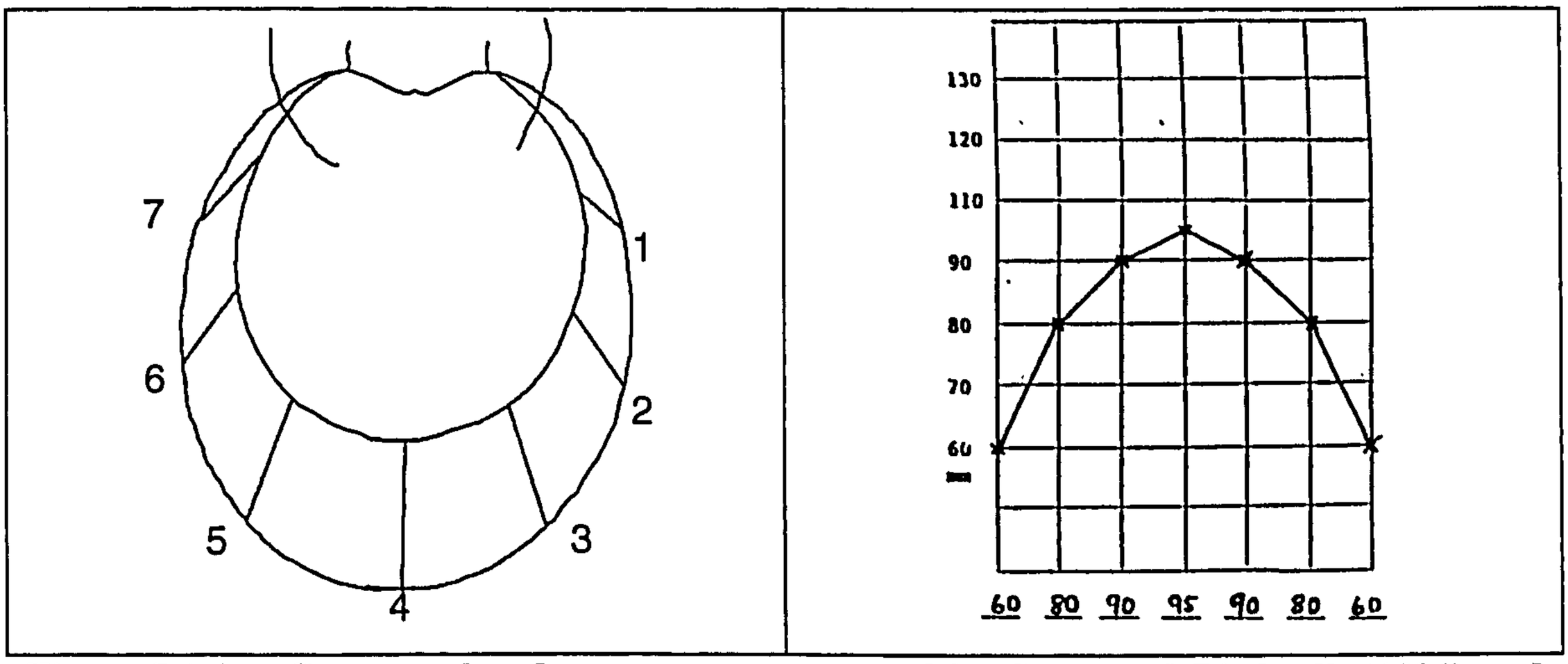


Figure 5.1.4.v Coronary band contours as measured by Snow and Birdwell, (1991) and Turner, (1992) *(adapted from Turner, 1992)*

Whilst this method visually illustrated the length of the hoof wall and any resultant distortions of the coronary band, (Snow and Birdwall 1991), in reality it is more likely to be both the bearing border and the coronary band, which are uneven but the two may not parallel each other in the distortion. In addition, it would be difficult to compare anatomical areas because due to medial /lateral imbalance the toe is often off centre and therefore the origin will vary.

The distal perimeter of the hoof in this thesis was measured using the delineation of Douglas, (1996). The reference points of the distal perimeter were the most caudal contact that the wall had with the ground and therefore available for weight bearing. The proximal perimeter reference points were delineated by following the tubules from the distal points to the roots of the hairline at the coronary band

Defining the sole

The anatomical points were defined as described in chapter two, *section 2.1.1*

Sole length

Kane *et al.* (1998) defined sole length in two ways. Firstly from the solar base, the distance parallel to the long axis of the hoof from the toe to a line between the corners of the heels was measured; secondly the length of the capsule was measured from the lateral view along the ground surface from the toe to a line drawn vertically from the most palmer aspect of the heel bulb. There is likely to be a discrepancy between these two measurements and they recorded a difference of 1.5 - 2.5cm between the two sole measurements. The heels may have asymmetry and it may be more accurate to take the measurement actually from the sole rather than projected to a parallel line, *figure 5.1.4.vi*. In addition the precise position of the anatomical point described as the toe had not been identified by Kane *et al.* (1998), which may have resulted in measurement differences rather than true differences. In several capsules the weight bearing part of the wall is not the same as the entire length of the sole, underrun heels will result in the palmer aspect being in a different position to upright heels but this is not reflected at the bearing border.

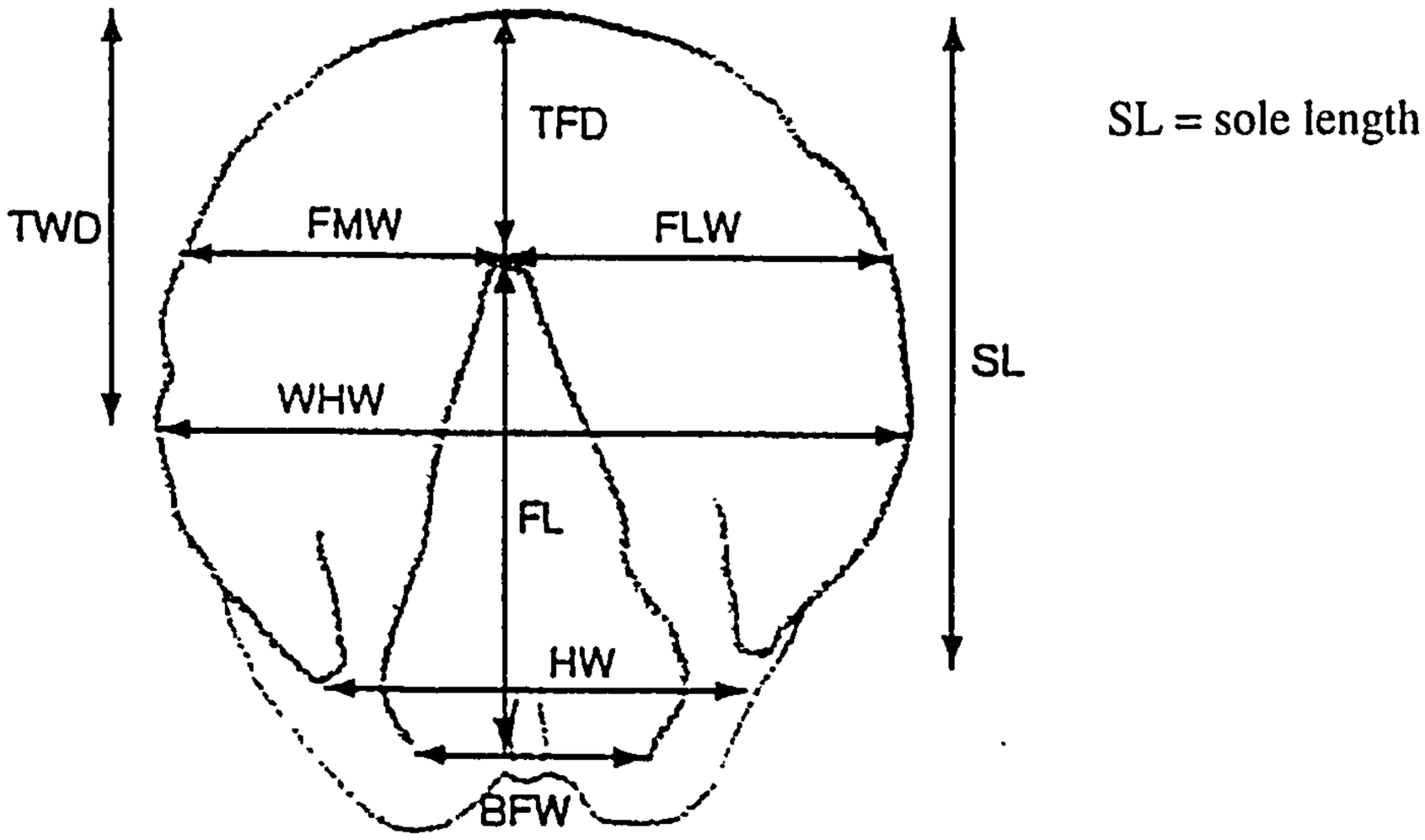


Figure 5.1.4.vi Measurements taken by Kane *et al* (1998) to describe the sole of the hoof capsule

In order to ensure consistent measurements for the base of the sole, one length was used to represent the length of the sole length and one length to represent the length of the weight bearing hoof capsule. The length of the capsular base from the point of the toe to the line which bisects the bulbs of the frog was defined as the capsular base. In addition a second length was measured and defined as the capsular depth, (section 2.1.1). The capsular width was measured at 50% of the capsular depth (CW50%) and at its widest diameter, (CWWP).

Defining the photographic technique

Because the hoof is a complex shape, it is difficult to adapt instrumentation and taking photographs, which then can be digitised, may be an easier option. Glade and Salzmann, (1985) used photographs to capture the image of a cow's hoof; the camera and hoof were placed in a wooden frame, which standardised distances from each other and photographs were developed so that the images were life-size. Bergsten, (1993) standardised photography of the sole of dairy cows; the camera, (50mm lens and 50ASA colour film) was positioned 40cm from the foot with lens parallel to the sole, so that the sole filled the field of view. However, there was no scale included in the photographs, without which it is impossible to standardise for size. In order to fill the field of view for smaller feet, the camera would have to be moved closer to the foot Measurements taken directly from the claw were not reported and therefore no comparisons between photographs and direct could be made.

Vermont and Greenough, (1996) used a similar technique to take lateral views of cattle claws, a 200mm lens was used and a 14cm scale positioned immediately parallel to the wall of the claw. The inclusion of a scalar in the photograph meant that measurements could be compared as the sizing can be standardised. The photos were developed as 35mm slides, projected onto a digitising tablet, loaded onto a computer and the image analysed mathematically to give lengths and heights in centimetres and angles in degrees.

Bergsten, (1998) evaluated digital photography as a method for evaluating cattle claws for lesions and compared it to conventional photography and to direct visual scoring. The research concluded that 'the reliability of photo scoring appears to be comparable to direct visual scoring', unfortunately there appears not to have been any statistical comparison of the techniques. However, Leach *et al.* (1998) did compare visual and photographic techniques statistically and obtained a Spearman's rank correlation of only 0.65. The method evaluated was standardised by placing two 12mm discs on the distal surface near the toe and the heel for calibration; the photograph was taken perpendicular to the distal surface at a distance of 0.4m, so that the foot filled the field of view. The solar view was then digitised and the claw outline and lesions measured by an image analysis package. In this instance the shape of the claw was not being measured, rather the presence and severity of lesions, but it did illustrate that photographic measurements may not always reflect the 'on animal' measurement.

Kane *et al.* (1998) took measurements from photographs to evaluate not just the balance of the hoof, but also its size and shape, as possible risk factors contributing to muscular skeletal injuries in ninety five racehorses. The hooves were removed from the limbs of the horses and photographed using a standardised procedure. This included attaching the hoof to a square board and fitting it into a holding jig. The photographs were taken from a point parallel to the solar surface with a focal length of 50cm and a focal point centred on the ground surface of the hoof. A calibration ruler was included in each view. Toe length was measured from the hairline to the tip of the toe on the most dorsal aspect of the hoof from the medial and lateral views and then averaged. The precise position of the toe was not defined. It is likely that calculating a three dimensional angle from two dimensional photographs of the medial and lateral views introduced error, which was indicated by the fact that the two views, (Kane *et al.* 1998), gave different results and had to be averaged. In addition, unless the hair had been cut back to the roots, the hairline would be variable and difficult to identify on photographs. These factors were taken into consideration when choosing techniques to measure toe length in this thesis.

Kane *et al.* (1998) also used three medial and lateral lengths taken at equidistance from the toe which they measured from the photographs, but commented that 'surprisingly none of these measurements helped to distinguish cases from controls'. It is possible that the measurements taken from photographs, being two dimensional, did not take into account the curvature of the hoof wall with the ground surface. The 'real' distance may not be equidistance, should these measurements be taken *in vivo*, there are likely to be discrepancies.

Thomason, (1998) took lateral and dorsal photographs of equine hooves to describe shape, using a previously developed technique, which had not been published. The lateral view was taken parallel to the sole and centred on the midpoint of the bearing surface. Thomson *et al.* (1992) stated that it was impossible to take the dorsal view parallel to the solar surface, centred on the midline at the toe, instead the camera was placed 2m in front of the hoof and 0.6m from the ground, a scale was included. This will introduce a variable if hooves are of different sizes. The authors also noted that there was a parallax[g] error in the medial and lateral angles but they assumed the error to be consistent.

In a later paper, (Thomason *et al.* 2001), a subset of 23 measurements from Kane *et al.* (1998) was used to quantify external hoof shape. The linear measurements were made from plaster casts of the feet using a ruler; the effect of the plaster cast on size was not discussed. However some variability must be inherent in this method unless the cast was standardised for thickness on every hoof. The cast was photographed and the angles and the solar views were measured from digitised images of the casts using public domain software, no detail on standardising focal length was discussed. In addition, the accuracy of finding the hairline from a plaster cast would need to be questioned. Radiographs were taken of all the hooves, and there was an indication that the radiograph was used to identify the tip of the extensor process, but it is not clear how this was translated onto the plaster casts

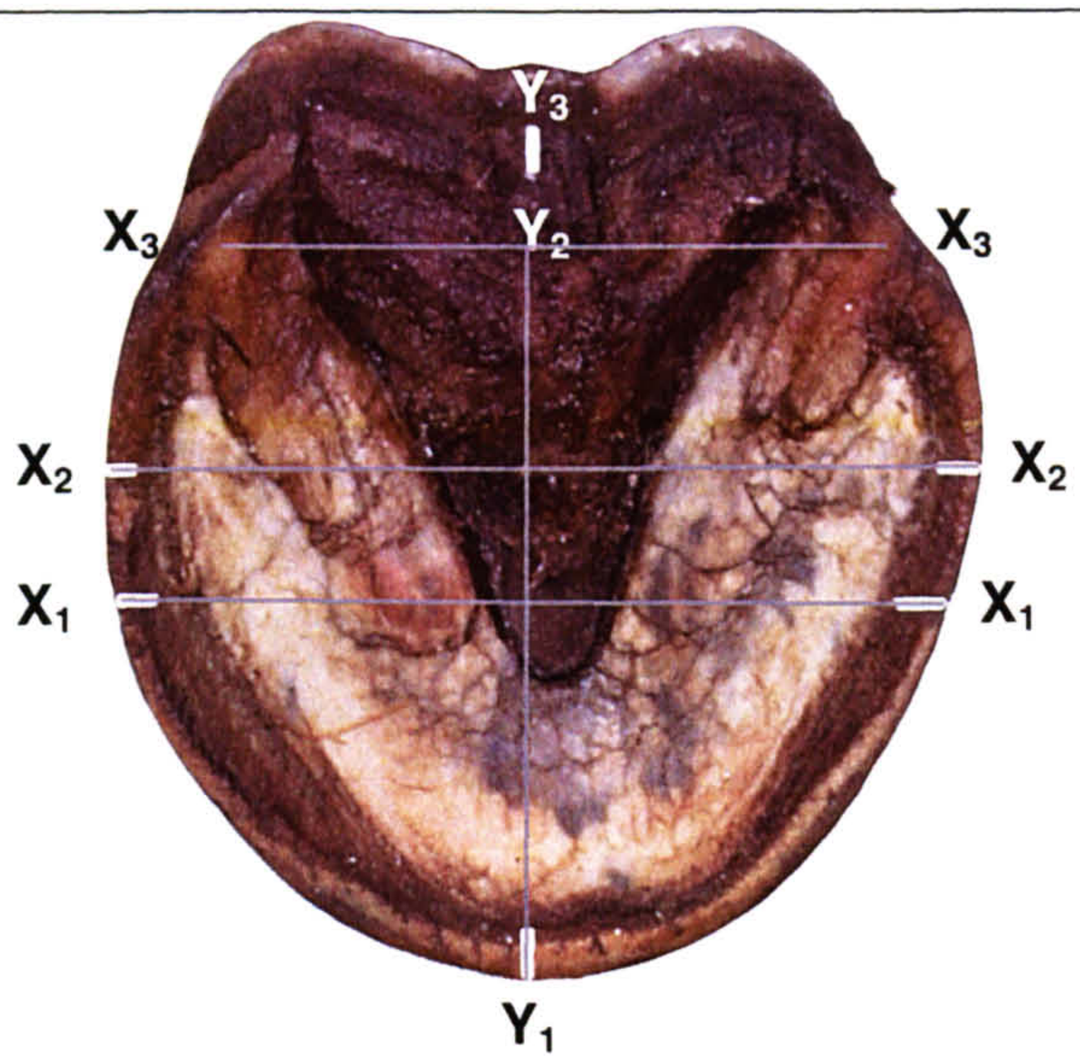
5.2 Methods

The specific aim of this part of the thesis was to measure the shape of the hoof wall using easily definable parameters and in addition to develop a series of ratios to allow comparisons between different sized hooves. This was achieved by the following:-

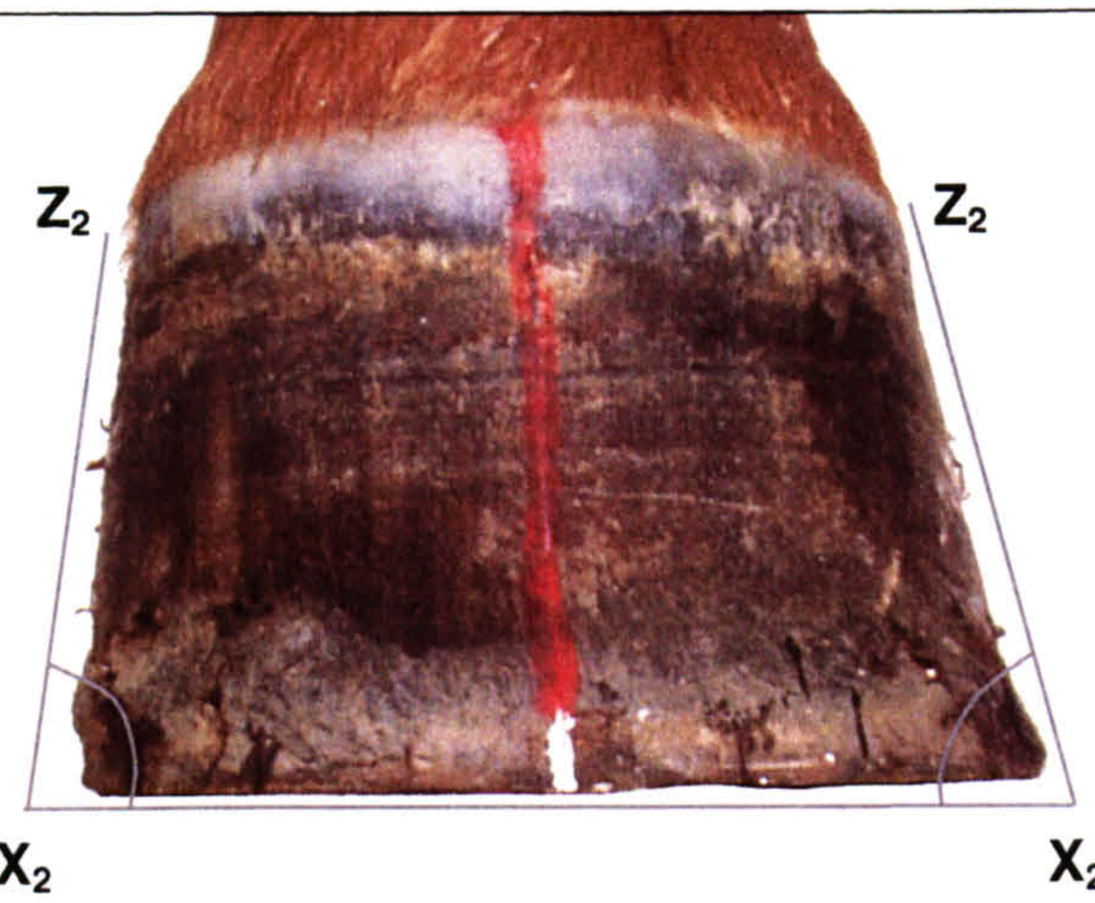
1. Measurement of easily definable parameters to obtain a data set of equine hoof wall shapes
2. Photography and digitisation of the hooves in order to compare *in vivo* measurements of the hoof capsule and the digitised photographs
3. Development of ratios of the defined measurements to distinguish between shape changes in hoof capsule shape

5.2.1 Identification of anatomical points

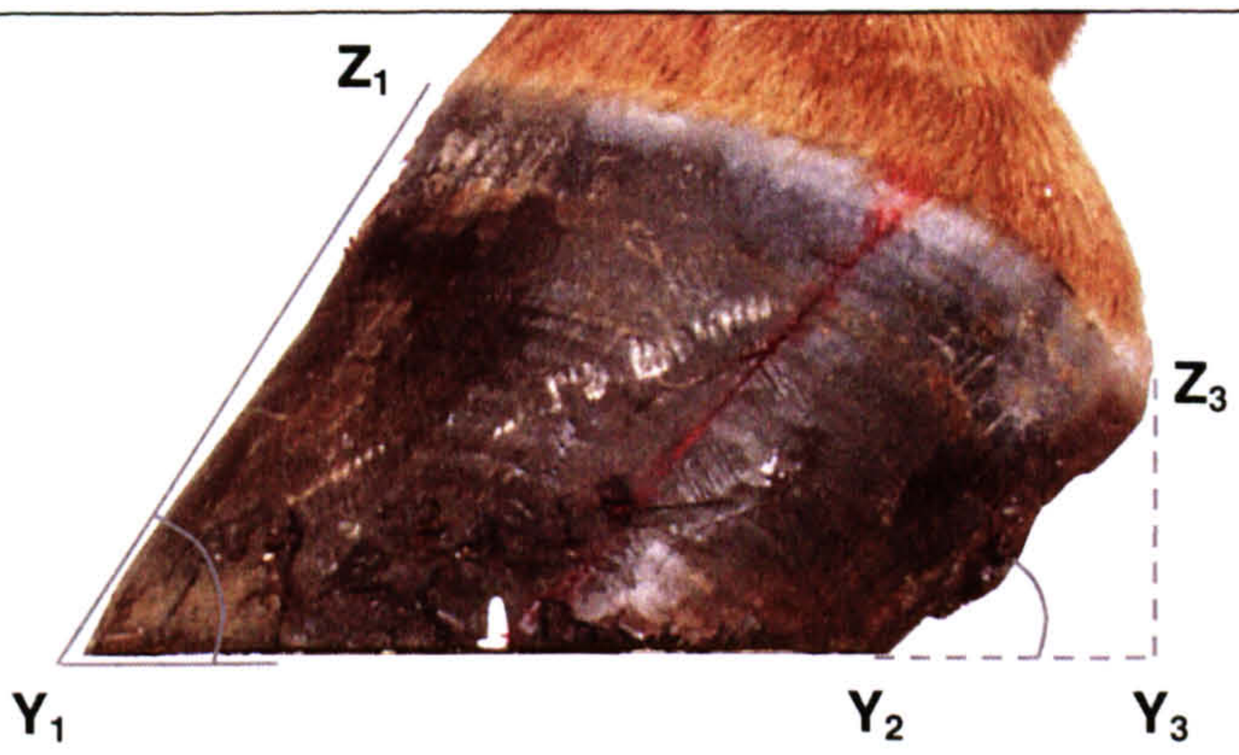
Some of the measurements chosen to describe shape were also used to designate points and regions from which samples were taken for trace element analysis or for crack mapping; these have been described in chapter 2, *section 2.1.1*. The anatomical points are summarised in *plate 5.2.1.i.*



CAPSULAR BASE / *TRANSVERSE*		
Position	Width	Depth
Y_1-Y_3 Y_1-Y_2		Capsular Base (CB) CD mdc
X_3-X_3	caudal wt bearing	
Y_1-X_2 X_2-X_2	Capsular width widest point CWWP	Capsular depth widest point CDWP
Y_1-X_1 X_1-X_1	Capsular width 50% CW50%	Capsular depth 50% CD 50%



FRONTAL		
Position	Length	Angle
Z_2-X_2	WP ¼ length	WP ¼ vertical angle



MEDIAL / LATERAL *SAGITTAL*		
Position	Length	Angle
Z_1-Y_1	Toe mdc length	Toe mdc angle
Z_3-Y_2	Heel length	Heel angle
Z_3-Y_3	Heel height	

Plate 5.2.1.i **Summary of the anatomical points and measurements chosen to determine the shape of the hoof capsule.**

5.2.2 Techniques for direct measurement of the hoof capsule

Measurements from the capsular base.

The length of the weight bearing capsular depth, (CDmdc), the capsular width at 50% (CW50%) of the CDmdc and the capaular width at the widest point of the capsular depth (CWWP) were measured from the graph paper using a ruler to the nearest mm and the widths and length recorded for every capsular base. String was used to trace the length of the distal perimeter on the hoof between the two caudal points. A ruler was used to measure the length of the string and this was noted.

Measurement of hoof wall lengths

The length from each designated reference point at the distal surface to the most distal roots of the hairline at the coronary band was measured along the scored line of the tubules. A set of dividers was used and then measured against a ruler; the lengths of the lines were noted. In addition the length of the capsular wall in the sagittal plane was measured at a 90^0 incline distally/proximally from the bearing border at the widest point of the capsule to the coronary band, (WP ¼ length).

The height of the heel was also measured from the most caudal point of the capsule at the distal hair roots to the ground, *plate 5.2.1.i.*

Measurement of hoof wall angles

A specifically designed angle gauge, (Peacometer) was used to measure the angle that the toe mdc made with the ground. The measurement was taken at 50% hoof wall height, (HWH) to avoid any discrepancies due to farriery at the bearing surface, *figure 5.2.2.i.* The equipment relied on gravity for its accuracy. The hoof was placed on a horizontal surface and the Peacometer was adjusted prior to using on each individual hoof. The Peacometer was designed so that when it was placed on a level surface and the metal button released, the weight fell vertically. The protractor scale was adjusted if required so that the red line and arrows were aligned specifically at the 180°. The Peacometer was then placed on the hoof toe mdc at 50% HWH and the metal button in the middle released to allow the weight to fall. The reading obtained was then taken away from 90° to provide the angle that the toe made with the bearing border

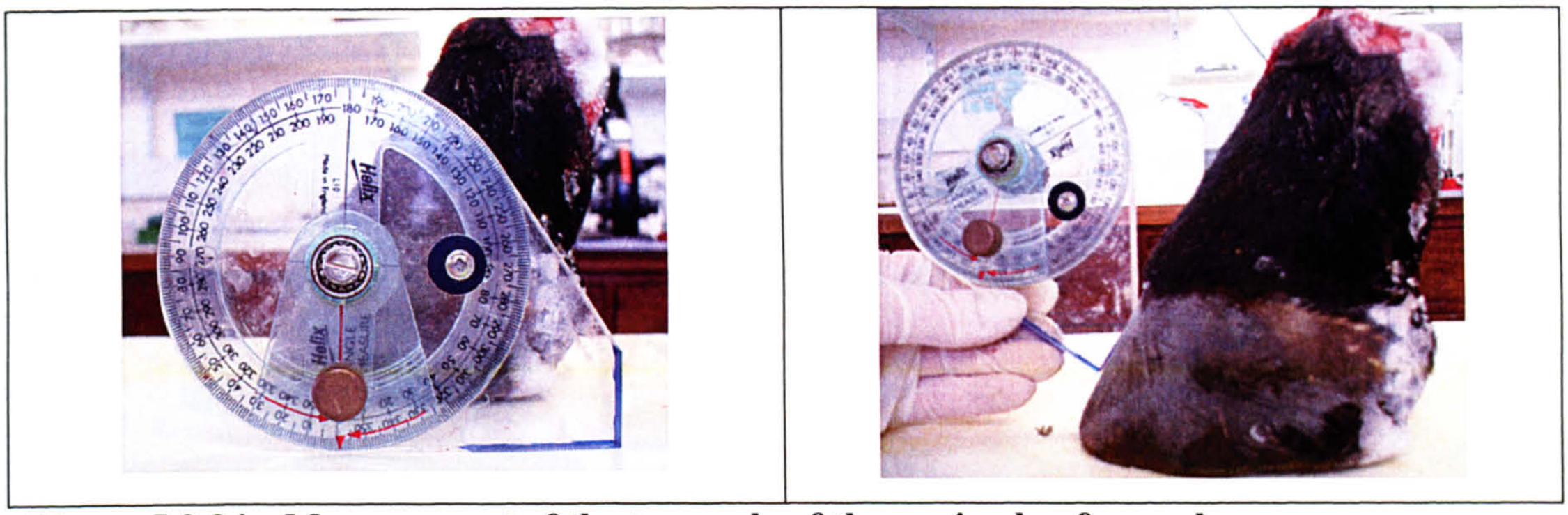


Figure 5.2.2.i Measurement of the toe angle of the equine hoof capsule

A protractor was used to measure the angle that the scored tubules of the wall made with the ground at the heels and the widest point of the quarters on both medial and lateral walls. The hoof was placed on a piece of graph paper, so that the base of the protractor was parallel to the base of the hoof and the 90° line was aligned to the bottom of the scored line. There was some difficulty in reading the protractor accurately at the heels. A back check was made by calculating a number of heel angles using the measured heel lengths. The lengths were substituted into the equation *sine* θ = *opposite/hypotenuse.*

The angle that the medial and lateral walls at the widest point of the quarters (WP ¼ vertical angle) made with the ground surface was measured using specifically designed equipment (Peacometer), *figure 5.2.2.ii*. The angle was recorded at 50% hoof wall height. The protractors were adjusted to 90° using a set square before a hoof was placed between them. The two protractors were then slid towards the hoof and the positioners placed against the hoof wall at the widest point of the quarter at 50% HWH. The angles that the walls made with floor were read directly from the Peacometer.

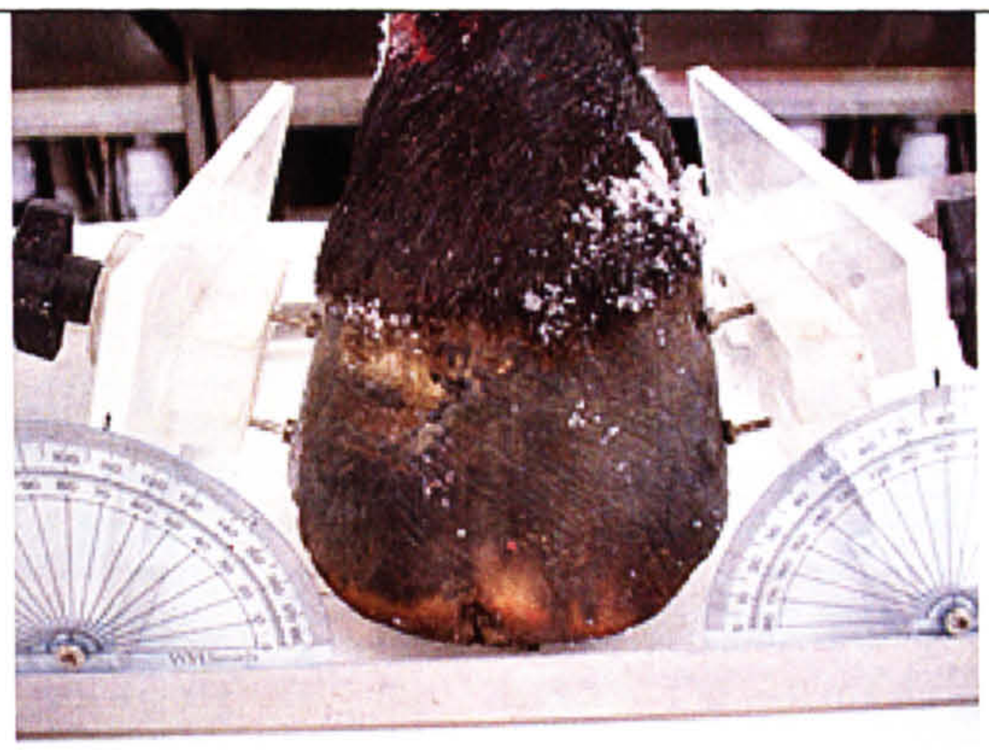


Figure 5.2.2.ii Measurement of the angle at the widest point of the quarters of the equine hoof capsule

A summary of all the measurements taken to investigate ways of measuring the shape of the hoof capsule are tabulated in *table 5.2.2.iii*.

Table 5.2.2.iii Summary of anatomical reference points and measurements taken to capture the shape of the hoof capsule

Measurement	Definition
Capsular base	
Capsular base, midline dead centre (CBmdc)	Midsagittal line of the solar base drawn from the caudal mid succulus of the heel through the centre of the frog if present to the dorsal wall.
Capsular Depth (CD)	A line drawn on the solar aspect of the hoof from the point of toe along the capsular base mdc to bisect the line drawn between the 2 most caudal weight bearing points on the distal perimeter
50% Capsular depth (CD50%)	Capsular depth at 50% of its length.
Capsular width 50% (CW50%)	The width of the capsular base at CD50% measured along a line, which bisects the capsular base mdc at 90^0. The points at which the line intercepts with the hoof wall at the distal surface were designated the point of the quarters
Capsular width widest point(CWWP)	The width of the capsular base at its widest point, measured along a line which bisects the capsular base mdc at 90° . The points at which the line intercepts the hoof capsule were designated the widest point of the quarters
Capsular depth widest point(CDWP)	The distance from the point of toe along the capsular base mdc to its bisect with CWWP.

Measurement	Definition
Toe	
Point of toe (Pt toe)	Intercept of the capsular base mdc with the dorsal wall at its distal surface
Toe midline dead centre (Toe mdc)	The line dissecting the dorsal hoof wall from the point of toe to the coronary band at the hairline parallel to the tubules.
Toe angle	The angle that the toe mdc makes with the ground, measured at 50% hoof wall height.
Quarters	
Quarter angle (WP ¼ angle) WP ¼ Vertical angle	The angle that the hoof wall makes with the ground at the widest point of the quarters. The tubular angle is the angle that the widest point of the quarters makes with the ground in the plantar/palmer aspect and is measured along the tubules. The vertical angle is the angle that the widest point of the quarters makes in the medial lateral aspect, measured at 50% HWH
Quarter length WP ¼ vertical length	The length from the widest point of the quarters to the hairline. The line measured along the tubules is the tubular length The length measured proximo/distally from the ground surface vertically to the hairline.
Proximal limit of the hoof wall	All measurements were taken from the junction of the hairline with the proximal border of the hoof wall at the coronary band. The hairline was defined as the distal hair roots, which are independent of hair length.
Proximal perimeter(PP)	The perimeter of the hoof wall measured around the hairline from the most caudal weight bearing aspect of the wall at the heel to the other heel.
Distal perimeter(DP)	The circumference of the hoof wall which is in contact with the ground; measured from the most caudal weight bearing point on one side of the capsule to the most caudal weight bearing point on the other side of the hoof capsule.
Heel	
The heel	The most caudal cornified aspect of the hoof wall at the distal aspect
Heel angle	The angle the heel makes with the ground along the line of the tubules from the cornified caudal edge of the hoof wall to the root base of the hairline
Heel height	The vertical height of the cornified caudal edge of the heel to the root base of the hairline
Heel length	The length of the wall from the caudal cornified wall at the distal surface along the line of the tubules to the root base of the hairline.

5.2.3. Techniques for photographic measurement of the hoof capsule`

It is not possible to develop a method of describing shape if measurements cannot be taken. There is a lack of instrumentation available to measure aspects of the hoof wall *in vivo* except for a hoof protractor. It is time consuming and difficult for the client, vet or farrier to take linear measurements of the hoof capsule simply because easy to use equipment has not been developed. Because of the potential limitations of photographic measurements as discussed above, it was necessary to compare photographic measurements taken under standardised

conditions with *in vitro* measurements made directly from the hoof capsule. A number of hooves were therefore measured using both these techniques.

Choosing a camera to use

As part of the method development in this thesis, two cameras were assessed to decide which minimised the influence of parallax referred to by Thomason, (1998). A Technical Monorail camera used by architects to remove parallax effects of tall buildings was fitted with a grided lens, which allowed reproducibility and repeatability because it was possible to centre the image. The lens can move independently which means that it was possible to alter the angles of the plane to distort the field of view to allow greater focal depth, which in turn increases accuracy. A digital camera was also assessed. A SLR Olympus E_{10} with 4.1million pixels was positioned 61.7cm from the base of the platform with a focal length of f13mm. The bellows extension was placed 13.2 cm from the platform base, a daylight balanced flash 500watt was used with an f32.5aperture to restrict light in order to improve focus, but to ensure adequate background light a multiple flash was used.

The base plate of the platform was taped in position, the camera tripod was taped in position and the focal length remained the same. This removed any variables which may have been introduced should any of these aspects have been altered. Rulers were put in the plane of measurement rather than a distance from the foot, this removed perspective and minimised magnification differences. One ruler was placed horizontally and another ruler vertically, to remove converging verticals. The base of the platform was used as the eye level for focus.

The pictures from the digital camera were downloaded straight onto the computer, which ensured that pixel for pixel remained constant. The Technical monorail camera required hard copies to be scanned, which could have resulted in distortion and variation introducing scale effects. In addition the digital camera produced colour photographs, which might be useful for looking at cracks at a later date. The digital camera was used for all further photographs.

Standardising the photographic procedure

The standardisation of the procedure is essential to remove error due to parallax and also to allow comparison of small hooves with big hooves whilst maintaining scale. All reference points on the hooves were renewed with correction fluid, to ensure visibility on photographs. The hair at the hairline was trimmed so that the distal roots would be clearly seen at the proximal end of all the scored lines. Any build up of ice was removed and the hooves were semi defrosted to minimize reflection from ice during photography. Hooves were photographed on a specifically designed mounting platform. When taking radiographs of the foot, a specific anatomical site is chosen for the camera's centre of focus, this is generally the centre of rotation

of the phalanx (Colles and Cripps). There is no easily identifiable anatomical site on the external surface of the hoof. If 50% of hoof wall height had been chosen, then the centre of focus would have varied depending on whether a hoof had a short toe or a long toe and the error due to a long toe would be greater than that due to a short toe. In order to minimise this variability, the centre of focus was standardised on the middle of the base of the platform. This removed any focal error due to size. The square platform was scored at 50% of both of its widths dividing its surface into 4 equal quarters, so a hoof could be aligned precisely to the same central point every time. The point of toe was placed on the vertical line and the widest point was aligned to the bisecting point, *(plate 5.2.3.i)* so that the length of toe was not influencing the positioning of the foot.

Each hoof was photographed from a dorsal, medial, lateral and solar view. In each instance the point of toe and the widest points were aligned on the platform using the bisecting lines, so that the positioning was reproducible. For the solar photograph the point of toe was placed centrally on the grid. The images were saved as Tiffs (10.7Mb) and numbered with the hoof number directly onto a laptop computer.

NIH imaging

NIH image* is a public domain image processing programme developed for Mac computers; a PC version is also available. The programme was used to measure linear and angular components of the hoof capsule. The control photograph for each set of hooves was used to calibrate the pixel scale. Both rules, vertical and horizontal, were used for the calibration in the first data set; however there was no significant difference between them, so other than for the first data set, the vertical rule was used. The same magnification was used throughout, so the calibration did not alter and the images were opened as TIFFS. The lines were measured between the marks which had been used for the measurements of the real hoof and the angles were measured by drawing two lines where the toe and heel met the ground surface.

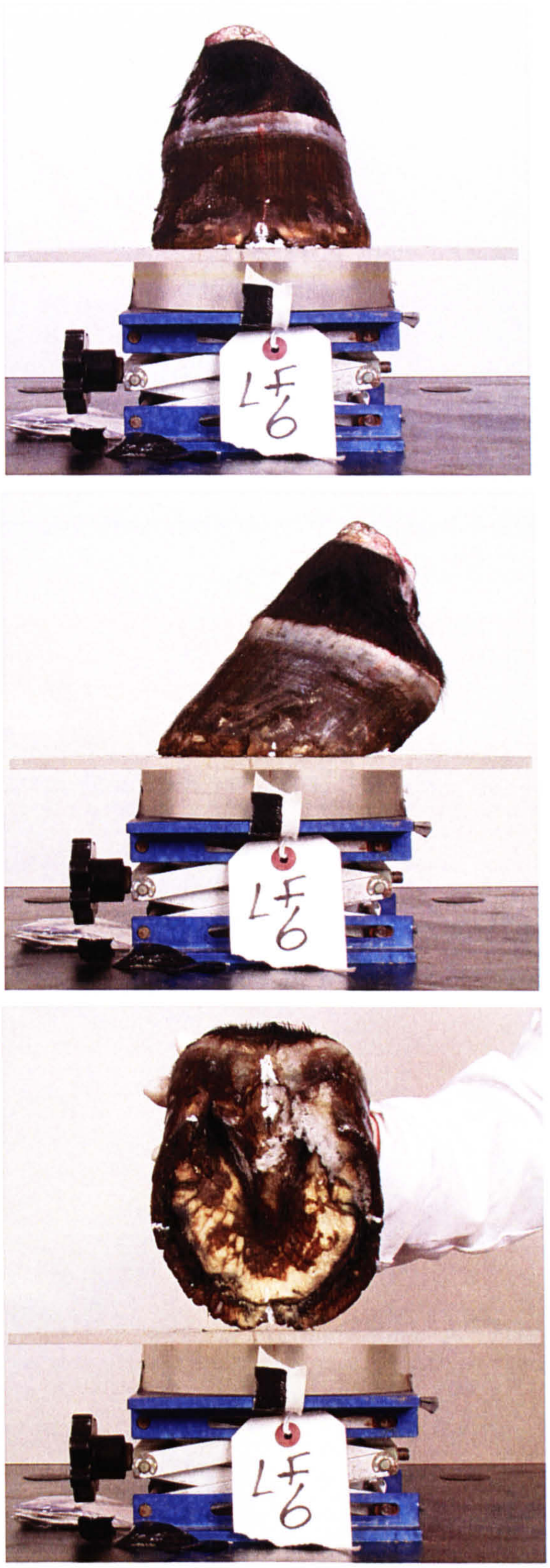


Plate 5.2.3.i **The positioning of the hoof on the platform as part of the standardisation procedure for photography**

5.2.4. Establishment of ratios to distinguish differences in shape

Various ratios, *plate 5.2.4.ii* were investigated in order to determine which captured best the shape of the hoof capsule.

The aims of using the ratios were: -

1. to establish an accepted number of easily recognisable descriptors of different hoof wall shape in the same way, that three measurements, (length, width, height), can define both size and distinguish between the shape of a cube and a rectangle
2. to capture the shape of the capsule in three planes, the capsular base, (transverse) , the dorsal, (frontal) and the sagittal
3. to remove size differences

It was decided *a priori* to use ratios which resulted in significant differences between measurements other than those chosen to group the hooves and which reduced the within group variation as determined by standard deviation compared to the whole population of hooves. The ratios chosen were required to distinguish the differences in shapes so that hooves could be categorised into the groups, *figure 5.2.4.i.*

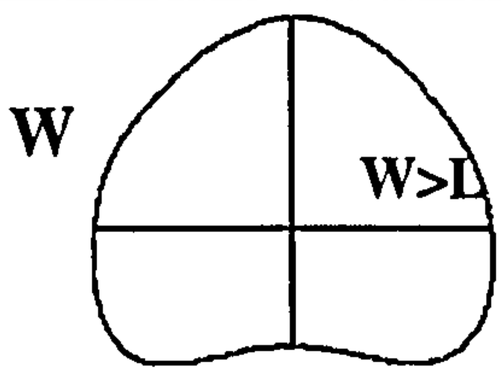
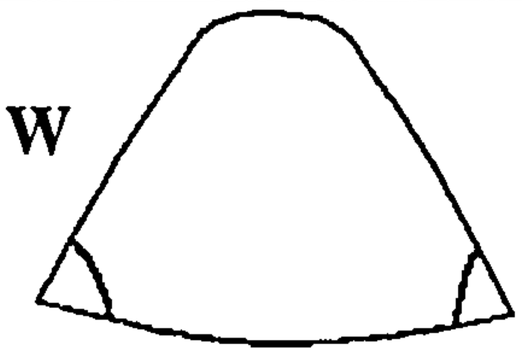
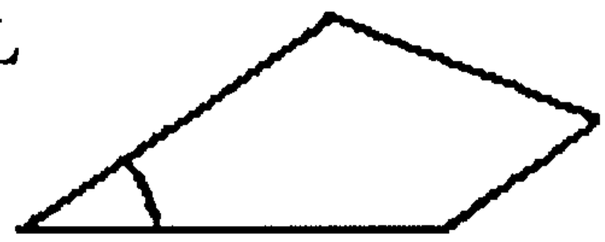
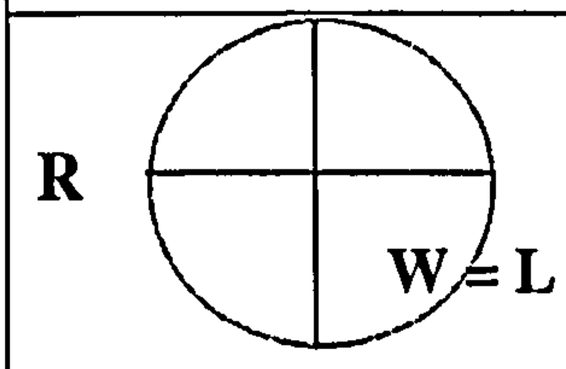
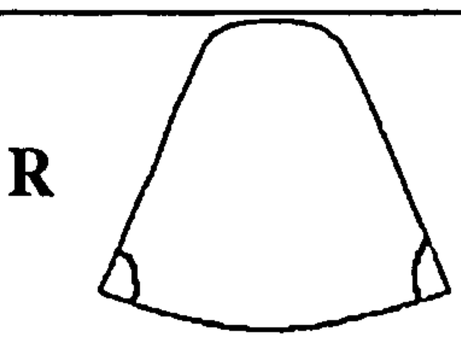
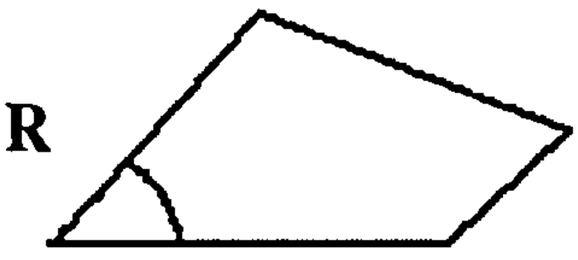
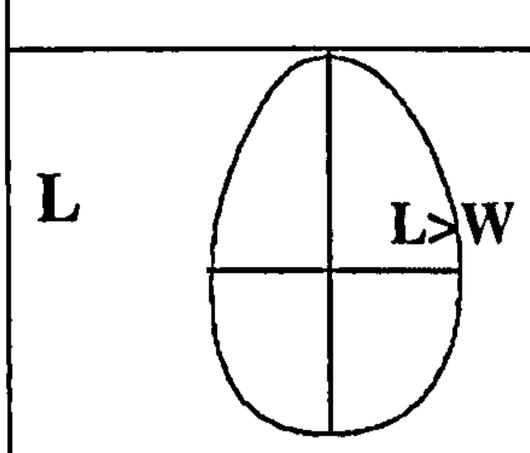
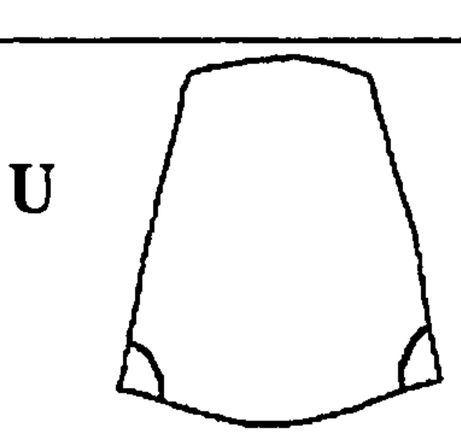
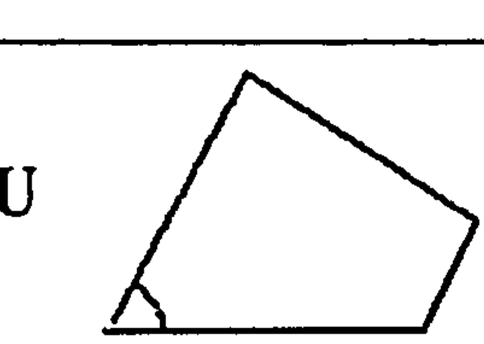
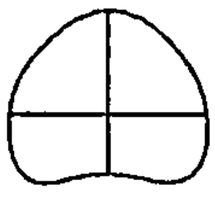
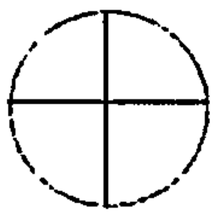
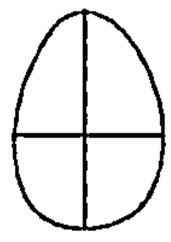
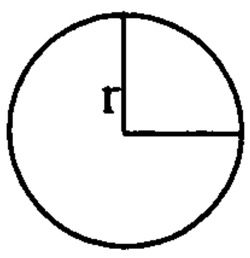
Base view *transvere plane*	**Front view** *frontal plane*	**Side view** *sagittal plane*
		
		
		

Figure 5.2.4.i Visual shapes which required quantification, R = regular, L= long, U = upright, W=wide

VISUAL	GEOMETRY	RATIOS INVESTIGATED

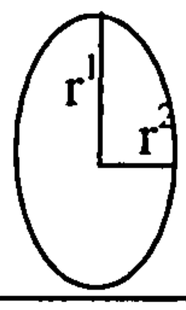
Establish capsular base plate

	Wide
	Roun d
	Long



$r = r \approx 1:1$

perimeter $= 2\pi r$



$r^1 > r^2 \approx 1 : < 0.95$

$r^1 < r^2 \approx 1 : > 0.95$

perimeter $= \pi(r^1 + r^2)$

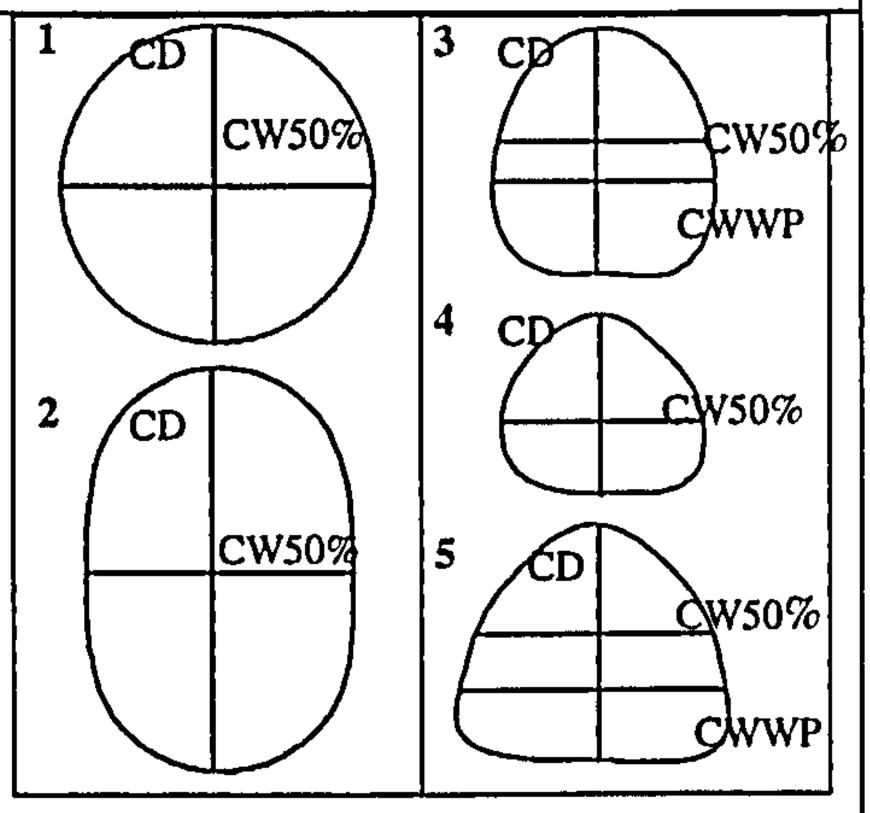
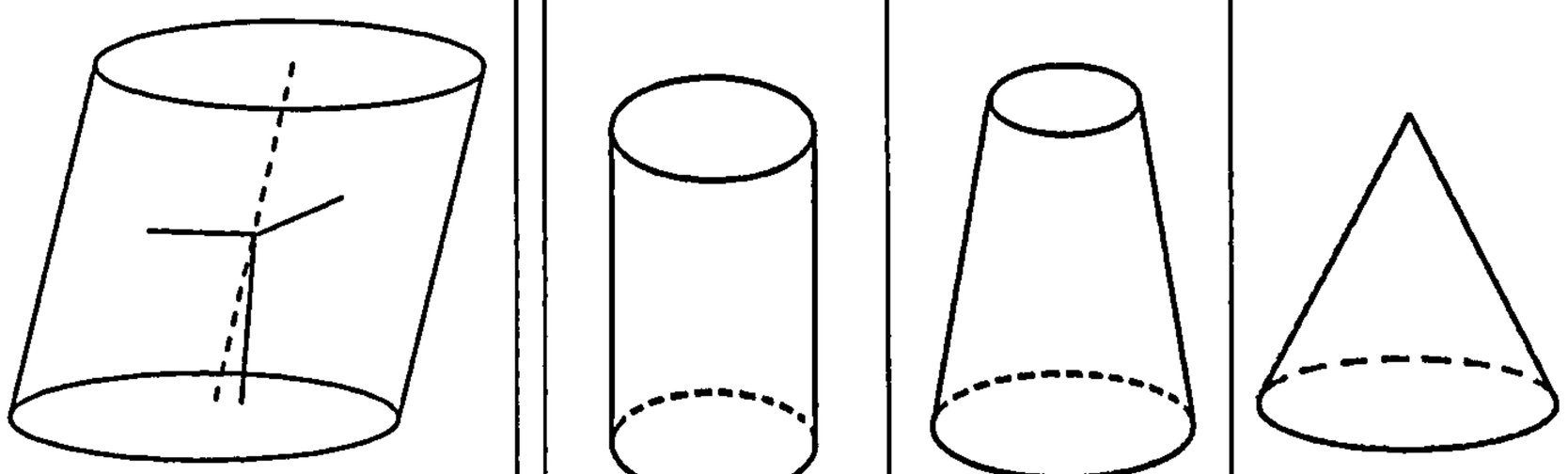


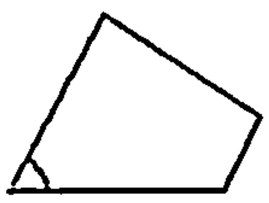
Diagram	1	2	3	4	5
CD:CW50%	1:1	1:<1	1:<1	1:>1	1:>1
CW50% : CWWP	1:1	1:1	1:<1	1:1	1:>1

Establish slenderness by investigating use of aspect ratios of width to length, capsular depth : toe length



Toe Length :CD
Toe length : CW 50%
WP ¼ vertical angle : toe angle

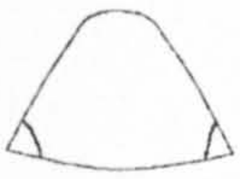
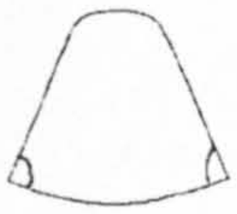
Establish Inclination by toe angle and heel angle

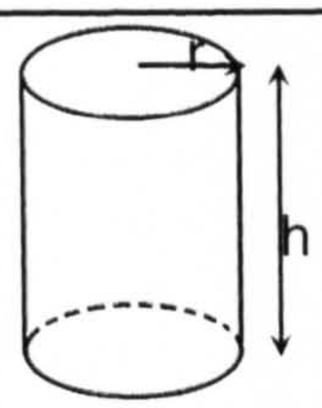
	Upright
	Regular
	Low

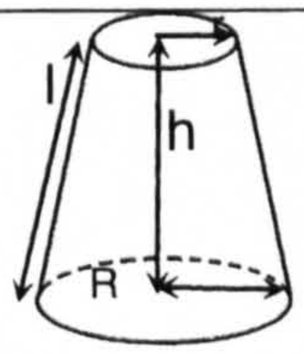
Toe angle should be ± 5° of heel angle. (Turner, 1982)

Toe angle : heel angle

Establish conical or cylindrical

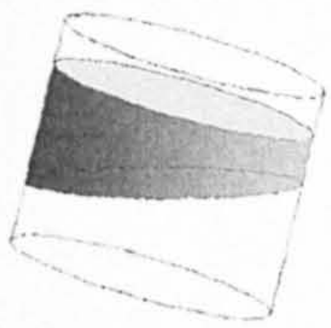
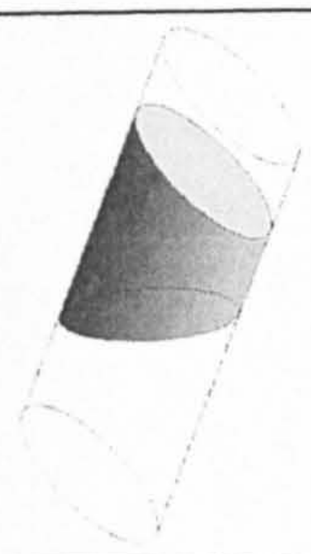
	Wide
	Regular
	Upright

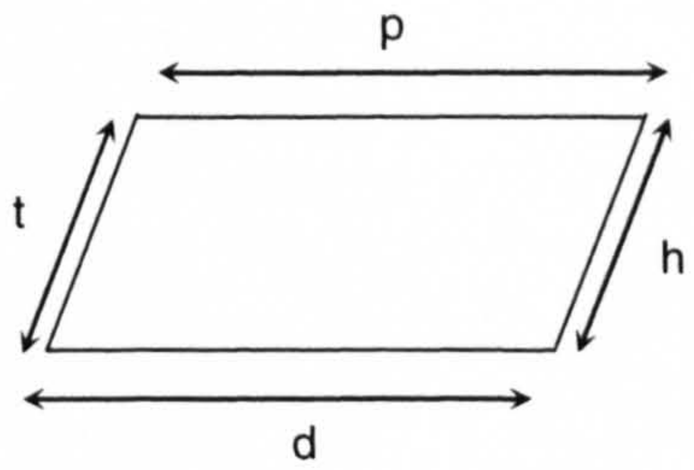




90° : WP ¼ angle

Establish level of truncation

	Wide
	Thin
	Cone



toe length : heel length dorsal
: proximal perimeter

Plate 5.2.4.ii A summary of the visual appearance of the hoof wall and ratios investigated to quantify the shape of the capsule *artwork © D.Gibson*

5.2.5 Statistical Analysis

All statistical analyses were performed using Minitab[13].

Summary data including the mean, standard deviation and range of measurements from:-

i) the whole hoof and ii) photographs of all the hooves are collated and presented in appendix *tables 5.3.1A.i – viii.*

All the analyses were performed on both data sets. The data from the mixed set of hooves was analysed initially and the data from the set of 28 left fores was subsequently analysed to test the assumptions reached with the mixed feet; however for ease of comparison and presentation, the results from both data sets are presented alongside each other.

All measurements were tested for normality by plotting histograms and confirmed using the Anderson Darling test of Normality at a significance level of $p > 0.05$.

5.3 Results

Measurements taken from the frontal plane were of the same order in both data sets, toe lengths were between 7.4cm -15.0cm. The widest point of the quarter vertical lengths were between 5.4cm-13.8cm and the vertical angles at the widest point of the quarter ranged from 54° to 103°.

Measurements taken from the transverse plane were more variable which was probably due to the hind feet in the data set of mixed feet. The capsular width widest point,(CWWP) ranged from 8.9cm -15.2cm in the group of mixed feet but between 11.5cm -18.8cm in the group of 28 left fores; capsular width at 50% of the capsular depth (CW50%) ranged from 8.3cm -14.9cm in the mixed feet and from 10.7 to 18.8cm in the group of 28 left fores. Toe angles measured from the sagittal plane varied from 35° to 60°.

Summary data of the mean measurements taken from the dorsal, (frontal) medial, lateral, (sagittal) and solar, (transverse) aspects of the hoof capsule and from photographs of the hoof capsule are presented in *table 5.3.1A.i* to *table 5.3.1A.viii* pages XXXIII - XXXVI in the appendix.

5.3.1 Comparisons of photographic and direct measurement techniques

There were no differences in the variances of any of the measurements being compared between the photographs and the capsules in either data set, ($p>0.05$).

Linear measurements taken from the actual capsule were significantly greater those taken from photographs but actual angles were not significantly bigger compared to those measured from photographs, ($p<0.05$). The differences were dependant upon whether the measurements were taken from the transverse plane of the capsular base or from any of the other planes of view.

Comparisons of measurements taken from the transverse plane (capsular base)

The measurements taken from the capsular base were not significantly greater than those taken from photographs of the capsular base in both data sets, ($p>0.05$).

Comparisons of measurements taken from the frontal plane

The mean length of toe and the length of the capsular wall at the widest point of the quarters measured from the capsule were significantly bigger than the same measurement from photographs, ($p<0.05$). However the difference was not significant when comparing the mean vertical ¼ angle at the widest point of the quarters measured from photographs or from the actual capsule, ($p>0.05$) in both data sets.

Comparisons of measurements taken from the sagittal planes (medial and lateral)

The mean medial toe length measured directly from the capsule was significantly bigger compared to the photograph, ($p<0.05$) in both data sets; but the difference in the mean toe length from the lateral view of 28 left fore feet was not significantly different between the two methods. When the two methods for measuring the length of the wall at the widest point of the quarters along the tubular length were compared, the length taken from the capsule was not significantly bigger compared to the photograph, ($p>0.05$) except for mean lateral length of the 28 left fore feet, ($p<0.05$). The mean toe angle from the capsule was not significantly bigger compared to that measured from the photograph, ($p<0.05$) in both data sets.

All mean heel measurements taken from the capsule were significantly bigger compared to those taken from photographs, ($p<0.05$) in both data sets.

Effect of precision on results

Although all measurements were accurate, it was not possible to be precise to more than ± 10% of the measurement being taken. Thus some of the differences which were shown to be statistically significant would not be biologically significant when this error was taken into account. The differences which were biologically and statistically significant in both data sets were reduced to those summarised in *table 5.3.1.i* when the measurement error was taken into account.

Table 5.3.1.i Summary of measurements shown to be significantly bigger taken direct from the actual hoof capsule compared to photographs with adjustment for measurement error of ± 10%

	mixed feet		28 left fores		
measurement	**photo mean**	**actual mean**	**photo mean**	**actual mean**	**significance difference if real difference is greater than measurement error**
frontal view					
toe mdc lgth	7.6	9.4	8.0	9.6	yes
mwp ¼ length	6.4	7.5	6.9	8.1	yes
lwp ¼ length	6.2	7.6	6.6	8.1	yes
sagittal view lateral					
toe mdc lgth	9.0	9.4	-	-	no

	mixed feet		28 left fores		
measurement	**photo mean**	**actual mean**	**photo mean**	**actual mean**	**significance difference if real difference is greater than measurement error**
heel length	4.2	4.5	4.8	5.4	no
heel angle	43.8	39.9	47.2	41.0	yes
heel height	2.9	3.5	3.6	4.0	yes
sagittal view Medial					
toe mdc length	8.9	9.4	9.4	9.6	no
heel length	4.3	4.8	4.8	5.8	yes mixed group of feet ; no 28 left fores
heel angle	43	36.6	46.1	39.0	yes
heel height	2.9	3.5	3.5	4.0	yes

Note 1 all length measurements are in centimetres **Note 2** all angle measurements are in degrees **Note 3** biological significance if differences between the measurements are greater than the ± 10% allowed for measurement precision.

Correlations between photographic and capsular measurements

Correlation coefficients between both methods of taking measurements chosen to describe shape were above 0.5, *tables 5.3.1.ii and 5.3.1.iii.* Photographic measurements taken from the capsular base in both data sets could be used to predict the actual measurements and in the data set of fore feet, 90% of the differences in actual measurements were matched by the differences in photographic measurements.

Table 5.3.1.ii Summary of correlation coefficients and regression equations between measurements taken from mixed feet and their corresponding photographs

Measurement	Pearson's correlation	p	Regression Equation	R^2 %
Plane				
frontal				
MWP ¼ lgth	0.523	0.0001		
LWP ¼ lgth	0.500	0.0001		
LWP ¼ angle	0.588	0.0001		
MWP ¼ angle	0.644	0.0001		
transverse				
CWWP	0.866	0.0001	CWWP= 1.45 + 0.879cwwpphoto	75.0
CW50%	0.890	0.0001	CW50% =0.8794 + 0.9309cw50%	79.2
sagittal				
Toe angle	0.632	0.0001		
toe length	0.750	0.0001		
M heel length	0.772	0.0001		
L heel length	0.679	0.0001		
L heel angle	0.533	0.0001		

Table 5.3.1.iii Summary of correlation coefficients and regression equationsbetween measurements taken from 28 left fore feet and their corresponding photographs

Measurement	Pearson's Correlation	P	Regression Equation	R^2
transverse				
CD	0.924	0.0001	CD actual = 2.024 + 0.827 cd photo	85.5%
CWWP	0.993	0.0001	CWWP actual = 0.5169 + 0.939 cwwp photo	98.6%
CW50%	0.991	0.0001	CW50% actual = 0.421 + 0.947 cw50% photo	98.3%
sagittal				
Toe angle	0.778	0.0001		
Toe length	0.880	0.0001	TOE LENGTH = 0.626 + 0.961 toe length photo	77.5%
Heel length	0.672	0.0001		
Heel height	0.698	0.0001		
frontal				
MWP ¼ angle	0.783	0.0001		
LWP ¼ angle	0.835	0.0001	LWP ¼ ANGLE = -9.818 + 1.14 LWP ¼ angle photo	69.8%
MWP ¼ length	0.480	0.011		
LWP ¼ length	0.611	0.001		

5.3.2 Effect of grouping on strength of relationships between variables describing shape

Effect of grouping by shape

The measurements and ratios which best captured the shape of the hoof capsule were the ratio of capsular width at 50% of the capsular base or at the widest point to capsular depth (CW50%/CWWP: CD) and the vertical inclination, (vertical angle at the widest point of the quarter when expressed as a proportion of 90°). Using these ratios hooves were grouped into shapes with long, wide or round capsular bases and wide, upright and regular vertical inclinations, tables *5.3.2.i – 5.3.2.ii*. Hooves were therefore either wide, wide, (WW), wide regular, (WR), wide upright (WU) or round wide, (RW), round regular, (RR), round upright, (RU) or long wide (LW) long regular, (LR) long upright (LU) where the first adjective refers to the shape of the base and the second adjective to the inclination of the angles in the frontal plane.

Table 5.3.2.i Allocation of mixed feet into groups according to the ratio of capsular depth (CD) to capsular width (CW50%&CWWP) and vertical inclination at the widest point of quarters

	Vertical Inclination		
Capsular base shape	Wide angle as a % of 90^0 <0.83	Regular angle as a % of 90^0 ≥0.83 & ≤0.89	Upright angle as a % of 90^0 > 0.89
Wide CD : CW >1 : 1.1	1,7, 8, 13, 16, 17, 19, 20, 21, 31, 32, 34, 48, 59, 71H, 78, 81, 85, 89, 92,95, 97, 99, 101	10, 11, 12, 15, 25, 33, 37, 40, 55, 56, 63, 65H, 80, 83, 90, 93, 98, 100H	18, 60, 61, 88H
Round CD:CW ≤1:1.1 & ≥1: 0.97	4,14, 26, 28, 35, 47, 49, 69H, 70, 72, 74H, 82, 86, 102	5,9, 22, 35, 36, 54, 57, 62, 68, 76, 84, 96H	6, 64, 75H
Long CD:CW < 1:0.97	*77,(laminitic), 91,(large part of wall missing)*	29H, 30H, 46, 50H, 53H, 57, 58, 73H,87H	2H,27H, 52H, 66H, 67H,94H, 79H, 92

Note 1 H denotes a hind foot
Note 2 Round base geometry based on ratio of radii 1:1 at both radii with a ± 10% measurement error
Note 3 Each tabulated number represents an individual hoof

The ratios captured the subjective visual appearance of the hoof. In the second data set of the 28 left fores, no hoof fell into the base long group. When a comparison was made with the data set of mixed feet, over 80% of the hooves in the long base group were hind feet. Two fore feet in the mixed group were allocated into long, wide on the basis of their ratios but hooves 71 and 91 had been highlighted as having known pathologies.

Table 5.3.2.ii Allocation of 28 left fore feet into groups according to the ratios of capsular depth to capsular width and vertical inclination at the widest point of the quarters

	Vertical Inclination		
Capsular base	Wide angle as a % of 90^0 < 0.83	Regular angle as a % of 90^0 ≥ 0.83 & ≤ 0.89	Upright angle as a % of 90^0 > 0.89
Wide CD :CW > 1 : 1.1	1a, 4a, 7a, 8a, 16a, 19a, 20a, 21a, 22a, 28a,	3a, 12a, 15a, 17a, 26a,	
Round CD:CW ≤ 1:1.1 & ≥ 1: 0.97	5a, 10a, 11a, 23a,24a,	6a, 9a, 13a, 18a, 27a,	2a, 14a, 25a,
Long CD:CW < 1:0.97			

Note 1 Round base geometry based on ratio of radii 1:1 at both radii with a ± 10% measurement error
Note 2 Each tabulated number represents an individual hoof

The summary data of the mean measurements of the different shaped hooves and the mean ratios of these hooves in the different shape groups are presented in the appendix in *tables 5.3.2A.i, iii, vi, viii.*

When hooves were allocated to different shape groups using the ratios CW50%/CWWP: CD and vertical angle at the widest quarter, there were significant differences, ($p<0.05$), between both mean measurements and median ratios depending upon the shape of the hoof, *tables 5.3.2.iii – vi*. The vertical inclination of RW was significantly less compared to RR, RU and WR and the vertical inclination of WW was significantly less than WR, RR and RU in both data sets. The CD: CW50% was significantly less in RR compared to WW in both data sets and significantly smaller in RU compared to WR and WW. The ratio of CD: CWWP of WR and WW was significantly greater than both RU and RR and the ratio of RW was significantly less than WW and WR in both data sets. The mean vertical angle at the widest point of the quarters varied in both data sets: - WW was significantly less than RR and both WW and RW were significantly less than RU. These differences are summarised in the *tables 5.3.2.v.* and *5.3.2.vi.*

Table 5.3.2.iii Summary of mean measurements and median ratios for mixed feet grouped by capsular base and dorsal angle into different shape groups

Caps Base vertical angle	long/ regular n=8	long/ wide n=2	long/ upright n= 2	round/ regular n=13	round/ wide n=11	round upright n=4	wide regular n=19	wide wide n=23	wide upright n=4	P
Measure	lgths=cms	angles=°								
CW50%	[b] 10.5	11.0	[b] 10.2	[b] 10.6	12.5	11.1	11.7	[a]12.6	11.1	p<0.001
CD	11.5	11.7	11.3	10.5	[a] 12.0	11.1	[b] 10.28	11.3	9.9	p<0.05
CWWP	[b] 10.9	12.1	[b] 10.7	[b] 10.9	12.6	11.3	12.11	[a]13.1	12.0	p<0.001
Distal Perim	29.4	29.0	29.4	28.4	32.4	29.4	29.4	31.1	27.7	
Toe angle	[bc]46.4	[b]39.0	52.1	[ca]50.1	47.3	48.8	49.3	49.4	[a] 56.0	p<0.001
MWP ¼ Vert angle	[ad]77.4	74.5	[a]85.5	[bg]74.5	[bc]68.5	77.0	[bcd]74.9	[bf]70.2	[acdg]84.2	p<0.001 * table5.3.2.v
Mean WP ¼ Vert angle	77.6	71.8	86.0	76.5	69.1	79.4	77.0	68.8	83.3	p<0.001
ratio	summary of diffs in ratios in table 5.3.2.v									diffs in table 5.3.2.v
CD:CW50%	0.92	0.94	0.94	1.0	1.03	0.99	1.12	1.11	1.14	p<0.001
CD: CWWP	0.96	1.0	0.97	1.05	1.06	1.01	1.17	1.15	1.19	p<0.001
toe lgth: CWWP	1.18	1.15	1.10	1.20	1.34	1.25	1.29	1.43	1.29	p<0.001
Vertical Inclination	0.86	0.8	0.96	0.86	0.78	0.9	0.86	0.78	0.92	p<0.001

Note 1 from the data set of mixed hooves
Note 2 measurements in cms
Note 3 angles in degrees
Note 4 different superscripts indicates differences at a significance level of $p<0.05$ unless otherwise stated, differences in ratios summarised in table 5.3.2.v
Note 5 measurements reported to one decimal place, ratios to two decimal places

Table 5.3.2.iv Summary of mean measurements and median ratios of hooves grouped according to the shape of their base plate and WP ¼ angles. (28 feet)

shape of capsule Base Angle	**Round Regular** N=6	**Round Upright** N=3	**Round Wide** N=4	**Wide Regular** N=5	**Wide Wide** N=10	P
Ratios	summary of ratio differences in table 5..3.2.vi					diffs in table 5.3.2.vi
CD:CW50%	1.01	1.01	1.02	1.12	1.17	p<0.001
CD:CWWP	1.04	1.02	1.05	1.16	1.20	p<0.0001
Toe length:CWWP	1.37	1.33	1.50	1.40	1.50	
Vertical Inclination	0.85	0.92	0.78	0.86	0.78	p<0.0001
measurements	measurement ± SD					
CD	13.0±2.2	11.0±0.7	13.0±1.7	11.5±1.9	12.3±1.4	
CW50%	13.0±1.8	11.9±0.5	13.3±1.2	12.6±1.2	14.5±2.0	
CWWP	13.6±2.1	12.2±0.5	13.8±1.6	13.3±1.9	14.9±1.7	
MWP ¼ vertical angle	77.1±4.6	85.0±1.7	69.0±3.1	71.4±3.4	69.1±5.1	p<0.0001
LWP ¼ vertical angle	76.1±2.4	81.0±1.7	69.7±3.8	82.8±2.8	68.8±7.4	p<0.0001
Toe angle	51.2±4.6	51.0±5.3	52.0±4.5	54.0±4.7	50.1±5.3	

Note 1 from the data set of 28 left fore hooves
Note 2 measurements in cms
Note 3 angles in degrees
Note 4 p<0.05 differences summarised in table 5.3.2.vi
Note 5 measurements reported to one decimal place, ratios to two decimal places

Table 5.3.2.v Summary of measurements which showed significant differences between different shaped hooves from the mixed feet data set

Base Vert Inclination	**Long Regular**	**Long Upright**	**Long Wide**	**Round Regular**	**Round Upright**	**Wide Regular**	**Wide Upright**	**Wide Wide**
shape groups		measurements						
LU	vertical Incline Mean WP ¼ vert angle							
LW		Mean WP ¼ Vert angle toe angle						
RR	CD:CW WP CD:CW 50%	vertical Incline MWP ¼ vert angle Mean WP ¼ vertical angle CD:CW50% CD:CWWP						
RU	CD:CW 50% CD:CW WP			Mean WP ¼ vert angle				

Base Vert Inclination	Long Regular	Long Upright	Long Wide	Round Regular	Round Upright	Wide Regular	Wide Upright	Wide Wide
RW	CD:CW 50% Mean WP ¼ vert angle CD:CW WP vertical Incline MWP ¼ vert angle	CD:CW50% CD:CWWP vertical Incline MWP ¼ vert angle Mean WP ¼ vert angle		vertical Incline Mean WP ¼ vert angle	Mean WP ¼ vert angle vertical Inclin	CD:CWW P vertical inclin CD:CW50 % CD Mean ¼ angle	vertical inclin CD:CW WP mean ¼ angle	MWP ¼ angle CD:C WWP
WR	CD:CW 50% CD:CW WP toe lgth	CD:CW50% CD:CWWP vertical Incline MWP ¼ vert angle Mean WP ¼ vert angle toe lgth	CD:CD 50% CD:CW WP vertical Inclin	CD:CW50 % CD:CWW P	CD:CW50 % CD:CWW P		vertical inclin	vert inclin mean ¼ angle
WU	CD:CW 50% vertical Inclinati on Toe Angle	CD:CW50% CD:CWWP	Toe angle Mean WP ¼ vert angle vertical inclin CD:CW 50	CD:CWW P CD:CW50 % vertical Inclination	CD:CW50 % CD:CWW P			
WW	CD:CW 50% CD:CW WP Toe length : CWWP vertical Inclinati on CW50% CWWP MWP ¼ vert angle Mean WP ¼ vert angle	CD:CW50 % CD:CWWP Toe length : CWWP vertical Inclination CW50% CWWP MWP ¼ vert angle Mean WP ¼ vert angle	CD:CW 50% CD:CW WP	CD:CW50 % CD:CWW P Toe length : CWWP vertical Inclination CW50% CWWP Mean WP ¼ vert angle	CD:CW50 % CD:CWW P Mean WP ¼ vert angle toe lgth:CWW P vertical inclin		vertical inclin M ¼ WP angle mean ¼ angle	

Note 1 all differences are significant at $p<0.05$

Table 5.3.2.vi Summary of measurements which differ significantly between different shaped hooves (28 feet)

28 feet Base/Vertical Inclination	Round Regular	Round Upright	Round Wide	Wide Regular
Round Upright	vertical inclination			
Round Wide	vertical inclination	Mean WP ¼ angle MWP ¼ angle vertical inclination		
Wide Regular	CD:CWWP	Mean WP ¼ angle MWP ¼ angle vertical Inclination CD:CW50% CD:CWWP	vertical inclination CD:CWWP LWP ¼ angle	
Wide Wide	CD:CW50% CD:CWWP vertical inclination Mean WP ¼ vert angle MWP ¼ angle	Mean WP ¼ vert angle LWP ¼ vert angle vertical inclination CD:CW50% CD:CWWP MWP ¼ angle	CD:CW50% CD:CWWP	LWP ¼ vert angle vertical inclination CD : CWWP

Note 1 all differences are significant at $p<0.05$

Effect of grouping by toe angle

Both sets of hooves were also categorised into groups dependant upon their toe angle, so that inter-literature comparisons could be made. A *post hoc* decision was taken to allocate the toe angles based on the interquartile ranges, *(table 5.3.2.vii)* measured in this thesis, because of the discrepancy regarding toe angles within the literature. The differences between the mean measurements of hooves grouped by toe angle are summarised in *tables 5.3.2A vii* and *5.3.2A.viii*. in the appendix

Table 5.3.2.vii Allocation of toe angle groups based on the median value obtained from the whole group and on values quoted in the literature

Toe	N	Mean	Median	St. Deviation	Min.	Max.	**Q1**	**Q3**
Angle	91	49.2	50.0	5.2	35.0	60.0	**45.0**	**53.0**

Note 1 angle measured in degree

There were no differences between any of the other measurements except for toe angle in the data set from the 28 left fores. In the group of mixed feet, the vertical angle of the upright toe group was significantly greater than the vertical angle in both the low and normal toe angle groups, $p<0.05$. This difference was reflected in the vertical inclination ratio.

Effect of grouping by either base plate or vertical inclination

Both data sets of hooves were divided into groups according to the shape of their base plate or their vertical inclination. The differences in the measurements between these groups were compared before characterising the hooves by both their base plate and vertical angle. There was a concern that the final shape groups would only contain a small numbers of hooves increasing the likelihood of a type II error[g] and therefore the effect of the individual shape parameters may have been a better indication of true differences.

When hooves were divided into groups based on the differences in the shape of their base plates, (long, wide or round), there were significant differences between some of the measurements and the ratios used to describe the geometry of the capsular base but the differences were dependant on the data set. In the data set of mixed hooves, wide base hooves were significantly wider at both their widest point and at 50% of their capsular width compared to long capsules,($p<0.05$). The vertical angles at the widest point of the quarters were significantly more upright in the long capsular base groups compared to both round and wide based groups,($p<0.05$). These differences are summarised in *tables 5.3.2A.i – iii* in the appendix.

Correlations between measurements of the whole data set and within shape groups

In the whole data set of hooves, the correlation coefficient between the capsular widths and capsular depth was greater than 0.7 at a significance of $p<0.001$ as was the correlation between the vertical angles at the widest point of the medial and lateral quarters. However allocating hooves by their shapes increased the correlation between the linear measurements of capsular depth and width, in both data sets, *tables 5.3.1A.ix, 5.3.2A.x* (round) and *5.3.2A.xi* and *5.3.2A.xii* (wide). Hooves with long capsular bases had a higher correlation of capsular depth and width compared to the whole group as summarised in *table 5.3.2A.xiv.*

Allocating hooves by their toe angles actually reduced the correlations when compared to the whole group of hooves in both data sets, *tables 5.3.2A.xv* (data set of 91 feet) and *table 5.3.2A.xvi*, (data set 28 left fores). Toe angle did not correlate with any other measurement in either of the data sets regardless of the shape groups used to allocate the hooves.

5.4 Discussion

A system to quantify the shape of the hoof capsule by using ratios to distinguish the base plate into long, round or wide and the vertical inclination of the medial and lateral angles to distinguish whether the capsular wall was upright or sloping successfully divided the hooves into groups which were visually and statistically different. It is appreciated that the quantified measurements chosen, depended partially upon subjective decisions to define what and how the capsule should be measured and that the resulting measurements might be accurate but not necessarily true.

However the chosen measurements were based on geometric formulas used to capture one dimensional shape; they were chosen because changes in them, reported in the literature, had a functional effect and they were measured from and to defined points which had been checked for repeatability. In addition for the first time, measurements taken from photographs were compared with those taken from the hoof capsule to improve accuracy. If morphological differences can be used as the practical basis for recognizing, classifying, describing, and naming leaves from one taxa of plants, (Barlow-Irick 2002), then using well defined points, angles and lines which are measurable can provide a tool to differentiate shape in the equine hoof capsule.

5.4.1 Methods

Measurements

Detecting when an object's three dimensional shape has changed requires the ability to model shape from images at different times and to distinguish significant from insignificant differences, (Leclerc *et al.* 2000). Some of the ratios considered such as the ratio of capsular depth50% to capsular depth widest point only provided information on insignificant differences and increased the groups into which the hooves were put, so that there were no apparent visual distinctions.

One of the main criticisms of traditional morphometrics is that measurements taken to capture and summarise the information are taken on ad hoc basis, (Adams *et al.* 2002). The measurements used in this work were not taken on an ad hoc basis but were based on the principles that Biewener, (1992) recommended. For example measurements were based on parameters or indices of structural shape which were shown to be critical to mechanical function by evaluation of research, discussed in chapter one. However the entire hoof wall is involved in both load bearing and protection and therefore any aspect could be considered to be important. Certain parameters such as toe angle have been studied in more detail compared to other parameters of structural shape, and whilst the toe is a major determinant of how the hoof functions as a load bearer, it is easier also to measure. Secondly practical measurements of these indices were then developed by adapting methods already used in research. Finally a range of different sized hooves were used from a cross selection of breed types. It needs to be emphasised that this work was not attempting to correlate the shape of the capsule with any aspect of function; the morbidity of the specimens and inappropriate laboratory facilities were not conducive to such a study. The aim of the work was to use the rudiments of morphometrics to distinguish between different shaped hoof capsules.

Kane *et al.* (1998) studied only one breed, the thoroughbred and noted that measurement variation and differences between controls and case means were small. It is important that a large enough sample of hooves is used to develop shape measurements which encompass not only size differences but breed differences. Hood and Jacobson, (1997) expressed concern that the shape of the hoof cannot be realistically contained in a simple set of measurements and that should there be less emphasis on one or two linear or angular parameters and more on the actual shape of the hoof. This is because an ideal template of hoof shape will not exist across the breeds; a 'normal' shape for a shire horse is unlikely to be 'normal' for a thoroughbred horse, (Hood and Jacobson, 1997). A limitation of this research was that the breeds of the horses were unknown due to the way that the hooves had to be collected, therefore at this stage it is not possible to say if the wide, wide hooves were predominantly from a specific breed such as the cob. An interesting future study would be to investigate if different shapes correlate to different breeds. The simple method of measuring the shape of the hoof developed in this work can now be used for a full epidemiological study of shape in the field across a range of breed types, during which bodyweights and heights should be collected.

A second criticism of morphometrics is that there is likely to be an unavoidable bias in the choice of landmarks used, (Chen *et al.* 2000) and this might be a criticism that could be levied in the light of toe angle results which will be discussed later. However as long as the landmarks

allow quantitative measurements such as lengths to be taken and the landmarks are comparable and homologous across the shapes and can be defined with a name, (Adams *et al.* 2002), then the bias is contained. All the anatomical points used in this work were defined, were comparable across a range of sizes and all can be defined by a name and a description of how they were obtained, thus the bias was minimised.

One of the limitations of the method used in this work is that it defines shape in two planes only and failed to define shape in the sagittal plane. This might mean that it will be restricted to distinguishing differences in average shapes across the population but will not be sensitive enough to distinguish differences within breeds. The method differentiated hind feet from fore feet and when the set of 28 left fores were grouped according to their shapes using the system developed with the mixed group of feet, none fell into the base long group, (*section 5.3.2*). When the data set of mixed feet was checked, it was noted that over 80% of hooves in base long were hind feet. To develop the next stage and describe the sagittal plane in terms of ratios will require a much larger group of feet. An attempt was made to include the toe angle to heel angle ratio, but it increased the number of shape groups to twenty seven and multivariante analysis would have been required to process the data, *plate 5.4.1.i*

An initial principal component analysis showed that the measurements used in the ratios were highly discriminating and cluster analysis put the hooves into twenty five groups. Multivariante analysis should be used to further investigate the measurements and ratios which best describe the shape of the hoof capsule. Fifteen ratios from measurements taken from leaves were used to distinguish between thirty four species within one taxa, (Meade and Parnell, 2003). The principle components and discriminate analysis showed that the best discriminator was the shape of the leaf base. The best discriminator of hoof capsule shape was also the base.

It is difficult to compare the methods used to group the hooves into shape groups with others because although various indices of shape such as lines, angles and medio-lateral imbalances have been described in the equine hoof capsule, (Kane *et al.* 1998; Thomason 1998; Kaneps *et al.* 1998), except for toe angle, very few of them are described by anything more rigorous than geometric adjectives and little attempt has been made to investigate relationships between parameters measured. Recently, Roland *et al.* (2003) developed a very detailed three dimensional system with clear definitions of origins and axes, focusing on the sole of the hoof. An (x, y, z) co-ordinate system was used with the (0, 0, 0) origin at the bisect of, as described in this thesis, CD and CW50%. The surface area of the sole was mapped with the z axis being perpendicular to the ground surface to give a topographical map of the entire structures of the sole. Their results agreed with the results from others, (Kane *et al.* 1998) that there were

differences in the medio-lateral widths and that the widest point of the capsule was dorsal of the point of frog. In addition Roland *et al.* (2003) reported that the radius of the wall increased palmarly, that sole depths were greater palmarly than distally and illustrated that the sole could be accurately mapped. It would be of interest to see if the correlation between the increased information obtained and clinical relevance is greater than the correlation between an (x, y) measured hoof shape and similar information.

The intention of this thesis was to develop a set of ratios which provided enough information to distinguish between different shaped hooves and this was achieved. In the development of the ratios, sufficient information has been obtained to provide a three dimensional z, y, z co-ordinate system to define the shape of the hoof in more detail. The centre of origin in this thesis is the intercept of CD with CWWP. The next stage of development in the measurement of shape should be to develop the three dimensional co-ordinate system, use it to allocate hooves to different shape groups and then compare the allocation of the hooves by the ratio system and the three dimensional system. The measurements taken in this thesis were used to make a model of a hind foot and a front foot using finite element analysis and this is discussed in chapter six.

5.4.2 Use of photography for obtaining measurements

Using photographs to take measurements of the hoof capsule which can be used subsequently to define shape provides a quick, objective record of the three plan views of the hoof and allows retrospective and objective evaluations of changes in shape. Over fifty full size image files can be stored onto a CD and the method can be used easily in the field as well as in the research environment.

Helman *et al.* (2002) highlighted a need for the serial documentation of hoof shape changes and developed a method of making a mould of a hoof in dental plastic from which a model was taken. It took less than an hour to make, but the practicality of this being adopted in the field is limited and will increase call time considerably compared to photographs. Redden, (2002) argued that models are superior to photographs He pointed out that photographs are only two dimensional, methods such as stereoscopic photogrammetry which is a specific technique used by surveyors to convert two dimensional photographs to three dimensional maps are available and might be adapted. However a three dimensional model may not provide any additional information which cannot be obtained from photographs.

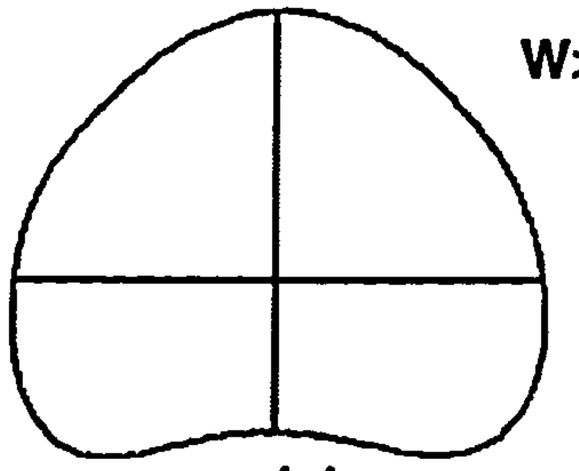
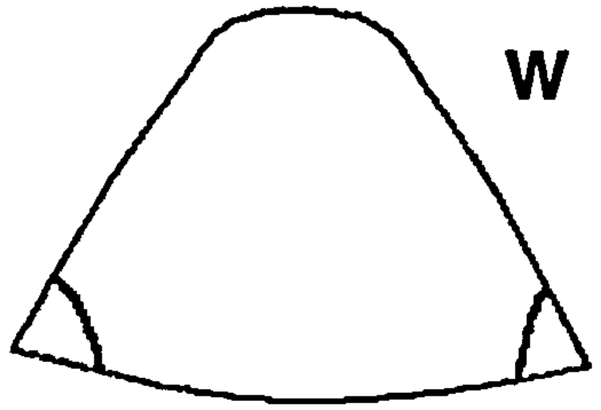
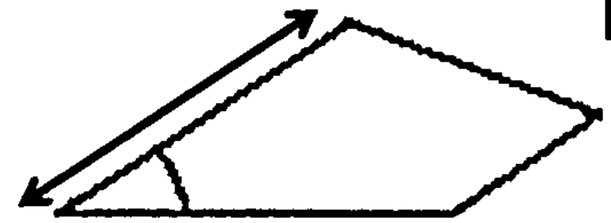
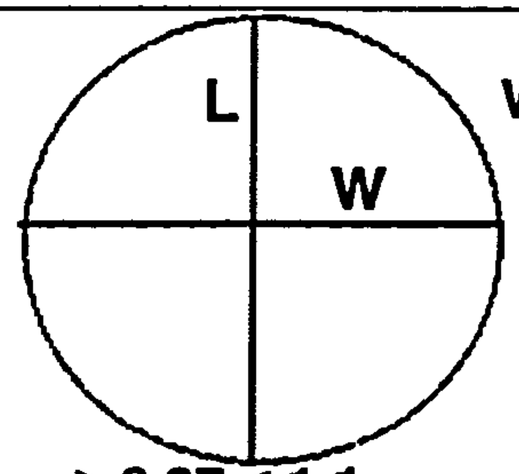
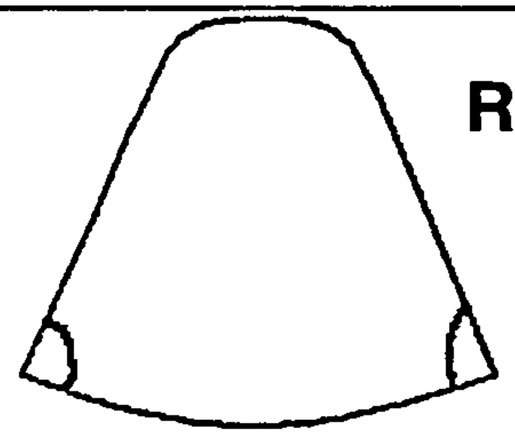
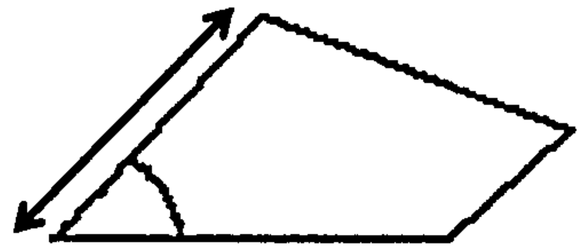
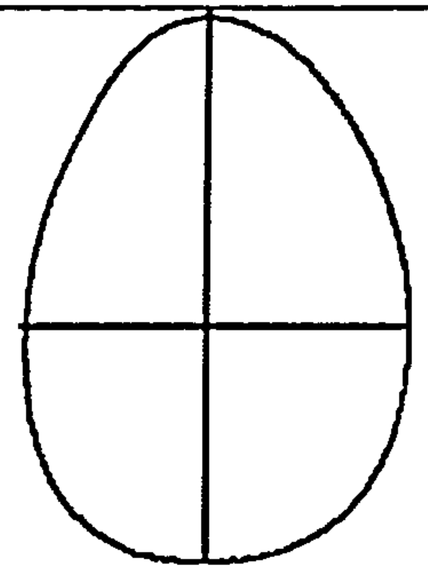
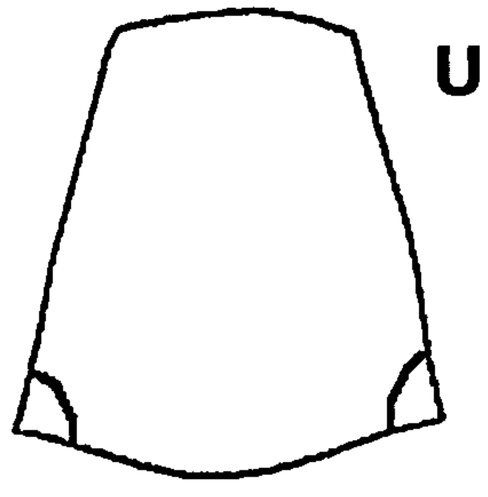
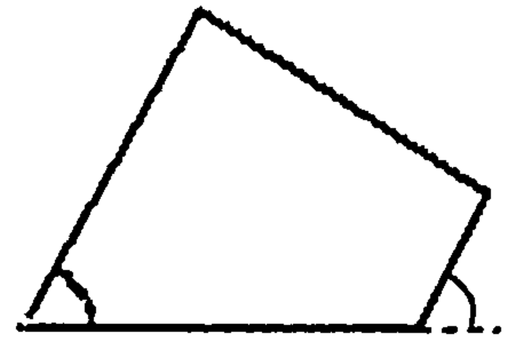
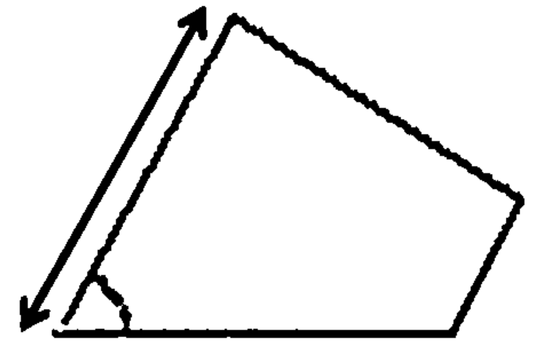
Base Plate	Vertical Inclination	Sagittal Inclination	Toe length : Heel length
W  W>L > 1.1	 W < 0.83	 L heel angle < 5° toe angle	 L < 0.3
R  L W W=L ≥ 0.97 ≤ 1.1	 R ≥ 0.83 ≤ 0.89	 R heel angle ± 5° toe angle	 R ≤ 0.3 ≥ 0.5
L  L>W < 0.97	 U > 0.89	 U heel angle>5° toe	 U > 0.5

Plate 5.4.1.i Categorising hooves into shapes according to their transverse, frontal and sagittal plane.

This thesis has shown that by using measurements taken from photographs in the three views, the three planes of the hoof can be adequately described with correlations as high as 0.99 with actual measurements from the transverse plane. Redden, (2002) also argued that whilst computerised tomography can build a three dimensional picture it can only be viewed in two dimensions, (not strictly true) and that it is inaccessible and expensive. Taking photographs with a digital camera in three planes overcomes those concerns. Consideration should be given to the type of information required from either the photographs or the model. Using a model will show how the capsule has changed with time, but this assessment will stay subjective unless measurements are taken from the capsule, thus photographs from which measurements can be taken will be as useful.

It must be noted that photography will only provide meaningful information if a calibration scale is included. In this thesis, a scale was included in only one of a set of photographs at the beginning of each session when photographing cadaver hooves as conditions could be tightly standardised. The camera was set up and secured in a set position at known distance, the focus and focal length was therefore standardised. The hooves were positioned on a specific platform ensuring repeatability and reproducibility. It will be important to ensure that a scalar is included in all pictures taken in the field to ensure repeatability as standardisation maybe difficult.

Photographs are used in forensic science and a scalar is included for two reasons, (Krauss, 1984) to provide a relative size and perspective and to allow accurate enlargement. There are stipulations to the characteristics of the ruler: - it must be rigid to ensure positive total parallelism and the graduation marks must be machine marked. Krauss, (1984) expressed concern regarding the accuracy of taking measurements of a curved surface from a flat print. This was confirmed in this thesis, with measurements from the curved surfaces (frontal and sagittal planes) being significantly different to those from the photograph. To minimise these discrepancies, Krauss, (1984) recommended that the camera and ruler should be parallel for maximum accuracy, which is why a horizontal and vertical ruler was included in photographs used to take shape measurements from the hoof. The shape of the hoof is a continuous population and the divisions chosen are arbitrary, based on the need to measure what is described in the literature. It needs to be appreciated that an error in measurement may result in a hoof being categorised into the wrong shape group. Krauss, (1984) showed that a 40° curvature introduced an error of 5mm; this is greater than the precision of 1mm worked to in this thesis. A curved scalar and a straight scale should be included in future work as the distortion of the curved scale will be proportional to the distortion of the curve of the hoof capsule.

5.4.3 Comparison of measurements with those reported in the literature

Comparison of toe angle

The mean toe angle in the mixed group of hooves was 49.0° ± 5.2°; mean toe angle in the group of 28 left fores was 51.0°± 4.8°. Many researchers have measured toe angle, some researchers have recommended specific toe angles based on the premise that toe angle affects the way that the hoof is able to weight bear; but opinion on fore toe angle is divided; White, (1802) recommended 45°—50° whereas Fleming, (1872) recommended between 50° and 55°. Barrey, (1990) measured the effect of toe angle on weight distribution and showed that a toe angle of less than 55° resulted in unequal weight bearing between the toe and the heel; consequently he also recommended that toe angle should be between 50° and 55°. Singer, (2001) and Turner and Stork, (1988) recommended that the fore toe angle should be between 50° and 55° but this recommendation appears to be based on observation of sound horses in the field rather than scientific study.

Originally the aim of this thesis was to group feet by toe angle using the recommended range as normal so as to be able to make comparisons with the literature; toe angles less than 50° were to be grouped as low and above 55° as high. This decision accepted the fact that some of the recommendations of these researchers were based on published measurement, others on popular opinion. However, more recently fore toe angles *(table 5.4.3.i)* have been accurately measured, (Turner 1992; Kane *et al.* 1998; Thomason 1998; Thomason *et al.* 2001; 2002) and these results varied considerably from the recommendations referred to above, with toe angle falling between 42°and 58°. Thomason *et al.* (2002) considered a toe angle of 48° to be normal and 42° to be low. Glade and Salzman, (1985) showed that toe angles of 45° resulted in contraction of the heels over a period of one hundred and twenty six days, measured by the difference in angles of a series of radii measured around the hoof from the point of frog, the width of the frog also contracted, this is likely to reduce the surface area of the wall and the load bearing area.

Although research has suggested that fore toe angle should be consistent between 50° and 55°, this is not reflected in the field nor are researchers in agreement. It may be that the accurate measurement of the toe angle both in the field and in research is not important if measured in isolation. Thomason *et al.* (2002) allocated hooves into toe angle groups based on visual

observation only and reported the standard deviation of this visual assessment as only ± 1°. There was only a 1° difference between the toe angle of 50.7° of control horses and the 49.7° of those which had suffered catastrophic musculoskeletal injuries, (Kane *et al.* 1998). Perhaps the significance of a 1° difference in toe angle, is irrelevant and not the major correlate to the incidence of injury. Bushe *et al.* (1987) expressed concern that the recommended toe angle of 45° to 50° was not appropriate. The group used x-rays to investigate the effect of changing the toe angle using wooden blocks on the angulation of the distal joints of ten horses; the joints were in line when hoof angles ranged from 45° to 65° with a mean of 55°. If the aim of altering the toe angle of the hoof is to ensure the alignment of the distal joints, then this relationship should be further investigated both in the field and also by artificially changing the toe angle. In this thesis, toe angle was not correlated with any other measured parameter. Toe angle might be valuable as an independent predictor of shape changes. However if a relationship does not exist between toe angle and any other parameter, it would be difficult to use the measure to allocate hooves to shape groups as the allocation would be inconsistent. All the inference that toe angle changes the way the hoof deals with load has been investigated by artificially altering toe angle, (Balch *et al.* 1991; Thompson *et al.* 1993) or by measuring the toe angle *in vivo*,(Barrey 1990; Douglas *et al.* 1998; Thomason *et al.* 1992).

Table 5.4.3.i Summary of linear and angular measurements taken from the hoof capsule and reported in the literature.

Author and date	toe length	toe angle	MWP¼ angle	LWP ¼ angle	CWWP	CD	Distal Perim	Proximal P
	cms	degrees	degrees	degrees	cms	cms	cms	cms
Kane *et al.* (1998)	n= 5 8.89 n= 70 8.93 n= 43 8.92 n=10 9.03	50.7 49.7 49.2 48.4	77.0 75.4 74.8 75.6	72.8 73.4 73.0 73.1	12.4 12.5 12.5 12.5	11.6 11.6 11.6 11.7		
Roland *et al.* (2001)	n=20				12.2 ±6.52	11.5 ±6.2		
Thomason *et al.* (2001)	n=5 9.7± 0.6 n=5 10.2±0.4	48.0± 1 42.0± 1	78.0±4 68.0±5	79.0± 3 69.0±6	7.6± 0.3 7.8± 1.8		34.3± 2.6 38.2± 2.8	34.9± 2.5 38.2± 3.7
Kaneps *et al.* (1998) foals	4.6± 0.5→ 5.9± 0.5	51.3 ±2.6 → 56.1±2.1						
Thomason (1998)	6.9 →9.4	48.0→57.0	80.0→89.0	72.5→84.0				
Turner *et al.* (1998)		50.0 – 55.0						
Bushe *et al.* (1987)		42.0 – 67.0						
Clayton (1987)		48-55						
Balch (1980)	7.6 - 8.9	front 50.0 54.0 hind 53.0 57.0						
Adams (1974)		>45.0						
Fleming (1872)		50 .0- 60.0						
White (1802)		45.0						
Glade (1985)		front 45.0 hind 52.0-53.0						
Turner (1992)		front 48.0 55.0 hind 53.0 57.0						
Hinterhofen (2000)		50.0	80.0	80.0				
Thomason *et al.* (2002)n =9	8.5– 9.9	47.5 – 58.3	72.3 – 90.0	67.7 – 79.8				
Leach (1980)		47.0						
Barrey (1990)	n = 20	39.0 – 55.0						
Lungwitz (1891)		83.0 - 36.0	52.0, 61.0	52.0, 61.0				
Balch (1991)		53.0						
Thompson (1993)		52.0- 55.0						
Verschooten (1993)	8.5 – 11.5	32.0 – 55.0					35.0 – 49.0	31.0 – 43.0

Author and date	toe length	toe angle	MWP¼ angle	LWP ¼ angle	CWWP	CD	Distal Perimeter	Proximal Perimeter
this thesis								
n=91	7.3-11.4	35.0-60.0	57.0-95.0	57.0-103.0	8.5-15.2	7.4-14.5	19.0-39.0	17.5-36.0
n=28	8.1-15.0	41.0-60.0	58.0-87.0	54.0-86.0	11.5-18.8	9.4-15.0	25.3-45.5	23.5-39.7

Medial and lateral angles as well as toe angles have been measured (Thomason, 1998) but correlations between the angles were not investigated. A number of the papers reviewed investigated the effect of changing toe angle on other linear or angular measurements of the hoof capsule, (Thomason *et al.* 2001) but not in relation to load bearing and some researchers measured toe and heel angles and looked at loading effects (Verschooten, 1993) but no correlation details were available. Singer, (2001) and Turner and Stork, (1988) recommended a relationship between toe and heel angles of ±5°, Thomason *et al.* (2001) measured correlations between measurements and only found a correlation between toe angle and toe length. No correlations were found between toe angles in this work.

In order to further investigate the relationships with toe angle, hooves were divided into low, medium and upright toe angles to determine if the same difference existed between measurements of each group as those found by Thomason *et al.* (2001). The median toe angle in this research was 50° and the upper and lower quartiles 53° and 45° respectively A *post hoc* decision was taken to use these angles to allocate hooves to the groups distinguished by normal, low and upright toe angles, because of the lack of consensus on the normal fore toe angle. Whilst Thomason *et al.* (2001) found a significant difference between the medial and lateral angles and the distal perimeter between the normal and low toe angle groups, this work did not. The vertical angles were significantly bigger in the upright toe group compared to the other toes but the two measurements were not correlated. Originally it was thought that these hooves would be all hind feet, but only 14% of the hind feet fell into this category. Farriery intervention was also considered as a contributory factor but both this work and Thomason *et al.* (2001) used abattoir specimens and therefore had no control over farriery. However Thomason *et al.* (2001) chose their hooves by visual assessment and this might have introduced a bias towards a symmetrical hoof, selection should always be carried out randomly.

One of the reasons that it proved problematical to find a ratio to describe the sagittal plane was because of the lack of relationship of any other parameter with toe angle. It was thought that toe angle would be related to capsular depth in the same way that there is a relationship between capsular width and dorsal inclination. It is possible that toe angle also needs expressing as an inclination to remove the effect of the measured variable. Toe angle is the parameter most altered by farriery and it is often considered in isolation or at least subjectively. If the shape of the hoof alters depending upon its load, then other parameters would change proportionally, but if every six weeks the toe is altered independently through farriery, then this would prevent proportional changes in this parameter. Cattle research has shown that there is a relationship between toe length and angle; Hahn *et al.* (1984) found a negative correlation between angle

and length of toe, longer toes had shallower angles. Neither data set in this work showed correlation of any measurement with toe angle.

Vermunt and Greenough, (1995) indicated that claw shape is in part dependant on claw trimming; it would be interesting to investigate if there was a significant inter-farrier influence on hoof shape in the equine hoof capsule and to measure hooves which have not had any farriery intervention. Recommendations on shape and aspects of shape should be based on objective measures and scientific evaluation, not just subjective views.

Comparison of photographic measurements

There have not been any studies evaluating the relationship between subjective, objective and photographic measurements of shape in the equine hoof capsule, so results from this thesis can only be compared with cattle work. Hahn *et al.* (1984) and Vermunt and Greenough, (1995) compared objective and subjective methods of measuring shape of cattle claw and concluded that traditional 'eyeball' methods of classifying cow claws were inaccurate and that objective evaluation picked up much more detail than subjective. Vermunt and Greenough, (1996) used photography as an objective method of measuring shape in cattle claws, but did not analyse the correlation between measurements taken from the photographs and those taken from the claw. Bergsten, (1993) correlated measurements of lesions from the sole of the claw with photography and obtained Spearman Rank correlations of between 0.78 for the front hooves, 0.85 for hind hooves ($p<0.001$), he also quoted Pearson's correlations of 0.85 and 0.88 respectively. Leach *et al.* (1998) using a similar system obtained a correlation between photographs and measurement of 0.6.

The correlations obtained in this thesis depended upon which plane was being measured and the data set. Measurements which did not differ significantly between the hoof and the photograph also correlated more strongly between the two methods. Measurements taken from the capsular base were mainly non significant in their differences and had the highest correlations of between 0.89 and 0.99. Linear measurements from the sagittal and frontal planes differed significantly between photographs and hooves; correlations varied between the data set of left fores (0.83-0.78) and the random group of hooves, (0.58-0.64). Generally linear measurements taken from photographs were smaller compared to capsular measurements. Angles were greater taken from photographs compared to the capsule, but the difference was only significant for heel angles emphasising the usefulness of specific apparatus for measuring angles.

Depending upon the degree of accuracy to which other workers adhered, it may be that using photographs without calibration accounted for the lack of difference between groups of hooves measured, (Kane *et al.* 1998). Thomason *et al.* (2002) used photographs to take measurements

of hooves from which he subsequently developed several FEA models to investigate the effect of hoof shape on loading. They expected to see differences in strain related to differences in the indices of shape that they measured, however no correlations were found.

5.4.4 Use of ratios to group hooves into different shapes

Linear measurements and ratios are used in morphometry, which uses powerful statistical methods to convert simple linear measurements to graphical representation, to describe structural features and shape. The ratios investigated were those whose geometry best captured the descriptors commonly used to describe the visual appearance of the equine hoof capsule.

Expressing measurements as a ratio of each other rather than absolute values removed size as a variable and allowed shape comparisons. Hooves were systematically filtered according to the ratios and then *post hoc* decisions on the most appropriate ratios were taken by noting the visual appearance of the individual capsules within each group. Two sets of ratios were used to describe the capsular base; CD: CW50% and CD: CWWP. This was because the widest point of the capsule was often very close to the heels and weighted the ratio too much towards the depth. Originally it was thought that the radii CW50% and CDWP would be the best descriptors of a circle or oval; however *figure 5.4.4.i* illustrates why CDWP was not used, because of the bias it put on the length of the CD.

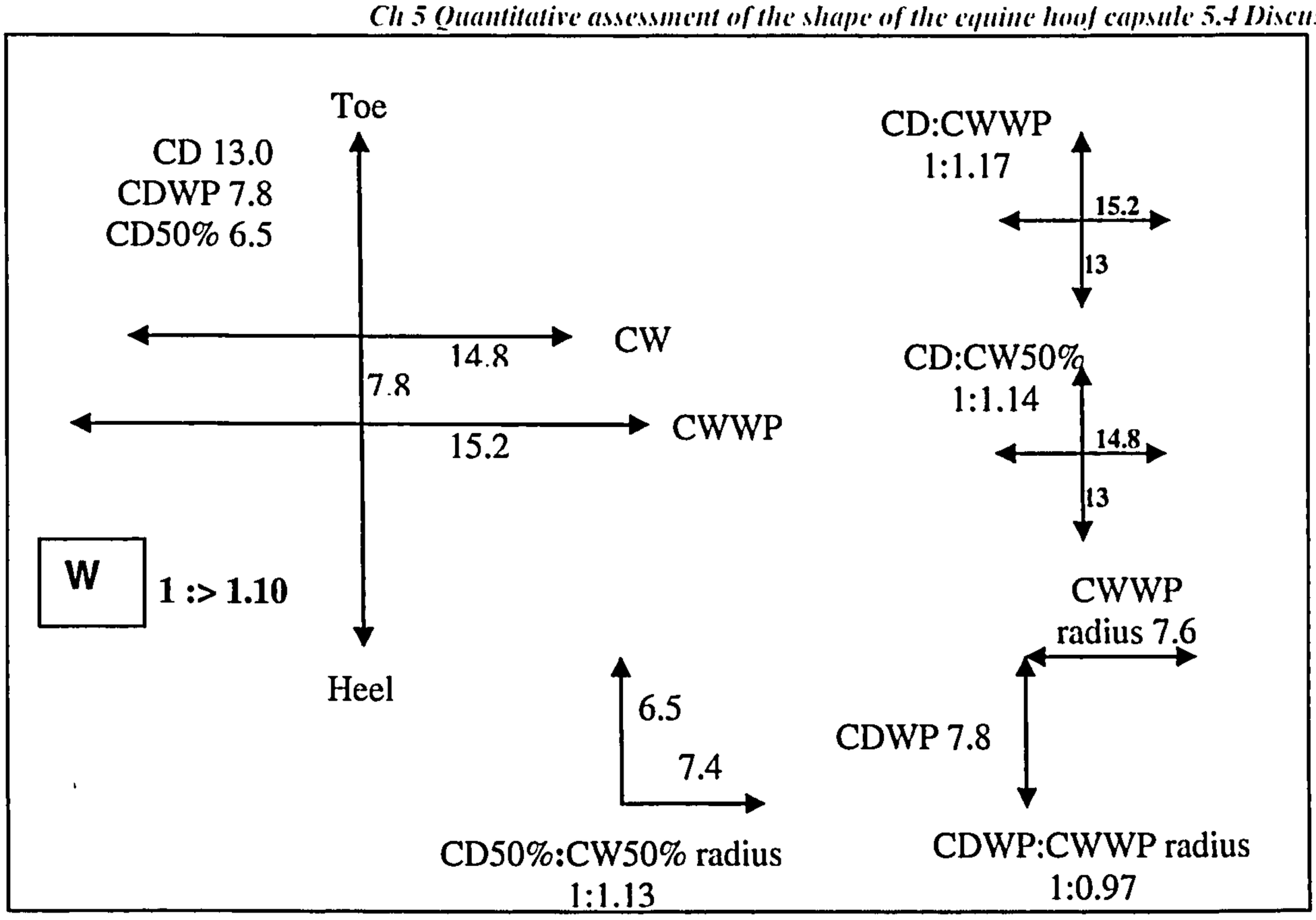


Figure 5.4.4.i The use of CD: CW ratios to best capture the shape of the capsular base plate

note 1 all numbers are cms except ratios
note 2 hoof has wide base, therefore ratio needs to be 1: >1.1

Using the ratios to group hooves according to their shape increased the correlations between the actual measurements within the shape groups compared to the correlations between the actual measurements in the whole data set for both sets of hooves, *tables 5.3.2A.xi—xvi*. In addition it reduced the within-population variance relative to the between-population variance in most groups as shown by the standard deviation in *table 5.4.4.ii*. There were significant differences between shape groups in vertical inclination and the ratios of capsular depth to capsular width in both data sets. In addition in the data set of 91 hooves there were significant differences in CW50% and CWWP between hooves in WW group and those in LR, LU and RR groups. The toe length: CWWP was significantly bigger in WW group compared to LR, LU, RR ($p<0.0001$) and RU, ($p< 0.05$) groups. All the differences appeared to be a reflection of the wide base plate.

Table 5.4.4.ii Comparisons of the variations within all hooves and within shape groups

28 left fores	ALL	RR	RU	RW	WR	WW
	measurement ± SD					
CD	12.4 ± 1.7	13.0 ± 2.2	11.0 ± 0.7	13.0 ± 1.7	11.5 ± 1.9	12.3 ± 1.4
CWWP	13.9 ± 1.9	13.6 ± 2.1	12.2 ± 0.5	13.8 ± 1.6	13.3 ± 1.9	14.9 ± 1.7
vertical angle M/4	72.9 ± 6.7	77.1 ± 4.6	85.0 ± 1.7	69.0 ± 3.1	71.4 ± 3.4	69.1 ± 5.1
vertical angle L/4	74.3 ± 7.5	76.1 ± 2.4	81.0 ± 1.7	69.7 ± 3.8	82.8 ± 2.8	68.8 ± 7.4

91 mixed feet	ALL	WW	WS	WR	RR	RW	RS
CD	11.0± 1.4	11.2± 0.9	9.0± 0.9	10.2± 1.4	10.5± 1.6	11.9± 1.6	11.1± 1.8
CWWP	12.0± 1.6	13.1± 1.0	12.0± 1.4	12.1± 1.8	10.9± 1.5	12.6± 1.6	11.3± 1.9
vertical angleM/4	75.4± 8.0	70.2± 6.1	84.2± 1.5	74.9± 2.9	74.5± 4.6	68.4± 4.9	77.0± 7.7
vertical angle L/4	74.3± 7.1	67.4± 6.1	82.2± 3.6	79.3± 3.5	78.3± 4.6	69.8± 4.2	81.7± 7.0

Roland *et al.* (2003) measured capsules which would have been grouped as base round 1:1.05 (12.2÷11.6) in this thesis as did Kane *et al.* (1998). In this work less than half the capsules in both data sets were base round. This is most likely to be a reflection of the sampling. Mixed breeds have capsular bases with different shapes, whereas thoroughbred horses have the same shape of capsular base, (Kane *et al.* 1998; Roland *et al.* 2003); highlighting that the ratios appear to be sensitive to differentiate between breed differences in base plate.

Conclusion

It is possible that the shape groups chosen only partially describe the three dimensional geometry of the hoof capsule, however the need to detect and describe differences in shape has been commented upon but to date the elucidation has been ignored. As already stated, shape can only be described and categorised if an outline or area can be delimited by an arrangement of points or lines, which form interrelated elements with each other. The lines and points must be unambiguous, and the inclination or angle of the two lines specific, so that the combination results in a repeatable and recognisable shape. This thesis has achieved this. To date this interrelationship has been missing and the dorsal and lateral measurements which have been taken, (Douglas *et al.* 1998; Thomason, 1998), cannot describe shape unless they are related to each other via the shape of the base plate. Measurements of capsular width and capsular depth are scantily recorded in the recent literature.

Researchers may feel that the classification of shape in this thesis is oversimplified and a better classification must exist. It is acknowledged that hoof shape covers a continuous and overlapping pattern of variation in measurements but this should not prevent a system to describe shape materialising. Without objective and clearly-defined morphological landmarks, the description of hoof shape was likely to remain ambiguous, arbitrary, and inadequate.

Whilst huge steps have been taken in the science of morphometry in other aspects of biology, it needs appreciating that landmark-based geometric morphometric methods begin with the collection of two or three dimensional coordinates of biologically definable landmarks. The work completed in this thesis provides a useful source of these coordinates. The developed method of using ratios to differentiate hooves according to shape does not provide enough information to reproduce a precise replica of the individual hoof. On the other hand it does objectively capture what till now has been a subjective characterisation of hoof shape and it removes the confounding factor of size. Many researchers have taken numerous measurements which they described as shape measurements but none of them result in an outline of the hoof capsule that can be visually differentiated.

5.4.5 Future work

- Verification that the measurements used adequately describes the shape of the hoof.

The current measurements were developed in order to objectively quantify the shapes of hooves commonly recognised and subjectively described in both the literature and used clinically. Hooves were allocated to groups on the basis of the ratios chosen and then compared with their photographs as a guide to the adequacy of the grouping. The ratios only captured the frontal and transverse planes of the capsule and it would be useful to describe the toe and heel angles as a proportion in order to describe the caudal/cranial perspective in the sagittal plane.

An objective confirmation that the measurements used are adequate could be achieved with the use of laser topography. A laser model needs developing which can be computerised to collaborate whether the measurements used capture shape adequately.

- Develop the measurement of shape by utilising the science of morphometry.

The use of geometric morphometric programmes which allow principal components and other statistical parameters to be calculated from the digitised images should be investigated. Direct analysis of the coordinates used in this thesis as variables would be inappropriate as the effects of variation in position, orientation and scale of the hoof are still present. Therefore, the non-shape variation must be mathematically removed prior to the analysis of such variables. Once non-shape variation has been eliminated, the variables become shape variables and may be used to statistically compare samples, and graphical representations of shape may be generated for comparison. The results from this type of analysis would lend themselves to research but maybe too complex for field application.

- To adapt the method for use in the field and compare actual measurements with those taken from a standardised photographic technique.

Having developed a method of measurement within a controlled environment, it is important that the technique is applicable within the field situation; otherwise epidemiological studies will not be possible. The photographic method needs standardising, so that farriers and vets can use the technique to compare shape changes over time. It will be especially useful in terms of remedial farriery to monitor changes and also to clarify to the owner that changes, which can take up to 12months to appear, are being implicated. Many of the problems in the foot such as under-run heels, navicular disease and laminitis are associated with poor foot conformation. Sequential accurate measurements over a period of time, will allow monitoring of relatively subtle changes in capsular shape before catastrophic changes occur.

- The descriptors need testing against the clinical judgement of farriers and vets to see if the measurements distinguish the differences that are perceived visually.

The ability to measure shape should not be used to try and make all hooves an ideal shape. However it will be useful to have an objective method of measuring angles and lengths, which in most instances are still being measured by eye. Whilst advice indicates that fore toe angles should be between 50°and 55° this study has shown that in data set of hooves used for this work, that this is not the case. The measurement of the angles should not discourage farriers from continuing to trim to the most suitable angle for that horse; however it will mean that true angles can be compared rather than perceived angles.

- Effect of farriery.

An investigation into intra and inter farriery effects on the shape of the hoof capsule and a comparison with the shape of hooves which have not received farriery. This would establish if correlations between measurements exist in hooves which are removed by farriery.

6 Interactions between shape, trace element concentration, fracture toughness and cracks scores

6.1 Introduction

There is evidence that deficiencies or excesses of trace elements have an effect on the development and maturation of the keratinocyte and on the process of cornification. Whilst little is known about the function of single nutrients in the process of keratinisation in the hoof, (Mulling *et al.* 1999), the literature provides support for the argument ,as discussed in chapter one, (Tomlinson *et al.* 2004) that specific trace elements influence aspects of development in the keratinocyte and cornification. Changes in dietary zinc, (Coenen and Spitzlei 1997; Siciliano *et al.* 2003) are recorded as changing the hardness of the hoof wall but the relationship between the trace element composition of the hoof wall and its visual appearance in terms of cracks was not investigated.

Josseck *et al.* (1995) in a survey of 152 horses noted that lateral quarters were particularly brittle and chipped. As part of the same survey, the tensile strength of the hooves were measured, (Zenker *et al.* 1995) and the quarters had a greater mean tensile strength of 36N/mm^2 compared to the 46N/mm^2 of the dorsal wall, Douglas *et al.* (1996) measured modulus of elasticity in compression and tension and found that the toe had a greater mean modulus of elasticity (955±199MPa) compared to the quarters, (607±100MPa) and described the toes as being stiffer than the quarters. The quarters flare inwards and downwards in response to load, (Leach and Zoerb 1983), and Douglas *et al.* (1996) concluded that the quarters needed to be more flexible than the toe in order to fulfil that aspect of their function.

If low copper and zinc are associated with a reduction in tensile strength, then it is hypothesised that hooves with a high crack score might have lower zinc and copper compared to hooves with less severe cracks. However if the cracks are indicative of an inflammation event at time of formation, then the hoof wall might actually have higher levels of copper as copper is reported to increase in inflamed tissues. Comparison with tumorous breast tissue and normal breast

tissue, (Geraki, *et al.* 2004) showed that tumours had significantly increased levels of both copper and zinc. Baggott *et al.* (1988) found that zinc levels in the horn of lame cattle were lower and magnesium and copper was higher compared to sound animals.

In addition the trace elements do not work in isolation and many trace elements are known to inhibit the action of others. Some of the pathological changes seen in keratinised tissues associated with zinc deficiency maybe a secondary effect and may not be directly attributable to the zinc but to the inhibitory effect that zinc has on other elements. Brewer *et al.* (1979) proposed that zinc inhibited calmodulin function and therefore had an inhibitory effect on calcium within the cell. Results showed that at low levels (40μm) zinc inhibited the calmodulin-complexed Ca-ATPase in erythrocyte membranes by binding to calmodulin. In sickle cell anaemia, the membrane becomes very permeable to calcium and the stimulation of calmodulin by this excess calcium leads to membrane damage. Zinc has been shown to protect against membrane damage in the sickle cell, (Brewer *et al.* 1979) possibly by complexing with calmodulin and preventing its action. Substantial quantities of firmly bound zinc stabilise the structures of RNA, DNA, ribosomes, (Vallee 1982) and is incorporated into the polypeptide chain forming a stable complex with the protein which will have a characteristic function. Alternatively it is possible that the pathological changes might be because the differing availability of zinc at proliferation alters the characteristic of the keratin polypeptides.

It is hypothesised that the ratios of calcium and zinc and copper will vary between hooves with either cracked or uncracked hoof wall epidermis. Expressing trace elements as a ratio to each other will remove the variation in intakes and final concentration in the hoof wall and may better reflect relationships between mechanical properties and cracks in the hoof wall. Whilst there may not be a correlation between dietary intakes and the concentrations of trace elements in epidermal tissue, there is likely to be some relationship between the mechanical properties and visual appearance of the tissue and the levels of trace elements within it. In humans twenty percent of zinc is concentrated in the epidermis and epidermal structures and it is generally considered that hair acts as a reservoir for excess zinc in the body, (Lansdown 1995). It is thought that zinc is absorbed from the circulation at sites of tissue injury and the amount absorbed is proportional to the injury. Thus it would be interesting to investigate whether hooves from laminitic animals have increased zinc in the hoof horn compared to normal hooves. If the levels are not higher then one might hypothesise that those hooves are likely to have higher crack scores as there could have been repair impairment at the time of formation. Laminitis is considered an inflammatory condition, (Neville *et al.* 2004), it may be reasonable to expect higher levels of copper in hooves from laminitic animals. Having developed methods to

objectively quantify trace elements, fracture toughness crack scores and categorise the shape of the equine hoof capsule, the work described in this chapter investigates if there are any differences or interactions between all the measured properties.

6.2 Methods

All statistical analyses were performed using Minitab[13]. Summary data including the mean copper, calcium, zinc, fracture toughness and crack scores for different shaped hooves were collated. The data from the 28 left fores, (28lfs) and mixed feet were kept separate for the purpose of analysis but are presented consecutively for ease of comparison.

6.2.1 Comparisons in trace elements, fracture toughness and crack scores between different shaped hooves

Non parametric tests were used to investigate the differences between the properties of the hooves grouped according to their shapes because of the small size of the various data sets. In addition differences were investigated between hooves with either different vertical inclinations or different shaped bases.

The Kruskal-Wallis test was used to investigate differences between crack scores and trace elements to test the hypothesis that different shaped hooves do not vary in their crack score or trace element concentration. If a difference was established, then multiple comparisons between anatomical positions and shape groups were carried out using the Wilcoxon Rank Sum test at a significance level of $p<0.05$ with a Bonferroni correction to reduce the likelihood of type 1 errors[g].

A two way ANOVA test using the General Linear Model with block design was used to investigate between and within shape group differences of fracture toughness in all data sets to test the hypothesis that there are no differences in fracture toughness between different shaped hooves or between anatomical positions within a group of hooves of the same shape.

6.2.2 Correlations between trace elements, fracture toughness and crack scores in the whole data set and within different shape groups

Linear relationships between all the measured parameters were investigated by calculating coefficient correlations. Spearman's Rank Correlation coefficients between trace element concentrations and crack scores and trace elements and fracture toughness between all hooves were calculated for the data set of 28 left fores and between trace elements and crack scores between all hooves from the data set of mixed feet. Pearson's Correlation coefficients between trace elements and fracture toughness were calculated between all hooves from the data set of mixed feet. Spearman's Rank Correlation coefficients between trace elements, fracture toughness and cracks cores within all shape groups from both data sets were calculated.

6.3 Results

Summary data of the mean crack scores, fracture toughness and trace elements of different shaped hooves are presented in *tables 6.3.1A.i – 6.3.1A.v.*

6.3.1 Relationships between cracks and shape

Differences in crack scores of different shaped hooves

The differences in crack scores between both data sets of hooves grouped into the shape groups recommended by this thesis were not significant, ($p>0.05$). In the data set of mixed hooves, trends were apparent *tables 6.3.1.i* and the geometric area and number scores, (GSA and GSNo) and severity number score (SSNo) of the hooves with round bases and regular vertical inclination, (RR) group were considerably less than the other shaped hooves. Hooves with

round bases and upright vertical inclination in the group of 28lfs had significantly smaller areas of cracks at the toe, ($p<0.05$) *table 6.3.1.ii figure 6.3.1.i* and appendix *table 6.3.1A.v.*

Table 6.3.1.i Descriptive summary of crack scores of mixed feet grouped according to their shape

Descriptive summaries of crack scores in different shaped hooves									
	long	regular		round	regular		round	wide	
Variable	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3
M/4no	1.5	1.0	2.5	1.0	0.0	2.0	2.0	0.5	3.5
M/4 SSNo	4.0	3.0	9.0	3.0	0.0	5.0	7.0	3.0	12.0
M/4GSNo	8.0	3.8	22.0	4.0	0.0	6.0	12.0	5.0	23.0
M/4 mm	85.6	27.7	231.3	15.0	0.0	68.4	23.0	1.3	153.3
SSAM/4	414.0	32.0	925.0	44.9	0.0	158.0	69.0	3.0	577.0
GSAM/4	899.0	34.0	2835.0	59.9	0.0	179.2	92.0	3.0	1361.0
toe no	1.5	0.8	4.3	1.0	0.0	3.0	1.0	0.5	2.0
SSNoT	3.0	1.5	9.5	0.0	0.0	10.0	5.0	0.0	6.0
GSNoT	3.0	1.5	12.5	0.0	0.0	13.0	6.0	0.0	21.0
SSAT	121.0	52.0	481.0	0.0	0.0	121.2	45.0	11.0	3730.0
toe mm	79.6	26.2	190.4	0.0	0.0	54.9	16.0	3.0	653.0
GSAT	162.0	53.0	679.0	0.0	0.0	132.6	57.0	22.0	19353.0
L/4 no	2.0	1.5	3.5	1.0	0.0	2.0	1.0	0.0	3.5
L/4SSNo	8.0	3.5	11.3	2.0	1.0	6.0	4.0	0.0	12.0
L/4GSNo	13.0	3.5	24.5	2.0	1.0	8.0	8.0	0.0	42.0
SSAL/4	243.0	68.0	686.0	58.1	24.3	193.7	92.8	0.0	245.8
L/4 mm	97.7	33.4	182.8	24.3	9.7	89.8	23.2	0.0	52.4
L/4GSA	375.0	70.0	1558.0	77.5	24.3	207.8	186.0	0.0	926.0
TCNo	6.5	3.3	9.5	3.0	0.5	6.5	4.0	2.0	8.0
SSNo	15.5	10.3	28.8	6.0	1.0	20.5	17.0	7.0	28.5
GSNo	25.0	14.0	48.5	6.0	1.0	27.0	44.0	13.0	85.0
TCA	398.7	148.6	542.0	69.0	12.2	195.9	111.0	30.0	895.0
SSA	672.0	302.0	2068.0	206.9	24.3	421.4	445.0	118.0	4496.0
GSA	828.0	306.0	5242.0	275.9	24.3	450.9	1643.0	234.0	20914.0
	wide	regular		wide	wide				
Variable	Median	Q1	Q3	Median	Q1	Q3			
M/4no	0.5	0.0	5.0	2.0	1.0	3.0			
M/4SSNo	7.5	0.0	16.0	8.0	4.0	13.0			
M/4GSNo	12.0	0.0	43.3	16.0	7.0	44.0			
M/4 mm	15.2	0.0	90.0	89.0	18.9	186.5			
SSAM/4	30.3	0.0	282.7	274.0	54.0	812.0			
GSAM/4	30.0	0.0	414.0	385.0	59.0	2896.0			
toe no	1.5	0.8	2.3	2.0	1.0	4.0			
SSNoT	3.0	0.0	7.3	6.0	4.0	12.0			
GSNoT	3.0	0.0	36.0	16.0	4.0	48.0			
SSAT	320.0	74.0	1672.0	279.0	151.0	1577.0			
toe mm	131.4	36.8	294.0	120.1	62.5	340.0			
GSAT	1097.0	74.0	7118.0	467.0	184.0	8411.0			
L/4 no	1.0	0.8	2.5	2.0	1.0	4.0			
L/4SSNo	3.5	0.8	12.0	9.0	4.0	12.5			
L/4GSNo	9.0	0.8	28.0	22.0	6.0	44.0			

	wide	regular		wide	wide	
Variable	Median	Q1	Q3	Median	Q1	Q3
SSAL/4	258.0	3.0	1291.0	358.0	119.0	1032.0
L/4 mm	113.2	3.3	229.1	127.5	31.3	197.7
L/4GSA	290.0	3.0	5566.0	448.0	204.0	4617.0
TCNo	4.0	1.8	8.5	7.0	4.5	8.0
SSNo	15.5	5.3	32.5	24.0	17.5	32.5
GSNo	47.0	5.3	96.8	54.0	21.0	132.0
TCA	304.6	167.1	457.1	420.0	200.0	771.0
SSA	1228.0	333.0	2252.0	1286.0	543.0	3710.0
GSA	4645.0	333.0	10042.0	1890.0	657.0	15831.0

Note 1 data set of mixed hooves

Table 6.3.1.ii Descriptive summary of crack scores of 28 left fores grouped according to their different shapes

Summary of crack scores in different shaped hooves									
shape	round	round		round	wide		round upright		
crack score	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3
M/4 no	3.0	2.0	5.3	4.5	1.8	7.3	3.0	1.0	4.0
M/4 SSNo	13.0	7.5	19.5	17.0	7.0	33.8	11.0	4.0	14.0
M/4 GSNo	23.0	14.0	52.0	42.0	17.0	109.0	20.0	8.0	24.0
M/4mm	174.7	124.7	269.8	230.0	76.0	521.0	235.0	113.3	249.0
M/4SSA	671.0	476.0	973.0	1058.0	287.0	2544.0	808.0	434.0	996.0
M/4 GSA	1228.0	905.0	1869.0	3120.0	619.0	9191.0	1352.0	830.0	1992.0
Toe no	3.5	1.5	4.5	2.5	2.0	5.3	2.0	1.0	5.0
SSNoT	12.5	3.8	17.8	11.0	6.0	25.0	7.0	4.0	14.0
GSNoT	17.0	4.5	50.0	40.0	8.0	90.0	12.0	8.0	18.0
Toe mm	206.0	25.0	518.0	103.0	30.0	404.0	103.7	62.3	144.8
GSAT	749.0	97.0	6647.0	2295.0	119.0	11521.0	498.0	282.0	735.0
SSAT	569.0	74.0	2381.0	521.0	89.0	2308.0	249.0	244.6	473.3
L/4no	4.5	1.5	5.0	3.5	2.3	4.8	3.0	2.0	4.0
L/4SSNo	16.5	6.0	20.8	11.0	7.8	21.0	14.0	9.0	18.0
L/4GSNo	32.0	12.0	51.0	26.0	13.0	78.0	28.0	24.0	96.0
L/4mm	251.8	88.3	321.7				110.6	81.8	286.4
L/4 SSA	892.0	389.0	1383.0	728.0	330.0	2715.0	664.0	334.0	1318.0
L/4GSA	1648.0	987.0	3444.0	2539.0	488.0	10813.0	3540.0	859.0	3673.0
TCNo	10.5	5.5	16.3	11.0	10.0	12.8	7.0	5.0	13.0
SSNo	42.5	21.5	58.0	47.0	34.0	58.5	27.0	22.0	42.0
GSNo	75.0	45.5	138.5	146.0	69.0	208.0	70.0	56.0	100.0
TCA mm	603.0	343.0	1110.0	699.0	297.0	1154.0	422.0	299.0	666.0
SSA	2114.0	1310.0	4888.0	3442.0	1105.0	6252.0	1909.0	1013.0	2599.0
GSA	4049.0	2504.0	11741.0	13785.0	2515.0	28269.0	5759.0	1971.0	6030.0

shape	wide	round		wide	wide	
crack score	Median	Q1	Q3	Median	Q1	Q3
M/4 no	4.0	3.0	5.5	2.0	1.0	3.0
M/4 SSNo	16.0	10.0	22.5	6.0	4.5	13.5
M/4 GSNo	32.0	16.0	86.0	20.0	8.0	40.0
M/4mm	441.0	190.0	769.0	250.7	49.6	325.3
M/4SSA	2326.0	638.0	3485.0	463.0	163.0	1535.0
M/4 GSA	4652.0	1087.0	12673.0	918.0	316.0	6602.0
Toe no	2.0	2.0	3.0	3.0	2.5	5.5
SSNoT	9.0	4.5	12.0	15.0	7.5	30.0
GSNoT	22.0	7.0	32.0	28.0	12.0	94.0
Toe mm	254.4	113.1	482.6	341.0	196.0	483.0
GSAT	3592.0	1631.0	3963.0	3498.0	1007.0	9509.0
SSAT	1212.0	528.0	1817.0	1327.0	701.0	2399.0
L/4no	2.0	0.5	4.0	3.0	3.0	5.5
L/4SSNo	5.0	2.0	13.0	14.0	11.5	17.5
L/4GSNo	14.0	2.0	22.0	32.0	20.0	42.0
L/4mm	34.2	15.1	132.5	142.7	83.6	410.3
L/4 SSA	170.9	30.3	404.9	571.0	260.0	1597.0
L/4GSA	383.0	30.0	743.0	1142.0	433.0	3678.0
TCNo	7.0	6.0	12.5	11.0	7.0	13.0
SSNo	33.0	18.5	44.0	39.0	27.0	53.5
GSNo	80.0	35.0	124.0	72.0	48.0	150.0
TCA	989.0	379.0	1185.0	658.0	426.0	1360.0
SSA	4398.0	1537.0	5107.0	2600.0	1120.0	6169.0
GSA	8776.0	3968.0	16834.0	6403.0	2414.0	22789.0

Note 1 data set of 28 left fores

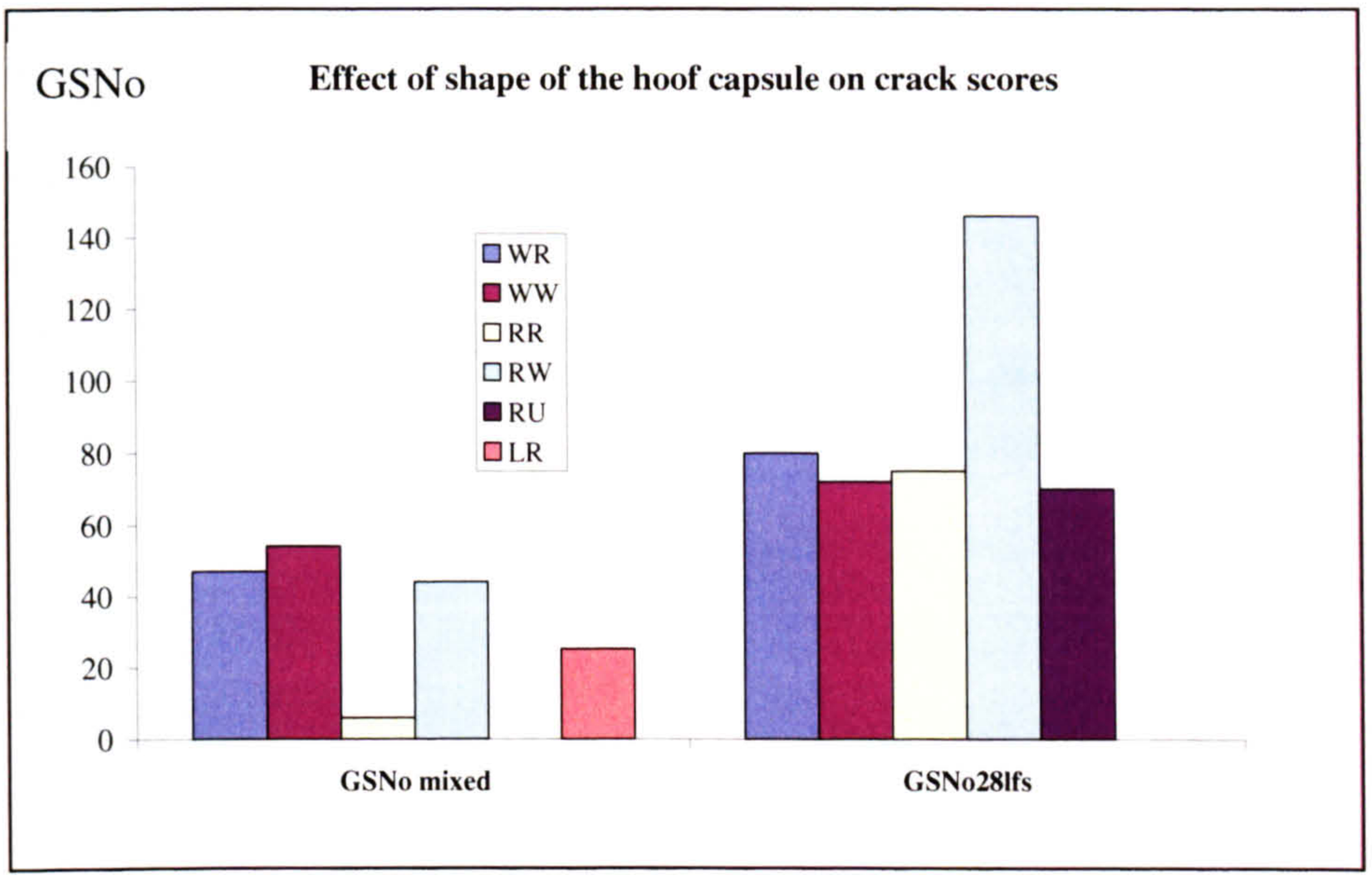
Crack scores in hooves grouped *just* by base plate *or* toe angle *or* vertical inclination

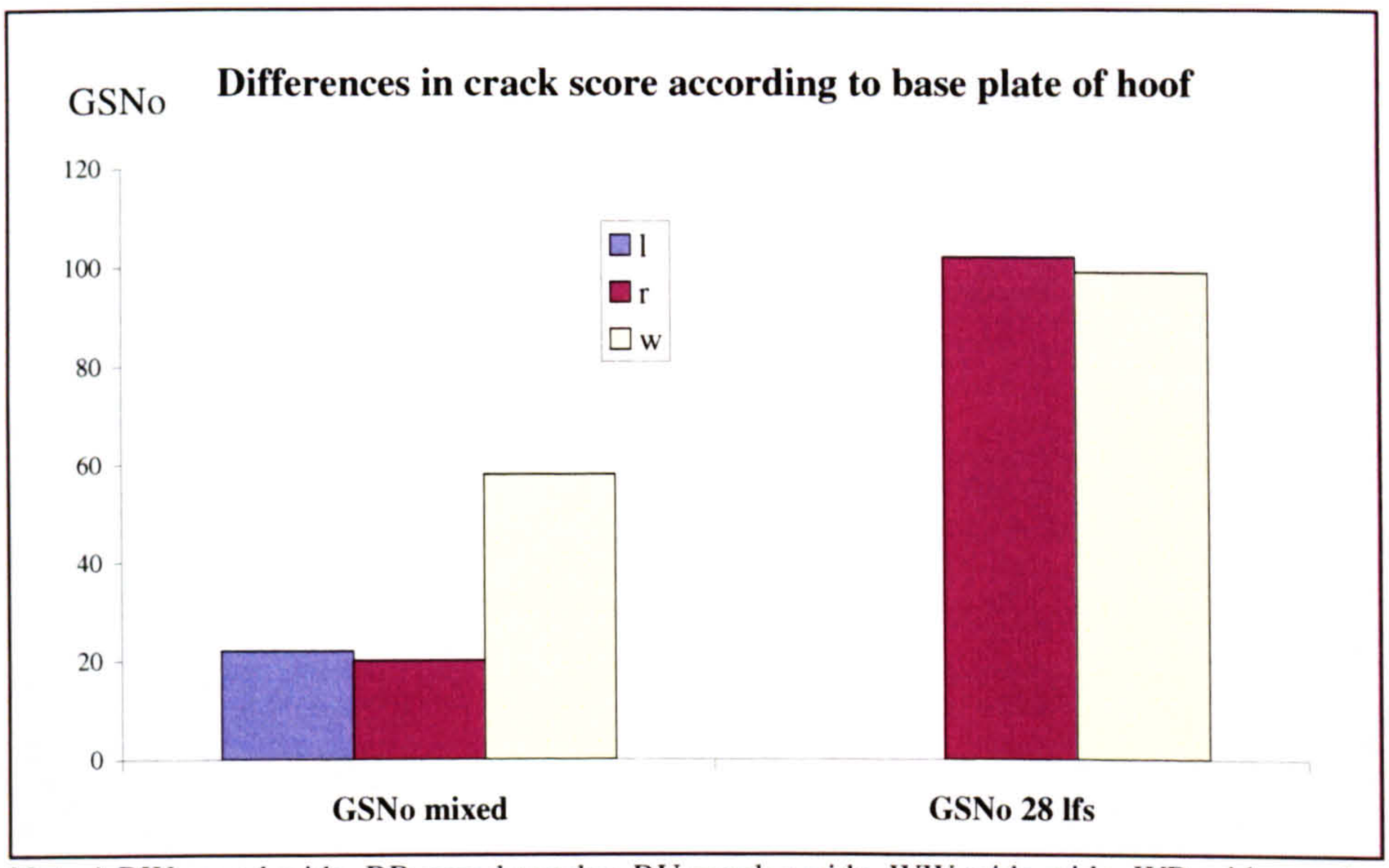
When the hooves were allocated to their shape groups, some groups contained only one hoof, for example in the mixed group of hooves there was only one hoof with an upright vertical inclination in base long group. This was because several of the original hooves had been used to develop techniques and although they had all been measured, not all were cracked scored or tested for trace elements. Therefore differences in crack scores in hooves grouped only by the shape of the base plate or by their vertical inclination or by toe angle were also investigated.

Crack scores in hooves with different shaped capsular bases

In the data set of mixed hooves, there was a significant difference in the severity area score (SSA) of the whole hoof, $p<0.05$, (*appendix, table 6.3.1A.i*); hooves with wide capsular bottoms had a greater area score compared to those with round capsular bottoms. This probably reflected the significantly higher, ($p<0.05$) area and adjusted area scores of the wide capsular base hooves

at their lateral quarters. Whilst the difference in the other scores were not significant, ($p>0.05$) all area scores were higher for the wide capsule compared to the other shapes and this was reflected in the number score. In the data set of left fores, hooves with round bases tended to have a smaller geometric area crack score at the toe and those with wide bases tended to have less cracks at the quarters, *(appendix table 6.3.1A.ii.), figure 6.3.1.i.*





Note 1 RW round wide, RR round regular, RU regular wide, WW wide wide, WR wide regular, WU wide upright, LR long round, LW long wide, LU long upright

Note 2 l =long, r = round, w = wide bases

Figure 6.3.1.i Graphical illustration of the difference in crack scores between different shaped hooves

Crack scores in hooves with different vertical inclination

In the data set of 48 hooves, there were no significant differences between crack scores in hooves with different vertical inclination, ($p>0.05$) *appendix table 6.3.1A.iii*. However hooves with wide vertical inclination had higher number scores compared to those with regular or upright vertical inclination; a comparison with area scores emphasised this difference. In the data set of left fores, there was a significant difference in geometric area crack score between the toe and the lateral quarter in hooves with an upright vertical angle, $p<0.05$ (appendix, *table 6.3.1A.v).*

Differences in crack scores between hooves with low, normal *or* upright toe angles

In the data set of mixed feet, the whole hoof area severity and geometric scores of hooves with a regular toe angle were significantly higher, ($p<0.05$) compared to hooves with an upright toe angle. The trend for increased area and number scores for hooves with a regular toe angle was reflected in all scores, *table 6.3.1A.iv*.

6.3.2 Relationships between fracture toughness and shape

The differences in fracture toughness between hooves of different shapes were not significant $p>0.05$. There was a trend in the group of mixed hooves for round wide (RW) and long upright, (LU) capsules to have higher values compared to the other shapes, (*table 6.3.2.i*). These differences were reflected in the individual values of fracture toughness for the vertical side angles and the capsular base, *table 6.3.2A.i*. In the data set of 28 left fores there was a trend for round upright hooves, (RU) to have lower fracture toughness compared to the other shapes, *table 6.3.2ii*.

Table 6.3.2.i Summary of mean fracture toughness of 48 mixed hooves grouped according to their different shapes

base plate & Vertical inclination	long, regular	long, upright	round regular	round upright	round wide	wide regular	wide upright	wide wide
	Fracture toughness ± SD							
IR	275.3 ± 105.3	361.6 ± 152.0	239.7 ± 125.6	273.9 ± 92.1	330.9 ± 158.9	265 ± 111.9	288.0 ± 24.0	272.5 ± 50.3
IS	26.5 ± 8.6	29.8 ± 8.3	26.6 ± 8.7	27.7 ± 7.5	32.0 ± 10.0	28.7 ± 11.0	26.6 ± 5.8	29.7 ± 5.7

Note 1 IR impact resistance J/m
Note 2 IS impact strength kJ/m^2

Table 6.3.2.ii Summary of mean fracture toughness of 28 left fore feet grouped according to their different shapes

shape group	ROUND REGULAR		ROUND UPRIGHT		ROUND WIDE	
Variable	Mean	StDev	Mean	StDev	Mean	StDev
M/4 IR	380.6	201.0	289.3	74.7	342.4	122.0
M/4 IS	43.7	11.3	38.2	12.7	46.1	20.8
mdc IR	312.3	58.7	224.6	61.3	254.6	67.0
mdc IS	33.3	7.0	23.0	9.0	27.1	1.9
L/4 IR	372.9	102.5	311.9	23.5	355.9	150.3
L/4 IS	43.5	12.6	42.9	5.3	48.7	22.0

shape group	WIDE REGULAR		WIDE WIDE	
Variable	Mean	StDev	Mean	StDev
M/4 IR	391.6	85.6	308.9	26.9
M/4 IS	45.4	13.4	37.4	4.7
mdc IR	340.4	62.6	277.4	68.4
mdc IS	31.6	7.8	26.8	6.2
L/4 IR	377.0	55.4	340.5	116.9
L/4 IS	44.0	4.9	39.8	9.6

Note 1 data set 28 left fores
Note 2 IR impact resistance J/m; IS impact strength kJ/m^2

Within shape group differences in fracture toughness due to anatomical positions

Differences between the fracture toughness at the toe and quarters within the different shape groups were tested in the group of 28 left fore feet. Within most of the shape groups, the impact strength at the quarters was significantly greater than the impact strength at the toe $p<0.05$, *table 6.3.2.iii, figure 6.3.2.iii*.

Table 6.3.2.iii Summary of mean impact strengths at different anatomical positions within shape groups

anatomical position	L/4	M/4	toe
shape	Impact strength kJ/m^2		
round regular	43.4	43.7	33.3
round upright	40.8	34.4	24.7
round wide	[b]42.9	40.3	[a]26.9
wide regular	[b]44.5	45.4	[a]28.5
wide wide	[b]37.2	[b]35.9	[a]27.7

Note 1 data set 28 left fores

Note 2 different superscripts indicate significant differences at $p<0.05$ except for WW $p<0.001$

Fracture toughness in hooves grouped *just* by base plate *or* toe angle *or* vertical inclination

The differences in fracture toughness between either data set of hooves with different shaped capsular bases, toe angles or vertical inclination were not significant, ($p<0.05$). However in the data set of 28 left fores there was a trend for wide bases to have lower fracture toughness compared to the round bases, *table 6.3.2A i and table 6.3.2Aii*

Regardless of which geometry was used to group the hooves, the fracture toughness at the toe was significantly less ($p<0.05$) than the fracture toughness at the quarters, (*table 6.3.2A.iii*).

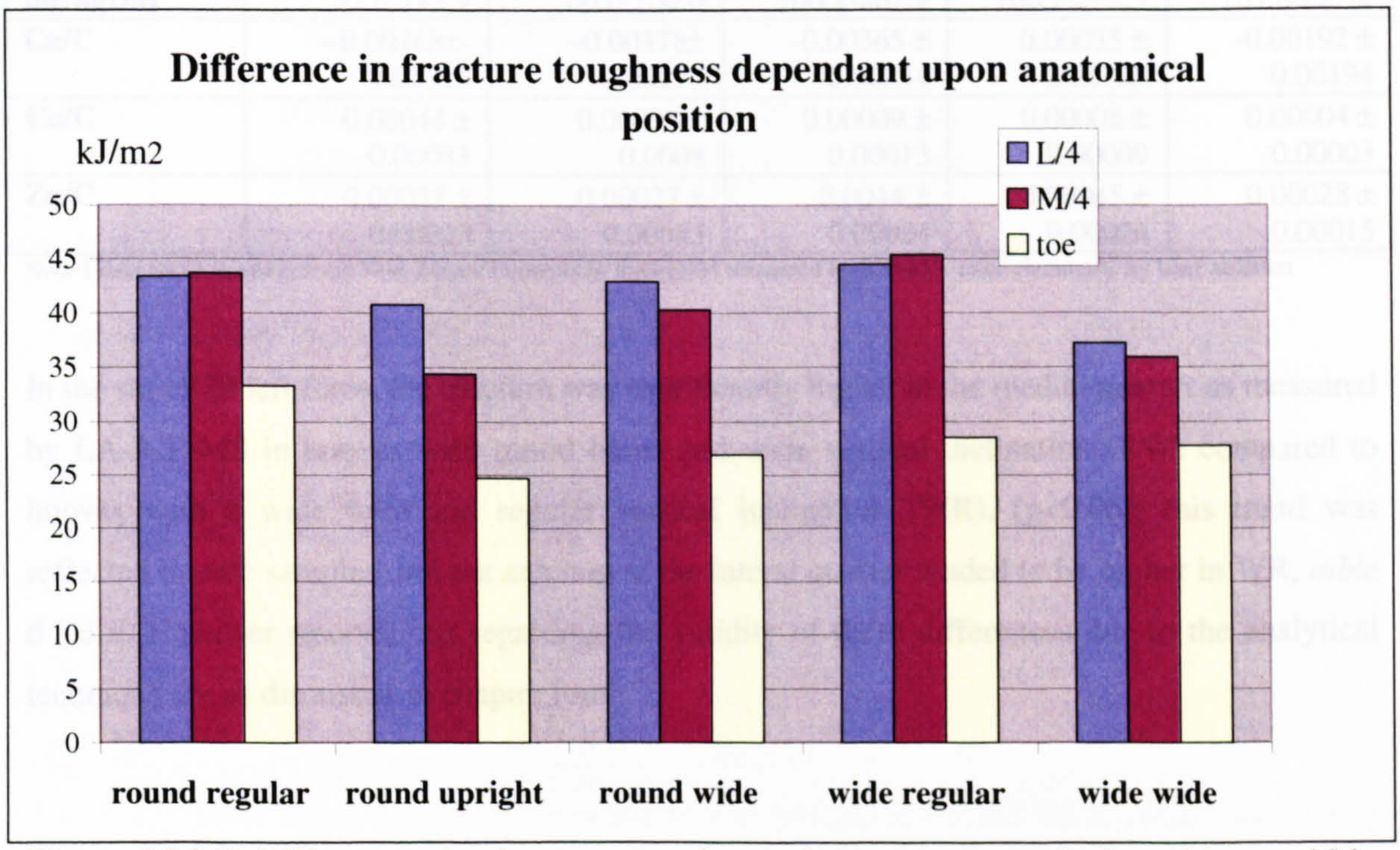


Figure 6.3.2.iii Differences in fracture toughness between anatomical positions within shape groups

Note 1 L/4 = lateral quarter; M/4 = medial quarter

6.3.3 Relationships between trace element concentrations and shape

The differences in trace elements measured by ICP-MS solution between hooves grouped into shape groups as recommended by this thesis were not significant, ($p>0.05$). In the data set of mixed hooves, there was a trend for hooves with round bases and regular vertical inclination, (RR) to have lower copper and zinc and for hooves with a round base and wide vertical inclination (RW) to have higher calcium compared to the other shapes, (*table 6.3.3. i*).

Table 6.3.3. i Summary of mean trace elements in hooves with different shapes

shape groups	LR	RR	RW	WR	WW
Copper mg/kgDM	25.2 ± 8.1 *21.1 -41.7*	18.2 ± 4.1 *12.6- 23.7*	26.9 ± 7.3 *20.8-39.5*	25.8 ± 7.8 *19.8 -41.2*	21.0 ± 1.9 *18.0-24.6*
Calcium mg/kgDM	422.0 ± 261.0 *128.0-695.0*	726.0 ± 329.0 *360.0-1160.0*	1034.0 ±461.0 *471.0-1747.0*	777.0 ± 317.0 *513.0-1378.0*	858.0 ± 414.0 *31.0-1256.0*
Zinc mg/kgDM	141.9 ± 73.1 *31.0-242.9*	133.6 ± 39.8 *80.0-180.0*	185.2 ± 65.3 *106.2-287.9*	165.8 ± 68.2 *100.7-277.5*	183.7 ± 34.6 *109.8-227.2*
Ca/C	–0.00468± 0.00314	–0.00378± 0.00273	-0.00365 ± 0.00334	0.00035 ± 0.00705	-0.00192 ± 0.00194
Cu/C	0.00044 ± 0.00083	0.00005 ± 0.0008	0.00009 ± 0.00013	0.00008 ± 0.00009	0.00004 ± 0.00003
Zn/C	0.00037 ± 0.00023	0.00027 ± 0.00023	0.0044 ± 0.00024	0.00045 ± 0.00026	0.00028 ± 0.00015

Note 1 data set of mixed hooves Note 2 trace elements in mg/kgDM measured by ICP-MS; trace element/C by laser ablation

In the set of 28 left fores, the calcium was significantly higher at the medial quarter as measured by LA-ICP-MS in hooves with round bases and wide vertical inclination (RW) compared to hooves with a wide base and regular vertical inclination (WR), ($p<0.05$); this trend was reflected in mdc samples, but the calcium at the lateral quarter tended to be higher in WR, *table 6.3.3.ii*. However reservations regarding the validity of these differences due to the analytical technique are as discussed in chapter four

Table 6.3.3.ii Descriptive summary of descriptive medians of trace element ppm /carbon ppm in 28 left fores

Median qualitative trace element measurements in hooves grouped according to their different shapes						
	round	regular		round	upright	
Variable	Median	Q1	Q3	Median	Q1	Q3
M/4 Ca/C	4.30E-04	0.00012	1.44E-03	3.00E-04	-3.60E-02	5.00E-04
M/4 Cu/C	1.00E-05	0.00001	4.00E-05	9.00E-05	1.00E-05	1.64E-02
M/4 Zn/C	2.10E-04	0.00005	6.00E-04	6.10E-04	2.70E-04	9.44E-03
L/4 Ca/C	-9.08E-03	-0.01421	4.07E-03	-8.45E-03	-1.31E-02	1.60E-03
L/4 Cu/C	5.80E-04	0.00013	3.36E-03	5.80E-04	1.80E-04	3.93E-03
L/4 Zn/C	4.26E-03	0.00257	7.82E-03	3.55E-03	3.05E-03	4.00E-03
mdc Ca/C	3.00E-04	-0.00007	3.50E-04	1.70E-04	-5.10E-04	1.30E-03
mdcCu/C	0.00E+00	0	2.00E-05	1.00E-05	0.00E+00	1.00E-05
mdc Zn/C	1.10E-04	0.00006	6.20E-04	2.60E-04	2.00E-04	3.40E-04
	round	wide		wide	regular	
Variable	Median	Q1	Q3	Median	Q1	Q3
M/4 Ca/C	1.15E-03[a]	9.40E-04	2.06E-03	1.00E-04[b]	-2.01E-01	5.00E-04
M/4 Cu/C	2.00E-05	1.00E-05	1.10E-04	4.00E-05	0.00E+00	1.65E-02
M/4 Zn/C	6.10E-04	1.20E-04	1.78E-03	8.00E-04	4.00E-04	4.41E-02
L/4 Ca/C	-1.43E-02	-1.73E-02	-3.20E–03	-1.23E-02	-1.40E-02	-2.98E–03
L/4 Cu/C	3.10E-04	1.10E-04	1.61E-03	2.10E-04	1.30E-04	1.95E-03
L/4 Zn/C	4.43E-03	2.84E-03	1.14E-02	3.84E-03	2.79E-03	4.15E-03
mdc Ca/C	-7.00E-05	-1.10E-04	8.00E-04	1.02E-03	-2.60E-04	1.87E-03
mdc Cu/C	0.00E+00	0.00E+00	1.00E-05	1.00E-05	1.00E-05	1.00E-04
mdc Zn/C	1.30E-04	4.00E-05	4.60E-04	6.50E-04	4.60E-04	8.30E-04
	wide	wide				
Variable	Median	Q1	Q3			
M/4 Ca/C	7.60E-04	2.90E-04	1.08E-03			
M/4 Cu/C	1.00E-05	1.00E-05	2.00E-05			
M/4 Zn/C	9.20E-04	9.00E-05	1.49E-03			
L/4 Ca/C	-8.69E-03	-1.04E-02	9.60E-04			
L/4 Cu/C	1.90E-04	1.40E-04	9.10E-04			
L/4 Zn/C	4.01E-03	2.95E-03	4.71E-03			
mdc Ca/C	2.70E-04	5.00E-05	8.50E-04			
mdc Cu/C	1.00E-05	0.00E+00	1.00E-05			
mdc Zn/C	3.00E-04	1.40E-04	8.40E-04			

Note 1 measurements are trace element ppm/Carbon ppm

Note 2 the different superscripts indicate differences between M/4Ca/C at RW and WR at a significance level of $p<0.05$

Note 3 data set 28 left fores

Differences between ratios of trace element concentrations in hooves of different shapes

In the data set of mixed hooves, hooves in the LR shape group had a significantly lower Zn: Ca ratio compared to hooves in the RW group, ($p<0.05$). There was a trend for hooves in the RR group to have a lower Cu: Ca ratio but higher Zn: Cu than the other shaped hooves and for hooves in the RW group to have a lower Ca: Zn ratio compared to the other shapes. WW hooves tended to have the highest Ca: Cu ratio out of all the shape groups, (*table 6.3.3.iii*).

In the data set of left fores, there were no significant differences in qualitative trace elements measured at the toe, medial and lateral quarters between hooves grouped according to their base shape and vertical inclination, ($p>0.05$), *table 6.3.3.iv*.

Table 6.3.3.iii Summary of mean trace element ratios in hooves with different shapes

shape	Long Regular	Round Regular	Round Wide	Wide Regular	Wide Wide
ratio	all ratios 1: mean ± SD	*interquartile range*			
Cu: Ca	19.4 ± 12.4 *6.0-32.7*	42.9± 18.7 *28.5-70.0*	39.6 ± 18.9 *20.0-69.1*	29.6 ± 4.5 *23.6-36.2*	40.6 ± 18.9 *1.4-61.3*
Ca : Cu	0.08 ± 0.06 *0.03-0.17*	0.02 ± 0.01 *0.01-0.04*	0.03 ± 0.02 *0.01-0.05*	0.03 ± 0.01 *0.03-0.04*	0.10 ± 0.22 *0.02-0.71*
Cu : Zn	6.4 ± 3.7 *0.74-11.5*	7.4 ± 2 *4.6-9.5*	6.8 ± 1.4 *5.7- 8.2*	6.4 ± 1.8 *4.1-9.5*	8.7 ± 1.55 *5-9.7*
Zn: Cu	0.35 ± 0.49 *0.09-1.30*	0.14 ± 0.048 *0.10-0.22*	0.15 ± 0.04 *0.22 – 0.13*	0.17 ± 0.04 *0.11-0.24*	0.12 ± 0.03 *0.10-0.20*
Zn : Ca	[a]2.4 ± 1.1 *1.2- 3.7*	5.1 ± 1.5 *3.7-7.3*	[b]5.7 ± 2.3 *4.00-9.5*	4.8 ± 1.0 *3.8-6.7*	4.2 ± 2.0 *0.28 -6.3*
Ca: Zn	0.52 ± 0.28 *0.27-0.86*	0.20 ± 0.05 *0.14-0.27*	0.19 ± 0.06 *0.27-0.14*	0.22 ± 0.04 *0.15-0.26*	0.64 ± 1.18 *0.16-3.60*

Note 1 data set of 48 hooves
Note 2 Different superscripts indicate a significant difference at $p<0.01$

Table 6.3.3.iv Summary of ratios of trace elements measured qualitatively by laser ablation in different shaped hooves

medians and differences in qualitative TE ratios between 28 left fores grouped according to their shapes						
	round	regular		round	upright	
Variable	Median	Q1	Q3	Median	Q1	Q3
M/4s						
Ca/C:Cu/C	0.04	0.02	0.07	0.05	-0.46	0.17
Ca/C:Zn/C	0.39	0.21	1.10	0.51	-0.26	2.04
Zn/C:Cu/C	0.16	0.02	0.25	0.34	0.02	1.74
Cu/C:Ca/C	27.54	14.92	47.04	5.80	-2.19	21.06
Zn/C:Ca/C	3.01	1.08	4.78	0.49	-3.82	1.94
Cu/C:Zn/C	6.50	3.90	54.10	3.00	0.60	43.00

	round	regular		round	upright	
Variable	**Median**	**Q1**	**Q3**	**Median**	**Q1**	**Q3**
L/4s						
Cu/C:Zn/C	10.00	3.41	21.94	6.87	0.78	19.31
Cu/C:Ca/C	-18.40	-82.20	3.10	-3.33	-14.52	8.68
Zn/C: Ca/C	-2.15	-3.26	0.67	-2.11	-4.28	0.45
Zn/C:Cu/C	0.13	0.06	0.46	0.15	0.05	1.29
Ca/C:Cu/C	-0.02	-0.16	0.25	-0.07	-0.30	0.12
Ca/C:Zn/C	-0.29	-0.45	1.15	-0.23	-0.47	2.22
toe						
Ca/C:Cu/C	0.01	-0.32	0.02	0.01	-0.02	0.03
Ca/C:Zn/C	0.22	-7.82	0.63	0.26	-0.51	1.21
Cu/C:Ca/C	60.10	-13.40	140.30	35.20	-46.40	97.40
Cu/C:Zn/C	26.62	22.85	39.08	25.71	23.68	42.69
Zn/C:Cu/C	18913.00	4074.00	35836.00	7749.00	5848.00	9761.00
Zn/C:Ca/C	1.62	-2.28	4.26	1.94	0.83	3.79
Variable	**Median**	**Q1**	**Q3**	**Median**	**Q1**	**Q3**
	round	**wide**		**wide**	**wide**	
M/4						
Ca/C:Cu/C	0.01	0.01	0.11	0.01	0.00	0.03
Ca/C:Zn/C	0.65	0.10	1.41	1.20	0.03	1.41
Zn/C:Cu/C	0.23	0.01	0.47	0.02	0.01	0.08
Cu/C:Ca/C	98.20	25.80	137.00	39.00	-78.00	81.00
Zn/C:Ca/C	2.17	0.74	29.98	0.71	-4.81	4.06
Cu/C:Zn/C	72.40	2.10	168.90	41.30	11.90	83.50
L/4						
Cu/C:Zn/C	14.56	8.31	28.67	20.44	4.52	27.89
Cu/C:Ca/C	-49.00	-124.80	-2.90	-7.60	-77.90	6.10
Zn/C: Ca/C	-2.71	-5.33	-0.38	-2.17	-2.67	0.32
Zn/C:Cu/C	0.07	0.04	0.13	0.05	0.04	0.28
Ca/C:Cu/C	-0.07	-0.76	-0.01	-0.01	-0.08	0.08
Ca/C:Zn/C	-0.51	-10.09	-0.19	-0.32	-0.46	1.56
toe						
Ca/C:Cu/C	-0.02	-0.06	0.00	0.02	0.00	0.07
Ca/C:Zn/C	-0.77	-1.34	0.33	0.82	0.69	1.82
Cu/C:Ca/C	-28.30	-48.70	70.30	20.70	11.50	170.30
Cu/C:Zn/C	35.30	14.50	54.80	40.90	17.70	129.90
Zn/C:Cu/C	17082.00	5647.00	83693.00	6698.00	2388.00	20471.00
Zn/C:Ca/C	-0.76	-3.19	0.22	1.16	0.37	1.30

Variable	**Median**	**Q1**	**Q3**
	wide regular		
M/4			
Ca/C:Cu/C	0.35	-0.04	0.65
Ca/C:Zn/C	6.40	0.00	39.00
Zn/C:Cu/C	0.06	0.02	0.22
Cu/C:Ca/C	2.40	-5.50	75.90
Zn/C:Ca/C	0.07	-2.30	2.63
Cu/C:Zn/C	18.00	8.80	93.40

Variable	Median	Q1	Q3
	wide regular		
L/4			
Cu/C:Zn/C	18.73	2.14	26.34
Cu/C:Ca/C	-60.00	-109.40	-1.00
Zn/C: Ca/C	-3.06	-4.33	-1.15
Zn/C:Cu/C	0.05	0.04	0.63
Ca/C:Cu/C	-0.01	-0.20	0.84
Ca/C:Zn/C	-0.31	-0.37	2.65
toe			
Ca/C:Cu/C	0.01	-0.04	0.03
Ca/C:Zn/C	0.43	-0.01	0.85
Cu/C:Ca/C	85.60	5.50	168.10
Cu/C:Zn/C	71.60	7.20	91.10
Zn/C:Cu/C	3068.00	2500.00	4339.00
Zn/C:Ca/C	2.27	-0.58	2.31

Note 1 data set 28 left fores
Note 2 all trace elements measured qualitatively by laser ablation and expressed as TEppm/Cppm

Differences in trace element concentrations and ratios in hooves grouped *just* by base plate *or* toe angle *or* vertical inclination

Differences in trace elements in hooves with round, long or wide capsular bases

In the data set of 48 hooves there were significant differences in the Zn: Ca ratios between hooves with long capsular bases having a lower ratio compared to hooves with wide or regular capsular bases, ($p<0.001$). There was a trend for hooves with a long capsular base to have higher copper but lower zinc and calcium compared to the other base shapes as well as a lower Ca: Cu ratio and a higher Zn: Cu ratio, (*table 6.3.3A.i*).

Differences in trace elements in hooves with low, upright or regular toe angle

In the data set of mixed hooves, Cu: Zn ratio was significantly lower ($p<0.05$) in hooves with a low toe angle compared to hooves with a regular or upright toe angle. Hooves with a low toe angle tended to have lower calcium and zinc compared to upright and regular toe angles. Low toe angled hooves also had lower Cu: Ca but higher Zn: Cu and Ca: Zn although the differences were not significant, ($p>0.05$) *table 6.3.3A.ii*. In the data set of mixed feet, the ratio of Ca/C: Cu/C was significantly higher in normal toe angle hooves compared to hooves with upright toe angles, ($p<0.05$). In the data set of 28 left fores, there was a significant difference in Zn/C between upright and normal toe angle hooves at the medial quarter only,($p<0.05$) and upright toe angles appeared to have less Zn/C, *table 6.3.3A.i.* There was a trend for higher medial Ca/C: Zn/C and higher lateral Cu/C: Zn/C but lower mdc Cu/C: Zn/C in hooves with a low toe angle, *table 6.3.3A.iv*

6.3.4 Correlations between trace elements, crack score, and fracture toughness

Correlations between crack scores and trace elements in whole data set

Calcium was the only trace element which showed any correlation with crack scores. Calcium was most strongly correlated with GSAT, *table 6.3.4.i*. A regression was carried out to see if crack scores would be a useful predictor of calcium content in the hoof, *table 6.3.4.ii*.

Table 6.3.4.i Summary of correlations between trace element concentrations and crack scores in a group of mixed hooves

Ca mg/kgDM with	Spearman's Rank Correlation p value		
SSAT	0.496 0.004	toe crack area mm^2	0.533 0.002
GSA	0.51 0.003	GSAT	0.61 0.0001
GSNoT	0.470 0.007		
Cu : Ca with		**Zn:Ca with**	
SSAT	0.508 0.003	0.545 0.002	
GSAT	0.531 0.002	0.574 0.005	
toe area mm	0.459 0.008	0.49 0.001	

Table 6.3.4.ii Polynomial relationships between crack scores and trace element concentration in a group of mixed feet

Regression equation	**p & R^2 values**
$SSAT = 1274.41 - 4.83821Ca + 0.0042271\ Ca^2$	$R^2 = 73.2\%$ $p = 0.0001$
$GSAT = 5564.71 - 24.4259Ca + 0.0219444Ca^2$	$R^2 = 72.1\%$ $p = 0.0001$
$toe\ mm^2 = 275.472 - 0.816717Ca + 0.0006868Ca^2$	$R^2 = 64.9\%$ $p = 0.0001$

Correlations between trace elements measured qualitatively by LA-ICP-MS and other measured parameters are reported in the appendix for interest pages LXXXI. A decision had been made that due to the fact that less than 0.01mm area was used to analyse the trace elements and because the analysis was qualitative, that any correlation would be purely speculative.. They were qualitative results and the aim of this thesis was to discuss objective measurements only as these qualitative measurements are not repeatable.

Correlations between fracture toughness and trace elements in whole data set

There were no correlations between fracture toughness and trace elements or between fracture toughness and trace element ratios in the group of mixed feet.

Correlations between fracture toughness and crack scores in hooves of different shapes

When hooves had been allocated to groups according to their shape, there were significant correlations between the fracture toughness and the crack score of that hoof in some of the groups. In the data set of mixed hooves impact strength had only been measured at the toe, therefore correlations with cracks were only carried out for this anatomical area and with the median crack score for the whole hoof.

There were correlations between fracture toughness and area scores in hooves with long bases and regular vertical inclination, (LR), in hooves with round bases and wide vertical inclination, (RW) and in hooves with wide bases and wide vertical inclination, (WW), *table 6.3.4.iii.*

Table 6.3.4.iii Summary correlations between fracture toughness and crack area scores in different shaped hooves

long base regular vertical inclination (LR)		
Impact resistance	**whole hoof**	**toe**
TCA	0.792 p = 0.499	0.795 p = 0.050
GSA	0.870 p = 0.024	0.820 p = 0.046
SSA	0.844 p = 0.035	0.854 p = 0.03
round base wide vertical inclination (RW)		
Impact resistance	**whole hoof**	**toe**
TCA	0.926 p = 0.024	0.822 p = 0.048
round base wide dorsal inclination (RW)		
Impact resistance	**whole hoof**	**toe**
SSA	0.893 p = 0.041	
impact strength	**whole hoof**	**toe**
TCA	0.924 p = 0.025	0.97 p = 0.035
GSA	0.898 p = 0.038	0.897 p = 0.039
SSA	0.913 p = 0.03	0.904 p = 0.035
GSNo		0.887 p = 0.045

wide base regular vertical inclination (WR)		
Impact resistance	**whole hoof**	
GSNo	0.851 p = 0.032	
GSA	0.736 p = 0.0499	
impact strength		
GSNo	0.893 p = 0.017	
GSA	0.816 p = 0.048	
wide base wide vertical inclination (WW)		
Impact resistance	**whole hoof**	**toe**
GSA		-0.737 p = 0.023
impact strength		
GSA	-0.76 p = 0.018	- 0.744 p = 0.022
SSA	- 0.786 p = 0.012	-0.74 p = 0.023

Note 1 each cell contains Spearman's Rank correlation coefficient and p value Note 2 data set of 48 hooves

Correlations between trace elements and fracture toughness in hooves of different shapes

In the data set of mixed feet, there were no correlations between trace elements and fracture toughness between different shaped hooves.

Correlations between trace elements and crack scores in different shaped hooves

Putting hooves into groups according to their shapes increased the correlations between trace elements and crack number scores compared to the whole data set, *table 6.3.4.iv* as well as crack area scores, *table 6.3.4A.vi*. Some of the increase in correlation could have been due to the fact that there were only small numbers of hooves in each group and therefore correlation coefficients were calculated for hooves grouped only according to their base shape or according to their toe or vertical inclination.

Correlations in fracture toughness and crack scores in hooves with different shaped bases *or* different vertical inclination *or* different toe angles

In the data set of 48 hooves, there were no correlations between the presence of cracks however measured and the impact resistance or strength of the hoof material in hooves with wide bases. Hooves with long capsular bases showed strong correlations between toe crack scores and impact resistance as did hooves with round bases, *table 6.3.4A.i*. There were no correlations between crack scores and fracture toughness in hooves with a low vertical inclination, nor hooves with a low toe angle. All the other groups showed correlations between some crack

scores and fracture toughness, *table 6.3.4A.i.* In the data set of 28 left fores, there were also correlations between fracture toughness and crack scores, *table 6.3.4A.ii.*

Correlations between trace elements and fracture toughness in hooves with different capsular base shapes *or* with different dorsal inclination *or* toe angles

When hooves were grouped by their toe angles, there was a strong correlation between calcium and impact strength in hooves with an upright toe angle in the data set of mixed hooves. All ratios between the trace elements were correlated with fracture toughness only in hooves with an upright toe angle, *(appendix, table 6.3.4A.iv).* There were no correlations between trace elements and fracture toughness in low toe angled hooves. Hooves with a regular toe angle or with a wide or regular vertical inclination had the most correlations between impact strength and trace elements.

When hooves were grouped according to the shape of their base plates, hooves with a round base or with a wide base had the most correlations between impact strength and trace elements, *table 6.3.6A.v.* In the data set of mixed hooves, there was a strong correlation between calcium and impact strength in hooves with round and long bases. Cu: Ca was strongly correlated with impact strength in hooves with long or round bases; however the inverse ratio of Ca: Cu was only correlated in base long hooves. Impact strength with Ca: Zn was correlated in base long hooves and with Zn: Ca with long and round base hooves. There were no correlations between trace element ratios and fracture toughness in hooves with wide bases, *table 6.3.4.v.*

Table 6.3.4.iv Summary correlations between crack number scores and trace elements (mixed feet)

	TCNo						toe no			GSNoT					SSNo				
	Zn	**Cu**	**Ca**	**Ca:Cu**	**Cu:Zn**	**Zn:Cu**	**Cu:Ca**	**Cu:Zn**	**Zn:Ca**	**Cu:Ca**	**Zn:Ca**	**Zn**	**Ca**	**Cu**	**Zn**	**Cu:Zn**	**Cu:Ca**	**Zn:Cu**	**Ca:Cu**
LR	0.918 0.01	–0.745 0.05			0.924 0.009	-0.762 0.05									0.812 0.05	0.81 0.05		-0.747 0.05	
RR			0.966 0.034	-0.978 0.02			0.994 0.006		0.974 0.026	0.974 0.026	0.967 0.033						0.947 0.05		-0.967 0.033
RW	0.933 0.021							0.894 0.041			0.874 0.05		0.882 0.048		0.936 0.011				
WR										0.799 0.057		0.981 0.001	0.914 0.011	0.803 0.055					

	SSNoT					GSNo				
	Zn	**Cu**	**Ca**	**Cu:Ca**	**Zn:Ca**	**Zn**	**Zn:Ca**	**Cu:Ca**	**Ca:Zn**	**Ca:Cu**
RR				0.977 0.023	0.969 0.031			0.941 0.059		-0.963 0.037
RW						0.97 0.006				
WR	0.923 0.009	0.827 0.042	0.87 0.024				0.919 0.01		-0.929 0.007	

Note 1 each cell contains coefficient correlation and p value

Correlations in trace elements and crack scores in hooves with different shaped bases *or* different vertical inclination *or* different toe angles

In the data set of mixed hooves, calcium was correlated with several crack scores in hooves with round, or wide capsular bases or normal toe angles. Zinc was only correlated with several crack scores in hooves with regular vertical inclination and copper was the least correlated with crack scores regardless of the shape of the hoof, *table 6.3.4A.vii.*

Correlations between trace element ratios and crack scores

Hooves with round bases or normal toe angles had the most correlations between trace element ratios and crack scores. Hooves with long bases had the least correlations followed by hooves with a low toe angle or upright vertical inclination. Cu: Ca was correlated with several crack scores in hooves with round or wide bases and inversely correlated with several scores in hooves with an upright vertical inclination. Zn: Ca was correlated with several crack scores in hooves with round bases and in hooves with a regular toe angle or a wide vertical inclination, *table 6.3.4A.viii.*

6.3.5 Relationships between cracks and trace elements

The copper content of hooves with a high GSNo crack score was significantly higher than those with a medium crack score, ($p<0.05$) and higher than those with a low crack score. The calcium content of hooves with a high GSNo crack score was significantly higher than those with a low crack score, ($p<0.05$) and higher than those with a medium crack score. High GSNo crack score hooves had significantly more zinc than those with a low crack score, ($p<0.001$) and more zinc than those with a medium crack score. Hooves with a high GSA had significantly more calcium than those with a medium crack score, ($p<0.05$) and more than those with a low crack score, *table 6.3.5.i.*

Table 6.3.5.i Summary of trace element concentration in hooves grouped according to their crack scores.

Crack Score	high	low	medium
GSNo	mg/kgDM		
Cu	[a] 28.0	23.0	[b] 21.7
Ca	[a] 1034.9	[b] 533.1	721.6
Zn	[a] 211.9	[b] 123.0	168.7
GSA			
Ca	[a] 1042.6	617.7	[b] 694.2
Cu	24.9	22.9	23.0
Zn	190.7	131.4	173.2

Note 1 means of trace element Note 2 different subscripts indicate significance at $p<0.05$, except for Zn which is at a significance of $p<0.001$ Note 3 Data set of mixed feet

Table 6.3.5.ii Summary of trace element ratios in hooves grouped according to their crack scores

GSNo	high	medium	low
Cu: Ca	31.60	34.30	28.10
Ca: Cu	0.03	0.03	0.04
Cu: Zn	7.30	8.10	6.00
Zn: Cu	0.14	0.12	0.17
Zn : Ca	5.00	3.80	4.10
Ca: Zn	0.20	0.26	0.24
GSA	**high**	**medium**	**low**
Cu: Ca	44.50	29.70	28.50
Ca: Cu	0.02	0.03	0.04
Cu: Zn	7.30	7.80	6.70
Zn: Cu	0.14	0.13	0.15
Zn : Ca	5.80	3.70	4.40
Ca: Zn	0.17	0.27	0.23

Note 1 data set of mixed hooves
Note 2 crack score division as described ch 3; division are lower and upper quartiles
Note 3 GSNo geometric number score; GSA geometric area score of whole hooves

6.3.6 Relationships between fracture toughness and trace elements

Hooves with low fracture toughness had significantly more copper compared to hooves with higher fracture toughness, *table 6.3.6.i*. This difference was not significant when copper was expressed as a ratio of the other trace elements, *table 6.3.6.ii*.

Table 6.3.6.i Summary of trace element concentration in hooves grouped according to their fracture toughness

Fracture toughness	high	medium	low
IR	mg/kgDM		
Cu	21.7	[a]19.7	[b]26.8
Ca	859.0	555.8	876.8
Zn	168.9	155.1	175.5
IS	**high**	**medium**	**low**
Cu	21.6	20.0	24.9
Ca	833.0	604.2	810.5
Zn	159.6	168.6	169.3

Note 1 IR impact resistance J/m
Note 2 IS impact strength kJ/m^2
Note 3 data set mixed feet
Note 4 low, medium and high fracture toughness allocated by the lower and upper quartiles

Table 6.3.6.ii Summary of trace element ratios in hooves grouped by their fracture toughness measurements

Fracture toughness	high	medium	low
IR			
Cu : Ca	36.60	29.40	33.40
Ca: Cu	0.02	0.04	0.03
Cu : Zn	8.20	7.40	6.90
Zn : Cu	0.12	0.14	0.14
Zn : Ca	5.20	3.90	4.40
Ca : Zn	0.19	0.26	0.23
IS			
Cu : Ca	39.20	28.10	33.10
Ca: Cu	0.03	0.04	0.03
Cu : Zn	8.20	7.10	6.90
Zn : Cu	0.12	0.14	0.14
Zn : Ca	5.50	3.90	4.30
Ca : Zn	0.18	0.26	0.23

Note 1 IR impact resistance J/m
Note 2 IS impact strength kJ/m^2
Note 3 data set mixed feet
Note 4 low, medium and high fracture toughness divided by the lower and upper quartiles

6.4 Discussion

6.4.1 Interactions between shape and other measured parameters

When considering possible interactions between material properties, visual appearance and composition of hoof wall epidermis, or any epidermal tissue, the variations due to bodyweight, age, sex, diet, environment, and treatments such as farriery are likely to outweigh any variability of comparable biochemical measurements unless steps are taken to minimise the biological variations. In addition the bioavailability of trace elements from the diet will be influenced by level of trace element intake, chemical form, overall diet digestibility, particle size, interactions with other trace elements, chelators, physiological state of the animal, and the processing of the other ingredients in the diet. The development of standardised methodology in this thesis helped to remove some of the measurement and collection variables but due to the multiplicity of the relationships any inter-actions are likely to be complex.

When hooves were grouped according to their shape, correlations increased between some of the measured parameters compared to correlations within the whole data sets and trends amongst others were apparent. There were no significant differences between different shaped hooves in trace elements, fracture toughness nor crack scores. There may be several reasons for this.

Firstly caution must be applied, when trying to correlate the differences in shape with other parameters. Farriery intervention will have an immediate effect on the shape of the equine hoof capsule, thus the shape of the hoof may have been immediate, but the development of cracks and the trace element content will have been historical; time lines need considering as they may confound the relationship between shape and trace elements.

Hood and Jacobson, (1997) emphasised that foot conformation or shape can be changed easily through farriery intervention by changing angles, widths and lengths of the hoof capsule, In this thesis single measurements of the parameters were taken and, as discussed in chapter three, it was not possible to record chronological changes because cadaver hooves were used. Although,

it is generally accepted that the shape of the hoof is an inheritable characteristic, it can be profoundly affected by alterations in weight bearing as well as farriery (Ellis 1998). Davis, (2002) in an editorial commented that the distortion of the hoof capsule during use affects the way it grows, so that within a few weeks a change in the shape of the capsule will show, however no evidence was offered to support the statement. The capsule grows at seven mm/month taking up to twelve months to renew, (Josseck *et al.* 1995), so any changes in shape due to growth will only change the shape of the entire capsule once the entire capsule has grown out. The measurements used in this work would not distinguish the shape changes referred to by Davis, (2002). However, Thomason *et al.* (2001) showed that the effects of change in shape due to loading are one of medium to long term and the measurements taken in this thesis would be able to differentiate these changes in shape, as they distinguish between whole capsule differences but not within capsule differences. Significant differences in shape were shown when hooves were allocated to different shape groups using ratios of the capsular base and vertical inclination.

Secondly, dividing the hooves into different shape groups meant that very small numbers of hooves were being compared. Multiple comparisons of small groups of data will increase type 1 errors and differences might have been recorded which were due to chance, it would have been more appropriate to make a *post hoc* decision to increase p to <0.01. However because of the concern regarding the affect of small data groups, comparisons were also made between and within hooves grouped according to either the shape of their capsular bases or their vertical inclination and also their toe angles so that comparisons could be made across the literature.

Thirdly, bodyweight must be taken into consideration when attempting to correlate cracks and shape. Two horses may have the same shape and indeed the same size of hoof, but one might be considerably heavier than the other, thus increasing the stress on the hoof wall. However biological load bearing structures must be reliable and be able to cope with loads larger than those they would normally experience, (Niklas, 1998). Biological material has a safety factor (the ratio of breaking stress to working stress) of between three and five, (Blickan *et al.*1993; Blickan and Full 1992). For example, mammalian skeletons experience peak locomotor stresses (force per area) that are 25 to 50% of their failure strength, indicating a safety factor of between two and four, (Biewener, 1990). The mechanism by which animals achieve a constant skeletal safety factor varies depending on the size of the animal. Larger mammals maintain uniform skeletal stress primarily by having a more upright posture, (Biewener, 1990) and smaller animal's skeletons are stiffer. Whilst bone is a different material to hoof wall, it would be interesting to see if the more upright hooves generally associated with lighter breeds are stiffer

than the WW shaped hooves. However this principle may not apply to the specific situation of the hoof, generally large horses are perceived to have the soup plate hooves, which are wide with shallow vertical inclination. It would be interesting to investigate the relationship between body size and hoof shape. At greater sizes, increased skeletal allometry and decreased locomotor performance are likely to maintain stresses constant, (Biewener 1990), certainly larger horses are not chosen for their speed. Hooves can easily accommodate the weight of the horse, but the effect of a continuous excess weight on the hoof capsule especially if the shape of the hoof may predisposes the hoof wall to additional increased stress. A relationship between bodyweight and crack development has been recorded in cattle claw, (Anderson and Landstrum 1981) with heavier animals suffering from more cracks. Barrey (1990) used instrumented hoof boots in the equine to measure force distribution and found that the distribution of force was greater at the quarters compared to the toe. This difference was greatest in the heavier horses; and might be attributed to the increased bodyweight or a difference in shape of hoof.

Interactions between cracks and the shape of the hoof capsule

In a normal environment a horse would rarely encounter a substrate which would allow a perfectly uniform distribution of stress, (Bertram and Gosline, 1986). Imperfections in the hoof wall may have a similar effect and localised stresses might reach a concentration great enough to provide energy of magnitude to cause fracture or crack. A defect on the distal surface of the hoof wall may not have enough strain energy to propagate cracks. However a defect combined with a poor overall shape, which changes the loading pattern of the force on the wall, together with a difference in tubular patterns or density, may predispose the wall to cracks.

The effect of the shape of the base plate

Leach and Zoerb, (1983) compressed hoof blocks laterally and vertically to investigate the modulus of elasticity. They found that blocks loaded laterally had a greater elasticity compared to those loaded vertically. They indicted that this might be due to the shape of the tubules. In laterally loaded blocks the tubules presented an elliptical shape to the force and might minimise the effect of lateral compression. If an elliptical shape facilitates load bearing then the effect of hoof capsule shape at a gross level may also be offering a similar protection against loading. There is a perception that fewer cracks are seen in hooves which are wider than they are long based on the premise that the shape presents an ellipse to the vertical load from the leg. However in this work it was found that wide based hooves had greater crack scores compared to long and round bases. It is possible that the presence of cracks on the wide bases were in part due to the low angles at the quarters and the shape model developed in this thesis may in the

long term, need to take into account medial-lateral balance and describe shape in the sagittal plane.

The effect of quarter angles

Wilson *et al.* (1998) used wedges to investigate the effect of altering the medial-lateral balance of the hoof. The centre of force was moved towards the side of the hoof which was elevated by the wedges. However when wedges were used on the medial quarter, the increase in force as measured on a force plate was greater on the medial side of the hoof compared to the force measured on the lateral side when wedges were placed under the lateral quarter. Thus cracks maybe more dominant on the side of the hoof which suffered the greater impact. In this thesis there was an effect of quarter angles on crack distribution, *(table 6.2A.ii).* Hooves with an upright vertical inclination had a significantly bigger crack area at the lateral quarters compared to the toe; those with a regular vertical inclination had smaller crack areas at the lateral quarters. Hooves with a larger crack area at the toes tended to be those with a wide vertical inclination. Thomason, (1998) noted that contrary to expectations hooves with more upright quarters were stiffer and provided less impact absorption, this may offer an explanation as to why in this thesis more cracks were recorded in hooves with upright quarter angles.

The effect of toe angle

Barrey, (1990) used instrumented hoof boots to measure force distribution and found that a low toe angle increased the distribution of force to the quarters compared to an upright toe angle and in comparison an upright toe angle increased force at the toe. Others, (Moyer, 2003; O'Grady, 2001) related the incidence of quarter cracks to long toes. In this thesis hooves with an upright toe angle had a greater crack area but not crack number at the toe compared to hooves with normal toe angle, (*table 6.3.1A.iv).* However, a comparison between the quarter and toe crack area of hooves with different toe angles did not show any significant differences, *(table 6.3.1A.v).* Hooves with low toe angles generally did not have an increase in numbers of cracks at the quarters in this thesis, *(table 6.3.1A.v).* Thomason *et al.* (2001) found a correlation between medial and lateral angles, (defined as vertical inclination in this thesis), lateral sole width, solar circumference and toe length in hooves with a normal toe angle, (48°). It is possible that the length of toe was also altered by changing the toe angle and it is the toe length as well as or rather than toe angle which is related to the increase in cracks. In cattle, researchers, (Smit *et al.* 1986) measured a correlation between toe length and lesion scores. Claws with long toes had more lesions whereas claws with an upright toe angle had fewer lesions. It is possible that a longer length toe is made of older material compared to a shorter toe and if the material has

been exposed to more loading cycles, then it could be more predisposed to cracks. However this work found no correlations between toe angle and any other measurement.

The use of Finite Element Analysis to investigate the effect of shape on stress distribution in the hoof capsule

A separate experiment was undertaken *post hoc* to investigate some of the hypothesis raised on the effect of toe angle and vertical inclination on crack distribution, (Newlyn and Collins, 2004). Two hooves measured in this thesis were modelled using Finite Element Analysis, (FEA) to isolate the effect of shape from those of bodyweight and material properties and to investigate if different shapes resulted in different stress patterns on the hoof capsule. The principles, methods and extended results are described in the appendix page LXXIV. One hind and one fore hoof were chosen at random from the data set of mixed hooves.

Results from the model suggest that the effect of toe angle on stress distribution and displacement are negated in hooves with steep quarter angles, whereas toe angle is more important when the hoof is more cone-like in shape. The differences in the distribution of tensile and compressive stress could have significance in the susceptibility of the hoof to damage. It could be argued that the increased region of tensile stress in the hind foot approximately 45^0 around from the toe might indicate increased susceptibility to cracking in this area. The front foot however has the most tensile stress near the heel. Similarly, the front foot has a more extensive region of compression in the toe region. This could have implications in the susceptibility to de-lamination particularly in a diseased or damaged foot. The results suggest that the vertical ¼ angles may have a greater effect on the load bearing capacity in terms of stress and strain distribution of the hoof than previously considered.

The emphasis on toe angle as the major parameter should be qualified by consideration of the lateral angles, (vertical inclination), which may play a significant role in assessing the hoof functionality. Therefore the investigation of the effect of shape on other characteristics of the hoof should involve just that; the measurement of a single geometric parameter is not measuring shape. Thomason *et al.* (2001) found correlations between measurements in the normal toe group indicating that linear and angular measurements do not change in isolation. However Thomason *et al.* (2001) only measured five hooves and it is possible that a spurious correlation may have been recorded due to chance or sampling error due to the sample size, in addition as previously discussed sampling was biased. The same differences were not found to be significant in this thesis nor were correlations found between the same measurements indicating that relationships between measurements of the hoof capsule are complex and emphasising the importance of minimising measurement differences in order to measure systemic differences.

The work in this thesis provides researchers with a method of defining shape. However the work needs developing and an investigation into shape and its relationship with function should follow; the foot must not be considered in isolation. Kane *et al.* (1998) measured aspects of shape of hooves in three groups of horses, one control group and two groups suffering from catastrophic musculoskeletal injury. If the shape groups recommended by this thesis were applied to Kane *et al.* (1998)'s results, all their hooves would be in the round base, regular vertical inclination group (RR), in addition they had normal toe angles. However, the researchers reported a significant difference in toe angles (48.4° and 49.2°) between the two groups; (the difference was not reported as significant in their results table). They also used separate one way analysis between the three groups, this might have increased the type 1 errors and identified differences which did not exist. The differences they subsequently linked to the incidence of injury. The hooves were collected from the abattoir and studied in isolation; factors other than the shape of the capsule may have had a greater effect on function. To characterise and compare shapes, a co-ordinate system is necessary with clear definitions of origins and axis relative to prominent hoof features. Both Kane *et al.* (1998) and Thomason *et al.* (2001) used measurements with clear definitions of origins, but neither attempted to delimit an outline of the hoof.

6.4.2 Interactions between trace elements and other measured parameters

Trace elements and hardness

Baggott *et al.* (1988) were some of the few researchers to sample from designated areas to obtain mechanical and chemical information from the epidermal cattle claw. The areas whilst repeatable may not have been reproducible as no information was given as to how the areas were defined; they were simply illustrated on a diagram. However they did show a relationship between mineral concentrations and the hardness of the epidermis. In addition they investigated the incidence of disease, (in some cases, lesions) within the claws. They concluded that the concentration of calcium and phosphorus were lower in the softer heel compared to the harder wall as were zinc and copper which were also lower in concentration in the sole. They hypothesised that copper increased in inflamed tissues and that this might explain the higher levels found in the hoof epidermis of lame cows. They concluded that reduced hardness was associated with claw disease and therefore the relationship between hardness and mineral

content differences (copper, zinc and magnesium) is due a change in generalised differences in the availability of elements to the keratin forming cells. More recently, (Siciliano *et al.* 2003) sampled from the same area of the equine hoof capsule for trace elements, hardness and tensile strength but did not find a relationship between zinc and copper and the mechanical properties nor between dietary intake and copper and zinc concentration in the hoof horn. Issues regarding repeatability were raised in the report but there was no information on the techniques used to test the mechanical properties nor any note of standardisation of DM contents. In a previous experiment, (Siciliano *et al.* 2001) the affect of lower levels of copper and zinc on the same mechanical properties were compared. Despite the fact that both sets of experiments showed that copper and hardness decreased with time and zinc increased with time, the authors came to two different conclusions. Siciliano *et al.* (2001) concluded that there was no relationship between zinc, copper and the mechanical properties, whereas Siciliano *et al.* (2003) concluded that there was a relationship. It is interesting to note that researchers are persisting in measuring hardness in the equine hoof wall, with no justification as to the suitability of the test, there was no indication that the researchers were attempting to correlate tensile strength with hardness. Specimens were air dried to normalise for water content in the first experiment. It is possible that the variability in methodology may have affected results and this emphasises why standardised techniques must be agreed upon and followed, before future workers challenge the techniques and develop new ones.

Trace elements and fracture toughness

High mineralization in bulla (ear) bone is related to a high modulus; it is very stiff, has a high modulus of elasticity, but as a trade off the bone is brittle with a low work of fracture. Antler bone on the other hand has a lower mineralization, (Currey, 1999) and normally deforms in a u shape but it is extremely difficult to break, it has a high work of fracture and impact resistance, (Currey, 1999). Currey, (1999) indicated that as mineral levels increased in bone, then water content decreased, thus making the bone more brittle; however there was no indication as to whether the tests were carried out under the same hydration conditions. One could hypothesise that this is similar to hoof. Hooves have a low degree of mineralisation and are extremely difficult to break in impact. Despite putting pieces of hoof cut with a Bandsaw into liquid nitrogen and attempting to grind with a coffee grinder, an industrial grinder and a hammer, it originally proved extremely challenging to process hoof into small enough pieces to dissolve for trace element analysis.

There were no correlations between fracture toughness and trace element levels in the data set of all the hooves in this thesis, nor within groups of hooves of different shapes. Ley *et al.* (1998)

were unable to quantitatively correlate minerals, (except sulphur) to the material properties of relative elasticity , defined as displacement in mm divided by the cross sectional area of each specimen and tensile strength (N/m^2). However as previously discussed, this result may have been compromised by testing different samples for trace elements compared to those tested for mechanical properties.

Moisture

The difference in modulus, (stiffness) across the hoof wall depth measured by Douglas *et al.* (1996) was inversely correlated to moisture as discussed in chapter three. Leach, (1980) and Bertram and Gosline, (1987) manipulated moisture content and found that horn with a high moisture had a low modulus which they described as stiffness and a lower work of fracture. This thesis is in agreement with this finding as normally hydrated blocks could not be broken using the Izod machine available, a fact which contributed to the decision to test fully hydrated blocks.

All keratinised tissues share this common relationship and it is a reflection of the composite nature of the material. At 100% humidity the matrix is highly hydrated, mechanically weak and exhibits viscoelastic behaviour; as the water content is reduced the matrix becomes progressively stiffer until at 0% humidity its properties approach that of the filaments, (Fraser and Macrae 1980). Fibres are tension resisting they can withstand a large amount of stress and they do not deform but because they do not deform under stress they can be brittle and susceptible to cracking. The matrix on the other hand can absorb energy by deforming under stress which adds toughness to the composite but they are not strong. By hydrating the hoof block, the mechanical properties were changed the matrix becomes less tough and absorbs less energy before breaking. The material in this thesis was 100% hydrated to remove the influence of moisture content. However despite this standardisation the fracture toughness of the quarters was greater than the toe. Douglas *et al.* (1990) found that the toe was stiffer than the quarters. A lower modulus (allows more stretch or displacement) at the quarters may be useful in material which was shown in the FEA model to displace more than the toe, the increase in fracture toughness would allow a greater absorption of energy before the quarters cracked.

Copper

It is possible that the hydration status is a reflection of the bound water in keratinised tissues and that this may be affected by the copper concentrations of the tissue or by the concentration of copper during differentiation of the tissue. A decrease in copper has been shown to result in a decrease in the water holding capacity of the extra – cellular matrix and to affect the adhesion in acinar cells in cattle pancreatic tissue, (Fell *et al.* 1985). Studies on dry and wet wool showed

that there is extensive swelling of the matrix when wet but x ray diffraction indicated that longitudinal swelling of the wool fibre in water is restricted to 0.5% by the microfibrils, (Gillespie 1967). Fraser and Macrae, (1980) reported a similar effect in porcupine quills, where the matrix shrinks much more than the filaments when water loss occurs. The differing copper levels may affect the amount of cross links which may in turn influence hoof's ability to cope with water. If there are less cross links then the water holding capacity of the matrix may not be so restricted by the microfilaments and water loss from the hoof might be more rapid. Dry materials tend to be brittle.

Weigmann and Dansizer, (1971) explored the relationship between cross linking on the mechanical properties of wool. Copper is required for cross linking of both IFs and IFAPs. They found that removal of thirty percent of the disulphide content of wool, (high sulphur is associated with matrix proteins), resulted in a lower modulus, (increased stiffness), subsequent replacement of some of the disulphide links with stable cross links reversed the result, thus it appeared that the disulphide bonds were correlated with mechanical properties.

A substantial proportion of the disulphide bonds are presumed to be between the intermediate filaments (IFs) and intermediate associated proteins (IFAPs), (Gillespie, 1964). It is not until the final stages of cornification that the IFs are aligned to allow cross linking between and within themselves to form bundles and links with the IFAPs. In copper deficiency only some of these links will occur and it is most likely that they will be between the IFs due to disorientation within the matrix, (Gillespie, 1964). Wool from copper deficient sheep contains more N terminal glycine and alanine and sometimes more N terminal serine and glutamic acid, indicating that lack of copper has interfered with the arrangement of the keratin polypeptide chains during synthesis. This together with the effect on the water holding capacity of the tissue may explain the increase in stiffness of copper deficient wool.

Early research, (Feughelman, 1959; 1961, quoted in Gillespie, 1961) concentrated on finding the effects of altering sulphur content on wool strength. Horn, fur and merino wool were sectioned 20μm, so that specimens were comparable in thickness to a single wool fibre; the low sulphur, high microfibril containing fibres of horn were stiffer than those of wool which had greater matrix content. In addition, it was shown that plasticity increased with increasing sulphur content of wool, (matrix, which has a higher water holding capacity), and with decreasing crystalline to amorphous ratio.

In this thesis, the copper concentration in hooves with low fracture toughness was significantly more than in hooves with medium fracture toughness and more than those with high fracture

toughness. This was reflected in the crack score results; hooves with high crack scores also had significantly more copper than those with lower crack scores. If 100% hydrated hooves have a lower fracture toughness compared to those at normal physiological moisture (Bertram and Gosline, 1987), it might be that lower copper is preferable if it reduces the water holding capacity and results in increased fracture toughness. High copper might be linked to an increase in the filament cross links indicating a higher filament to matrix ratio resulting in the tissue being stiffer, less elastic and therefore more brittle. Leeder, (1986) argued that abrasion resistance in wool has been shown to be improved by increasing cross links removing the preferred fission boundaries, but high levels of cross linking increased brittleness and resulted in severe loss of mechanical properties.

Alternatively a decrease in copper may reduce matrix to filament ratios resulting in a stiffer tissue with ultimately a more brittle hoof capsule. Copper is required as a co-enzyme for lysyl oxidase in the formation of cross links in collagen, for the formation of desmosomes in elastin and for the cross linking of the disulphide bonds of cysteine in both keratin and IFAPs, (Danks 1991). Deficiency in copper resulted in structural changes in hair with a subsequent reduction in elasticity, a reduction of copper in wool fibres resulted in a 35-45% decrease in strength, (Gillespie 1964). The decrease in elasticity has been hypothesised to be due to a decrease in the cross linking. The cross linking in structural units allows the fibrils to return to original shape once the load has been removed, (Hill *et al.* 1967). The change in mechanical strength in collagen and bone in copper deficient chicks was considered to be due to the decrease in cross linking amino acids, (Rucker *et al.* 1975; Opsahl *et al.* 1982). Bone from copper deficient birds had low tolerance to deformation and torsional force and required less energy to create a fracture as well as showing little plastic deformation (Opsahl *et al.* 1982).This bone contained substantially less reducible cross links compared to normal bone; however the viscoelastic properties of tendon were not affected. The authors hypothesised that this was because the collagen fibres in tendon are highly ordered and parallel and therefore reduction in cross links has to be severe before mechanical properties are affected. Rucker *et al.* (1975) introduced chemically derived cross linking into the organic matrix of bone from copper deficient chicks and found that it improved the mechanical properties of the bone.

A reduction in collagen cross links are generally associated with a decrease in stiffness and toughness in bone, (Currey, 1990). However Wang *et al.* (2001) denatured bone and measured the effect on work of fracture, fracture toughness and stiffness. Collagen denaturation was correlated with a decrease in fracture toughness and work of fracture but not with strength or stiffness. Copper deficiency and a reduction in cross links appear not to affect the strength of

skin, (Danks 1991). The apparent discrepancies in results indicate that different levels of copper are having tissue specific effects, which vary according perhaps not just on the keratinised tissue but also on its function. It would it be interesting to explore the relationship between dietary copper intakes and the reduction in cross links and also the degree to which defective cross-link formation is influenced and over what range of intakes and whether .mechanical properties of hoof keratin are influenced by any of these factors.

Finally the relationship between the amount of copper in hoof material and the association with low fracture toughness and a high crack score may not reflect a change in cross linkages but might be an indication of inflammatory events at time of formation. Copper as previously discussed is found in higher concentrations in inflamed tissue and if an increase is indicative of abnormal keratinisation, the relationship might be an indirect consequence of for example rapid proliferation. Wide base hooves had more copper compared to other shaped hooves in this thesis as did hooves with wide side angles. Flaring at the quarters is frequently found in laminitic hooves, (personal observation), this might be indicative of an underlying cause and effect.

Calcium

Calcium was perceived to play a role in improving tubular integrity, (Kempson 1987). Normally calcium is absent from the stratum corneum , but it has been shown in psoriasis that the stratum corneum contains intercellular calcium aggregates, (Menon *et al.* 1992). It is possible that hoof wall which is cracked or damaged contains higher than average concentrations of calcium. Hooves with a high crack score had significantly higher levels of calcium compared to hooves with medium crack scores and higher calcium compared to hooves with a low crack score. On the other hand, a decrease in calcium has been shown to reduce the rate of differentiation of the keratinocyte, (Hennings *et al.* 1980; Heenon *et al.* 1992). It would be interesting to investigate if hooves with a slow growth rate were correlated to calcium concentrations either in the final epidermal hoof horn or in cultured keratinocytes harvested from the hoof.

Calcium triggers the attachment of proteins to involucrin[g] which is a precursor protein responsible for accepting the large amounts of lipid molecules esterified to the envelope. Leeder, (1986) supported the argument that it is the cell membrane complex which determines the 'quality' of the wool fibre. Summarising research he concluded that when wool fibres break under repeated cyclic stress, laboratory abrasion tests, torsional fatigue or repeated freezing and thawing, they do so by preferential splitting along the cell boundaries. A decrease in calcium may be associated with flaky cells and dry brittle hoof horn as cadherins which are responsible for cell to cell adhesion are calcium dependant and adheren junctions appear to be important in maintaining stratification of keratinocytes during differentiation. Deficiency in Ca may result in

a lack of cell adhesion in the developing keratinocyte and change the mechanical properties of the final tissue, but little is known about the pathophysiological role of the cell envelope and its role in any disorders of keratinisation, (Hohl 1990).

Feeding calcium above NRC requirements to gilts had no effect on incidence of lesion severity and incidence, (Calabotta *et al.* 1982). However results were possibly confounded as the control still provided adequate calcium to meet requirements and the pigs were growing and therefore getting heavier. There are several references, (Goonewarde and Hand 1995; Andersson and Lundstrom 1981; Greenough and Vermunt 1991) to the fact that increasing bodyweight is correlated with an increase in lesions in claws. The shape and size of the claw appeared to be more significant than calcium intake on the incidence of lesions, (Calabotta *et al.* 1982). In this work hooves with round bases contained more calcium compared to long and wide bases and hooves with a normal toe angles contained more calcium compared to hooves with either low or upright toe angles. Increased concentrations of calcium have been reported in diseased dog claw compared to normal dog claw, (Harvey and Markwell 1996) this is similar to the situation measured in psoriasis but no account had been made of dietary intakes and there is a possibility that the differences may just reflect different dietary regimes.

Zinc

A simple linear relationship does not exist between tensile strength and zinc concentration in epidermal tissues. The concentration of zinc in the skin of rats fed low zinc diets for between fourteen and eighty days was no lower than those fed adequate zinc but the breaking strength of the 'zinc deficient' tissue was less than the control, (Agren and Franzen 1990). Research with cattle claw indicated that increasing zinc in the diet increased the tensile strength of the claw material, decreased the number of cracks and increased microscopic quality, (Stern and Geyer 1998). Unfortunately the zinc content of the epidermal tissue was not measured.

The lack of relationship between dietary zinc and the concentration within epidermal tissue is further supported by work done on wool, (White *et al.* 1994). Zinc deficiency induced histological changes such as retained nuclei in the cells, thirty percent reduction in cortical size and fibre distortion in some of the follicles in the wool fibres of sheep. In addition, the zinc content in the wool dropped when dietary intake was reduced, but there was no correlation between zinc content of wool and dietary intake until severe histological changes were recorded and then there was a subsequent decrease in zinc in the wool fibre. Epidermal tissue may be low in zinc but cracks may only appear in the tissue which is most accessible to mechanical influences. Zinc is an integral part of most tissues and is involved in over two hundred enzyme reactions; thus it was naïve to expect a less than complex interaction picture.

There appears to be a species difference in the response of epidermal tissues to zinc deficiency. Goat hair showed a concomitant decrease in zinc within two months of being fed a zinc deficient diet, (Ray *et al.* 1997). Lesions and fissures in inter-digital skin appeared in the claws of bulls four weeks after feeding a zinc deficient diet and healed as quickly when zinc was added back to the diet; however there was no report of any lesions or problems in the claw horn, (Demertzis and Mills 1973). It is probable that the effect of any trace element deficiency on hoof horn will depend upon how much of the keratinised tissue was affected at formation and the subsequent environmental, farriery and loading influences on the tissue. In lambs the effect of zinc deficiency is shown by distortion of the hoof due to a decrease in keratin hardening, (Vallee and Falchuk 1993).

Zinc deficiency has been noted in humans during rapid periods of growth such as pregnancy or growing children, (Prasad 1982). It would be interesting to see if zinc concentration in equine hoof horn correlated with either the increased growth rate of hoof horn in the laminitic or during pregnancy and lactation. Zinc deficiencies resulted in a reduced growth rate in young animals, (Underwood and Suttle 1999). It is possible that the only consequence of overt zinc deficiency in hoof horn is a decrease in growth rate. Subsequently the wall is subjected to a longer life cycle of stress and strain because renewal rate is reduced and perhaps the cracks appear due to fatigue of the material rather than a straightforward correlation with zinc concentration.

To further complicate the picture, Coenen and Spitzlei, (1996) found that equine hoof horn of lowered hardness contained significantly less zinc compared to 'normal' horn and that this reduction was similar in both hair and plasma but these associations were not related to inadequate dietary intakes of zinc. However the authors reported a concomitant increase of zinc in the hoof horn after dietary supplementation of zinc. On the other hand no association between plasma and hoof horn zinc in cattle was recorded, (Moore *et al.* 1989), although the researchers reported an increase in growth rate and an improvement in the visual scores in the cattle claws of cows fed supplemental zinc. It is highly likely that none of these experiments pair fed for energy or protein thus confounding the effect of zinc. Low protein diets, (Wallwork *et al.* 1983) regardless of whether zinc was adequate or not, decreased growth rates and the plasma zinc concentrations were dependant on the levels of both zinc and protein in the diet. Rats fed diets deficient in both zinc and protein had higher plasma zinc levels than those deficient only in zinc. Hay and straight fed horses, whilst receiving adequate crude protein in their diet, will be deficient in biological available protein, (personnel observation), if this reduced the growth rate of hooves, then zinc concentration will not be reduced and therefore there may not be a true correlation between zinc concentration and crack scores. Hooves with a low crack score had

lower zinc compared to hooves with high crack scores; hooves with a high fracture toughness had a lower zinc concentration compared to low and medium scores. Researchers report an increase in zinc in inflammatory tissues, (Landsdowne and Sampson, 1997), if the severity of cracks is related to an inflammatory event at time of formation, then this may be the explanation for this relationship.

Zinc deficiency can be induced in the face of adequate dietary intakes by stress, infection, and post operative trauma, (Chesters. 1982). Endotoxaemic stresses reduced the plasma levels of zinc and therefore the amount of zinc reaching dividing tissue, (Chesters 1982); but additional zinc did little to alleviate the problem; other researchers reported an increase in zinc in inflammatory tissues, (Landsdowne and Sampson 1997).

Interactions between trace elements

Trace elements which interfere with the metabolism of copper may result in decreased cross linkages, despite adequate dietary intakes of copper. So for example high levels of zinc may result in reduced absorption or tissue distribution of copper as indicated by wool work where the concentration of copper in the wool showed a progressive decline with increasing zinc in the diet, (White *et al.* 1994). There were no correlations between copper and zinc in this thesis, but that may have been because the horses were not being fed an excess or deficiency of either of the trace elements. There was a correlation, ($r = 0.637$, $p=0.0001$) between zinc and calcium in this thesis.

Kirchgessner *et al.* (1982) noted that interactions between trace elements may be either positive or negative. If an element requires the presence of another to function, then this is defined as a positive interaction, for example Ca: P in a 2:1 ratio. The interaction between zinc and copper is a negative interaction; an excess of one results in a deficiency in the other, because of competition at absorption sites in the gut wall. However during copper deficiency, absorption of copper is actually increased at the lumen wall, (Kirchgessner *et al.* 1982) highlighting the far from simple relationships and subsequent effects of trace elements on epidermal tissues. Absolute concentrations and ratios of one trace element to another varies greatly depending upon the tissue considered, its developmental condition and the state of the health of the individual', (Landsdown *et al.* 1995).

Forslind *et al.* (1999) and Forslind, (2000) using scanning nuclear microprobe have shown a difference in the concentration of calcium and zinc and their ratios between normal, dry and psoriatic skin. Four to five times higher levels of zinc were recorded in the stratum corneum of cracked skin compared to normal and the ratio of Ca: Zn changed from 12:1 in normal skin to 15: 1 in atopic skin and 8:1 in psoriatic but uncracked skin. Results from this thesis indicate that

wide based hooves have the lowest Ca: Zn of 1.5:1 compared to a mean of all hooves of Ca: Zn of 4.1: 1. Hooves with a high crack scores actually had higher ratios compared to all hooves, (GSA Ca: Zn ratio of 5.8:1; GSNo 5:1), similar to the situation found in human skin. Hooves with low crack scores had ratios similar to all hooves, (GSA and GSNo Ca: Zn = 4.3:1).

The measurement of individual trace elements within equine hoof horn epidermis maybe inappropriate due to the multifactorial interactions not just of the trace elements but also all the variables discussed above. In addition, most diets fed in the UK meet horses' requirements for the trace elements, (*table 6.4.1A.iv);* however it is probable that many diets do not provide the correct balance of trace elements.

When describing fracture behaviour in whiskers, (Cox, quoted in Gordon 1976), it was noted that steps due to irregular growth imparted as much stress concentration as a crack and that addition of impurities accumulate at boundaries causing them to become a line of weakness. It is possible that the correct ratio of trace elements ensures a good contact at molecular boundaries, between the cell envelopes and incorrect ratios cause irregular adhesions between the cells, introducing weaknesses at the cellular level. At a different level of magnitude ridges due to different growth rates of hooves may exert a similar influence and be influenced not just by the energy and protein intake but also by certain trace elements such as zinc.

Conclusion

It does not take clinical sophistication to determine that the hoof wall is cracked and that a dull surface looks different from a smooth surface. Quantification of this appearance is difficult and so is the determination of what molecular, biochemical or structural changes might be associated with this appearance and if indeed the association is actually a true cause and effect relationship.

The description of the appearance of the hoof capsule should be based on objective measurement of defined hoof characteristics, so that the measurements can be repeated, thus enabling investigations into correlations with other aspects of pathology. The mechanical strength of the epidermal hoof wall material is considered to depend primarily on the keratinisation of cells in the germinal layer of the epidermis, (Mulling *et al.* 1999). The mechanical strength of the hoof is determined by the integrity of the cell to cell interactions and the macromolecular organisation of the keratin filaments within the matrix, (Hendry *et al.* 1995). It has been shown that damage to the basement membrane, intercellular infiltration of amorphous material and cell damage is associated with poor quality hoof horn in cattle, (Kempson and Logue 1993). If it is possible to link underlying pathology with gross appearance then it maybe possible to influence keratinisation and decrease the incidence of poor quality

horn, commonly described as sand and grass cracks, brittle horn, seedy toe, thin walls, thin soles and white line disease by taking steps to correct the problem.

The developmental work presented in this thesis offers researchers in the field of equine hoof wall a series of standardised methods of obtaining gross, biochemical and mechanical measurements. Interactions between mechanical properties, visual appearance and the effects of nutrition can be measured accurately in order to minimise both the financial and welfare implications of a non integral equine hoof wall epidermis.

6.4.3 Future work.

A change in the functional integrity of the hoof in terms of cracks or mechanical properties can be catastrophic if the integrity of the hoof epidermis is compromised, further work is required in order to achieve:-

1. Identification of what alters or affects the biochemical processes to initiate the process of abnormal keratinisation and the relevance of trace element concentrations in epidermal tissue of differing integrities.
2. Sufficient appreciation that the mechanical properties of the material are also dependant upon its structure and shape.
3. International agreement in definition of anatomical nomenclature of the equine hoof capsule as has been achieved in the ruminant digit,(Weaver *et al.*1981; VIth Symposium on Diseases of the Ruminant Digit, Liverpool, 1990) and co-operation to define and measure the geometric shape of the equine hoof capsule.
4. The use of standardised protocol for collection of material and the scoring of the visual appearance of the hoof capsule.

7 Overall discussion and recommendations for future work

7.1 Conclusions

For a risk or predisposing factor to be considered one of cause and effect, consistent assessment from different data sources is essential, as lack of objective scoring methods can lead to scientific conflict which is merely an artefact of the means of assessment used, (Murray 1999). The discrepancies between perceived toe angle and that being measured in the field and the perception that quarter cracks have a higher incidence to toe cracks are apt illustrations which when quantified throw doubt on current knowledge.

It is vitally important that methodology is tightly defined and wherever possible, conditions controlled so as to minimise external contributions to variability when attempting to correlate relationships in any biological tissue.

1. There were no significant differences in the mean number, area or median severity of cracks between the toe and the quarters on the whole hoof.
2. All hooves had significantly more type 3 and 4 cracks compared to cosmetic (types 1, 2) and clinical significant (types 5, 6) crack types.
3. Geometric area scores, (GSA) were not significantly different to geometric number scores (GSNo). The scoring systems were strongly correlated. GSNo ranked hooves in the same order as GSA. The use of GSA and GSNo gave the same information about the fracture toughness of the toe. The use of GSNo to quantify cracks is recommended.
4. The fracture toughness of the quarters, (40kJ/m^2) was significantly higher compared to the toes, (28kJ/m^2) and this difference was consistent within groups of hooves grouped according to their crack scores, except the high crack group and within groups of different shaped hooves.
5. Low crack groups had lower fracture toughness, (FT) at the toe but higher at the quarters compared to high crack score groups.
6. The use of two different washing techniques did not result in the removal of significantly different quantities of trace elements, except for the calcium in the lateral

blocks which was 45% greater when washed in millipore water compared to a non ionic detergent. There were no differences in trace element concentrations between anatomical positions measured by ICP-MS or LA-ICP-MS when standardising sampling to minimise within hoof variability of chronological age.

7. Calcium and zinc varied in concentration proximo-distally along the hoof wall height.
8. All trace elements varied across the hoof wall depth; the highest concentration of calcium and zinc was measured closest to the dermis.
9. Zinc and calcium were correlated in all hooves and were present at a mean ratio of Zn: Ca of 1:4.2.
10. Linear measurements taken from the hoof capsule were significantly bigger compared to those taken from photographs in the frontal and sagittal views but not between those taken from the transverse plane.
11. Between 70-90% of the difference in actual measurements could be captured from photographs if measuring from the base plate.
12. CD:CW and vertical inclination divided hooves into six shape groups,(wide wide, wide regular, wide upright; regular wide, regular regular, regular upright; long wide, long regular, long upright) which had significant differences between the groups and an increased correlation of measurements within the groups compared to the whole data set.
13. Dividing the hooves by toe angle decreased correlations compared to the whole data set and the only significant difference was that the vertical inclination of the upright toe group was greater than the vertical inclination of the low and regular toe groups.
14. Toe angle was not correlated with any other measurement.
15. The difference in fracture toughness, trace elements and crack scores between shape groups was not significant.
16. Hooves with wide bases had more cracks compared to round bases.
17. Hooves with a wide vertical inclination had more cracks compared to regular or upright vertical inclination.
18. Hooves with a regular toe angle had significantly more cracks compared to hooves with low or upright toe angles.
19. There were no correlations between fracture toughness and crack scores or between trace elements and crack scores in the whole data sets of hooves. However when hooves were allocated to different shape groups, there were correlations within all the shape groups, indicating that differences in shape might be hiding relationships.

20. When hooves were allocated according to whether they had a low high or medium crack score, there were significant differences in their trace element content. Hooves with a high geometric number score had increased levels of copper, calcium and zinc.
21. Hooves with low fracture toughness had a high concentration of copper.

7.2 Future work

Having established relationships between crack scores, fracture toughness and trace elements in different shaped hooves, it is now necessary to use the standardised methods to look at affects on function in order to further the research. Reid, (1980) stated that the study of biological structure even in quantitative terms, is a sterile and obsolete science unless related to function. In addition to the future work which has been discussed within each chapter and the recommendations made at the end of each chapter, the following work would further elucidate the contribution of the quantified visual appearance in terms of cracks and shape, the mechanical properties and trace element concentrations to the epidermal integrity of the hoof wall.

1. Trace elements

- Analysis of trace elements in hair has been performed for almost 50 years, (Druyan *et al.* 1998). Hoof or hair epidermis is a good tissue for analysis as non invasive techniques can be used and the epidermis is relatively inert. If the analysis can be carried out quantitatively then it could be a useful tool for screening a population of horses or for following a disease process. In order for the analysis of trace elements in hoof to be meaningful, reference values are needed. As already discussed in chapter six, the levels of trace elements in epidermal tissue are subsequent to complex and varied interactions with numerous biochemical processes. However it should be possible to undertake a study to obtain reference values for trace elements in equine hoof horn in the UK. Whilst accepting the inevitable inter-laboratory variations, inherent in any analytical process, if standard methods and procedures were to be agreed upon and followed, then as long as data collection was standardised and exogenous variables such as age documented, reference ranges can and should be obtained for equine hoof horn. The establishment of reference ranges for human hair has been attempted, (Druyan *et al.* 1998) and preliminary studies have been undertaken on equine hair (Dunnett and Lees 2004) to obtain reference levels for drug residues. The

visual appearance of the hoof capsule in terms of cracks should be recorded at the same time using the method developed in this work as well as a note of diseases such as laminitis.

- The concentration of trace elements in the hoof maybe useful as an indicator of the overall health status of the horse; certainly the appearance of hair, nails and skin are considered a good indicator as to the health of the human body, (Lansdown 1995). The use of trace element analysis to predict the metabolic state of the horse will remain experimental and controversial until more information is collected on the effects of age, sex, environmental exposure, individual variation and diet and in addition agreement is reached on the adoption of standard techniques.
- The link with copper, fracture toughness and crack scores needs further investigation, in order to determine if the link is a function of increased water holding capacity or an indication of inflammatory events at time of formation.

Keratin intermediate filaments are similar in nail, hair, skin and hoof. However, response to nutrition in all epidermal tissues is very variable as all biological systems buffer increases and decreases in nutrients over a wide range of dietary intakes. In fact epidermal structures are often described as 'nutritional' dumps; hence the usefulness of using them to determine the history of drug use, (Dunnett and Lee 2004). The functionality of the keratinocyte and ultimately the epidermal tissues is determined by the mechanical properties of the filament, filament length, filament orientation and packaging, mechanical properties of the matrix, proportion of the matrix to filaments, adhesion of the matrix to the filaments and adhesion of the corneocytes to each other, (Fraser and Macrae 1980).

- It would be interesting to investigate if nail and hoof are hard and skin and hair are soft in material terms because of the difference in the proportions of the keratins to IFAPs and the structural relationships between them and between adjacent corneocytes. If this relationship exists, then trace elements may be of greater significance in the functionality of keratinised tissues than previously considered. Trace elements are intimately involved in the differentiation of the keratinocyte and may determine the coherence of the corneocytes. What might not be relevant is the relationship of the trace element within the mature corneocyte and its ultimate functionality.

To further investigate the relationship between mechanical function and the concentration of trace elements in the hoof wall, the techniques used in this work need refining in order to sample across the hoof wall depth.

- Hoof blocks should be cut longitudinally, proximo-distally. The most appropriate place to make the divisions should be investigated but this thesis recommends dividing the wall at 12.5%, 37.5% 62.5% and 87.5% hoof wall depth to reflect the change in tubular pattern,

(Reilly *et al.* 1996). Tubular pattern, trace element concentration and fracture toughness should be investigated at each hoof wall depth.

- In addition further work should be carried out into the effect of different washing techniques. Comparisons of washed with unwashed hoof material should be carried out, to establish not only differences in washing techniques but also differences between washed and unwashed hoof. This needs doing on material split at a common anatomical position as carried out in this thesis. Once a standardised technique has been established, work can be compared with confidence across research groups.

2. Crack scores

The crack scoring system developed in this work is incomplete and requires further development. A performance index such as lameness or loss of shoes should be tested and the observed outcome of the crack scores be tested against expected outcomes, the weighting may then need adjusting. It is possible that crack type 6 is of great functional significance regardless of its area or number and therefore any other crack score is irrelevant, thus the weighting to this type should be increased. For instance when weighting candidates for job applications, the top scores are often the only ones considered. Alternatively when the system is tested, it may be that the combination of types 4 and 5 are more clinically significant than type 6. Certainly the cumulative number of both these crack types in the equine hoof wall is greater than crack type 6, *(figures 3.3.1.i-ii; graphs B*), although only the cumulative area is greater in one data set, (*plates 3.3.1.iv-v.; graphs B*). Recent work, (Kempson and Robb, 2004) studied the effect of bacterial washes on the quality of equine hoof horn over a twelve month period. Whilst the authors claimed that bacterial wash improved the quality of the horn by sampling from non defined areas of the hoof over the time period and by making a subjective assessment of the wall, the research did little to contribute to distinguishing between the functionality of the hoof wall and its appearance. The scepticism and confusion over the use of both dietary and topical treatments to improve hoof horn will remain until the relationship between the presence of cracks and their effect on the function of the hoof wall is established through the use of an appropriate and evolving scoring system.

3. Shape

The use of statistical methods in allometry and morphometric changes is not new, (Siegal and Benson, 1982). If the use of mathematical models can distinguish between the growth changes of a microscopic marine crustaceans in order to determine if it is a male or female, (Siegal and Benson,1982) then the technique will be sensitive enough to distinguish shape differences of the equine hoof capsule due to breed, disease or farrier effects. This technique needs further investigation using the landmark measurements developed in this thesis as the foundation of the

shape variables. Wide based hooves and those with a wide vertical inclination were shown in this work to have higher geometric crack scores. Horses suffering from laminitis are described as having a flared shape, under-run heels and low toe angles, it would be useful to define this shape objectively and then to establish whether the presence of cracks is an attribute of the functionality of this disease state.

4 Applying the techniques and results established in this work to investigate the relationship between body weight and the shape and volume of the hoof capsule of the laminitic and obese horse

An understanding of the compositional and structural characteristics of hoof horn which is associated with structural weakness and possibly cracks is still poorly understood. Goonewardene and Hand, (1985) established that cows with cracks were older, heavier and fatter. They hypothesised that load on hooves could increase progressively and that the positive relationship between age, weight and condition to the prevalence of cracks implied that shear force of weight influences prevalence. Obese horses and ponies are more susceptible to laminitis, (Keen *et al.* 2004) which results in a failure of attachment of the sensitive and insensitive laminae and a loss of load bearing ability. Dyson, (1995) showed an increase in lameness in horses with small hooves relative to their body size; a similar relationship between hoof volume, bodyweight and susceptibility to laminitis may exist. Hood *et al.* (2001) showed that unshod horses maintained on a concrete surface for a week showed changed loading patterns from the hoof wall to an increase in pressure at the bars of the foot and the frog due presumably to the hoof wall being worn away by attrition on the hard surface. The rate of change was greatest for the heaviest horses. This illustrated that the effect of the shape of the hoof wall with the ground, (bearing surface) is transient and that change over a short period of time has a direct effect on the ground reaction forces. The weight of the horse appeared to override the effect of the shape of the bearing surface presumably by increasing the force on the wall compared to a lighter horse with the same shape hoof capsule.

Not all obese horses suffer from laminitis and although the most recent research is suggesting that individual susceptibility is due to abnormalities in glucose metabolism, (Keen *et al.*, 2004; Johnson *et al.* 2004), the relationship between bodyweight, condition score and shape of the hoof cannot be ignored. Biological principles state that it is expensive to produce more material to ensure the safety factors required for load bearing, (Taylor *et al.* 2000) and animals evolve a change in shape The fact that 'heavy breed' horses are considered to have soup plate shaped hooves, not dissimilar to the shape of the laminitic may mean that the horse is compensating by a change in shape of the hoof.

On the other hand maybe the increased weight: volume means that the hoof wall and the laminae fail due to prolonged fatigue because of excess weight and the development of micro cracks. Hoof volume maybe as relevant in predicting the propensity of a hoof to crack or develop laminitis because mechanical forces maybe involved in the aetiology of cracking. If hoof volume relative to body weight is low then increased stress on the weight bearing surfaces and wall may mean that the material exceeds its fracture toughness.

Alternatively Neville *et al.* (2004) have shown that chronic laminitics have high levels of free radicals indicative of an inflammatory response compared to normal ponies. If this inflammation results in an increase in copper at keratinogenesis, this too may contribute to tissue which is compromised in its load bearing abilities.

This thesis has quantifiably characterised relationships which might be of significance in the above aetiology and in addition provides a method of consistently measuring the shape of the hoof. These techniques will now be used to investigate some of the above hypotheses.

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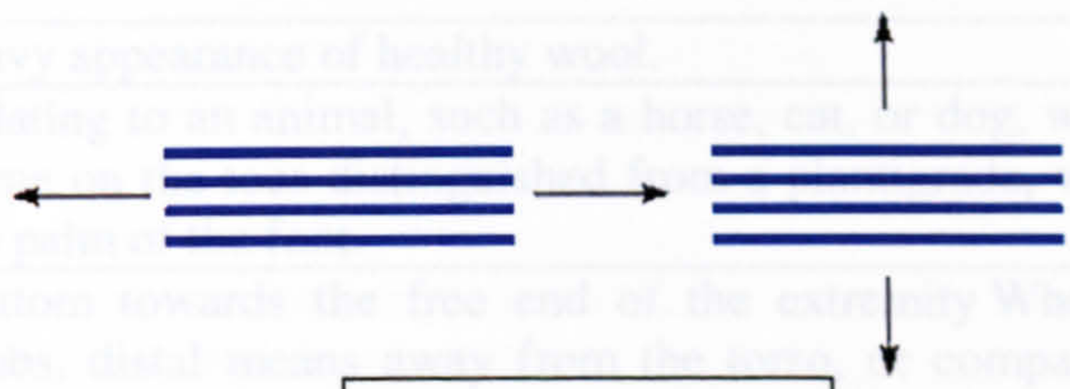
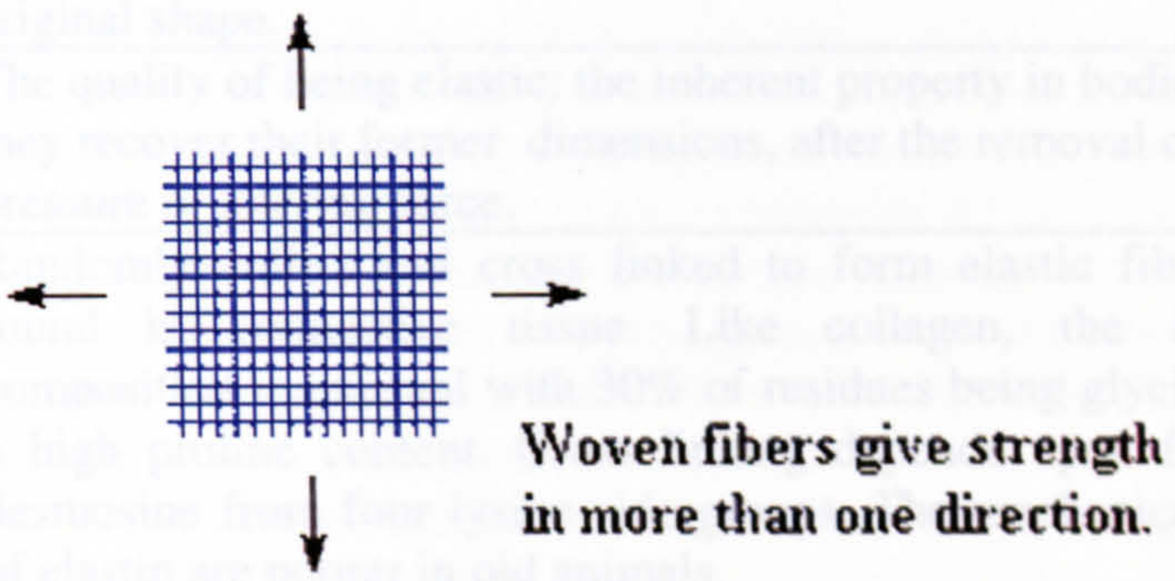
Glossary

adlib	Freely as in allowing animal free access to as much food as they want to eat.
amine	The functional group $-NH_2$ which is formed when one of the hydrogen atoms is replaced by an organic group.
amine oxidase	A group of enzymes including those oxidizing primary monoamines, diamines, and histamine. They are copper proteins.
amino acid	A group of water soluble organic compounds that posses a carboxyl (-COOH) and an amino group ($-NH_2$).
analysis	The separation of a compound substance, by chemical processes, into its constituents, with a view to ascertain either (a) what elements it contains, or (b) how much of each element is present. The former is called qualitative, and the latter quantitative analysis.
anisotropic	The tendency of a material to exhibit different properties in response to stresses applied along axes in different directions
apolar	The surface of a protein that becomes available for interaction with water during denaturising.
atomic mass	The number of neutrons and protons in the nucleus of an atom
atomic number	The number of protons in an atom which will equal the number of electrons
atomic weight of an element	the number of protons and neutrons of the mean weight of all isotopes of the element
bearing border	The part of the hoof wall which is contiguous with the ground.
calcium	99% of the body's calcium is stored in the bones. The remaining 1% is carried in the plasma; 50% of which is in the free ionised form, 40% protein bound and 10% anion bound. Because calcium is vital for most body functions, such as transmission of nerve impulses, muscle relaxation and contraction and the body has such a large store in the bones, overt deficiency signs in keratinised tissues are not seen. However several aspects of keratinisation are calcium dependant, (Fairley 1991) with calcium either acting as a co enzyme or as a catalyst.
calmodulin	A calcium binding protein which modulates the functional state of target proteins in response to changes in free calcium concentrations in the cytoplasm of the cell.
capsule	see hoof capsule
caudally	toward the posterior end of the body
caudally/ caudal	towards the tail
ceruloplasmin	A blood glycoprotein to which copper is bound during transport and storage.
collagen	An insoluble fibrous protein found extensively in the connective tissues of skin, bones and tendons. The polypeptides of collagen contain glycine and proline which form triple stranded helical coils which bind together to form fibrils which have great strength but limited elasticity.

composites , fibres and matrix	The strength of fibres is dependant upon the direction of the applied force as illustrated below:  Fibres are strong if force applied in same orientation If strength is required in more than one direction, then the fibres should be orientated in more than one direction. The keratin filaments change orientation throughout the hoof wall, (Kasapi and Gosline,1997)  **Woven fibers give strength in more than one direction.** The Matrix The matrix holds the fibres together and although fibres are strong, they can be brittle. The matrix can absorb energy by deforming under stress and adds *toughness* to the composite. Fibres have a high tensile strength (they are strong when pulled) but they usually have low *compressional* strength (they buckle when squashed). The matrix gives compressional strength to the composite.
Copper	The distribution of copper amongst tissues varies with the species, age and copper status of the animal, (Danks 1991). Up to 79% of body stores are found in the liver, 8-12% in the muscles, 9% in the wool and 2% in the skeleton. Copper is distributed throughout many subcellular components; 18% is found in the microsomal fraction, 20% in the nucleus, 20% in the mitochondria and lysosomes and 50% in the cytosol, where it is stored in metallothionein [g], copper-zinc superoxide dismutase and ceruloplasmin[g], (Danks,1991).

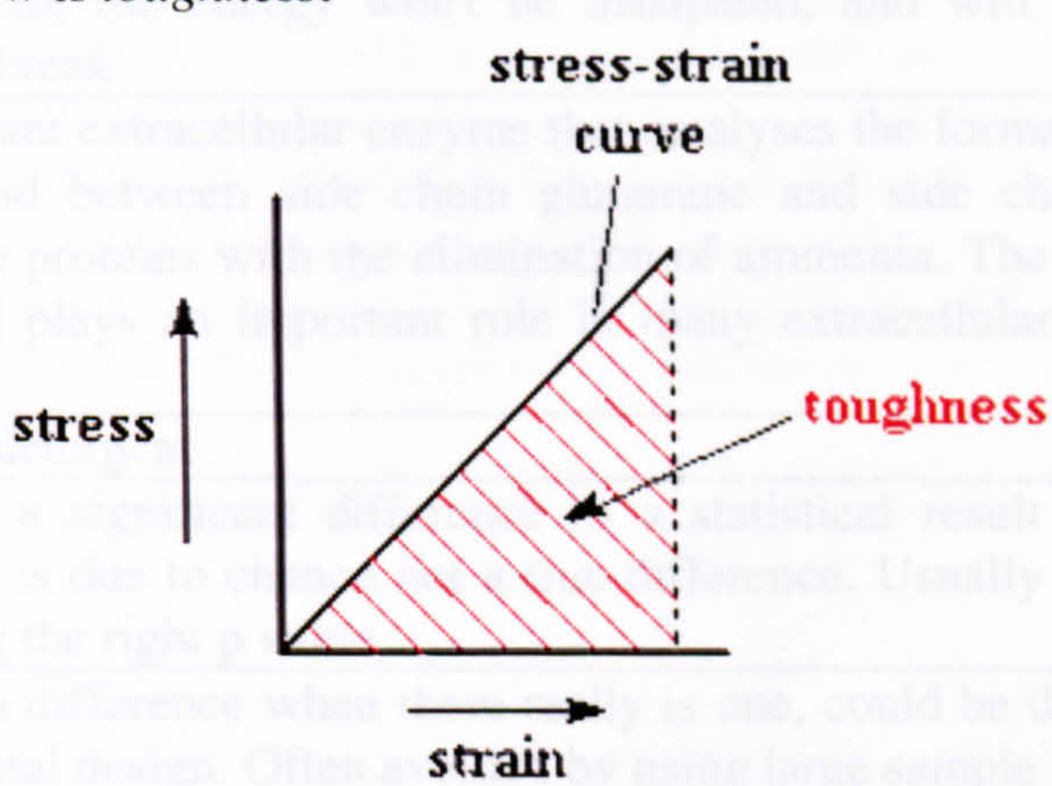
	Copper is an integral part of enzyme structure and is recognised as being a 'true metalloenzyme', (Danks 1991).
corneocyte	A functional unit of the stratum corneum which is an envelope of cross linked protein surrounding a network of keratin intermediate filaments.
cranially/ cranial	towards the head
crimp	Wavy appearance of healthy wool.
digitigrade	Relating to an animal, such as a horse, cat, or dog, whose weight is borne on the toes distinguished from a plantigrade, which walks on the palm of the foot.
distal	Bottom towards the free end of the extremity When referring to limbs, distal means away from the torso, or comparatively farther from the torso or centre of gravity. Opposite of proximal.
disulphide bond	a covalent bond between sulphur atoms The S= S linkage. A linkage formed between the SH groups due to oxidation.
divalent	An atom or radical group which has a valence of two, or which can combine with two (rather than one) different other atoms or molecules.
dorsal	[from Latin the dorsum, the back]: (1.) The front surface of the equine hoof and leg. (2.) When referring to the entire animal, dorsal means the spine or centre-line of the back.
EDTA	A chemical which complexes with metal ions and removes them from solution.
elastic	A material which when deformed (within limits) returns to its original shape.
elasticity	The quality of being elastic; the inherent property in bodies by which they recover their former dimensions, after the removal of external pressure or altering force.
elastin	Randomly coiled and cross linked to form elastic fibres that are found in connective tissue. Like collagen, the amino acid composition is unusual with 30% of residues being glycine and with a high proline content. Cross linking depends upon formation of desmosine from four lysine side groups. The mechanical properties of elastin are poorer in old animals.
electrons	Stable elementary particles having the smallest known negative charge, present in all elements; The numbers, energies and arrangement of electrons around atomic nuclei determine the chemical identities of elements.
ellipse	A plane curve, especially a conic section whose plane is not parallel to the axis base.
epitope	A portion of an antigenic macromolecule recognized and bound by a specific antibody.
error(s)	The difference between an observed value and the true value of a quantity. (b) The difference between the observed value of a quantity and that which is taken or computed to be the true value; -- sometimes called residual error
exon	The segment of a eukaryotic gene that is transcribed into a protein or incorporated into the structure of an RNA.
filaggrin	Is a inter-filamentous protein responsible for forming transient cross links with keratin filaments which allows them to align and form permanent disulphide bonds.

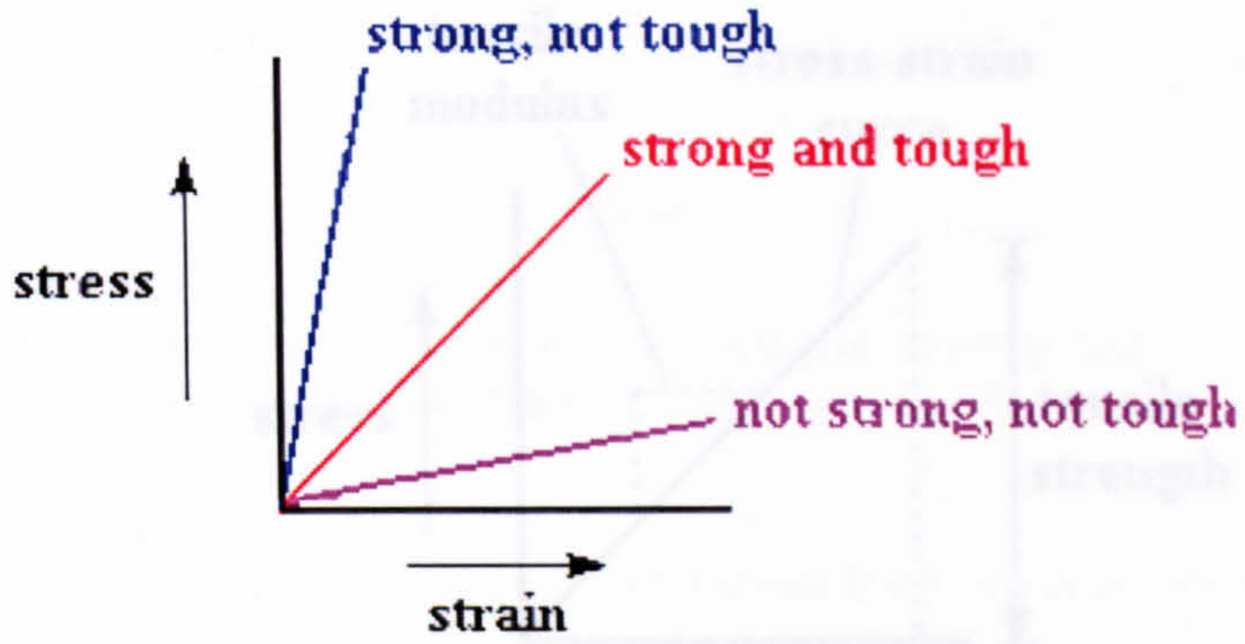
finite element analysis	see appendix 6.4.1, p LXXIV
force	The capacity to do work or cause physical change; energy, strength, or active power. A vector quantity that tends to produce an acceleration of a body in the direction of its application
fracture toughness	Measure of resistance to crack propagation. The quantity of energy required to break a given cross-section of a material is known as its fracture toughness.
function	Mode of action by which the hoof fulfils its purpose.
functionality	The serving of a function; which may be designed to be practical rather than attractive. Thus a cracked hoof can still be functional.
gauge(s)	A measure; a standard of measure; an instrument to determine dimensions, distance, or capacity; a standard
Genome	Sum total of genes, conveyed in a DNA molecule and encompassing coding for all of an organism's proteins. Represents the genetic potential of an organism.
geometric	Characterised by regular lines or shapes.
geometry	Branch of mathematics which is concerned with the properties of and relations of points, lines, surfaces and solids.
gilts	Baby pigs.
glass transition	temperature which after cooling the material becomes glass like and therefore more brittle
Golgi apparatus	A network of stacked membranous vesicles present in most living cells that functions in the formation of secretions within the cell. Stores proteins made in the endoplasmic reticulum; is involved with cell wall formation Also called Golgi body, Golgi complex.
ground reaction force	For every action, according to Newton's 3rd Law of Motion (Law of Reaction), there is an equal and opposite reaction. Due to the gravity, there is a constantly maintained contact with the ground, and in this process, there occur interactions between the body and the ground. The reaction force supplied by the ground is specifically called the **ground reaction force (GRF)**, which is basically the reaction to the force the body exerts on the ground. The GRF, along with the weight, is an important external force. The GRF is normally measured by a force-plate. The ground reaction force is equal in magnitude and opposite in direction to the force that the body exerts on the supporting surface through the foot.
hardness	The property of being rigid and resistant to pressure; not easily scratched.
hoof capsule	The hoof capsule is the part of the foot which can be seen when the foot is raised from the ground and includes the hoof wall and the sole and the frog. The hoof capsule completely surrounds the inner structures including the pedal bones and the dermal tissues of the foot.
hoof wall	The part of the hoof which is visible when the foot is on the ground.
horn	The tough, fibrous material of which true horns are composed, in the horse the term is often used instead of hoof wall.
Hyperkeratosis	Hypertrophy of the cornea or the horny layer of the skin. Also called *hyperkeratinization* A disease of cattle marked by thickening and wrinkling of the hide and formation of papillary outgrowths on the

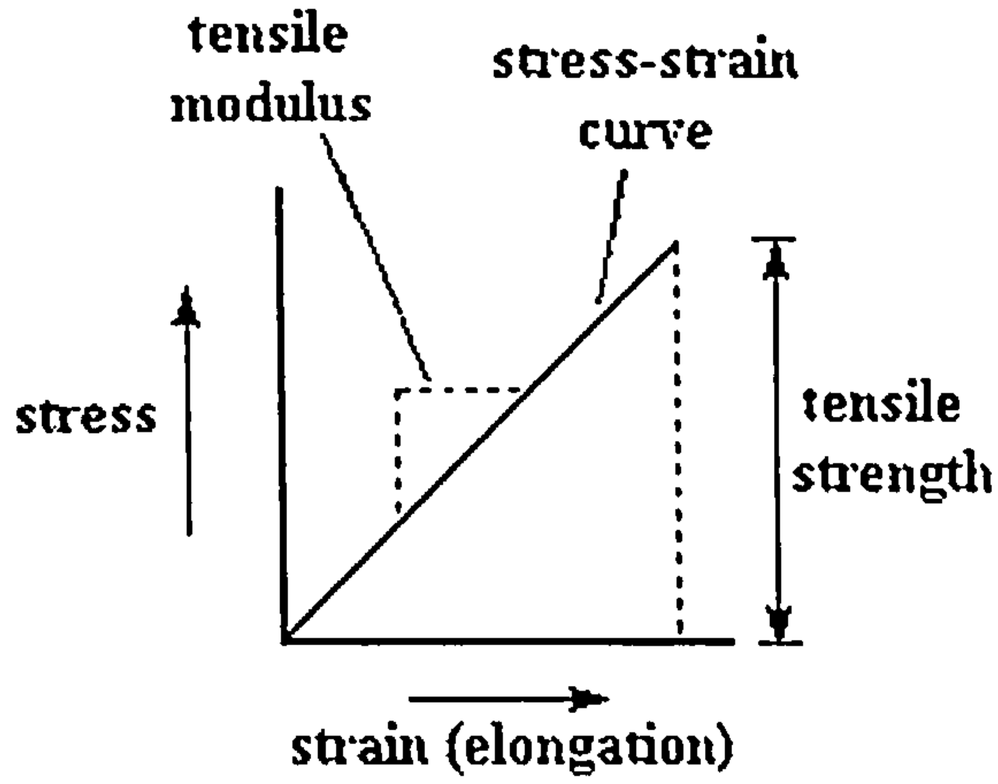
	buccal mucous membranes, often accompanied by watery discharge from eyes and nose, diarrhoea, loss of condition and abortion of pregnant animals.
hyperplasic	Growth of tissue through cellular multiplication not cellular enlargement.
hypocupraemia	Low plasma copper, deficiency of copper in the blood.
hysteresis	A lagging or retardation of the effect, when the forces acting upon a body are changed, as if from velocity or internal friction; a temporary resistance to change from a condition previously induced.
integrity	Wholeness or soundness
integument	The outermost body layer of an animal, usually consisting of a layer of differentiating cells and a layer of superficial protective cells which together are defined as the epidermis. Also dermis and subcutis The enveloping membrane of the body, including the dermis, epidermis, hair, nails, and sebaceous, sweat, and mammary glands.
involucrin	Marker protein for keratinocyte differentiation first appearing in the upper spinous layer of the epidermis. Together with trichohyalin forms the scaffold for the cell envelope.
ions	An atom or group of atoms that has lost or gained one or more electrons to become a charged species.
isotopes	A variant of an atom, chemically identical but with a different atomic mass. Isotopes have the same atomic number (same number of protons) but different atomic masses (different numbers of neutrons)
isotropic	Descriptive of a physical property that is independent of the angle of observation.
Kjeldahl	An industry standard procedure of oxidising the organic matter of feedstuffs with sulphuric acid and a selenium catalyst, so that the nitrogen can be measured and therefore the protein content of the food.
laminitic	Inflammation of the sensitive laminae of the hoof, especially in horses
lateral	Towards the side of the body.
lateral	Denoting a position farther from the median plane or midline of the body or of a structure.
lysyl oxidase	Copper metalloenzyme found primarily in connective tissue. It catalyses the oxidative deamination of peptidyl lysine residues in collagen and elastin to allow the formation of covalent cross links in these proteins.
mass-to-charge ratio	The atomic mass of an ion divided by the number of charges that the ion bears
Mass spectrometer	Determines the mass of ions from an atom or element. The element is ionised by for example argon gas. The detector measures the number of ions of a given mass which reach the detector in a given time. The heavier the ion the longer it takes to travel along the tube
match pairing	Matching animals of same age, height, bodyweight and sex to minimise biological variation.
matrix effects	in order to ensure the chemical environment does not affect analytical results, all unknowns and standards and stocks are made up using the same chemicals , so that any matrix effects are standardised across all solutions
medial	Towards the middle of the body. The side of the body or body part

	that is nearer to the middle or centre (median) of the body. For example, when referring to the knee, medial would mean the side of the knee that is closest to the other knee the opposite of medial is lateral.
Menkes	Kinky hair disease, congenital defect of copper metabolism manifested in short, sparse, poorly pigmented kinky hair; associated with failure to thrive, physical and mental retardation, and progressive severe deterioration of the brain; apparently a defect of copper transport; X-linked recessive inheritance.
metalloprotein	A protein having a metal ion as its prosthetic group.
metallo-thionein	A small metal-binding protein, rich in sulfur-containing amino acids, that is synthesized in the liver and kidney and important in ion transport.
modulus of elasticity	Stiffness Young's Modulus. The measure of the elastic force of any substance, expressed by the ratio of a stress on a given unit to the accompanying distortion, or strain. -- called also Young's modulus.
Molecular physiology	seeks to use approaches at a molecular level to address questions relating to the function of living organisms, and the cells and systems which comprise them. Such approaches might include the cloning and expression of key proteins, the introduction into cells or animals of molecular markers which can be visualised or physiological studies in cells or animals in which specific genes can be turned on or off. Molecular physiology explores how the complex cellular phenotypes that underlie the integrated functions of the tissues and organ systems comprising higher living organisms, emerge from the genomic code
morphometric morphometry	The use of quantitative data in the description of structure.
Nalgene	Branded plastic bottle made from PTFE, polytetrafluoroethylene. PTFE is particularly resistant to all chemicals and therefore will not contaminate solutions being stored within the bottles.
non ionic detergent	Detergent in which the hydrophilic head group is uncharged. In practice hydrophilicity is usually conferred by OH groups. Examples are the polyoxyethylene octyl phenols known as Tritons. Nonionic detergents can be used to solubilise intrinsic membrane proteins with fewer tendencies to denature them compared to charged or ionic detergents. They do not usually cause disassembly of structures such as microfilaments and microtubules that depend on protein- protein interactions.
oedematous	Watery; from oedema, an accumulation of fluid in tissue or cells often due to inflammation.
osteoblasts	Cells that arise from fibroblasts and which, as they mature, are associated with the production of bone.
oxidase	Any of a group of enzymes that catalyze oxidation, especially an enzyme that reacts with molecular oxygen to catalyze the oxidation of a substrate
pair fed	When comparing the effects of different levels of zinc in the diet, it is necessary to control for the effect that zinc has on appetite. Zinc deficiency suppresses appetite and therefore reduces energy intakes which may affect mitosis. Therefore a pair control diet is fed to

	provide an animal with the same energy intake that the zinc deficient animal ate the day before but supplemented with the highest level of zinc being fed on the experiments.
palmer	Pertaining to, or corresponding with, the palm of the hand, so palmer = front foot; plantar back foot.
Parakeratosis	thickening, fissuring and hardening of the skin
parallax	Parallax is defined as the apparent difference in the position of an object when viewed from different positions. One assumes that the distance between the camera and the hoof being photographed is small enough not to result in parallelism or converging focal points. However unless a scale is included measurements cannot be compared with the original object.
Peak vertical force	Ground reaction forces, which are usually resolved into vertical and horizontal (shear) components, measure the forces between the hoof and the ground. Summation of the ground reaction force throughout the stance phase is the impulse Vertical ground reaction force (GRF) represents the weight-bearing function of the limb, with peak vertical force and vertical impulse being the most useful measurements. The peak vertical force is the total weight borne by the limb of hoof
plastic	when loaded it dissipates the energy by changing shape, and does not return to its original shape
polypeptide	A long chain of amino acids (less than 100) linked by peptide bonds
proximal	Towards the top of the extremity
proximo-distally	from top to bottom
pseudogenes	are copies of specific genes and are considered to be non functional as they have accumulated vast numbers of mutations during evolution and have lost the ability to be transcribed. They are present on every mammalian chromosome.
repeatable	The repeatability of a method measures the extent to which the same observer obtains the same results following a prescribed method
reproducible	Measures how close the results are from two different observers who are following the same method. Repeatability of a method needs establishing first.
rigid	A material which when loaded , puts the load directly onto the molecules , so there is little shape change e.g. bone
shape	Total effect produced by the outlines of a thing, the external form or appearance
shear	An action, resulting from applied forces, which tends to cause two contiguous parts of a body to slide relatively to each other in a direction parallel to their plane of contact; also called shearing stress, and tangential stress. A strain, or change of shape, of an elastic body, consisting of an extension in one direction, an equal compression in a perpendicular direction, with an unchanged magnitude in the third direction.
sows	breeding female pig
standard deviation	The SD quantifies scatter — how much the values vary from one another
standard error of the mean	The SEM quantifies how accurately you know the true mean of the population. The SEM gets smaller as your samples get larger. This

	makes sense, because the mean of a large sample is likely to be closer to the true population mean than is the mean of a small sample.
stiffness	ratio of force to extension the ability of a material to resist deformation (or a measure of how hard it is to deform a material a certain amount) Measured as Young's modulus E = Stress ÷Strain for elastic part of curve or the slope of the elastic part of the curve.
strain	Change in length ÷ original length (no units) The length that a material extends to when under load as a proportion of its original length. A measure of how *far* the atoms at any point in a solid are being pulled apart.
strain gauges	An instrument for measuring strain in a surface. An extensometer
strength	is the stress needed to break the sample measured in units of force divided by units of area, usually N/m^2
strength	Strength is the amount of force required to break a sample.
stress	Force applied ÷area (N/m^2 or Pascals) a measure of how *hard* the atoms and molecules in a material are being pushed together or pulled apart.
sulphation sulfation	The addition of sulfate groups as esters to molecules
sulphydryl	Compounds that bind to SH groups.
thiol	The monovalent radical -SH
toughness	The area underneath the stress-strain curve, coloured red in the graph below is *toughness*.  Toughness is a measure of the energy a sample can absorb before it breaks. The height of the triangle in the plot is strength, and the base of the triangle is strain, then the area is proportional to strength times strain. Since strength is proportional to the force needed to break the sample, and strain is measured in units of distance (the distance the sample is stretched).
toughness versus strength	Strength is the amount of force required to break a sample. Toughness is the amount of energy absorbed before the sample breaks. Strength and toughness are not related.

	 The blue line is the stress-strain curve for a sample that is strong, but not tough and, it takes a lot of force to break this sample, but not much energy, as there isn't much area underneath the curve. The sample can't stretch very far before it breaks. A material like this which is strong, but can't deform very much before it breaks is called *brittle*. The red line is a stress-strain curve for a sample that is both strong and tough. This material is not as strong as the sample in the blue plot, but the area underneath its curve is a lot larger than the area under the blue sample's curve. So it can absorb a lot more energy than the blue sample can. The red sample can absorb more energy than the blue plot. The red sample elongates a lot more before breaking compared to the blue sample. Deformation allows a sample to dissipate energy. If a sample can't deform, the energy won't be dissipated, and will cause the sample to break.
transglutamase/ transglutaminase	An important extracellular enzyme that catalyses the formation of an amide bond between side chain glutamine and side chain lysine residues in proteins with the elimination of ammonia. The linkage is stable and plays an important role in many extracellular assembly processes
Triton-X;	non ionic detergent
type I error	allocating a significant difference to a statistical result when the difference is due to chance not a true difference. Usually controlled by picking the right p value
type II error	rejecting a difference when there really is one, could be due to poor experimental design. Often avoided by using large sample numbers
ungulate	herbivorous mammal with hoofed feet; evolved so only the toe bears weight. Comes from the latin word 'ungula' meaning hoof.
viscoelasticity	The property of a viscous material that also shows elasticity
viscoelastic	when loaded it changes shape semi permanently but will return to its original shape in time
Young's modulus.	A measurement of the stress exerted on the material

	 The plot is a stress-strain curve. The tensile modulus is the slope of this plot. If the slope is steep, the sample has a high tensile modulus, which means it resists deformation. If the slope is gentle, then the sample has a low tensile modulus, which means it is easily deformed.
zinc	99% of total body zinc is intracellular; about 30-40% of total cellular zinc is in the nucleus, 50% in the cytoplasm and its organelles and the rest in the cell membrane. Virtually all the zinc is bound to macromolecules in the form of zinc proteins/enzymes or the nucleotides RNA and DNA, (Vallee 1982; Vallee,1993; Ward ,1954). Cells with a high turnover are more sensitive to zinc deprivation than slow turnover ones, due to the essential role of zinc in cell division, development and differentiation, and up to 20% of the body's zinc is concentrated in the skin and other epidermal structures. Zinc carries out its role in keratinisation as in all other physiological process bound to enzymes and other proteins. Literature confirms that zinc has three functions in zinc enzymes, (Vallee 1982; Vallee and Falchuk 1993) i). Catalytic, i.e. zinc participates directly in enzyme catalyst, if zinc is removed the enzyme does not work, ii). Coactive, zinc enhances a catalytic function but is not essential for the enzyme activity, iii). Structural, zinc is required for the structural stability of the protein.

List of Appendix Tables

List of Appendix Figures

1 Introduction

Table 1 2.2A.ii Summary of selected research to illustrate the development of keratin nomenclature and elucidation of the various structures described as keratin

Nomenclature	Tissue/cell	Structure	Chemical composition	Reference
Keratin filaments/ fibrils adhesive cement keratin is considered to be a complex made of fine filaments embedded in an amorphous substance	hair	bundles of filaments which fuse to form fibrils with hexagonal packing dense amorphous material	α keratin, low sulphur, γ keratin , high sulphur, high cysteine	Birbeck and Mercer (1957)
Hard keratin soft keratin	horn, nail, claw, hoof skin and the medulla of hair, wool and feathers	whole cell whole cell	higher in arginine & cysteine equimolar lysine arginine	Ward and Lundgren (1954)
keratin intermediate filaments	epithelial cells	IFs of 10nm diameter	2 chains of aas one basic, one acidic from a dimer. 2 dimers form a protofilament and 4 protofilaments form the keratin IF. 30 different keratins	Suter *et al.* (1997)

Nomenclature	Tissue/cell	Structure	Chemical composition	Reference
'keratin pattern'/tonofibrils keratinised cells/ 'keratin pattern' keratin with α diffraction pattern	skin hair nail	fibril content of the horny cell filaments surrounded by dense matrix cytoplasmic filaments grouped into bundles	low sulphur	Montagna and Parakka, (1974)
hard keratin	hair, hoof, claw, nail	filamentous microfibrils embedded in non filamentous matrix	filaments low sulphur matrix high sulphur;2 gps one high in cysteine and one high in tyrosine and glycine	Marshall and Gillespie (1977)
hard keratins	specialised epidermal appendages	biphasic structure of filaments and non filamentous matrix	3 protein classes:- filaments α helical ,ow in S; matrix rich in cysteine ;matrix high in tyrosine	Gillespie and Frenkel (1974)
wool & fibrous keratins	3 interacting components	2 axially orientated α helical proteins; non helical component that is highly cross linked	α helical low sulphur proteins; non helical, globular matrix , high S proteins	Menefee (1971)

Nomenclature	Tissue/cell	Structure	Chemical composition	Reference
keratin molecules	keratinocyte of hair	450A long molecules alternated as coiled parts and non helical segments. Assembled longitudinally & laterally to from a complex cylindrical object about 75A called a microfibril	Bundles of microfibrils embedded in S rich matrix (intermicrofibrillar matrix) to from a macrofibril (0.4um in diam.)	Briki (2000)
keratins- precursors of intermediate filaments	keratinocytes	class of IF proteins; coiled, coil α helix	20 proteins mass 40-70 kDa, 2 classes type I acidic & type II basic. Rod domain is 45-48nm in length. Structure of keratin genes	Eckert (1989)
keratins	horn and fur	microfibrils and matrix	low sulphur microfibrils and high sulphur non fibrous osmiophilic matrix	Gillespie,(1967)
keratins	epithelial cells	intermediate filaments	Elongated α helix enriched proteins which form coiled coils. Obligate heteropolymers	Goldsmith, (1991)
keratins	hard and soft in keratinocytes	'filament –matrix' proteins of soft and hard keratins	filaments α x ray diffraction and non helical portion ; soft keratins contains glycine' hard keratins contain cysteine	Fraser and Macrae,(1980)

Nomenclature	Tissue/cell	Structure	Chemical composition	Reference
keratins	all products which result from the cornification of ectodermal cells such as modified cell membranes and intercellular cement	long filamentous strands set in an unorientated ground subs or matrix	filaments = low sulphur; matrix consistently high sulphur	Lee and Baden, (1975)
keratin fibres	wool fibre	crystalline microfibrils embedded in an amorphous highly cross linked matrix		Weigmann & Dansiezer,(1971)
keratin fibres	entire wool fibre	cuticle, epicuticle and cortex cells; microfibril –matrix structure		Peters & Bradley, (1976)
keratin	skin	tonofibrils	-SH containing fibrous α protein	Matoltsy, (1976)
horny keratins	hair, nail, claw, horn	composed of mainly cells ; biphasic structure, filaments of protein embedded in a non-filamentous matrix	low sulphur filament , high sulphur matrix. overall similarity of amino acids	Marshall, (1986)
hard keratin ;keratinised fibres	wool, hair, quills, hooves, horns, nails	contain polypeptides organised into IFs embedded in matrix of non helical cysteine rich proteins and a variety of lipid components	cysteine , glycine and tyrosine rich proteins; total no. of proteins exceeds 100	Marshall et al, (1991)

Nomenclature	Tissue/cell	Structure	Chemical composition	Reference
keratin	proteins which contain variable amounts of sulphur and are associated with lipid	hard and sort keratins	Tonofibrils made from protofibrils next to an amorphous matrix. Tonofibrils from the framework of all keratin structures	Giroud & Leblond (1952)
keratin	family of proteins , part of the keratinised nail	fibrous proteins	gp I acidic low mol wt (40-56.5K) gp II basic high mol wt,(52-68K) expressed in pairs	Baden, (1991)
keratin	hair proteins	acidic (type 1 mol wt 44-46K) & basic (type II mol wt 56-60K) keratins and associated 10-25K high S proteins	1: 1 ratio acidic: basic keratin polypeptides	{Rogers,1989}

3 The relationship between the appearance and mechanical properties of the equine hoof.

3.1.5 A Tests for hardness

Rockwell test for hardness

Rockwell hardness test is slightly more sophisticated than Shore D, however it cannot be performed on the whole hoof. A hoof block is indented by a known weight for a known period of time and the Rockwell hardness number noted. The number is derived from the net increase in depth impression as the load on the indenter is increased from a fixed minor load to a major load and then returned to the minor load. The indenter is a round steel ball of known diameter and the reading is taken after the force has been applied for 15sces and then released. A Rockwell hardness number, (ASTM, 1998) is directly related to the indentation hardness but this number is not generally considered a measure of the abrasion or wear resistance of the material. This together with the fact, that the hoof wall blocks showed very elastic and creep behaviour and did not stabilise when the first light load was put on the blocks meant that this was not a practical nor meaningful test to carry out.

Vickers

Vickers hardness test is a more consistent, operator independent test which can be related to mode of fracture under load, {Artunc, 2003}. The material to be tested was placed under the probe and the diamond tip was focused onto the block using the microscope, so that the diamond shape delineates the edges of the block. The probe was then released and load was placed on the block at constant pressure and for a set time, a reading was then taken from the machine. Vickers testing would not be appropriate in field conditions although it is a more rigorous test compared to Shore D.

Despite sandpapering the surface of the hoof block it was not possible to focus on the surface due to lack of light refraction. Bonsor,(1995) polished bone flat using 500 grit emery paper and then polished with abrasive alumina paste to obtain a glossy surface.
This modification would need to be adapted if further investigation was required.

Table 3.2.7A.i Criteria used to group feet into high medium and low crack score groups

48 feet			
Crack score	**low**	**medium**	**high**
TCNo	< 2.7	≥ 2.7 ≤ 8	> 8
TCA	< 81.4	≥ 81.4 ≤ 495.4	> 495.4
SSNo	< 6	≥ 6 ≤ 28	>28
GSNo	<9.5	≥ 9.5 ≤ 63	>63
SSA	< 236	≥ 236 ≤ 1646	>1646
GSA	< 376	≥ 376 ≤ 5242	> 5242
28 left fores			
crack score	**low**	**medium**	**high**
GSA R/4	<400	≥400 ≤ 4963	> 4963
SSA R/4	<364	≥364 ≤ 1596	>1596
GSAT	<258	≥258 ≤ 4124	> 4124
SSAT	< 152	≥152 ≤1939	> 1939
GSA L/4	<383	≥383 ≤3540	>3540
SSA L/4	<258	≥258 ≤1198	>1198
GSA	< 3004	≥3004 ≤16277	>16277
SSA	< 1408	≥1408 ≤4448	>4448
GSNoR/4	< 16	≥16 ≤ 48	> 48
SSNoR/4	<6	≥6 ≤ 16	> 16
SSNoT	<6	≥6 ≤ 17	> 17
GSNoT	<8	≥8 ≤ 68	> 68
SSNoL/4	<8	≥8 ≤ 17	> 17
GSNoL/4	<16	≥16 ≤ 40	> 40

3.3 Descriptive summary data

Table 3.3A.i Descriptive summary data of the number of crack types counted at different anatomical positions on mixed feet

position	whole hoof	R/4	toe	L/4
type	mean ± SD *min-max*			
type 1	0.4 ± 1.0 *0-4*	0.1± 0.3 *0-1*	0.2 ± 0.6 *0-2*	0.1 ± 0.4 *0-2*
type 2	1.5 ± 1.5 *0-6*	0.3 ± 0.5 *0-2*	0.7 ± 1.1 *0-5*	0.4 ± 0.6 *0-2*
type 3	1.9 ± 3.2 *0-11*	0.7 ± 1.4 *0-7*	0.4 ± 0.9 *0-4*	0.7 ± 1.3 *0-5*
type 4	1.0 ± 1.4 *0-5*	0.4 ± 0.7 *0-2*	0.2± 0.4 *0-2*	0.3 ± 0.6 *0-2*
type 5	0.3 ± 1.1 *0-5*	0.1 ± 0.5 *0-2*	0.0 ± 0.16 *0-1*	0.2 ± 0.6 *0-3*
type 6	0.7 ± 1.3 *0-5*	0.2 ± 0.5 *0-2*	0.2 ± 0.5 *0-2*	0.2 ± 0.6 *0-2*
total number	5.6 ± 3.9 *0-16*	1.8 ± 1.6 *0-8*	1.9 ± 1.6 *0-6*	1.9 ± 1.6 *0-7*
% of total number	100	32.0 ± 27.2 *0-100*	33.7 ± 26.8 *0-100*	31.4 ± 22.5 *0-100*
1 as % of all types	6.6 ± 19.0 *0-80*	4.8 ± 18.7 *0-100*	4.4 ± 13.3 *0-50*	5.9 ± 23.3 *0-100*
2 as % of all types	31.4 ± 34.5 *0-100*	15.9 ± 32.8 *0-100*	29.5 ± 38.9 *0-100*	14.8 ± 29.8 *0-100*
3 as % of all types	22.7 ± 32.9 *0-100*	21.9 ± 36.5 *0-100*	13.5 ± 37.3 *0-100*	22.4 ± 39.1 *0-100*
4 as % of all types	17.3 ± 25.8 *0-100*	17.2 ± 31.0 *0-100*	5.4 ± 14.6 *0-50*	14.2 ± 30.8 *0-100*
5 as % of all types	4.4 ± 14.3 *0-71*	3.9 ± 14.1 *0-66*	1.4 ± 8.3 0-50	9 ± 26.8 *0-100*
6 as % of all types	15.8 ± 31.9 *0-100*	11.1 ± 29.5 *0-100*	18 ± 38 *0-100*	15 ± 35.3 *0-100*

Table 3.3A.ii Descriptive summary data of the number of crack types counted at different anatomical positions on 28 left fore feet

.position	whole hoof	M/4	toe	L/4
	mean ± SD *min-max*			
total number of cracks	10.0 ± 3.6 *4-17*	3.4 ± 2.0 *1-9*	3.4 ± 1.8 *1-9*	3.6 ± 1.5 *1-7*
% number of total cracks	100	33 ± 16.0 *7 -66*	32.6 ± 14.5 *0-64*	33.1 ± 15.9 *0-66*
type 1	0.1 ± 0.4 *0 -2*	0.0 ± 0.2 *0-1*	0.0± 0.4 *0-12*	0 ± 0 *0 -0*
type 2	1.0 ± 1.7 *0-6*	0.3 ± 1.2 *0-6*	0.4 ± 0.7 *1-2*	0.2 ± 0.6 *0-2*
type 3	3.0 ± 3.2 *0-10*	1.0 ± 1.3 *0-4*	0.8 ± 1.0 *1-4*	1.1 ± 1.7 *1-5*
type 4	3.2 ± 2.4 *1-8*	1.0 ± 1.2 *1-4*	1.1 ± 1.2 *1-4*	1.2 ± 1.2 *1-4*
type 5	1.5 ± 1.6 *0-6*	0.6 ± 1.1 *0-5*	0.4 ± 0.8 *1-3*	0.4 ± 0.6 *0-2*
type 6	1.1 ± 1.7 *0-5*	0.3 ± 0.6 *0-3*	0.5 ± 1.0 *1-4*	0.3 ± 0.7 *0-3*
% type 1	1.6± 6.7 *0-33*	1.9 ± 9.6 *0-50*	3.7 ± 19.3 *0-100*	0 ± 0 *0-0*
% type 2	10.5 ± 15.2 *0- 42*	7.7 ± 22.0 *1-100*	12.5 ± 25.2 *0-100*	8.5 ± 23.6 *0-100*
% type 3	27.9 ± 29.9 *0-90*	25.9 ± 33.3 *0-100*	27.4 ± 32.4 *0-100*	25.2 ± 35.7 *0-100*
% type 4	33.3 ± 27.8 *0-88*	33.4 ± 39.3 *0-100*	33.3 ± 34.4 *0-100*	35.4 ± 36.4 *0-100*
% type 5	14.7 ± 17 *0-71*	20.6 ± 33.1 *0-100*	9.7 ± 18.4 *0-66*	15.1 ± 26.2 *0-100*
% type 6	11.9 ± 17.8 *0-60*	11.4 ± 24.3 *0-100*	11.7 ± 23 *0-66*	8.4 ± 21.8 *0-100*

Table 3.3A.iii Descriptive summary data of the area of crack types measured at different anatomical positions on mixed feet

position/type	whole hoof	R/4	toe	L/4
	mean ± SD *min-max*			
type 1 mm²	26.6 ± 87.6 *0-443*	5.7 ± 20.4 *0-93*	16.6 ± 49.2 *0-253*	3.7 ± 15.5 *0-80*
type 2 mm²	82.9 ± 96.5 *0-297*	8.4 ± 21.9 *0-94*	28.8 ± 41.8 *0-154*	22.0 ± 47.1 *0-179*
type 3 mm²	30.1 ± 63.2 *0- 335*	10.2± 21.6 *0-83*	6.8 ± 28.3 *0-167*	12 ± 30.8 *0-167*
type 4 mm²	45.4 ± 89.2 *0-393*	28.0± 68.1 *0- 321*	9.0 ± 34.4 *0-196*	16.9 ± 47.8 *0-250*
type 5 mm²	36.2 ± 108.9 *0-559*	6.6± 22.8 *0-99*	6.4± 38.0 *0-225*	24.1 ± 73.0 *0-334*
type 6 mm²	135.6 ± 395.1 *0-2054*	17.5 ± 62.8 *0- 343*	73.7 ± 221.0 *0- 1197*	17.7 ± 52.3 *0-216*
total crack area mm²	366.9 ± 418.3 *0-2054*	76.0 ± 90.0 *0- 343.1*	141.4 ± 218.0 *0- 1197.2*	95.5 ± 102.6 *0-447.2*
% of total area	100	25.3 ± 26.4 *0-100*	32.1 ± 28.3 *0-100*	32.6 ± 28.5 *0-100*
1 as % of all types	6.5 ±19.0 *0-86*	7.3 ± 25.0 *0-100*	11.7 ± 28.0 *0-96*	0.0 ± 0.2 *0-1*
2 as % of all types	37.4 ± 38.2 *0-100*	14.8 ± 32.9 *0-100*	29.7 ± 40.7 *0-100*	19.5 ± 34.7 *0-100*
3 as % of all types	12.8 ± 25.4 *0-100*	20.2 ± 37.4 *0-100*	9.0 ± 26.0 *0-100*	20.6 ± 39.0 *0-100*
4 as % of all types	13.3 ± 25.1 *0-100*	17.4 ± 33.7 *0-100*	9.0 ± 25.0 *0-100*	4.8 ± 20.3 *0-100*
5 as % of all types	6.4 ± 17.9 *0-100*	3.3± 11.3 *0-46*	2.0 ± 12.0 *0-71.8*	9.7 ± 28.7 *0-100*
6 as % of all types	17.8 ± 33.9 *0-73.88*	10.7 ± 30.5 *0-100*	16.0 ± 35.0 *0-100*	13.8 ± 34.5 *0-100*

Table 3.3A.iv Descriptive summary data of the area of crack types measured at different anatomical positions on 28 left fore feet

position	whole hoof	M/4	toe	L/4
	mean ± SD *min-max*			
total area of cracks mm²	747.4 ± 436.5 *169-1893*	264.4 ± 210.8 *24-955*	292.2 ± 279.2 *0-1265*	191.2 ± 169.1 *0-677*
% area of total cracks	100	36.4± 17.1 *4- 91*	37.9 ± 27.6 *0-108*	26.1 ± 15.2 *0-60*
type 1 mm²	11.7 ± 60.8 *0-315*	11.7 ± 60.8 *0-315*	0.5 ± 2.8 *0-14*	0 ± 0 *0-0*
type 2 mm²	28.5 ± 61.6 *0-254*	5.7 ± 17.6 *0-74*	21.5 ± 48.7 *0-170*	5.4 ± 20.2 *0-101*
type 3 mm²	122.2 ± 174.7 *0-716*	33.0 ± 58.5 *0-194*	48.6 ± 90.8 *0-341*	41.0 ± 88.4 *0-374*
type 4 mm²	273.4 ± 299.6 *0-1187*	108.0 ± 144.3 *0-581*	97.5 ± 184.0 *0-745*	80.0 ± 101.2 *0-342*
type 5 mm²	148.9 ± 209.9 *0-801*	54.0 ± 127.9 *0-561*	42.5 ± 91.0 *0-357*	42.4 ± 74.6 *0-284*
type 6 mm²	162.6 ± 300.9 *0-956*	51.3 ± 125.1 *0-484*	81.6 ± 206.0 *0-908*	22.5 ± 63.8 *0-298*
% type 1	2.2 ± 2.2 *0-59*	3.4 ± 17.9 *0-92*	3.7 ± 19.2 *0-100*	0 ± 0 *0-0*
% type 2	5.8± 2.4 *0-55*	5.0 ± 19.5 *0-100*	13.1 ± 29.1 *0-100*	7.1 ± 24.1 *0-100*
% type 3	22.8 ± 5.6 *0-100*	22.1 ± 34.3 *0-100*	26.3 ± 36.8 *0-100*	22.8 ± 37.0 *0-100*
% type 4	36.1 ± 6.1 *0-99.5*	39.3 ± 42.0 *0-100*	26.0 ± 34.7 *0-100*	35.2± 38.7 *0-100*
% type 5	18.3 ± 3.8 *0-63*	17.4 ± 33.6 *0-100*	11.9 ± 25.6 *0-91*	17.7 ± 28.6 *0-100*
% type 6	14.7 ± 4.6 *0-78*	12.7 ± 29.7 *0-100*	15.1 ± 30.9 *0-98*	9.9 ± 26.7 *0-100*

Table 3.3A.v Descriptive summary data of the geometric area and number of crack types at different anatomical positions on mixed feet

position	whole hoof	Medial ¼	toe	Lateral ¼
score	median score *range*			
GSA	5753 .0 *0-65747*	953.0 *0-10980*	2651.0 *0-38310*	1184.0 *0- 6926*
SSA	1500.0 *0-12328*	303.0 *0-2058*	605.0 *0- 7183*	378.0 *0-1985*
GSA type 1	27.3 *0-443*	5.8 *0-93*	33.4 *0-507*	3.5 *0-80*
GSA type 2	170.8 *0-595*	16.0 *0-188*	57.7 *0-308*	44.7 *0-358*
GSA type 3	120.4 *0-1340*	41.0 *0-333.7*	25.7 *0-607*	48.0 *0-668.8*
GSA type 4	363.0 *0-3150*	211.7 *0-2571*	72.1 *0-1571*	127.6 *0-2001*
GSA type 5	579.0 *0-8950*	100.1 *0-1594.9*	103.0 *0-3599.4*	386.0 *0-5351*
GSA type 6	4339.0 *0-65747*	529.0 *0-10980*	2358 .0 *0-38310*	537 .0 *0-6921*
SSNo	18.0 *0-43*	6.4 *0-25*	5.1 *0-14*	6.7 *0-19*
GSNo	44.5 *0-160*	15.9 *0-65*	13.5 *0-64*	17.2 *0-68*
% GSA type 1	5.9 *0-100*	6.0 *0-100*	8.0 *0-93.5*	5.0 *0-100*
% GSA type 2	11.9 *0-100*	11.9 *0-100*	24.4 *0-100*	16.8 *0-100*
% GSA type 3	19.8 *0-100*	19.8 *0-100*	9.7 *0-100*	21.0 *0-100*
% GSA type 4	16.9 *0-3150*	16.9 *0-100*	11.5 *0-100*	14.9 *0-100*
% GSA type 5	4.8 *0-76*	4.9 *0-76*	1.5 *0-56*	9.7 *0-100*
% GSA type 6	10.8 *0-100*	10.8 *0-100*	17.1 *0-100*	13.5 *0-100*

Table 3.3A.vi Descriptive summary data of the geometric area and number of crack types at different anatomical positions on 28 left fore feet

position	whole hoof	M/4	toe	L/4
score	median score *range*			
GSA	10331.0 *777-44384*	3531.0 *105-15507*	4309.0 *0-34781*	2212.0 *0-13100*
SSA	3239.0 *533-10277*	1135.0 *91- 4384*	1281.0 *0-7237*	800.0 *0-3369*
GSA type 1	11.7 *0-315.7*	11.7 *0-315.7*	0.53 *0-14.3*	0 ± 0 *0-0*
GSA type 2	57.1 *0-509*	11.5 *0-149.6*	43.1 *0-339.7*	10.7 *0-203.78*
GSA type 3	489.0 *0-2866*	131.9 *0-776.9*	194.3 *0-1367.7*	164.1 *0- 1498.8*
GSA type 4	2187.0 *0-9502*	870.0 *0-4652*	780.0 *0-5967*	639.0 *0-2736*
GSA type 5	2383.0 *0-12827*	864.0 *0-8988*	680.0 *0-5721*	679.0 *0- 4555*
GSA type 6	5204.0 *0-30613*	1642.0 *0-15507*	2611.0 *0- 29060*	720.0 *0 - 9553*
SSNo	38.4 *11-64*	13.1 *3-38*	13.0 *0-39*	12.9 *0-24*
GSNo	100.5 *14-264*	33.8 *3-124*	36.2 *0-160*	31.5 *0-96*
% GSA type 1	0.786 *0-21.2*	3.2 *0-86.8*	3.7 *0-100*	0 ± 0 *0-0*
% GSA type 2	2.64 *0-38.7*	4.7 *0-100*	10.5 *0-100*	5.8 *0-100*
% GSA type 3	16.3 *0-100*	18.8 *0-100*	24.4 *0-100*	19.1 *0-100*
% GSA type 4	32.5 *0-99*	40.0 *0-100*	24.8 *0-100*	32.6 *0-100*
% GSA type 5	24.2 *0-83*	17.7 *0-100*	13.7 *0-95*	22.3 *0-100*
% GSA type 6	23.4 *0-90*	15.7 *0-100*	19.0 *0-99*	12.7 *0-100*

Table 3.3A.vii Descriptive summary of weighted number scores of 28 left fore feet

	whole hoof		whole hoof
Severity number	median score *range*	**geometric number**	
SSNo type 1	0.11 *0-2*	**GSNo type 1**	0.11 *0-2*
SSNo type 2	2.2 *0-12*	**GSNo type 2**	2.2 *0-12*
SSNo type 3	9.0 *0-30*	**GSNo type 3**	12 *0-40*
SSNo type 4	12.9 *0-32*	**GSNo type 4**	25.9 *0-64*
SSNo type 5	7.4 *0-30*	**GSNo type 5**	23.7 *0-96*
SSNo type 6	6.9 ± 10 *0-30*	**GSNo type 6**	36.7 *0-160*

3.3.4A Ranking hooves in descending order of their crack scores

Hooves were ranked in descending order of GSA and SSA to see how well the regression equation predicted the GSA from SSA

Table 3.3.4A.i Summary table of mixed hooves ranked in descending order of their total severity and geometric area scores

Hoof	SSA	Hoof	GSA
22	0	22	0
82	5.36	82	5.36
57	48.64	57	48.64
56	65.06	56	65.06
87	84.8	87	84.8
13	193.37	13	215.68
86	206.94	86	275.92
28	231.08	**64**	365.59
16	237.84	**50**	379.64
64	329.86	**5**	410.26
5	351.31	**55**	422.58
50	373.86	**28**	462.16
55	422.58	**16**	466.64
14	445.14	**96**	491.38
96	491.38	**73**	652.33
61	491.46	**52**	803.22
73	652.33	**19**	847.84
29	691.52	29	1003.88
52	736.74	**33**	1192.68
19	847.84	**31**	1291.26
33	849.58	**2**	1340.36
31	967.19	**14**	1642.84
2	1005.27	**32**	1890.12
4	1106.7	4	2491.5
32	1285.65	**61**	2541.3

Hoof	SSA	Hoof	GSA
59	1470.34	59	4217.36
98	1605.51	**46**	4869.22
15	1767.6	**30**	6362.22
46	2035.89	**98**	8097.45
30	2165.62	**15**	9246.76
8	2520.36	**12**	12427.26
12	3706.18	**8**	13441.92
17	4899.07	17	18220.96
49	7886.23	49	39336.78
7	12327.6	7	65747.2

Note 1 hoof numbers in bold show where the ranking in GSA differs from SSA
Note 2 using SSA to predict GSA is erroneous in 63% of hooves

Table 3.3.4A.ii **28 left fore hooves ranked by descending order of SSA, GSA, SSNo and GSNo**

28 left fore hooves listed in descending order of value for geometric and severity area and number scores of the whole hoof

Hoof SSNo	SSNo	Hoof GSNo	GSNo	Hoof SSA	SSA	Hoof GSA	GSA
21a	64	21a	264	21a	10277	21a	44384
23a	61	23a	228	20a	7954.6	20a	36008.7
27a	61	**9a**	200	23a	6982.4	23a	31079.4
19a	60	19a	160	17a	5767.1	**5a**	19838.4
9a	57	**3a**	152	9a	5221	9a	18201.2
11a	51	**5a**	148	10a	4777.6	**3a**	17391.7
6a	48	**11a**	144	12a	4447.6	**17a**	16276.9
7a	47	**20a**	140	3a	4398.2	**10a**	9587.4
3a	45	**7a**	136	16a	4383.6	**8a**	9569.7
5a	43	**27a**	118	5a	4061.5	**12a**	8775.7
26a	43	**2a**	100	8a	2837.6	**16a**	7993.2
25a	42	**17a**	96	11a	2822.3	11a	7731.2
16a	39	**10a**	80	4a	2600.1	**7a**	6402.9
28a	39	**26a**	80	14a	2599.3	**2a**	6029.5
10a	37	**8a**	72	7a	2401.9	**14a**	5759.1
20a	35	**16a**	72	27a	2230.7	**4a**	5139.8
17a	33	**6a**	70	6a	1996.5	**27a**	5092.8
24a	31	**25a**	70	2a	1908.7	**26a**	4283.1
1a	27	**14a**	56	26a	1653.5	**15a**	3653.8
8a	27	**18a**	56	15a	1421.2	**19a**	3340
14a	27	**4a**	48	18a	1407.9	18a	3004.4
4a	26	**28a**	48	19a	1243.3	**6a**	2888
18a	25	**1a**	47	13a	1014.7	**25a**	1970.6
12a	23	12a	44	25a	1012.5	**1a**	1488.1
2a	22	**24a**	44	1a	997.3	**13a**	1352.9
15a	14	15a	26	28a	778.6	28a	919.4
13a	11	13a	14	24a	532.8	24a	776.8

Note 1 Hooves highlighted in bold indicate where the severity score ranks the hoof in a different order compared to the equivalent geometric score
Note 2 SSA severity score area; GSA geometric score area; SSNo severity score number, GSNo geometric score number
Note 3 Using SSNo to predict GSNo is 75% erroneous; using SSA to predict GSA is erroneous in 68% of the hooves

3.3.6

Table 3.3.6A.i Mixed hooves categorised into low, medium and high crack score groups and filtered according to different score

	ranked by					
Hoof number	**TCNo**	**SSNo**	**GSNo**	**TCA**	**SSA**	**GSA**
2	**tcnol**	mssno	**lgsno**	mtca	mssa	mgsa
4	tcnom	mssno	mgsno	mtca	mssa	mgsa
5	tcnom	mssno	mgsno	mtca	mssa	mgsa
7	**tcnom**	**mssno**	hgsno	htca	hssa	hgsa
8	**tcnom**	hssno	hgsno	mtca	hssa	hgsa
12	**tcnom**	hssno	hgsno	htca	hssa	hgsa
13	**tcnom**	**mssno**	**mgsno**	mtca	lssa	lgsa
14	**tcnoh**	**hssno**	**hgsno**	mtca	mssa	mgsa
15	**tcnol**	**mssno**	**mgsno**	mtca	hssa	hgsa
16	**tcnol**	mssno	mgsno	ltca	mssa	mgsa
17	**tcnom**	hssno	hgsno	htca	hssa	hgsa
19	tcnom	mssno	mgsno	htca	mssa	mgsa
22	tcnol	lssno	lgsno	ltca	lssa	lgsa
28	tcnom	mssno	mgsno	ltca	lssa	mgsa
29	**tcnoh**	**hssno**	mgsno	mtca	mssa	mgsa
30	tcnoh	**mssno**	hgsno	htca	hssa	hgsa
31	**tcnoh**	**hssno**	mgsno	mtca	mssa	mgsa
32	tcnom	mssno	mgsno	mtca	mssa	mgsa
33	tcnoh	**hssno**	mgsno	mtca	mssa	mgsa
46	tcnom	mssno	mgsno	htca	hssa	mgsa
49	**tcnom**	**mssno**	**mgsno**	htca	hssa	hgsa
50	tcnom	mssno	mgsno	mtca	mssa	mgsa
55	tcnom	mssno	**lgsno**	mtca	mssa	mgsa
56	tcnol	lssno	lgsno	ltca	lssa	lgsa
57	tcnol	lssno	lgsno	ltca	lssa	lgsa
59	tcnom	**hssno**	**hgsno**	mtca	mssa	mgsa
61	tcnom	mssno	**hgsno**	mtca	mssa	mgsa
64	**tcnoh**	**mssno**	**mgsno**	mtca	mssa	lgsa
73	tcnom	mssno	mgsno	htca	mssa	mgsa
82	tcnol	lssno	lgsno	ltca	lssa	lgsa
86	**tcnom**	**mssno**	**mgsno**	ltca	lssa	lgsa
87	tcnol	lssno	lgsno	ltca	lssa	lgsa
96	tcnom	mssno	**lgsno**	mtca	mssa	mgsa
98	**tcnom**	**mssno**	hgsno	mtca	mssa	hgsa

Note 1 tcnom total crack number medium; tcnol total crack number low; tcnoh total crack number high
Note 2 mssno severity number score medium; lssno low severity number; hssno high severity number
Note 3 mgsno geometric number medium; lgsno low geometric number; hgsno high geometric number
Note 4 mtca medium total crack area; ltca low total crack area; htca high total crack area
Note 5 mssa medium severity area; lssa low severity area; hssa high severity area
Note 6 mgsa medium geometric are; lgsa low geometric area; hgsa high geometric area
Note 7 using TCNo as a predictor of GSA is erroneous in 42% of the hooves; using SSNo wrongly predicts GSA 36% of the time and using GSNo wrongly predicts GSA 30% of the time.

Table 3.3.6A.ii Filtering of 28 left fore hooves according to low, medium or high geometric area or geometric number scores

GSA l m h	GSNo l m h	GSAM/4 l m h	GSNoM/4 l m h	GSAT l m h	GSNoT l m h	GSA L/4 l m h	GSNoL/4 l m h
lg	lg	lg	lg	lg	**mg**	mg	mg
mg	mg	mg	**lg**	mg	mg	mg	**hg**
hg	hg	hg	hg	mg	mg	lg	lg
mg	mg	lg	lg	mg	mg	mg	mg
hg	hg	mg	mg	hg	hg	hg	**mg**
lg	**mg**	mg	mg	lg	**mg**	mg	mg
mg	mg	mg	mg	mg	**hg**	mg	**hg**
mg	mg	hg	**mg**	mg	mg	lg	lg
hg	hg	mg	mg	hg	hg	hg	hg
mg	mg	mg	mg	hg	**mg**	mg	mg
mg	mg	mg	**hg**	lg	**mg**	mg	**lg**
mg	**lg**	mg	mg	mg	mg	lg	lg
lg	lg	lg	lg	mg	**lg**	lg	lg
mg	mg	mg	mg	mg	mg	hg	**mg**
mg	**lg**	mg	mg	lg	lg	lg	lg
mg	mg	mg	**lg**	mg	mg	mg	**hg**
mg	mg	hg	hg	mg	mg	mg	mg
mg	mg	mg	mg	lg	lg	mg	mg
mg	**hg**	lg	**mg**	mg	**hg**	mg	mg
hg	**mg**	hg	**mg**	hg	**mg**	hg	**mg**
hg	hg	hg	hg	hg	hg	hg	**mg**
hg	hg	hg	hg	hg	hg	hg	hg
lg	lg	mg	mg	lg	**mg**	lg	**mg**
lg	**mg**	mg	mg	mg	mg	mg	mg
mg	mg	lg	lg	mg	mg	mg	mg
mg	mg	mg	**hg**	lg	**mg**	mg	**hg**
lg	**mg**	lg	**mg**	mg	**lg**	lg	**mg**

Note 1 (low, lg ; medium, mg ; high, hg) geometric scores
Note 2 GSA geometric score area; GSNo geometric score number
Note 3 T toe; M/4 medial quarter; L/4 lateral quarter
Note 4 Using GSNo wrongly predicts GSA 25% of the time on the whole hoof; using GSNo at the medial quarter wrongly predicts GSA 29% of the time; at the toe and lateral quarter 40% of the time

4 An evaluation of two methods for measuring trace element concentration

Repeatability of the method

Table 3.3.6A.iii Levene's test of differences between the variances in blocks taken from the same anatomical position and subsequently split into two.

Anatomical positions	F test P value
A vs. B	P = 0.942
mdc A vs. mdc B	P = 0.556
L/4A vs. L/4B	P = 0.993
M/4A vs. M/4B	P= 0.530
L/4s vs. M/4s	P= 0.304
mdc vs. 1/4s	P= 0.193

P > 0.05 in all of the above, the null hypothesis cannot be rejected and there is no evidence of inequality between the variances; the assumptions underlying the paired t test are therefore valid.

Table 3.3.6A.iv Differences between the fracture toughness of blocks A and B

Anatomical position	All As	mdc A	L/4A	M/4A
All Bs	Mean A 48.41± 16.76 Mean B 45.77± 17.06			
mdc B		Mean A 39.94± 15.04 Mean B 41.1 ± 19.87		
L/4B			Mean A 51.33 ± 20.36 Mean B 42.93 ± 19.38	
M/4				Mean A 53.47 ± 12.62 Mean B 53.76 ± 9.37

Note 1 there was no significant differences between fracture toughness of blocks A or B (p>0.05)

The null hypothesis could not be rejected; there are no statistically significant differences between the means of the blocks at any anatomical position indicating that the methods are repeatable.

Appendix Trace element chapter four

Table 4.3.1A.i Descriptive summary of zinc, calcium and copper concentration measured by IPC-Laser ablation in blocks from six hooves taken from the toe, medial and lateral quarters and subsequently split into two

Hoof position	Mean Zinc	Median	Q1	Q3
	ppm/ppmC			
mdc A	0.00003	0.00000	0.00000	0.00005
mdc B	0.00000	0.00000	0.00000	0.00000
M/4A	0.00000	0.00000	0.00000	0.00000
M/4B	0.00000	0.00000	0.00000	0.00000
L/4A	0.00001	0.00000	0.00000	0.00001
L/4B	0.00000	0.00000	0.00000	0.00000

Hoof position	Mean Calcium	Median
ICPlaser	ppm/ppmC	
mdc A	-0.00007	-0.00007
mdc B	-0.00007	-0.00007
M/4A	-0.00007	-0.00007
M/4B	-0.00006	-0.00007
L/4A	[a]-0.00004	-0.00006
L/4B	[b]-0.00007	-0.00007

Hoof Position	Mean Copper	Median	Q1	Q3
ICPlaser	ppm/ppmC			
mdc A	0.00000	-0.0	-0.0000	0.0000
mdc B	–0.00000	–0.00000	–0.00000	0.0000
M/4A	0.00000	0.00000	-0.00000	0.0000
M/4B	0.00000	0.00000	-0.00000	0.0000
L/4A	0.00000	0.00000	–0.00000	0.0000
L/4B	–0.00043	0.00284	–0.00235	0.00138

Note 1 mdc is toemdc at 50% hoof wall height
Note 2 M/4 medial quarter
Note 3 L/4 lateral quarter
Note 4 A and B block split into two to compare washing techniques
Note 5 N = 6
Note 6 superscripts within column indicate difference in TE between washing techniques at a significance,$p<0.05$

Table 4.3.1A.ii Descriptive summary of zinc, calcium and copper concentration, measured by IPC-MS in blocks from six hooves taken from the toe, medial and lateral quarters and subsequently split into two

all hooves	Mean mg/kgDM	StDev	Minimum	Maximum
Zn	165.0	53.9	120.0	430.0
Ca	429.4	110.6	260.0	740.0
Cu	2.9	6.4	-3.3	26.6
	Mean	StDev	Minimum	Maximum
Zinc by block	Trace element mg/kgDM			
Zn mdcA	155.0	26.6	120.0	200.0
Zn mdc B	167.5	17.25	140.0	190.0
Zn M/4A	196.7	116.6	130.0	430.0
Zn M/4B	150.0	21.6	120.0	170.0
Zn L/4A	166.0	42.2	140.0	240.0
Zn L/4B	151.6	24.0	120.0	180.0
Ca by block				
Ca mdc A	406.7	101.1	270.0	520.0
Ca mdc B	400.0	122.1	260.0	560.0
Ca M/4A	446.7	81.4	310.0	550.0
Ca M/4B	500.0	173.4	350.0	740.0
Ca L/4A	510.0	86.0	410.0	640.0
Ca L/4B	350.0	50.2	270.0	400.0
Cu by block				
Cu mdc A	3.4	6.7	-3.3	15.3
Cu mdc B	1.6	2.9	-0.8	7.4
Cu M/4A	5.7	11.0	-2.2	26.6
Cu M/4B	0	0	0	0
Cu L/4A	[a]7.4	7.80	0	16.5
Cu L/4B	[b]1.0	0.28	-0.99	6.1
Zn by hoof				
Hoof_	Mean	StDev	Minimum	Maximum
34	160.0	32.7	120	200
62	140.0	10.9	120	150
76	188.0	31.1	160	240
91	145.8	13.5	130	165
92	148.3	22.2	120	180
94	211.7	108.2	150	430
Ca by hoof	Mean	StDev	Minimum	Maximum
34	460.0	129.9	270	560
62	393.3	120.4	260	550
76	454.0	61.1	390	540
91	413.3	173.6	300	740
92	401.7	70.8	290	480
94	468.3	97.9	370	640
Cu by hoof				
Hoof_	Mean	StDev	Minimum	Maximum
34	0.8	0.8	-0.002	1.9
62	1.5	2.8	0	7.1

Hoof_	Mean	StDev	Minimum	Maximum
76	3.7	7.0	-3.3	15.1
91	0.6	2.1	-0.8	4.8
92	-0.5	1.1	-2.2	0.9
94	10.9	10.4	0	26.6
TE anatomical position	**Mean**	**StDev**	**Minimum**	**Maximum**
Zinc				
mdc	161.2	22.3	120	200
M/4	178.0	91.0	120	430
L/4	158.1	32.5	120	240
Calcium				
mdc	403.3	107.0	260	560
M/4	468.0	120.3	310	740
L/4	422.7	105.8	270	640
Copper				
mdc	2.5	5.1	-3.3	15.3
L/4	2.8	8.1	-2.2	26.6
M/4	3.4	6.3	0	16.5

Note 1 superscripts indicate difference within column between trace elements measured in blocks which have undergone different washing techniques

Table 4.3.1A.iii Descriptive summary of trace element concentration of zinc, copper and calcium at toe mdc from mixed feet measured by ICP-MS or ICP-Laser ablation

Trace elements concentration in mixed hooves				
Trace element	**Mean**	**+/_StDev**	**Min**	**Max**
65Cu mg/kgDM	22.8	5.6	12.6	41.7
43Ca mg/kgDM	679.4	434.3	9.3	1899.2
66Zn mg/kgDM	160.4	54.8	31.0	287.9
Laser ppm/C ppm	**Mean**	**Median**	**Q1**	**Q3**
calcium/ C	–0.00518	–0.00340	–0.00777	–0.00107
copper/C	0.00011	0.00003	0.00002	0.00007
zinc/C	0.00039	0.00036	0.00014	0.00050

Table 4.3.1A.ivSummary of median trace element at the toe mdc, medial and lateral quarters of 28 feet measured by laser ablation

median TEppm/Cppm of the toe mdc, L/4 and M/4 in 28 left fore feet				
		Ca ppm/Cppm	Cu ppm/Cppm	Zn ppm/Cppm
position	**N**	Median	Median	Median
mdc	28	[a]2.82E-04	[a]6.05E-06	[a]2.90E-04
M/4	28	[a]5.88E-04	[b]1.67E-05	[a]5.72E-04
L/4	28	[b]-8.90E-03	[c]2.70E-04	[b]3.89E-03

Note 1 all were ablated at 12.5% hoof wall depth from block 2
Note 2 superscripts indicate significant differences down the column between anatomical positions at a significance, $p<0.05$

Table 4.3.1A.v Summary median trace element down the hoof wall height at toe mdc blocks at 12.5% hoof wall depth

Median TEppm/Cppm down hoof wall at toe mdc				
HWH block		Median **N**	**copper**	**calcium**	**zinc**
	2	11	6.33E-06	1.00E-03	5.61E-04
	3	11	7.49E-06	7.05E-04	3.65E-04
	4	11	6.85E-06	[a]1.04E-03	[a]4.80E-04
	5	11	3.57E-06	[b]3.62E-04	[b]8.19E-05

Note1 all blocks ablated at 12.5% hoof wall depth
Note 2 superscripts indicate differences down a column between TE at different positions down the HWH at a significance of $p<0.05$

Table 4.3.1A.vi Summary median trace element across the hoof wall depth of toe mdc blocks from 50% hoof wall height

TEppm/C ppm across HWD at 50% HWH mdc				
percent HWD	medians **N**	**calcium**	**copper**	**zinc**
12.5	11	[a]0.001003	[a]6.33E-06	[a]5.61E-04
37.5	11	[a]0.001984	[b]1.58E-05	[b]2.97E-03
62.5	11	[a]0.001876	[b]1.59E-05	[b]2.87E-03
87.5	11	[b]0.004505	[b]1.63E-05	[b]4.45E-03

Note 1 percentages across the hoof wall depth were calculated after measuring the full depth whilst the block was under the laser
Note 2 percentage depth represents the change in tubular pattern across the hoof wall, (Reilly, 1998)
Note 3 Superscripts within a column indicate differences in TE across the hoof wall depth at a significance of $p<0.05$

Table 4.3.1A.vii Summary median trace element dependant on whether mean obtained from hoof wall depth, height or from 12.5% hoof wall depth ablation.

Median TEppm/Cppm depending on position of analysis at mdc of 28 left fores using laser				
		Median		
analytical position	N	**Calcium**	**Copper**	**Zinc**
mean HWD	11	[a]2.46E-03	[a]1.65E-05	[a]2.84E-03
mean HWH	11	[b]8.79E-04	[b]8.50E-06	[b]3.98E-04
mdc single laser	11	[b]1.00E-03	[b]6.33E-06	[b]5.61E-04

Figure 4.3.6A.i Scatter plots of 6 hooves

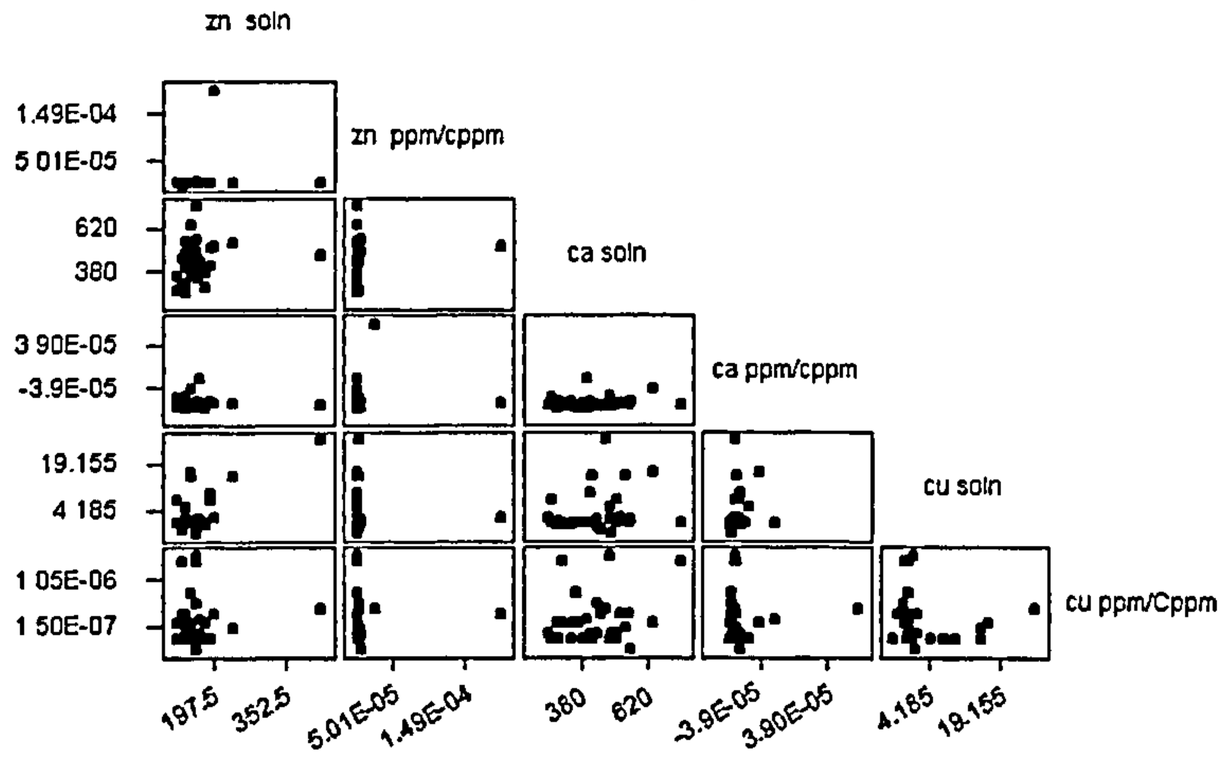


Table 4.3.7A.ii Correlations between trace elements sampled across the depth of the hoof wall blocks within each sampling depth and between each depth

within hoof wall depth	**12.5%**	**37.2%**	**62.5%**	**87.2%**
correlation between TEppm/Cppm	spearman's correlation p value			
Ca/C Cu/C	0.726 0.011			
Zn/C Ca/C		0.616 0.044	0.603 0.05	0.774 0.005
Ca/C Zn/C	0.641 0.033			
Cu/C Zn/c		0.599 0.051		
	Ca	**Cu**	**Zn**	
Correlations between hoof wall depths	spearman's correlation p value			
12.5% 37.2 %	0.801 0.003		0.728 0.011	
12.5% 62.5%	0.803 0.003		0.755 0.007	
12.5% 87.2%	0.728 0.011			
37.2% 62.5%	0.965 0.0001	0.697 0.017	0.816 0.002	
37.2% 87.5%	0.79 0.004		0.638 0.035	

Table 4.4A.i Differences in counts from ICP-MS for standard three over the analytical period

trace element	zinc	calcium	copper
counts /sec	27770.89	3980.88	191683.9
	35949.42	5228.95	249680.5
	40761.66	6073.73	282770.8
	41750.81	5968.02	287928.9
% difference between first and last counts	66.5	66.7	66.5

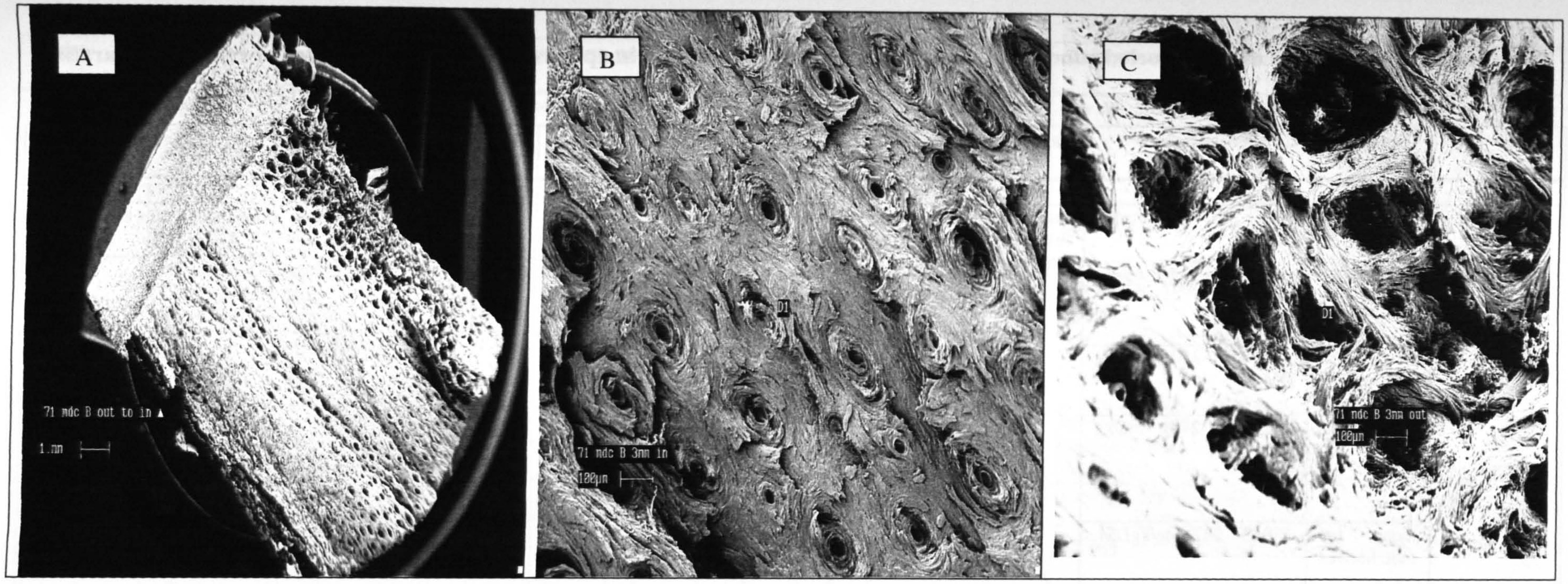


Plate 4. 4.1A.i Hoof block 71mdcB to show tubular damage along the fracture plane after IZOD test to illustrate standardisation of technique to allow the same block to be resampled

Note 1 photo A is full block with outer wall closest and inner wall furthest away, magnification 18X, focus 30nm, z=61
Note 2 photo B is 3mm in from outer wall and 2mm in from notch
Note 3 photo C is 3mm in from inner wall and 2.03mm in from notch
Note 4 photos B and C, magnification 200X; 5kv, 150pA, tilt 8.4

Table 4.4.2A.i. A summary of the reported concentrations of zinc, copper and calcium in epidermal tissues and methods of sample preparation and analysis

Trace element	Tissue type	Concentration	Sampling procedure	Washing procedure	Analytical technique	Author
ZINC						
Equine hoof pilot study	median 6 hooves	165 (120-430) mg/kg DM	same chronological age full hoof wall depth, 50% HWH toe mdc	ionic detergent and millipore water	Total digestion, microwave, nitric acid, ICP-MS	this thesis
	mean toe mdc	161 (120-200) mg/kg DM				
	median M/4	178 (120-430) mg/kg DM				
	median L/4	158 (120-240) mg/kg DM				
48 hooves	mean toe mdc hooves	160 (31-287) mg/kgDM		millipore water, 5 washes 5 mins each		
Equine	hoof wall	174-215 mg/kgDM				Coenan,1996
	hoof sole	93-106 mg/kg DM				
	hair	137-155 mg/kgDM				
Dog	footpad	32.7 µg/g Fresh/DM?	not chronological	none	flame atomic spectrophotometer dry ash, nitric acid	Lansdown,1997
	skin	21.2 µg/g Fresh/DM?				
Sheep	wool	50-110 mg/kgDM	new growth at set intervals	standardised technique	dry weight digestion atomic absorption	White,1994
Rats	skin	34 µg/g DM	skin removed, one sampling	none, cut with razor blade and tested on Instron	dried and then dry ashed, nitric acid flame AAS	Agren, 1990

Trace element	Tissue type	Concentration	Sampling procedure	Washing procedure	Analytical technique	Author
Dog claw	healthy	129 mg/kg Fresh/DM?	whole nail	unwashed	dry ash, dissolved in HCL, AAS	Harvey,1996
	diseased	151 mg/kg Fresh/DM?				
Goat's hair	diseased	73 ppm	individual samples of hair	unwashed	complete digestion (nitric, perchloric & sulphuric) AAS	Ray,1997
	healthy	128-131 ppm				
Pigs	toe wall Cornwall Kahyb	mg/kgDM 162± 5.4 147 ± 4.3	not specific	washed water and detergent, standardised procedure	dry weight complete digestion , nitric and perchloric AAS, except CU by photometry	Kovacs,1973 a
	sole Cornwall Kahyb	mg/kgDM 92 ± 2.2 116 ± 2.9				
Pigs	claw wall	92 mg/kgDM	not specific	washed water and detergent, standardised procedure	dry weight complete digestion , nitric and perchloric AAS, except CU by photometry	Kovacs 1973b
Cattle		142 mg/kgDM				
Sheep		88 mg/kgDM				
Equine	hoof wall	mg/kgDM good 195 ± 22 poor 171 ± 29.5	during routine hoof care clippings	no details	oven dry, dry ash, AAS	Coenen, 1997
	sole	mg/kgDM good 103 ±10 poor 96 ± 14				
	hair	mg/kgDM good 150 ± 14 poor 141 ± 11				
Bulls	hoof wall	80 g/kg fresh	portion of abaxial wall	Vortexed with distilled water for 60 secs.	0.5g ashed overnight, digested with HCL, AAS duplicate assays	Sugg, 1996
Beef cattle	hoof sole	yr 1 41.6 mg/kg yr 2 64.4 mg/kg	25-50g from unspecified area of sole	rinsed with distilled water	digested nitric, perchloric & sulphuric AAS	Hidiroglou, 1986
Equine	hoof wall	mg/kg DM/fresh? pasture 143 controlled 133 pasture & concentrates 114	toe and heel region to obtain 25g	rinsed with deionised water	dried , complete digestion nitric acid for 12hrs and filtered , ICP. analysed in duplicate , spikes used	Ley, 1998

Trace element	Tissue type	Concentration	Sampling procedure	Washing procedure	Analytical technique	Author
Cows	hoof wall	mg/kgDM good 128.4 lame 92.9	specified areas and mechanical measurements taken from same sites	none, trimmed with a stainless steel blade	dry weight ashed AAS	Baggot, 1988
	hoof sole	mg/kgDM good 65.8 lame 46.6				
	hoof heel	mg/kgDM good 57.6 lame 44.2				
	lateral claw	mg/kgDM good 83.1 lame 59				
	medial claw	mg/kgDM good 84.8 lame 63.5				
Human	skin	% DM of tissue $2X10^{-3} - 6.7 X 10^{-2}$	hair 1" from distal end nail specific weight	nail and hair, standardised wash technique; 30 mins shaken in tween ,rinsed 3 X in distilled water for 10mins	dry weights, digested in pyrex dishes in double distilled nitric acid. Spectograph , light emission , compared with standards	Goldblum, 1953
	nails	$1x 10^{-2} - 3.0 X 10^{-1}$				
	hair	$6.4 X10^{-3} - 5 X10^{-2}$				
Human	nails	µg/gDM women 222 men 178	nail clippings	several standardised washing techniques compared on longitudinally split nails	digestion in nitric and perchloric duplicate samples AAS & spark source mass spec	Harrison,1971
Dogs	hair	µg/gDM fox terrier (brown) 222 schnauzer(black)258 mini schnauzer (fair)222	3cm hair samples nearest to skin cut with stainless steel scissors homogenised	5 standardised procedures deionised water 2 organic solvents complexing agent non ionic detergent	0.5g wet acid digestion analytically pure nitric acid & perchloric 5 replicates ICP	Chyla, 2000
Human	hair	g/kgDM male 242 female 329	nape of neck with stainless steel scissors	distilled water and EDTA, no time parameters	dried in a vacuum oven, digested nitric acid & perchloric, stored in Nalgene bottles AAS	Medeiros, 1995
Pigs	toe wall Cornwall Kahyb	mg/kg DM 981 +/- 42 775 +/- 35	not specific	washed water and detergent, standardised procedure	dry weight complete digestion , nitric and perchloric AAS, except CU by photometry	Kovacs,1973 a
	sole Cornwall Kahyb	mg/kgDM 1141 +/- 34 821 +/- 26				

Trace element	Tissue type	Concentration	Sampling procedure	Washing procedure	Analytical technique	Author
CALCIUM						
Equine hooves Pilot study	median 6 hooves	mg/kg DM 429 (260-270)	50%HWH toe mdc same chronological age, whole block	millipore and non ionic detergent comparisons	microwave total digestion, nitric acid ICP-MS	this thesis
	mean mdc	403 (260-560)				
	mean M/4	468 (310-740)				
	mean L/4	422 (270-640)				
main study	mean 48 hooves mdc	mg/kgDM 657 (9.3- 1899)		millipore water 5 washes for 5mins each		
Dog claw	healthy	771 +/- 83.4 Fresh/DM?	whole nail	unwashed	dry ash, dissolved in HCL, AAS	Harvey,1996
	diseased	2535 +/- 915 sig diff Fresh/DM?				
Dog	footpad	423 mg/kg Fresh/DM?	not chronological	none	flame atomic spectrophotometer dry ash, nitric acid	Lansdown,1997
	skin	213 mg/kg Fresh/DM?				
Pigs	claw wall	1141 mg/kgDM	not specific	washed water and detergent, standardised procedure	dry weight complete digestion , nitric and perchloric AAS, except CU by photometry	Kovacs 1973b
Cattle		1267mg/kgDM				
Sheep		1431 mg/kgDM				
Bulls	hoof wall	1109g/kg fresh	portion of abaxial wall	vortexed with distilled water for 60 secs.	0.5g ashed overnight, digested with HCL, AAS duplicate assays	Sugg, 1996
Beef cattle	sole	yr1 953 mg/kg yr 2 1555 mg/kg	25-50g from unspecified area of sole	rinsed with distilled water	digested nitric, perchloric & sulphuric AAS	Hidiroglou, 1986
Cows	hoof wall	mg/kgDM good 1481 lame 1360	specified areas and mechanical measurements taken from same sites	none, trimmed with a stainless steel blade	dry weight ashed AAS	Baggot, 1988
	hoof sole	mg/kgDM good 635 lame 664				
Dogs	hair	µg/gDM fox terrier (brown) 381 schnauzer(black)1010 mini schnauzer (fair)610	3cm hair samples nearest to skin cut with stainless steel scissors homogenised	5 standardised procedures deionised water 2 organic solvents complexing agent non ionic detergent	0.5g wet acid digestion analytically pure nitric acid & perchloric 5 replicates ICP	Chyla, 2000

Trace element	Tissue type	Concentration	Sampling procedure	Washing procedure	Analytical technique	Author
	hoof heel	mg/kgDM good 747 lame 755				
Equine	hoof wall	mg/kg DM/fresh? pasture 951 controlled 1269 pasture & concentrates 1115	toe and heel region to obtain 25g	rinsed with deionised water	dried , complete digestion nitric acid for 12hrs and filtered , ICP. analysed in duplicate , spikes used	Ley, 1998
Human	skin	% DM of tissue $1.2X10^{-3} - 4.9 X 10^{-2}$	hair 1" from distal end nail specific wt	nail and hair, standardised wash technique; 30 mins shaken in tween,rinsed 3 X in distilled water for 10mins	dry weights, digested in pyrex dishes in double distilled nitric acid. Spectograph , light emission , compared with standards	Goldblum, 1953
	nails	$9.4x 10^{-2} - 5.9 X 10^{-1}$				
	hair	$7 X10^{-2} - 4.9 X10^{-1}$				
Human	nails	µg/gDM women 821 men 904	nail clippings	several standardised washing techniques compared on longitudinally split nails	digestion in nitric and perchloric duplicate samples AAS & spark source mass spec	Harrison,1971
Human	hair	g/kgDM male 1179 female 2106	nape of neck with stainless steel scissors	distilled water and EDTA, no time parameters	dried in a vacuum oven, digested nitric acid & perchloric, stored in Nalgene bottles AAS	Medeiros, 1995
COPPER						
Equine hooves Pilot study	median 6 hooves	mg/kg DM 2.92 (- 3.3 – 26)	50%HWH mdc same chronological age, whole block	millipore and non ionic detergent	microwave total digestion, nitric acid ICP-MS	this thesis
	median mdc	2.56 (-3.3 – 15.3)				
	median M/4	3.39 (0 – 16.5)				
	median L/4	2.86 (-2.2 – 26.6)				
main study	median 48 hooves mdc	mg/kgDM 22.8 (12.6 – 41.6)		millipore water, 5 washes for 5 mins each		
Dog	footpad	2.03 µg/g Fresh/DM?	not chronological	none	flame atomic spectrophotometer dry ash, nitric acid	Lansdown,1997
	skin	1.19 µg/g Fresh/DM?				

Trace element	Tissue type	Concentration	Sampling procedure	Washing procedure	Analytical technique	Author
Equine	hoof wall	4.2 – 5.1 mg/kgDM				Coenen,1996
	hoof sole	3.3 – 4.2 mg/kg DM				
Steers	hair	depletion 14.2 +/- 2.2 µmol/kgDM repletion 97.6 +/-14 µmol/kgDM	shaved hair, therefore chronological	none	AAS	Suttle, 1983
Lambs	fleece	depletion 56.7 +/- 5.5 µmol/kgDM repletion 53.5 +/- 15µmol/kgDM				
Pigs	toe wall Cornwall Kahyb	mg/kgDM 9.5 +/- 0.4 12.7 +/- 1.1	not specific	washed water and detergent, standardised procedure	dry weight complete digestion , nitric and perchloric AAS, except CU by photometry	Kovacs,1973 a
	sole Cornwall Kahyb	 5.1 +/- 0.2 9.3 +/- 0.6				
Pigs	claw wall	5.1 mg/kgDM	not specific	washed water and detergent, standardised procedure	dry weight complete digestion , nitric and perchloric AAS, except CU by photometry	Kovacs 1973b
Cattle		3.8 mg/kgDM				
Sheep		16 mg/kgDM				
Bulls	hoof wall	4.03g/kg fresh	portion of abaxial wall	vortexed with distilled water for 60 secs.	0.5g ashed overnight, digested with HCL, AAS duplicate assays	Sugg, 1996
Equine	hoof wall	mg/kg DM/fresh? pasture 6.14 controlled 10.05 pasture & concentrates 9.67	toe and heel region to obtain 25g	rinsed with deionised water	dried , complete digestion nitric acid for 12hrs and filtered , ICP. analysed in duplicate , spikes used	Ley, 1998

Trace element	Tissue type	Concentration	Sampling procedure	Washing procedure	Analytical technique	Author
Beef cattle	sole	yr1 1.5mg/kg yr 2 2.7 mg/kg	25-50g from unspecified area of sole	rinsed with distilled water	digested nitric, perchloric & sulphuric AAS	Hidiroglou, 1986
Cows	hoof wall	mg/kgDM good 8.29 lame 9.76	specified areas and mechanical measurements taken from same sites	none, trimmed with a stainless steel blade	dry weight ashed AAS	Baggot, 1988
	hoof sole	mg/kgDM good 6.28 lame 7.47				
	hoof heel	mg/kgDM good 6.32 lame 8.48				
	lateral claw	mg/kgDM good 6.97 lame 8.81				
	medial claw	mg/kgDM good 6.95 lame 8.32				
Human	skin	% DM of tissue $7.43X10^{-4} - 8.8 X 10^{-3}$	hair 1" from distal end nail specific wt	nail and hair, standardised wash technique; 30 mins shaken in tween, rinsed 3 X in distilled water for 10mins	Dry weights, digested in Pyrex dishes in double distilled nitric acid. Spectrograph , light emission , compared with standards	Goldblum, 1953
	nails	$9.4x 10^{-4} - 8.1 X 10^{-3}$				
	hair	$3.1 X10^{-3} - 1.28 X10^{-2}$				
Human	nails	µg/gDM women 44 men 62	nail clippings	several standardised washing techniques compared on longitudinally split nails	digestion in nitric and perchloric duplicate samples AAS & spark source mass spec	Harrison,1971
Dogs	hair	µg/gDM fox terrier (brown) 18.6 schnauzer(black)14.2 mini schnauzer (fair) 18.4	3cm hair samples nearest to skin cut with stainless steel scissors homogenised	5 standardised procedures deionised water 2 organic solvents complexing agent non ionic detergent	0.5g wet acid digestion analytically pure nitric acid & perchloric 5 replicates ICP	Chyla, 2000
Human	hair	g/kgDM male 49 female 64	nape of neck with stainless steel scissors	distilled water and EDTA, no time parameters	dried in a vacuum oven, digested nitric acid & perchloric, stored in Nalgene bottles AAS	Medeiros, 1995

5 Quantitative assessment of the shape of the equine hoof capsule

Appendix shape chapter

Descriptive summaries of measurements taken from hoof capsules and their photographs

Table 5.3.1A.i Descriptive summary of measurements taken from the dorsal view of hoof capsule and from photographs of mixed feet

Summary of mean measurements from the dorsal view of mixed random feet from actual hooves and photos				
Measurement	**Mean**	**StDev**	**Min**	**Max**
TOEmdc LENGTH	9.4	1.0	7.4	11.4
toemdc length photo	7.6	0.9	5.4	9.8
MWP ¼ VERTICAL length	7.5	0.9	5.5	10.0
mwp ¼ vertical length	6.4	0.8	4.8	8.0
LWP ¼ VERTICAL LENGTH	7.6	0.9	5.8	10.6
lwp ¼ vertical length	6.2	0.7	4.4	7.9
LWP ¼ VERTICAL ANGLE	74.4	7.2	57.0	103.0
lwp ¼ vert angle	75.3	5.4	66.0	96.0
MWP ¼ VERTICAL ANGLE	74.9	7.2	60.0	89.0
mwp ¼ vertical angle	75.7	5.8	64.0	89.0

Note 1. Measurements of real hooves are in capitals and the same measurements taken from photographs in lower case.
Note 2 All lengths are in centimetres and angles in degrees
Note 3 N = 68

Table 5.3.1A.ii Descriptive summary of dorsal measurements taken from the dorsal view of 28 left fore feet

Summary of mean measurements taken from the dorsal view of the hoof capsule and photographs of 28 left fore feet						
Variable	**Mean**	**± SD**	**Min**	**Max**	**Q1**	**Q3**
TOEmdc LENGTH	9.6	1.3	8.1	15.0	8.9	10.0
toemdc length	8.0	1.0	6.7	10.6	7.3	8.4
MWP¼VERTICALLENGTH	8.1	1.4	6.6	13.8	7.1	8.6
mwp ¼ vertical length	6.9	0.9	5.7	10.3	6.3	7.3
LWP¼VERTICALLENGTH	8.1	1.3	6.3	12.6	7.3	9.0
lwp ¼ vertical length	6.6	0.8	5.4	9.0	6.0	6.9
MWP¼ VERTICAL ANGLE	72.9	6.6	58.0	87.0	68.2	76.7
mwp ¼ vertical angle	72.6	5.2	64.8	84.2	68.4	76.7
LWP ¼ VERTICAL ANGLE	74.3	7.4	54.0	86.0	70.0	80.0
lwp ¼ vertical angle	73.9	5.5	63.4	87.5	70.5	77.9

Note 1. Measurements of real hooves are in capitals and the same measurements taken from photographs in lower case.
Note 2 All lengths are in centimetres and angles in degrees
Note 3 N = 28

Table 5.3.1A.iii Descriptive summary of measurements taken from the capsular base of mixed hooves and from photographs of those hooves

Summary mean measurements taken from the capsular base of mixed hooves				
measurement	Mean	±SD	Min	Max
CWWP	12.2	1.4	8.9	15.2
cwwp	12.2	1.4	9.6	15.3
CW50%	11.7	1.5	8.3	14.9
cw50%	11.7	1.4	9.1	14.7
CDWP	7.1	1.1	5.0	9.4
cdwp	7.0	1.0	5.1	9.4
CD	11.3	1.2	8.8	14.5
cd	11.7	2.1	8.1	16.0

Note 1. Measurements of real hooves are in capitals and the same measurements taken from photographs in lower case.
Note 2 All lengths are in centimetres and angles in degrees
Note 3 N = 68

Table 5.3.1A.iv Descriptive summary of mean measurements of the capsular base of 28 left fore feet taken from the sole and from photographs

Descriptive summary of measurements from the capsular base of 28 left fores taken from photos and actual hooves				
Measurement	mean	± SD	min	max
CD actual	11.6	1.5	9.4	15.0
cd photo	11.6	1.7	9.0	15.8
CDWP actual	7.3	1.3	5.1	10.1
cdwp photo	7.2	1.4	5.2	10.3
CWWP	13.9	1.9	11.5	18.8
cwwp photo	14.2	2.0	11.6	19.4
CW50%actual	13.4	1.8	10.7	18.8
CW50% photo	13.7	1.9	10.8	19.4

Note 1. Measurements of real hooves are in capitals and the same measurements taken from photographs in lower case.
Note 2 All lengths are in centimetres and angles in degrees
Note 3 N = 28

Table 5.3.1A.v Summary of mean measurements of the lateral view of mixed hooves taken from the capsule and from photographs

Descriptive summary of mean measurements taken from the lateral view of mixed hoof capsules and their photographs				
Variable	**Mean**	**±SD**	**Min**	**Max**
TOE mdc LENGTH	9.4	1.0	7.4	11.4
toemdc length	9.0	1.1	7.2	12.2
TOEmdc ANGLE	49.4	5.2	35.0	60.0
toemdc angle	49.7	4.0	35.0	61.0
LHEEL LENGTH	4.5	0.9	2.5	7.5
lheel length	4.2	0.8	2.4	6.9
LHEEL ANGLE	39.9	9.0	21.0	60.0
lheel angle	43.8	7.0	28.0	61.0
LHEEL HEIGHT	3.5	0.9	1.5	6.0
lheel height	29.0	0.5	1.7	4.4
LWP TUBULAR LENGTH	7.7	1.1	5.8	11.9
lwp tubular length	7.6	1.1	5.3	9.5

Note 1. Measurements of real hooves are in capitals and the same measurements taken from photographs in lower case.
Note 2 All lengths are in centimetres and angles in degrees
Note 3 N = 68

Table 5.3.1A.vi Summary of mean measurements taken from the lateral view of 28 left fore capsules and their photographs

Descriptive summary of mean measurements taken from the lateral view of 28 left fore feet capsules and their photographs				
Variable	**Mean**	**±SD**	**Min**	**Max**
TOE MDC LENGTH	9.6	1.3	8.1	15.0
toe mdc length	9.5	1.2	7.8	13.5
TOE ANGLE	51.4	4.8	41.0	60.0
toe angle	52.1	3.1	47.7	59.2
HEEL LENGTH	5.4	0.8	4.0	7.5
heel length	4.8	1.0	3.0	7.0
HEEL HEIGHT	3.9	0.8	2.6	5.9
heel height	3.5	0.8	2.2	5.2
HEEL ANGLE	41.0	7.3	25.0	55.0
heel angle	47.2	7.2	30.4	58.8
WP1/4 TUBULE LENGTH	8.7	1.4	7.0	14.0
wp1/4tubule length	8.4	1.2	6.8	11.7

Note 1. Measurements of real hooves are in capitals and the same measurements taken from photographs in lower case.
Note 2 All lengths are in centimetres and angles in degrees
Note 3 N = 28

Table 5.3.1A.vii Summary mean measurements taken from the medial view of mixed hooves and their photographs

Descriptive summary of mean measurements taken from the medial view of mixed hooves' capsules and their photographs				
Measurement	**Mean**	**± SD**	**Min**	**Max**
TOE MDC LENGTH	9.4	0.98	7.4	11.3
toe mdc length photo	8.9	1.1	7.2	11.3
TOE MDC ANGLE	49.5	5.0	35.0	60.0
toe mdc angle	49.7	3.9	37.0	60.0
MHEEL LENGTH	4.8	0.9	2.8	7.5
mheel length	4.3	0.9	2.7	7.5
MHEEL ANGLE	36.6	8.2	20.0	55.0
mheel angle	43.0	7.0	31.0	66.0
MHEEL HEIGHT	3.5	0.9	1.8	6.5
mheel height	2.9	0.6	2.0	4.2
MWP TUBULAR LENGTH	7.2	1.1	5.8	11.7
mwp tubular length	7.7	1.0	5.7	9.2

Note 1. Measurements of real hooves are in capitals and the same measurements taken from photographs in lower case.
Note 2 All lengths are in centimetres and angles in degrees
Note 3 N = 68

Table 5.3.1A.viii Summary of medial measurements from the capsule and photographs of 28 left fore feet

Descriptive summary of medial measurements of 28 left fores taken from photographs and actual hooves				
Variable	**Mean**	**±SD**	**Min**	**Max**
TOE MDC LENGTH	9.6	1.3	8.1	15.0
toemdc length	9.3	1.2	7.7	13.1
TOE ANGLE	51.3	4.8	41.0	60.0
toe angle	51.4	2.9	46.5	57.8
WP ¼ TUBULAR LENGTH	8.4	1.1	6.8	12.4
wp tubular length	8.4	1.4	6.6	13.0
HEEL LENGTH	5.8	1.3	3.3	9.7
heel length	4.8	1.1	2.9	7.3
HEEL HEIGHT	3.9	0.8	2.2	5.5
heel height	3.4	0.7	2.0	5.0
HEEL ANGLE	39.0	7.8	25.0	52.0
Heel angle	46.1	8.2	33.8	73.7

Note 1. Measurements of real hooves are in capitals and the same measurements taken from photographs in lower case.
Note 2 All lengths are in centimetres and angles in degrees
Note 3 N = 28

Descriptive summaries of measurements from hooves with different shapes

Table 5.3.1A.ix Summary of mean liner and angular measurements of hooves with different shaped capsular bases

Descriptive summary of liner and angular measurements of a group of mixed hoof capsules allocated according to the shape of their base plates as described by the ratio of capsular width to capsular depth				
Variable	**Mean**	**StDev**	**Min**	**Max**
Group LONG n=17				
Frontal				
toe length	9.5	1.2	7.4	11.3
toe angle	47.9	5.5	35.0	58.0
heel:toe length	2.2	0.5	1.6	3.4
calculated heel angle	49.3	7.9	37.0	66.0
mean heel angle	40.3	6.0	31.0	52.5
mean heel length	4.5	1.0	3.3	7.0
mean heel height	3.4	1.0	2.3	6.3
Lateral/medial/sagittal				
MWP 1/4 lgth	7.4	1.0	5.9	10.1
LWP 1/4 lgth	7.2	1.2	5.9	9.6
MWP 1/4 vertical angle	80.4	7.8	72.0	103.0
LWP 1/4 vertical angle	80.4	8.2	65.0	95.0
Vertical inclination	0.9	0.1	0.8	1.1
proximal perimeter	26.5	3.8	20.5	36.0
distal perimeter	29.4	3.7	22.3	36.5
Group ROUND n=28				
Frontal				
toe length	9.3	0.9	7.8	11.3
toe angle	48.8	5.9	35.0	60.0
proximal perimeter	27.1	4.5	17.6	35.8
distal perimeter	30.1	4.8	20.9	39.0
mean heel length	4.7	0.9	3.2	7.5
mean heel height	3.6	1.0	2.0	6.0
calculated heel angle	49.0	10.4	34.0	77.0
mean heel angle	39.6	8.1	21.0	55.0
Lateral/medial/sagittal				
RWP 1/4 lgth	7.8	1.0	5.8	10.6
LWP1/4 lgth	7.6	1.0	6.0	10.0
vertical angleRWP 1/4	72.5	6.1	62.0	85.0
vertical angleLWP 1/4	75.5	6.7	63.0	89.0
mean vertical angle	74.0	5.6	62.5	86.5
vertical inclination	0.8	0.1	0.7	1.0

Variable	Mean	StDev	Min	Max
Group WIDE n=46				
Frontal				
toe length	9.3	1.1	7.3	11.4
toe angle	49.9	4.5	35.0	58.0
proximal perimeter	27.3	3.5	17.5	33.1
distal perimeter	30.2	4.3	19.0	38.0
mean heel length	4.7	0.9	3.0	7.2
mean heel height	3.4	0.8	1.8	5.8
calculated heel angle	47.8	8.9	30.0	77.0
mean heel angle	47.8	8.9	30.0	77.0
Medial/lateral/sagittal				
RWP 1/4length	7.7	1.1	5.8	10.8
LWP1/4 length	7.7	1.3	5.0	12.0
Vertical angleRWP 1/4	73.2	6.3	57.0	85.0
Vertical angle LWP 1/4	73.3	8.1	57.0	87.0
mean vertical angle	73.3	6.2	58.0	86.0
vertical inclination	0.8	0.1	0.6	1.0

Table 5.3.1A.x Summary of mean capsular base measurements from a group of mixed hooves with different shaped base plates

Descriptive summary of mean capsular base measurements of mixed hooves allocated to groups according to the shape of their base plate as described by ratios				
Variable	**Mean**	**SD**	**Min**	**Max**
Group LONG				
CD	11.6	1.2	9.4	13.8
CW 50%	10.6	1.2	8.4	12.5
CW WP	11.1	1.1	8.7	12.5
CDWP	7.5	1.0	5.1	8.9
CD:CW50%	0.9	0.0	0.8	1.0
CD:CWWP	1.0	0.1	0.9	1.1
Group ROUND				
CD	11.2	1.7	7.8	14.5
CW 50%	11.4	1.8	8.0	14.7
CW WP	11.7	1.8	8.2	14.7
CDWP	6.8	1.4	5.0	10.0
CD:CW50%	1.0	0.0	1.0	1.1
CD:CWWP	1.0	0.0	1.0	1.1
Group WIDE				
CD	10.8	1.3	7.4	13.4
CW 50%	12.1	1.5	8.2	14.9

Variable	Mean	SD	Min	Max
CW WP	12.6	1.5	8.4	15.2
CDWP	7.0	1.8	4.6	8.9
CD:CW50%	1.1	0.1	1.0	1.3
CD:CWWP	1.2	0.1	1.1	1.4

Table 5.3.1A.xi Summary of mean measurements of 28 left fore hooves grouped according to the difference in the shape of their base plates

Descriptive summary of measurements from 28 left fores hooves allocated to groups according to their base plate ratio				
Measurement	**Mean**	**StDev**	**Min**	**Max**
Group ROUND n=13				
Capsular base				
CD	12.7	1.8	10.8	16.0
CW50%	12.9	1.4	10.7	15.4
CWWP	13.3	1.7	11.5	16.3
CD:CW50%	1.0	0.0	0.9	1.1
CD:CWWP	1.0	0.0	1.0	1.1
CDWP	7.7	1.7	5.5	10.8
Proximal Perimeter	29.0	3.6	24.6	36.8
Lateral, medial and sagittal planes				
TOE mdc LGTH	9.4	0.9	8.1	11.2
TOE ANGLE	51.4	4.3	45.0	58.0
Lateral heel length	5.4	1.0	4.0	7.5
lateral heel height	3.9	0.8	2.6	5.5
Lateral heel angle	42.0	8.5	25.0	55.0
calc lateral heel angle	47.2	4.7	41.0	55.0
Medial heel length	5.4	0.9	4.2	7.0
Medial heel height	3.9	0.8	2.2	5.0
medial calc heel angle	47.1	7.5	29.0	58.0
Medial heel angle	40.9	6.1	35.0	52.0
Frontal Plane				
Medial WP 1/4 length	7.6	0.9	6.6	9.7
Lateral WP 1/4 length	7.8	1.0	6.5	9.1
medial WP 1/4 vert angle	76.5	7.0	66.0	87.0
lateral WP 1/4 vert angle	75.3	5.0	67.0	82.0
Group Wide n= 15				
Capsular Base				
CD	12.0	1.6	9.8	15.0
CW50%	13.9	2.0	11.3	18.8
CWWP	14.4	1.9	11.7	18.8
CD:CW50%	1.2	0.1	1.0	1.3
CD:CWWP	1.2	0.1	1.1	1.3
CDWP	7.3	1.3	5.2	10.3
Prox Perimeter	29.4	4.7	23.5	39.7
Distal Perimeter	32.7	4.8	26.8	45.5
Medial/lateral plane/sagittal plane				

Measurement	Mean	StDev	Min	Max
TOE LGTH	9.8	1.6	8.1	15.0
TOE ANGLE	51.4	5.3	41.0	60.0
Lateral heel lgth	5.5	0.7	4.1	6.6
Lateral heel height	4.0	0.9	2.9	5.9
Lateral heel angle	40.1	6.3	30.0	52.0
calc lateral heel angle	48.0	9.9	36.0	72.0
medial heel lgth	6.2	1.6	3.3	9.7
medial heel height	4.0	1.0	2.5	5.5
medial heel angle	37.5	9.0	25.0	51.0
calc medial heel angle	41.5	6.6	30.0	51.0
Frontal plane				
Medial WP1/4 lgth	8.6	1.6	7.1	13.8
Lateral WP 1/4lgth	8.4	1.5	6.3	12.6
medial WP 1/4 vert angle	69.9	4.7	58.0	77.0
lateral WP 1/4 vert angle	73.5	9.2	54.0	86.0

Comparisons between measurements of hooves with different shapes

Table 5.3.2A.i Summary mean measurements and median ratios of shapes of the hoof capsule grouped according to whether they have round, long or wide capsular bases in a group of mixed feet

shape of capsular base	Long	Round	Wide	significance level
Measurement				
CW50%	[a]10.4	[a,b]11.4	[b]12.1	$p < 0.001$
CD	11.4	11.1	10.8	
CWWP	[a]10.9	[a] 11.6	[b] 12.6	$p<0.001$
Mean Vertical angle	[a] 80.4	[b] 74.0	[b] 73.0	$p< 0.0001$
toe length	9.5	9.3	9.3	
toe angle	47.9	48.8	49.9	
Proximal Perimeter	26.5	27.1	27.3	
Distal Perimeter	29.3	30.1	30.1	
Ratios				
CD:CW50%	[c] 0.94	[a] 1.0	[b] 1.1	$p<0.00001$
CD:CWWP	[c] 0.97	[a] 1.0	[b] 1.1	$p<0.00001$
Vertical Inclination	[b] 0.88	[a] 0.8	[a] 0.8	$p<0.0001$
Toe lgth:CD	1.20	1.2	1.2	
Distal P: Prox P	0.91	0.9	0.9	
Heel :toe lgth	2.20	1.9	2.0	
toe lgth :CWWP	[c] 1.10	[a] 1.2	[b] 1.4	$p<0.05$

Note 1 linear measurements are in centimetres
Note 2 angles are measured in degrees
Note 3 different subscripts indicate differences between measurements at a significance level of $p<0.05$ unless otherwise stated
Note 4 Measurements compared using the ANOVA test; ratios compared using the Kruskal Wallis test

XL

Table 5.3.2A.iii Summary of significant differences between the mean measurements and ratios of 28 left fore with either round and wide base plate capsules

Shape of capsular base	round	wide	P
	measurement ± SD		
CD	12.8 ±1.8	12.0 ±1.6	
CW50%	12.7 ±1.4	13.8 ± 1.9	
CWWP	13.3 ± 1.7	14.3 ±1.9	
MWP ¼ Vert angle	74.5 ± 1.9	69.7 ± 4.7	p<0.05
LWP ¼ vert angle	75.2 ± 5.0	73.4±2.4	
CD: CD50%	1.01	1.15	p< 0.0001
CD : CWWP	1.01	1.19	p< 0.00001
Vertical Inclination	0.84	0.79	

Note 1 all length measurements are in centimetres
Note 2 all angle measurements are in degrees
Note 3 measurements compared using t test; ratios compared using the Mann Witney test

5.3.2A.vii Descriptive summary and comparisons of mean measurements of mixed hooves grouped according to their toe angle

Toe angle group	Low <45° n=15	Normal ≥45° ≤53° n=57	Upright >53° n=19	P
Measurement	measurement ± SD			
CW50%	11.0 ± 1.9	11.8 ± 1.7	11.3 ± 1.3	
CWWP	11.4 ± 1.9	11.8 ± 1.7	11.8 ± 1.3	
Toe length	9.5± 1.2	9.4 ± 1.0	9.2 ± 1.1	
Toe angle	[a] 40.9 ± 3.2	[b] 49.2 ± 2.8	[c] 55.4 ± 1.7	p<0.0001
Distal Perimeter	28.2 ± 4.7	30.6 ± 4.2	29.4 ± 3.9	
MWP ¼ vert angle	71.9 ± 6.1	73.9 ± 7.5	77.6 ± 5.7	
LWP ¼ vert angle	[a] 73.0 ± 7.4	[a] 74.0 ± 8.0	[b] 80.5 ± 6.6	p<0.01
ratios				
CD:CD50%	1.03	1.08	1.02	
CD:CWWP	1.08	1.10	1.08	
Vertical Inclination	[a] 0.81	[a] 0.83	[b] 0.89	p<0.01

Note 1 all linear measurements are in centimetres
Note 2 all angles are measured in degrees
Note 3 Measurements to one decimal place; ratios to 2 decimal places
Note 4 Different subscripts indicate significant differences at p<0.05 unless otherwise stated
Note 5 The ANOVA test was used to compare measurements; the Kruskal Wallis test was used to compare ratios

Table 5.3.2A.viii Summary mean measurements of shape and median ratios for normal and upright toe angle groups (28 feet).

toe angle	Normal ≥ 45° ≤ 53° n = 14	Upright >53 n = 14	P
Measurement	measurement ± SD		
CD	11.9±1.5	12.6 ± 1.7	
CW50%	13.1± 1.3	13.2 ± 1.7	
CWWP	13.5±1.3	13.8 ± 1.9	
toe angle	[a] 48.1± 2.8	[b] 55.1± 2.5	0.0001
M ¼ WP angle	72.0 ± 6.9	74.2 ± 6.5	
L ¼ WP angle	74.5 ± 7.5	74.6 ± 7.7	
Proximal perimeter	27.8 ± 3.2	29.8 ± 4.2	
Distal perimeter	31.8 ± 3.0	32.1 ± 3.8	
ratios			
Vertical Inclination	0.8± 0.0	0.8 ± 0.0	
CD:CW50%	1.1± 0.0	1.1 ± 0.1	
CD:CWWP	1.5 ±0.1	1.1 ± 0.0	

Note 1 all linear measurements are measured in centimetres and all angles are measured in degrees
Note 3 Measurements to one decimal place; ratios to 2 decimal places
Note 4 Different subscripts indicate significant differences at $p<0.05$ unless otherwise stated
Note 5 The t test was used to compare measurements; the Mann Witney test was used to compare ratios

5.3.2A.xiRound intra-group correlations between measurements which showed significant differences compared to correlations between all hooves from the data set of mixed hooves.

RW / RS	RR / ALL	Capsular Depth		CW50%		CWWP		CD: CW50%		CD:CWWP		RWP ¼ Vert angle		Mean vert angle WP ¼	
		Correlation coefficient p value													
CW50%		0.963 0.000	0.975 0.000												
		0.993 0.007	0.743 0.0001												
CWWP		0.967 0.000	0.973 0.000	0.989 0.000	0.975 0.000										
		0.993 0.007	0.701 0.0001	1.000 0.000	0.973 0.0001										
CD: CW50%															
					0.486 0.0001		0.49 0.0001								
CD: CWWP								0.805 0.003	0.576 0.039						
									0.925 0.000						

RW / RS	RR / ALL	CD		CW50%		CWWP		CD:CW50%		CD:CWP		MWP¼ angle		Mean WP ¼ vert angle	
Toe lgth: CWWP		0.755 0.007	0.732 0.004	0.819 0.002	0.747 0.003	0.816 0.002	0.694 0.009								
					0.628 0.0001		0.621 0.0001		0.542 0.0001		0.488 0.0001				
Toe angle			0.574 0.040				0.582 0.037								
Mean WP ¼ Vert angle												0.904 0.000			
												0.999 0.001	0.842 0.000		
Dorsal Inclination												0.907 0.000	0.535 0.055	0.998 0.000	0.995 0.000
					-0.470 0.0001		-0.440 0.0001					0.999 0.001	0.745 0.0001	1.000 0.000	0.999 0.0001

Note 1 Each cell contains Pearson's Correlation for measures or Spearman's Rank coefficient for ratios and P value for groups RW RR RS and ALL
Note 2 Data set of mixed feet

5.3.2A.x Round intra-group correlations between measurements which showed significant differences compared to correlations between all hooves from the data set of 28 left fore feet

ALL / RR	RU / RW	Capsular Depth		CW50%		CWWP		CD: CW50%		CD:CWWP		MWP ¼ Vert angle		LWP ¼ vertical angle		Distal Perimeter		Toe Length: CWWP
			Correlation coefficient p value															
CW50%		0.774 0.000	0.945 0.212															
		0.954 0.003	0.973 0.027															
CWWP		0.833 0.000		0.960 0.000														
		0.987 0.003	0.998 0.002	0.976 0.001	0.983 0.017													
CD: CWWP								0.901 0.000										
								0.795 0.059	0.968 0.032									

Note 1 data set of 28 left fores
Note 2 each cell contains Pearson's Correlation coefficient for linear measures and Spearman's Rank coefficient for ratios and their P value.

Table 5.3.2A.xi Wide intra-group correlations between measurements which showed significant differences compared to correlations between all hooves from the date set of mixed hooves

WW / WS	WR / ALL	Capsular Depth		CW50%		CWWP		CD: CW50%		CD:CWWP		RWP ¼ Vert angle		Mean vert angle WP ¼	
				Correlation coefficient p value											
CW50%		0.819 0.0001	0.933 0.0001												
			0.774 0.0001												
CWWP		0.822 0.0001	0.916 0.0001	0.939 0.0001	0.984 0.0001										
			0.753 0.0001	0.992 0.008	0.973 0.0001										
CD: CW50%				0.576 0.003	0.517 0.028	0.460 0.024	0.516 0.028								
					0.486 0.0001		0.473 0.0001								
CD: CWWP							0.488 0.040	0.766 0.0001	0.893 0.0001						
							0.447 0.0001		0.932 0.0001						
Toe lgth: CWWP															
					0.628 0.0001		0.621 0.0001		0.542 0.0001		0.488 0.0001				
Mean WP ¼ Vert angle												0.782 0.0001			
					-0.468 0.0001								0.842 0.0001		
Dorsal Inclination												0.783 0.0001	0.382 0.117	0.998 0.0001	0.991 0.0001
					-0.470 0.0001								0.844 0.0001	0.994 0.006	0.999 0.0001

Note 1 Each cell contains Pearson's Correlation for measures and Spearman Rank coefficient for ratios and P value for groups WW, WR, WS and ALL
Note 2 Data set of mixed feet

Table 5.3.2A.xii Wide intra-group correlations between measurements which showed significant differences compared to correlations between all hooves from the data set of 28 left fores

ALL / WR	WW	Capsular Depth		CW50%		CWWP	CD: CW50%		CD:CWWP		Toe angle		MWP ¼ Vert angle		LWP ¼ vertical angle		Toe Length: CWWP	
		Correlation coefficient p value																
CW50%		0.774 0.000	0.952 0.000															
		0.967 0.000																
CWWP		0.833 0.000	0.926 0.000	0.960 0.000	0.978													
		0.994 0.001		0.976 0.004														
CD: CWWP							0.901 0.0001	0.621										
Toe angle																		
		0.893 0.041		0.902 0.036					-0.879 0.050									
MWP ¼ Vert angle				-0.556 0.002		-0.612 0.001	-0.489 0.008	-0.78	-0.631 0.0001	-0.8								
Distal Perimeter		0.676 0.0001	0.702 0.024	0.720 0.0001	0.744 0.0	0.693 0.0001	0.6											

ALL / WR	WW	Capsular Depth		CW50%		CWWP		CD: CW50%		CD:CWWP		Toe angle		MWP ¼ Vert angle		LWP ¼ vertical angle		Toe Length: CWWP	
Toe length: CWWP													0.720 0.019	-0.621 0.00011					
								-0.927 0.024		-0.970 0.006		0.845 0.072							
Vertical Inclination				-0.631 0.0001		-0.592 0.001		-0.561 0.002	-0.657 0.039	-0.550 0.002				0.782 0.0001		0.819 0.0001	0.776 0.008	- 0.488 0.008	

Note 1 Each cell contains Pearson's Correlation for measures and Spearman Rank coefficient for ratios and P value for groups WW, WR, WS and ALL
Note 2 Data set of 28 left fore feet

Table 5.3.2A.xiv Long intra-group correlations between measurements which showed significant differences compared to correlations between all hooves from the data set of mixed hooves

LS / LR	ALL	Capsular Depth		CW50%		CWWP		CD: CW50%		CD:CWWP		RWP ¼ Vert angle		Mean vert angle WP ¼	
		Correlation coefficient p value													
CW50%		0.904 0.005	0.979 0.0001												
			0.774 0.0001												
CWWP		0.944 0.001	0.976 0.0001	0.977 0.0001	0.992 0.0001										
			0.753 0.0001		0.973 0.0001										

LS	LR	Capsular Depth		CW50%		CWWP		CD: CW50%		CD:CWWP		RWP ¼ Vert angle		Mean vert angle WP ¼	
	ALL														
CD: CW50%															
					0.486 0.0001		0.473 0.0001								
CD: CWWP		–0.796 0.032						0.829 0.021	0.813 0.014						
									0.932 0.000						
Toe lgth: CWWP									0.830 0.011		0.713 0.047				
					0.628 0.000		0.621 0.000		0.542 0.000		0.488 0.000				
Mean WP ¼ Vert angle												0.888 0.008			
					-0.468 0.0001		-0.439 0.000						0.842 0.000		
Vertical Inclination												0.891 0.007		0.999 0.0001	0.995 0.0001
					-0.470 0.0001								0.844 0.0001		0.999 0.0001

Note 1 Each cell contains Pearson's Correlation for measures and Spearman's Rank for ratios and P value for groups LS,LR and all
Note 2 Data set for mixed feet

Table 5.3.2A.xv Correlations of shape measurement within low, normal and upright toe angle groups compared with correlations of measurements of all hooves: mixed hooves

L / U	N / ALL	Capsular Depth		CW50%		CWWP		CD: CW50%		CD:CWWP		Toe lgth: CWWP		RWP ¼ Vert angle		LWP ¼ angle	
CW50%		0.936 0.005	0.736 0.000														
		0.645 0.003	0.774 0.0001														
CWWP		0.924 0.001	0.714 0.0001	0.970 0.0001	0.976 0.0001												
		0.598 0.007	0.753 0.0001	0.952 0.0001	0.973 0.0001												
CD: CW50%					0.553 0.0001		0.544 0.0001										
		–0.470 0.042			0.486 0.0001		0.473 0.0001										
CD: CWWP							0.498 0.0001	0.800 0.0001	0.943 0.0001								
								0.941 0.0001	0.932 0.0001								
Toe lgth: CWWP		0.619 0.011		0.714 0.002	0.634 0.0001	0.697 0.003	0.627 0.0001		0.667 0.0001		0.604 0.0001						
					0.628 0.0001		0.621 0.0001		0.542 0.000		0.488 0.0001						
LWP ¼ Vert angle					-0.466 0.0001		-0.463 0.0001										
				-0.605 0.006		–0.610 0.006								0.553 0.014	0.484 0.000		

L / U	N / ALL	Capsular Depth		CW50%		CWWP		CD: CW50%		CD:CWWP		Toe lgth: CWWP		RWP ¼ Vert angle		LWP ¼ angle	
Vertical Inclination					-0.499 0.0001		-0.481 0.0001		-0.500 0.0001				-0.507 0.0001	0.718 0.002	0.847 0.000	0.801 0.000	0.866 0.000
					-0.470 0.0001	-0.512 0.025								0.866 0.000	0.844 0.000	0.894 0.000	0.876 0.000
Distal Perimeter		0.948 0.0001	0.853 0.0001	0.942 0.0001	0.884 0.0001	0.931 0.0001	0.853 0.0001					0.740 0.001					
		0.849 0.0001	0.873 0.0001	0.825 0.0001	0.890 0.0001	0.782 0.0001	0.865 0.0001										

Note 1 Each cell contains Pearson's Correlation coefficient for measures and Spearman Rank coefficient for ratios and P value for groups L,N,U and all
Note 2 Data set of 91 feet
Note 3 N = normal, U = upright, L = low toe angles

Table 5.3.2A.xvi. Correlations between hoof measurements within hooves grouped according to their toe angle (28 left feet)

ALL N	U	Capsular Depth	CW50%	CWWP	CD: CW50%	CD:CWWP	MWP ¼ Vert angle	LWP ¼ vertical angle	Toe Length: CWWP
CW50%		0.774 0.0001							
		0.713 0.006							
CWWP		0.833 0.0001	0.960 0.0001	0.9 58					
		0.754 0.003	0.932 0.0001						
CD: CW50%									
		–0.594 0.032							
CD: CWWP					0.901 0.0001	0. 85			
		-0.637 0.019			0.899 0.0001				
MWP ¼ Vert angle				–0.612 0.001	–0.6 75	-0.489 0.008	–0.631 0.000	–	
				–0.591 0.033		-0.558 0.047	–0.643 0.018		
Distal Perimeter		0.676 0.0001	0.720 0.0001	0.693 0.0001					
		0.908 0.0001	0.829 0.0001	0.779 0.002					

ALL N	U	Capsular Depth		CW50%		CWWP		CD: CW50%		CD:CWWP		MWP ¼ Vert angle		LWP ¼ vertical angle		Toe Length: CWWP	
Toe length: CWWP			0.565 0.035		0.757 0.002		0.761 0.002					–0.621 0.0001	–0.919 0.0001				
						0.521 0.068											
Vertical Inclination				-0.631 0.0001	-0.679 0.008	-0.592 0.001	-0.606 0.022	-0.561 0.002		-0.550 0.002		0.782 0.0001	0.784 0.001	0.819 0.0001	0.845 0.0001	–0.488 0.008	–0.799 0.001
				-0.573 0.041		-0.540 0.057		-0.631 0.021		-0.583 0.037		0.754 0.003		0.781 0.002			

Table 5.3.2A.xvii Summary of Correlation coefficients between relationships identified from matrix scatter plots between measurements taken from mixed data set of feet and their ratios considered for allocating hooves to groups based on their geometry

	CD	50%CW	CWWP	CD: CW50%	CD:CWWP	CD: toelgth	toelgth: proxP	LWP1/4 vert angle	RWP1/4 vert angle	Proximal perimeter
		Spearman's Rank P								
50%CW	0.743 0.0001									
CWWP	0.701 0.0001									
CD:CW50%		0.486 0.0001	0.49 0.0001							
CD:CWWP			0.462 0.0001							
CD: toelgth	-0.551 0.0001									
toelgth: ProxP		0.489 0.0001				-0.781 0.0001				
toelgth: CWWP		0.627 0.0001	0.629 0.0001	0.553 0.0001	0.509 0.0001	-0.684 0.0001	0.717 0.0001			
LWP1/4 vert angle			-0.475 0.0001							
MWP ¼ vert angle								0.758 0.001		
Vertical Inclination		-0.505 0.0001	-0.507 0.0001					0.894 0.0001	0.745 0.0001	
Distal Perim	0.873 0.0001	0.89 0.0001	0.865 0.0001							0.888 0.001
Proximal Perim	0.837 0.001	0.834 0.0001	0.821 0.0001							

6 Interactions between shape, trace element concentration, fracture toughness and cracks scores

Appendix interaction chapter

6.3.1A Crack scores

Table 6.3.1A.i Summary of crack numbers, areas and median scores, for mixed feet grouped by their base shape

base shape	long	round	wide
whole hoof	median score *range*		
SSA	1001.0 *85.0-2166.0*	[a]1009 .0 *0.0-7886.0*	[b]2104.0 *65.0-12328.0*
GSA	2009 .0 *85.0- 6362.0*	4139.0 *0.0-39337.0*	8771.0 *65.0 – 65747.0*
TCNo	5.7 *1.0-11.0*	4.6 *0.0-10.0*	5.7 *1.0-13.0*
GSNo	22.0 *8.0- 44.0*	[a]20.0 *2.0-44.0*	[b]58.0 *16.5- 120.0*
TCA mm²	349.5 *42.4 – 547.7*	243.0 *0.0-1459.0*	462.0 *35.0 - 20550*
medial quarter			
M/4no	1.6 *0.0-4.0*	1.5 *0.0-4.0*	2 .0 *0.0-8.0*
SSNoR/4	4.6 *0.0-12.0*	4.6 *0.0-15.0*	8.6 *0.0-25.0*
GSNoM/4	10.4 *0.0-34.0*	7.7 *0.0-34.0*	24 .0 *0.0-65.0*
M/4 area mm²	104.4 *0.0-312.4*	50.7 *0.0-222.4*	79.4 *0.0-343.0*
SSAM/4	423.0 *0.0-1286.0*	151.2 *0.0-829.0*	364.0 *0.0-2059.0*
GSAM/4	1149.0 *0.0-3627.0*	301.0 *0.0-2096.0*	1362.0 *0.0-10980.0*
toe			
toe no	2.0 *0.0-5.0*	1.7 *0.0-6.0*	1.8 *0.0-4.0*
SSNoT	4.3 *0.0-14.0*	4.4 *0.0-14.0*	5.6 *0.0-12.0*
GSNoT	5.4 *0.0-20.0*	7.7 *0.0-32.0*	18.7 *0.0-64.0*
toe mm²	116.3 *0.0-298.0*	140.0 *0.0-1197.0*	157.0 *0.0-425.0*
SSAT	303.0 *0.0-996.0*	717.0 *0.0-7183.0*	691.0 *0.0-2550.0*
GSAT	452.0 *0.0-1788.0*	3570.0 *0.0-38310.0*	3136.0 *0.0-13601.0*
lateral quarter			
L/4no	2.1 *0.0-5.0*	1.4 *0.0-5.0*	1.9 *0.0-4.0*
SSNoL/4	7.1 *2.0-15.0*	4.5 *0.0-16.0*	7.3 *0.0-19.0*
GSNoL/4	12.9 *2.0-32.0*	10.7 *0.0-68.0*	22.3 *0.0-64.0*
SSAL/4	375 .0 *20.0-922.0*	[a]110.3 *0.0-302.9*	[b]575.0 *0.0-1986.0*
L/4 mm²	111.0 *6.5- 184.4*	[a]36.4 *0.0-151.4*	[b]129.3 *0.0-447.2*
GSAL/4	796.0 *26.0-2950.0*	[a]250.0 *0.0-1434*	[b]2056.0 *0.0-6926.0*

Note 1* No diffs after Bonferroni correction and Wilcoxon Rank Sum
Note 2 different superscripts indicate differences at a significance level of $p<0.05$

Table 6.3.1A.ii Summary of crack scores in 28 left fores grouped according to the shape of their capsular base

	Round			Wide		
Variable	**Median**	*range*		**Median**	*range*	
SSA	2813.0	*533.0*	*6982.0*	3654.0	*779.0*	*10277.0*
GSA	8716.0	*777.0*	*31079.0*	11831.0	*919.0*	*44384.0*
TCA mm	647.5	*169.3*	*1299.0*	840.0	*277.0*	*1893.0*
SSAM/4	894.0	*250.0*	*2820.0*	1358.0	*91.0*	*4384.0*
GSAM/4	2303.0	*372*	*10600*	4671.0	*105.0*	*15507.0*
M/4mm	219.3	*75.1*	*568.1*	306.4	*24.6*	*955.6*
toe mm	204.9	*0.0*	*751.2*	373.2	*14.3*	*1266.0*
SSAT	867.0	*0.0*	*3010.0*	1666.0	*14.0*	*723.07*
GSAT	2785.0	*0.0*	*13888.0*	5723.0	*14.0*	*34781*
SSAL/4	984.0	*0.0*	*3369.0*	630.0	*0.0*	*2188.0*
GSAL/4	3086.0	*0.0*	*13100.0*	1401.0	*0.0*	*5360.0*
L/4mm	221.2	*0.0*	*677.6*	163.3	*0.0*	*506.0*
SSNo	39.7	*11.0*	*61.0*	37.29	*14.0*	*64.0*
GSNo	102.2	*14.0*	*228.0*	98.9	*26.0*	*264.0*
TCNo	10.31	*4.0*	*17.0*	9.8	*6.0*	*15.0*
M/4no	3.8	*1.0*	*9.0*	3.0	*1.0*	*6.0*
GSNoM/4	35.2	*8.0*	*124.0*	32.5	*3.0*	*116.0*
SSNoM/4	14.9	*4.0*	*38.0*	11.4	*3.0*	*26.0*
toe no	3.1	*0.0*	*6.0*	3.4	*2.0*	*9.0*
SSNoT	11.2	*0.0*	*28.0*	14.4	*2.0*	*39*
GSNoT	29.2	*0.0*	*96.0*	42.6	*2.0*	*160.0*
L/4 no	3.2	*0.0*	*5.0*	3.3	*0.0*	*7.0*
SSNoL/4	13.7	*0.0*	*24.0*	12.1	*0.0*	*24.0*
GSNoL/4	38.5	*0.0*	*96.0*	25.0	*0.0*	*48.0*

Table 6.3.1A.iii Summary of crack numbers and areas and crack scores for mixed feet grouped according to their vertical angles at the widest point of quarters,

Vertical inclination	regular	upright	wide
medial quarter	number/score, *range*		
M/4 no	1.7 *0.0-8.0*	1.0 *0.0-2.0*	2 .0 *0.0-4.0*
SSNoM/4	5.8 *0.0-25.0*	3.0 *0.0-8.0*	8.0 *0.0-18.0*
GSNoM/4	12.6 *0.0-65.0*	11.7 *0.0-34.0*	21.0 *0.0-64.0*
M/4mm	65.0 *0.0-321.4*	41.3 *0.0-74.0*	95.0 *0.0-343.1*
SSAM/4	236.0 *0.0-1285.6*	112.0 *0.0-262.0*	435.0 *0.0-2059.0*
GSAM/4	559.0 *0.0-3627.0*	464.0 *0.0-1317.0*	1589.0 *0.0- 11980.0*
toe			
toe no	2 .0 *0.0-5.0*	2.3 *0.0-6.0*	2.2 *0.0-4.0*
SSNoT	4.3 *0.0-14.0*	3.7 *0.0-11.0*	6.0 *00.-12.0*
GSNoT	8.8 *0.0-48.0*	3.7 *0.0-11.0*	18.7 *0.0-64.0*
SSAT	352.0 *0.0-1726.0*	208.0 *0.0-503.0*	1033.0 *0.0-7183.0*
toe area mm^2	98.2 *0.0-313.2*	88.4 *0.0-167.5*	209.1 *0.0-1197.2*
GSAT	1216.0 *0.0-9205.0*	288.0 *0.0-607.0*	5076.0 *0.0-38310.0*
lateral quarter			
L/4 no	1.6 *0.0-5.0*	1.3 *1.0-2.0*	2.0 *0.0-5.0*
SSNoL/4	5.6 *0.0-15.0*	4.7 *3.0-6.0*	7.6 *0.0-19.0*
GSNoL/4	12.0 *0.0-64.0*	14.0 *4.0-32.0*	22.7 *0.0-68.0*
SSAL/4	351.0 *0.0-1672.0*	289.0 *135.0 -502.0*	442.0 *0.0-1986.0*
L/4 area mm^2	93.7 *0.0-334.4*	85.0 *38.3 – 167.2*	100.0 *0.0-447.2*
GSAL/4	1035.0 *0.0-6210.0*	688.0 *170.0-1224.0*	1540.0 *0.0-6926.0*
whole hoof			
TCNo	5.0 *0-13*	4.7 *2-9*	5.9 *1-10*
SSNo	15.7 *0.0-43.0*	12.3 *6.0-17.0*	22.1 *2.0-39.0*
GSNo	32.7 *0.0-129.0*	30.7 *8.0-66.0*	64.7 *2.0-160.0*
TCA mm^2	268.6 *0.0-757.2*	214.7 *88.0-335.0*	521.0 *3.0-2055.0*
SSA	913.0 *0.0-3706.0*	609.0 *330.0-1005.0*	2459.0 *5.0-12328.0*
GSA	2708.0 *0.0-12427.0*	1416.0 *366.0-2541.0*	10734.0 *5.0-65747.0*

Table 6.3.1A.iv Descriptive summary of crack numbers and areas crack scores for mixed feet grouped according to their toe angles,

toe angle	low	regular	upright
medial quarter	number/area *range*		
M/4 no	1.0 *0.0-2.0*	2.0 *0.0-8.0*	1.6 *0.0-4.0*
SSNoM/4	5.6 *0.0-13.0*	7.0 *0.0-25.0*	5.1 *0.0-15.0*
GSNoM/4	17.4 *0.0-65.0*	16.9 *0.0-64.0*	12.1 *0.0-34.0*
M/4mm	26.8 *0.0-89.0*	93.4 *0.0-341.0*	53.0 *0.0-343.1*
SSAM/4	81.4 *0.0-2744.0*	400.0 *0.0-2059.0*	177.0 *0.0- 829.0*
GSAM/4	112.1 *0.0-385.3*	206.0 *0.0-1971.0*	512.0 *0.0-1317.0*
toe			
toe no	2.0 *0.0-4.0*	1.7 *0.0-5.0*	2.0 *0.0-6.0*
SSNoT	4.4 *0.0-12.0*	5.1 *0.0-14.0*	4.7 *0.0-14.0*
GSNoT	6.4 *0.0-20.0*	15.9 *0.0-64.0*	5.9 *0.0-18.0*
SSAT	147.0 *0.0-441.2*	890.0 *0.0-7183.0*	106.6 *0.0-277.6*
toe area mm^2	58.2 *0.0-163.0*	190.8 *0.0-38310.0*	53.3 *0.0-109.4*
GSAT	473.0 *0.0-1995.0*	324.0 *0.0-607.0*	136.2 *0.0-395.2*
lateral quarter			
L/4 no	1.8 *0.0-4.0*	1.9 *0.0-5.0*	1.4 *0.0-4.0*
SSNoL/4	6.6 *0.0-13.0*	6.9 *0.0-19.0*	4.4 *0.0-12.0*
GSNoL/4	8 .0 *0.0-64.0*	12.0 *0.0-68.0*	9.1 *0.0-32.0*
SSAL/4	301.0 *0.0-1164.0*	293.0 *0.0-1986.0*	48.6 *0.0-229.5*
L/4 area mm^2	96.3 *0.0-194.0*	118.8 *0.0-447.2*	21.8 *0.0-49.0*
GSAL/4	325.0 *0.0-6210.0*	410.0 *0.0-6926.0*	50.0 *0.0-1224.0*
whole hoof			
TCNo	4.8 *1.0-10.0*	5.6 *1.0-13.0*	5.0 *0.0-9.0*
SSNo	15.0 *2-32*	16.5 *2-43*	17.0 *0-26*
GSNo	20.0 *2.0-129.0*	36.0 *2.0-160.0*	30.0 *0.0-66.0*
TCA mm^2	202.7 *2.7-373.7*	482.0 *35.0-2055.0*	128.1 *0.0-331.8*
SSA	423.0 *5-1606*	[a]927.0 *65-12328*	[b]330.0 *0-1187*
GSA	423.0 *5.0-8097.0*	[a]1492.0 *65.0-65747.0*	[b]336.0 *0.0-2541.0*

Note 1 different superscripts indicate significant differences at $p<0.05$

Table 6.3.1A.v Summary of median crack scores at different anatomical positions of 28 left fores grouped by their different shapes

capsular base	L/4 GSA	M/4 GSA	toe GSA
round	1921.5	1316.7	498.0
wide	723.0	2256.7	3544.6
toe angle			
normal	1229.0	1710.0	1907.0
upright	1137.0	1354.0	3545.0
side angle			
regular	939.9	1430.7	3247.0
upright	[b]3539.5	1351.6	[a]498.0
wide	1142.0	1278.0	3498.0
base and angle group			
round regular	1648.0	1227.0	748.8
round upright	[b]3540.0	1357.0	[a]499.0
round wide	2539.0	3120.0	2295.0
wide regular	382.7	4651.7	3591.5
wide wide	1141.8	918.2	3497.6
capsular base	**L/4 GSNo**	**M/4 GSNo**	**mdc GSNo**
round	28	22	12
wide	26	20	24
toe angle			
normal	24	20	24
upright	32	24	20
side angle			
regular *	9	24	22
upright	28	20	12
wide	32	20	28
base and angle group			
round regular	32	23	17
round upright	28	20	12
round wide	26	42	40
wide regular	14	32	22
wide wide	32	20	28

Note 1 data set 28 left fores
Note 2 * no significant differences once Bonferroni correction used
Note 3 different superscripts indicate significance at $p<0.05$

6.3.2A Fracture toughness

Table 6.3.2A.i Descriptive summary of mean impact measurements of mixed hooves subsequently grouped according to a) their base plate shape, b) toe angles, or c) side angles

Base plate	long	round	wide
	Fracture toughness±SD *range*		
IR	304.0±120.8 *162.7 - 528.8*	283.4±132.6 *122. –569.5*	270.8±76.7 *81.4–420.4*
IS	27.6 ± 8.1 *15.0- 39.0*	29.0 ± 8.8 *17.2 – 48.2*	29.0 ± 7.9 *9.9 – 44.2*

toe angles	low	normal	upright	side angles	regular	upright	wide
IR	227.8±109.5 *95.0- 366.0*	291.6±100.2 *81.4 –569.5*	270.8±98.5 *122.0- 423.1*	IR	261.9±108.4 *81.4 – 447.5*	304.9±102.2 *208.0–528.8*	291.0±91.7 *203.4– 569.5*
IS	26.2 ± 9.95 *13.0-38.0*	29.4 ± 8.13 *9.9 – 48.2*	27.9 ± 7.1 *17.2 – 38.7*	IS	27.5 ± 9.4 *9.9 – 44.2*	28.5 – 5.9 *22.3 – 39.0*	30.3 ± 7.0 *20.0–48.2*

Note 1 IR impact resistance J/m
Note 2 IS impact strength kJ/m^2

Table 6.3.2A.ii Descriptive summary of impact scores at different anatomical positions in 28 left fores with different shaped capsular bases

base shape	round	wide
	fracture toughness ±SD *range*	
M/4 IR	347.8±151.3 *217-786.5*	338.4±66.3 *271.2-515.3*
M/4 IS	43.2±14.0 *27.9- 75.3*	40.2±9.2 *32.6- 68.9*
mdc IR	274.3±68.2 *155.9- 366.1*	300.0±71.2 *161.4 – 433.9*
mdc IS	29.0±7.4 *13.2 – 44.5*	28.5±6.9 *15.4 – 43.8*
L/4 IR	353.6±103.6 *244.1- 569.5*	353.6±98.4 *244.0-556.0*
L/4 IS	44.9±14.0 *23.0- 78.5*	41.3±8.3 *29.7 – 57.8*

Note 1 IR impact resistance J/m
Note 2 IS impact strength kJ/m^2
Note 3 M/4 medial quarter; L/4 lateral quarter; mdc toe

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Table 6.3.2A.iii **Descriptive summary of impact scores within anatomical positions of hooves with different shaped capsular base or dorsal inclination or toe angle**

capsular base	L/4 IS	M/4 IS	mdc IS
round p <0.001	[a]44.9 ± 14.0	[a]43.2 ± 14.0	[b]28.9 ± 7.4
wide p < 0.0001	[a]41.3 ± 8.2	[a]40.2 ± 9.2	[b]28.5 ± 6.9
toe angle			
normal p <0.01	[a]43.1 ± 14.8	[a]43.3 ± 11.7	[b]29.5 ± 5.9
upright p <0.01	[a]42.2 ± 7.9	[a]40.0 ± 12.2	[b]28.8 ± 7.7
Vertical inclination			
regular	43.7 ± 9.4	[a]45.0 ± 11.6	[b]32.5 ± 7.0
upright	[a]40.8 ± 5.2	34.4 ± 12.7	[b]24.7 ± 9.0
wide	[a]42.5 ± 14.1	40.0 ± 11.8	[b]26.0 ± 5.2

Note 1 data set of 28 left fore
Note 2 superscripts indicate significant differences at P<0.05 unless otherwise noted

6.3.3A Trace elements

Table 6.3.3A.i Descriptive summary of copper, zinc, calcium and ratios in mixed hooves grouped according to the shape of their capsular base.

capsular base shape	long	round	wide
	mean ± SD *range or* median *quartiles*		
copper mg/kgDM	24.6 ± 7.6 *20.7- 41.7*	22.6 ± 6.8 *12.7 – 39.5*	23.6 ± 5.8 *18-41.2*
calcium mg/kg DM	457.0 ± 249.0 *128.0- 695.0*	837.0 ± 435.0 *300.0-1747.0*	834.3 ± 357.8 *308.0 -1378.3*
zinc mg/kgDM	153.1±73.1 *31.0–242.9*	158.5 ± 54.9 *80.0- 287.9*	179.2 ± 50 *100.7 – 277.5*
Ca/C	- 0.000482 *-0.00942 -0.00084*	-0.0035 *-0.00863 – 0.00009*	-0.00114 *-0.00574 – 0.01398*
Cu/C	0.00038 *0.00001 – 0.0021*	0.00007 *0.00001- 0.00031*	0.00005 *0 – 0.00023*
Zn/C	0.00039 *0.00013- 0.00085*	0.00036 *0.00005- 0.00071*	0.00033 *0.00002 – 0.00077*
Cu: Ca	21.30 *6.0- 32.7*	38.30 *13.3 – 70.0*	35.80 *1.4 – 61.3*
Ca: Cu	0.07 *0.03 – 0.17*	0.03 *0.01 – 0.08*	0.07 *0.02 – 0.71*
Cu: Zn	7.00 *0.74 – 11.5*	7.10 *4.5 – 9.6*	7.70 *4.1 – 9.7*
Zn : Cu	0.31 *0.09 – 1.3*	0.15 *0.1- 0.22*	0.14 *0.1 – 0.24*
Zn: Ca	[a]2.50 *1.2 – 3.7*	[b]5.10 *2- 9.5*	[b]4.50 *0.28- 6.7*
Ca: Zn	[a]0.49 *0.27 – 0.86*	[b]0.23 *0.1 – 0.5*	[b] 0.40 *0.15 – 3.6*

Note 1 different subscripts indicate significant differences at p<0.05

Table 6.3.3A.ii Descriptive summary of trace elements and ratios measured by ICP-MS in mixed hooves grouped according to their toe angle

toe angle	low	regular	upright
	mean ± SD ,*range* Median ,*quartiles*		
copper mg/kgDM	21.9 ± 2.6 *17.8- 24.5*	24.4 ± 6.8 *18.0– 41.7*	21.6± 6.5 *12.6-33.2*
calcium mg/kg DM	481.0±272.0 *3.0- 716.0*	823.0 ±398.8 *128.0-1747.0*	800.0 ±373.0 *300.0 -1256.0*
zinc mg/kgDM	116.1± 16.3 *10.7–141.5*	177.7 ± 59.2 *31.0- 287.9*	170.1 ± 50.0 *80.0 – 227.5*
Cu: Ca	2.5 *1.4 - 40.3*	35.1 *6.0– 69.2*	38.2 *1.4 – 61.3*
Ca: Cu	0.17 *0.02– 0.70*	0.04 *0.01– 0.170*	0.03 *0.01 – 0.08*
Cu: Zn	[a]5.4 *4.1 – 8.0*	[b]7.60 *0.74– 11.5*	[b]8.2 *6.3 – 9.5*
Zn : Cu	0.31 *0.13 – 0.24*	0.19 *0.09 -1.30*	0.13 *0.10 – 0.16*
Zn: Ca	4.10 *0.28 – 6.7*	4.2 *1.2 - 9.5*	4.5 *2.0- 7.3*
Ca: Zn	0.88 *0.15 – 3.6*	0.3 *0.1 – 0.86*	0.25 *0.14 – 0.50*

Note 1 different subscripts indicate significant differences at p<0.05

Table 6.3.3A.iii Descriptive summary of qualitative trace element ratios in mixed hooves grouped according to their toe angle

anatomical position and ratio TEppm/Cppm	low toe angle	normal toe angle	upright toe angle
medial	TEppm/Cppm : TEppm/Cppm		
Ca/C: Cu/C	0.12	[a]0.047	[b]0.011
Ca/C : Zn/C	2.50	0.040	0.890
Zn/C : Cu/C	0.05	0.094	0.023
Cu/C : Ca/C	8.20	13.300	41.90
Zn/C : Ca/C	0.40	1.900	0.74
Cu/C : Zn/C	20.2	10.600	42.20
lateral			
Cu/C : Zn/C	28.9000	10.900	15.3000
Cu/C : Ca/C	-167.3000	-8.000	-19.1000
Zn/C : Ca/C	-5.7900	-1.700	-2.3000
Zn/C : Cu/C	0.0340	0.100	0.0650
Ca/C: Cu/C	-0.0006	-0.002	-0.0011
Ca/C : Zn/C	-0.1700	-0.318	-0.2800
mdc			
Ca/C: Cu/C	-0.0048	0.01	0.006
Ca/C : Zn/C	-0.8900	0.54	0.440
Cu/C : Ca/C	-20.7000	38.60	28.600
Cu/C : Zn/C	18.5000	37.70	24.200
Zn/C : Cu/C	1398.0000	7774.00	5657.000
Zn/C : Ca/C	-0.0300	1.30	0.920

Note 1 different subscripts indicate differences at a significance level of p<0.05

Table 6.3.3A.iv Descriptive summary of qualitative trace elements in 28 feet grouped according to their toe angle

medians and differences in qualitative trace elements in 28 left fores grouped according to toe angle						
	Normal			Upright		
Variable	Median	Q1	Q3	Median	Q1	Q3
	TEppm/Cppm					
M/4 Ca/C	0.0005	0.0001	0.0009	0.0008	-0.0045	0.0014
Cu/C	0.0000	0.0000	0.0001	0.0000	0.0000	0.0001
Zn/C	[a]0.0002	0.0000	0.0006	[b]0.0009	0.0004	0.0019
L/4 Ca/C	-0.0086	-0.0135	0.0017	-0.0104	-0.0128	-0.0003
Cu/C	0.0005	0.0002	0.0013	0.0002	0.0001	0.0016
Zn/C	0.0039	0.0030	0.0060	0.0038	0.0028	0.0045
mdc Ca/C	0.0003	0.0000	0.0008	0.0003	-0.0001	0.0010
Cu/C	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Zn/C	0.0003	0.0001	0.0004	0.0004	0.0001	0.0008

Note 1 different subscripts indicate significant differences at $p<0.05$

Correlations

6.3.4 Between fracture toughness and crack scores

Table 6.3.4A.i Summary of correlations between fracture toughness and crack scores in hooves with different base shapes or different angles

with long capsular bases		
Impact resistance	**whole hoof**	**toe**
TCA	0.77 p= 0.043	0.803 p = 0.03
SSA		0.86 p= 0.013
impact strength		
TCA	0.738 p = 0.058	

with round bases		
Impact resistance	**whole hoof**	**toe**
TCA	0.807 p = 0.003	0.806 p = 0.003
GSA		0.706 p = 0.015
SSA		0.726 p = 0.012
GSNo		0.806 0.003
impact strength		
TCA		0.75 p = 0.008
GSA		0.715 p = 0.013
SSA		0.729 p = 0.011
GSNo		0.806 0.003

Regular vertical inclination		
Impact resistance	**whole hoof**	**toe**
TCA	0.556 p = 0.021	0.576 p = 0.015
SSA	0.497 p = 0.042	
GSNo	0.553 p = 0.028	
TCNo		0.513 p = 0.049
SSNo		0.495 p = 0.059
impact strength		
TCA	0.569 p= 0.017	0.503 p = 0.04
GSA	0.502	

Wide vertical inclination	
Impact resistance	**toe**
TCA	0.711 p = 0.004
GSA	0.655 p = 0.011
SSA	0.677 0.008
impact strength	

	p = 0.04				
SSA	0.501 p = 0.041		TCA	0.574 p = 0.032	
GSNo	0.577 p = 0.015		SSA	0.529 0.052	
regular toe angle			**upright toe angle**		
Impact resistance	**whole hoof**	**toe**	**Impact resistance**	**whole hoof**	**toe**
TCA	0.431 p = 0.045	0.692 0.0001	GSA		0 772 p = 0.042
GSA		0.591 p = 0.004	SSA		0.858 p = 0.014
SSA		0.637 0.001	SSNo	0.874 p = 0.01	
impact strength			**impact strength**		
TCA		0.559 p = 0.007	SSNo	0.822 0.023	
GSA		0.498 p = 0.018			
SSA		0.528 p = 0.012			

Note 1 data set of mixed feet
Note 2 cell contains Spearman's rank correlation coefficient and p value

Table 6.3.4A.ii Summary correlations between fracture toughness and crack scores in hooves with different shapes

shape	toe angle normal	toe angle upright	base round	base wide	vert inc regular	vert inc upright	vert inc wide	round regular	round upright	round wide	wide wide
Impact score vs crack score	spearman's Rank correlation coefficient p value										
M/4 IS		none	none		none		none	none		none	none
M/4 area	-0.584 p=0.048										
SSAM/4	-0.603 p = 0.038										
M/4 no				0.682 p= 0.007		-0.997 p=0.047			-0.997 p=0.047		
GSNoM/4						-1.0 p=0.011			-1.0 p=0.011		
SSNoM/4						-0.999 p=0.024			-0.999 p=0.024		
M/4 IR		none			none		none	none		none	
GSAM/4						1.0 p=0.004			1.0 p=0.004		
M/4 mm						0.886 p=0.054					
SSNo M/4			-0.57 p=0.042								
M/4 no				0.725 p=0.003		-0.991 p=0.088					-0.673 p=0.047
mdc IR			none	none	none	none	none	none	none	none	none
GSAT	0.593 p =0.042										
toe no		-0.602 p = 0.023									
SSNoT		-0.528 p= 0.053									
mdc IS			none	none	none		none	none			none

shape	toe angle normal	toe angle upright	base round	base wide	vert inc regular	vert inc upright	vert inc wide	round regular	round upright	round wide	wide wide
SSAT	0.695 p =0.012										
GSAT	0.683 p =0.014										
SSNoT	0.652 p =0.022					-0.998 p=0.038			-0.998 p=0.038	-0.998 p=0.03	
GSNoT	0.869 0.0001					-0.999 p=0.034			-0.999 p=0.034	-0.999 p=0.034	
toe no		-0.659 0.01									
L/4 IR	none		none	none	none	none	none	none	none	none	none
L/4 no		-0.526 p = 0.053									
SSNoL/4		-0.577 0.053									
L/4 IS	none	none	none	none		none	none		none		none
GSAL/4					0.616 p=0.044			0.767 p=0.059			
SSAL/4					0.657 p=0.028			0.875 p=0.023			
L/4 mm					0.638 p=0.035			0.835 p=0.039			
L/4 no										-0.952 p=0.048	

Note 1 data set 28 fore feet

Trace elements and fracture toughness

Table 6.3.4A.iii Correlations between trace elements and fracture toughness within groups of hooves of different shapes, (28 fore feet)

shape groups	N toe angle	U toe angle	RR	RU	RW	WR	WW
correlations vs. tes and FT	Spearman's Rank correlation p value						
M/4 Impact resistance							none
M/4 Calcium/C	-0.775 p=0.003				1.0	-0.872 p=0.054	
M/4 copper/C		0.678 p=0.008		1.0			
M/4 Zinc/C			-0.841 p=0.036				
M/4 Impact strength							none
M/4 calcium/C	-0.72 p = 0.008		-0.829 p= 0.042		1.0	-0.9 p=0.037	
M/4 copper/C				1.0			
L/4 Impact resistance	none			none		none	
L/4 zinc/C		0.673 p=0.008	0.870 p=0.024		1.0		
L/4 Copper/C			0.986 p=0.0001		1.0		-0.833 p=0.005
L/4 impact strength	none	none	none	none		none	none
L/4 zinc/C					1.0		
L/4 copper/C					1.0		
mdc impact strength	none	none	none		none	none	none
mdc calcium/C				1.0			

Table 6.3.4A.iv Summary of correlations between trace elements and fracture toughness at the toe in mixed hooves with different toe angles and vertical inclination

grouped by angles	regular vertical inclination	wide vertical inclination	upright toe
impact resistance			
Zn/C	-0.477 p = 0.029	0.522 p=0.041	
Cu/C		0.811 p =0.0001	
Ca/C		-0.668 p=0.009	
Ca mg/kgDM			0.737 p=0.015
Cu: Ca			0.755 p = 0.05
Zn: Ca			0.696 p = 0.059

grouped by angles	regular vertical inclination	wide vertical inclination	upright toe
impact strength			
Zn/C	-0.445 p= 0.043	0.531 p=0.051	
Cu/C	-0.999 p= 0.035	0.708 p=0.005	
Ca mg/kgDM			0.854 p=0.002
Cu: Ca			0.818 p= 0.025
Cu : Zn			0.807 p = 0.028
Zn : Cu			-0.789 0.035

Table 6.3.4A.v Summary of correlations between trace elements and fracture toughness of mixed hooves grouped according to the shape of their capsular bases

base shapes	long	round	wide
impact resistance	spearman's rank coefficient p value		
Ca mg/kgDM		0.8 p= 0.005	
Ca/C		-0.597 p=0.052	
Zn/C			-0.548 p= 0.028
Cu: Ca		0.779 p=0.008	
Zn: Ca		0.785 p= 0.007	
impact strength			
Ca mg/kgDM	-0.967 p=0.002	0.783 p= 0.007	
Zn/C	-0.804 p=0.029		-0.548 p= 0.028
Cu: Ca	-0.968 p= 0.002	0.779 p=0.008	
Ca :Cu	0.908 p= 0.014		
Zn : Ca	-0.842 p= 0.035	0.818 p= 0.004	
Ca : Zn	0.859 p= 0.028		

Note 1 data set of mixed hooves

Trace elements and crack scores

Table 6.3.4A.vi Summary correlations between trace elements and crack area scores, mixed feet

	TCA					toe mm				GSAT				SSA				
	Zn;Ca	Cu	Ca	Cu:Ca	Ca:Cu	Cu:Ca	Zn	Ca	Zn:Ca	Cu:Ca	Zn:Ca	Zn	Ca	Ca	Cu	Zn	Cu:Ca	Ca:Cu
RR				0.981 0.019	-0.971 0.029	0.979 0.021				0.987 0.013	0.963 0.037						0.948 0.052	-0.98 0.02
RW	0.936 0.019		0.883 0.047	0.925 0.024		0.898 0.039		0.871 0.05	0.946 0.015	0.877 0.051	0.943 0.016		0.867 0.05	0.885 0.046			0.909 0.032	
WR		0.961 0.002	0.930 0.007			0.835 0.039	0.811 0.05	0.843 0.035		0.912 0.011		0.81 0.051		0.96 0.002	0.932 0.007	0.813 0.049		

	SSAT				GSA				
	Zn	Ca	Cu:Ca	Zn:Ca	Ca	Zn:Ca	Cu:Ca	Zn:Cu	Ca:Cu
RR			0.984 0.016	0.958 0.042					-0.975 0.025
RW		0.869 0.059	0.885 0.046	0.945 0.015	0.88 0.049	0.939 0.018	0.888 0.044		
WR	0.891 0.017	0.815 0.04	0.901 0.014		0.831 0.041				

Note 1 cells contain Spearman's Rank correlation coefficient and p value
Note 2 RR round regular; RW round wide; WR wide, regular.

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Table 6.3.4A.vii Summary of correlations between crack scores at the toe and of the whole hoof and trace elements measured at the mdc of mixed hooves grouped according to their base plate or angles.

TE & Crack score	base long	base round	base wide	toe angle regular	toe angle upright	vertical inclination regular	vertical inclination upright	vertical inclination wide
Ca mg/kgDM								
TCA	-0.868 p = 0.025	0.75 p = 0.013		0.464 p= 0.039				
SSA		0.767 p = 0.01		0.572 p= 0.008				0.515 p = 0.059
GSA		0.754 p= 0.012	0.561 p = 0.024	0.583 p = 0.007			0.998 p = 0.036	0.533 p = 0.05
toe mm		0.745 p = 0.013	0.469 p = 0.059	0.609 p= 0.004				0.625 p = 0.017
SSAT		0.745 p = 0.014	0.581 p = 0.018	0.691 p = 0.001				0.673 p = 0.008
GSAT		0.737 p = 0.015		0.715 p = 0.0001				0.681 p = 0.007
GSNoT		0.743 p = 0.014	0.483 p = 0.058	0.58 p = 0.007		0.1637 p = 0.011		
GSNo				0.51 p= 0.018				
Zn mg/kgDM								
GSA						0.496 p = 0.043		
SSA						0.531 p = 0.028		
toe mm						0.571 p = 0.017		
GSAT						0.562 p = 0.019		
SSAT						0.607 p = 0.01		

TE & Crack score	base long	base round	base wide	toe angle regular	toe angle upright	vertical inclination regular	vertical inclination upright	vertical inclination wide
GSNoT						0.704 p = 0.002	0.999 p = 0.03	
SSNoT						0.524 p = 0.031	-0.999 p =0.03	
GSNo				0.51 p = 0.018				
SSNo				0.48 p = 0.059				
Cu mg/kgDM								
GSA						0.468 p = 0.058		
SSA						0.503 p =0.04		
GSNo					0.74 p = 0.057		1 p = 0.01	

Note 1 cells contain Spearman's Rank correlation coefficient and p value
Note 2 RR round regular; RW round wide; WR wide, regular.
Note 3 data set mixed feet

Table 6.3.4A.viii Summary of correlations between trace element ratios and crack scores in mixed hooves with different base plates or angles

correlations ratios and crack scores	base long	base round	base wide	vert inclination steep	vert inclination wide	toe angle low	toe angle normal
TCA							
Cu :Ca	- 0.894 p = 0.016	0.593 p = 0.071			0.519 p = 0.057		0.486 p = 0.03
Zn : Ca		0.718 p = 0.019					0.476 p = 0.039
SSA							
Cu: Ca		0.6 p = 0.059			0.559 p = 0.039		0.584 p = 0.007
Zn : Ca		0.749 p = 0.013					0.557 p = 0.013
GSA							
Cu :Ca		0.582 p = 0.07			0.558 p = 0.038		0.607 p = 0.005
Zn : Ca		0.744 p = 0.014		0.996 p = 0.055			0.568 p = 0.01
toe mm							
Cu :Ca		0.607 p = 0.058			0.615 p = 0.019		
Zn : Ca		0.748 p = 0.013			0.696 p = 0.008		0.667 p = 0.002
SSAT							
Cu :Ca			0.566 p = 0.022		0.641 p = 0.013		
Zn : Ca		0.753 p = 0.013			0.738 p = 0.004		0.735 p = 0.0001
GSAT							
Cu :Ca			0.587 p = 0.017		0.643 p = 0.013		0.691 p = 0.001
Zn : Ca		0.745 p = 0.013			0.744 p = 0.004		0.766 p = 0.0001
GSNoT							

correlations ratios and crack scores	base long	base round	base wide	vert inclination steep	vert inclination wide	toe angle low	toe angle normal
Cu :Ca		0.731 p = 0.016	0.491 p = 0.053	-0.997 p = 0.046			
Ca :Cu				1.0		0.903 p = 0.036	
Zn : Ca		0.721 p = 0.019					0.458 p = 0.048
Ca : Zn						0.918 p = 0.028	
SSNoT							
Cu :Ca				-0.977 p = 0.046			
Ca :Cu				1.0			
TCNo							
Cu: Ca				-0.998 p = 0.038			

Table 6.3.4A.ix Summary correlations between qualitative trace elements and crack scores in hooves with different shapes

correlations between qualitative TEs & crack scores	toe angle normal	toe angle upright	round upright	round wide	wide regular	wide wide
GSA M/4						
M/4 Cu/C	0.692 p = 0.013		1.0			
GSNo M/4						
M/4 Cu/C	0.590 p = 0.044		- 1.0			
GSAL/4						
L/4 Cu/C					-0.9 p = 0.037	
GSNoL/4						
L/4 Cu/C		-0.666 p=0.009			-0.9 p = 0.037	
GSAT						
mdc Ca/C			1.0			
mdc Cu/C						-0.8 p=0.01
mdc Zn/C				- 1.0		
GSNoT						
mdc Zn/C				- 0.949 p=0.051		
mdc Cu/C					0.9 p=0.037	-0.686 p=0.041
mdc Ca/C						0.787 p=0.012

Note 1 data set of 28 fore feet

6.4.1 The use of Finite Element Analysis as an adjunct to gross measurements to investigate shape

Finite element analysis has been developed to model the effect of loading on the hoof wall. Finite element analysis, (FEA), is used in biomechanics to evaluate mechanical stresses on biological structures. It is a numerical tool, which is used to obtain approximate solutions to complex problems. (Wichtmann 1990; Wichtmann, 1991) used FEA to develop a 2 dimensional model to measure force distribution through the digit during static loading; (Hinterhofer 1998) developed a three dimensional model to investigate dynamic loading in the hoof capsule and more recently(Hinterhofer 2000) used the same model to measure the effect of different shoeing techniques on static loading. This model showed similar deformation and stresses as (Newlyn et al. 1998) who first used FEA to show the deformation of the donkey hoof wall under static loading and all models gave similar magnitude of results as those obtained *in vivo* and *in vitro*.

In vivo and *in vitro* results can be obtained using strain gauges; uniaxial (Colles 1989) and rosette (Thomason, 1992; Thomason 1998) which measure strain at one specific position on the hoof capsule or by photoelasticity (Dejardin 1999), which measures strains over the whole surface of the hoof. The results are specific to the hoof on which the measurements are being recorded. FEA, however, is a mathematical modelling technique that enables a geometric representation of the hoof (taken from *in vivo* measurements) to be subjected to an idealised system of loads and restraints (based on *in vivo* measurements) in an attempt to simulate the *in vivo* conditions, which exist. FEA does not try and simulate exactly what happens, but attempts to identify the effect and relevance of certain parameters in order to develop a better understanding of the functioning of the system. Indeed due to the material complexity of the hoof and the multifactorial effects on load bearing, an individual hoof can only be representative of the diversity of the system and therefore no methodology can be truly exclusive.

FEA can help elucidate the displacements, (deformations), the strains, (displacement gradients), and the stresses (force intensities), within the hoof capsule.

The aim of this study was to investigate using Finite Element Analysis, whether the difference in shape between the fore and hind feet affects the way the hoof capsule deforms when loaded under the same conditions. The hypothesis that when loaded under the same conditions, that size and shape differences between fore and hind feet affect the magnitude of stress and strains was investigated.

Materials and Methods

Hooves

The hooves were determined to be of normal configuration on the basis of being free from gross capsular abnormalities by clinical assessment (regular and symmetrical shape).

A series of measurements, which captured the reported differences described in the literature were used to describe the shape of the hooves. The measurements, (table 6.4.1.i) taken were as described in chapter 2.

Table 6.4.1A.i Basic geometric measurements of the hooves which were modelled using FEA

Measurement	front foot	hind foot
CD	12.3	10.5
CWWP	12.9	10.6
Widest point from toe	6.1	7.1
Toe angle	48 °	58°
Toe mdc length	9.5	7.4
WP M/4 length	8.0	6.1
WP L/4 length	8.2	6.1
WP M/4 angle	81°	86°
WP L/4 angle	75°	85°
Medial heel angle	42°	40°
Medial heel length	5.5	3.7
Lateral heel angle	45°	26 °
Lateral heel length	3.6	4.9
Proximal circumference	28.8	26.5
Distal circumference	32.8	28.0

Note 1 all angles are measured in degrees, all linear measurements in cm

To make a FEA model, four steps are required. Firstly the shape of the hoof is generated from the measured parameters, using curves and surfaces. The surfaces are then meshed to create the Finite Elements. In this analysis, the structure of the hoof was represented by constant thickness plate elements.

Secondly, having drawn the shape, it is necessary to give material properties to the model. In the hoof it is difficult to specify the material properties precisely as in common with many biological structures, the hoof has material properties, which may vary with location, (inhomogeneous), direction, (anisotropic) and loading rate. However there has been sufficient research in hoof material properties, (Douglas et al. 1998; Landeau *et al.* 1983; Leach and Zoerb 1983) to provide a Young's modulus and Poisson ratio. The Young's modulus and Poisson's ratio used were considered representational values.

Thirdly the load has to be defined. Force plate (Leach and Zoerb,1983) experiments have produced physiological load magnitudes and enable a load to be selected that is representative. However this study is a comparative one and the absolute load is not important, as it is the differences created by the same load that is being assessed.

The analysis is a linear elastic analysis and assumes the same modulus in compression and tension and is valid for small displacements. Finally the model has to be fixed, (known as boundary conditions) to prevent rigid body movement of the model, the boundary constraints should be away from primary areas of interest as stress artefacts can be created around the constraints.

Material properties may vary in different shapes therefore it is important to maintain all variables constant except shape, which we were seeking to model.

In order to generate data, which could clarify the shape, the two selected geometries were developed in identical ways using the parameters measured. They were then meshed, loaded and restrained in a similar manner.

Consistency of the dorso- concavity displacement between (Newlyn *et al.* 1998) and (Hinterhofer 1998; Hinterhofer *et a*l. 2001; Wichtmann *et a*l. 1990) supports the continued use of a model of the hoof wall in isolation as it generates as accurate simulation of *in vivo* conditions as models which incorporate sole, frog and laminar junctions.

Models generated

The lateral regions suffer considerable distortion. The effects of the lateral angles on the stress distribution can be assessed by looking at the bending and membrane stresses in these regions. The hind hoof had consistently greater bending stress down the wall, but consistently less bending around the circumference, it is possible that a low toe angle in the fore hoof made it more susceptible to bending as illustrated in figure 6.4.1.ii.

- The front foot exhibited much greater deflection at the coronary broader at MDC compared to the hind foot. The vertical deflection was nearly three times greater. This was mainly a consequence of the lower toe angle of the front foot.
- The maximum compressive stress in the toe region was approximately 50% greater in front foot compared to the hind foot
- The distribution of strain along the MDC indicated that the compressive strain in the hoof surface varied to a much larger degree in the front foot compared to the hind foot. This was as a consequence of the bending which was greater in the front hoof and this was illustrated by the displacement in figure 6.4.2.ii
- The front foot absorbed approximately 60% more energy under the loading conditions compared to the hind foot due to the greater displacements.
- The front foot which had a lower toe angle compared to the hind foot exhibited nearly twice the deflections. This may be a result of the toe angle but may also be a result of the wider dorsal inclination of the front foot. This may be because the load extended a distance back from the toe where the angle of the wall imposed a rotation on the wall and encouraged squat.

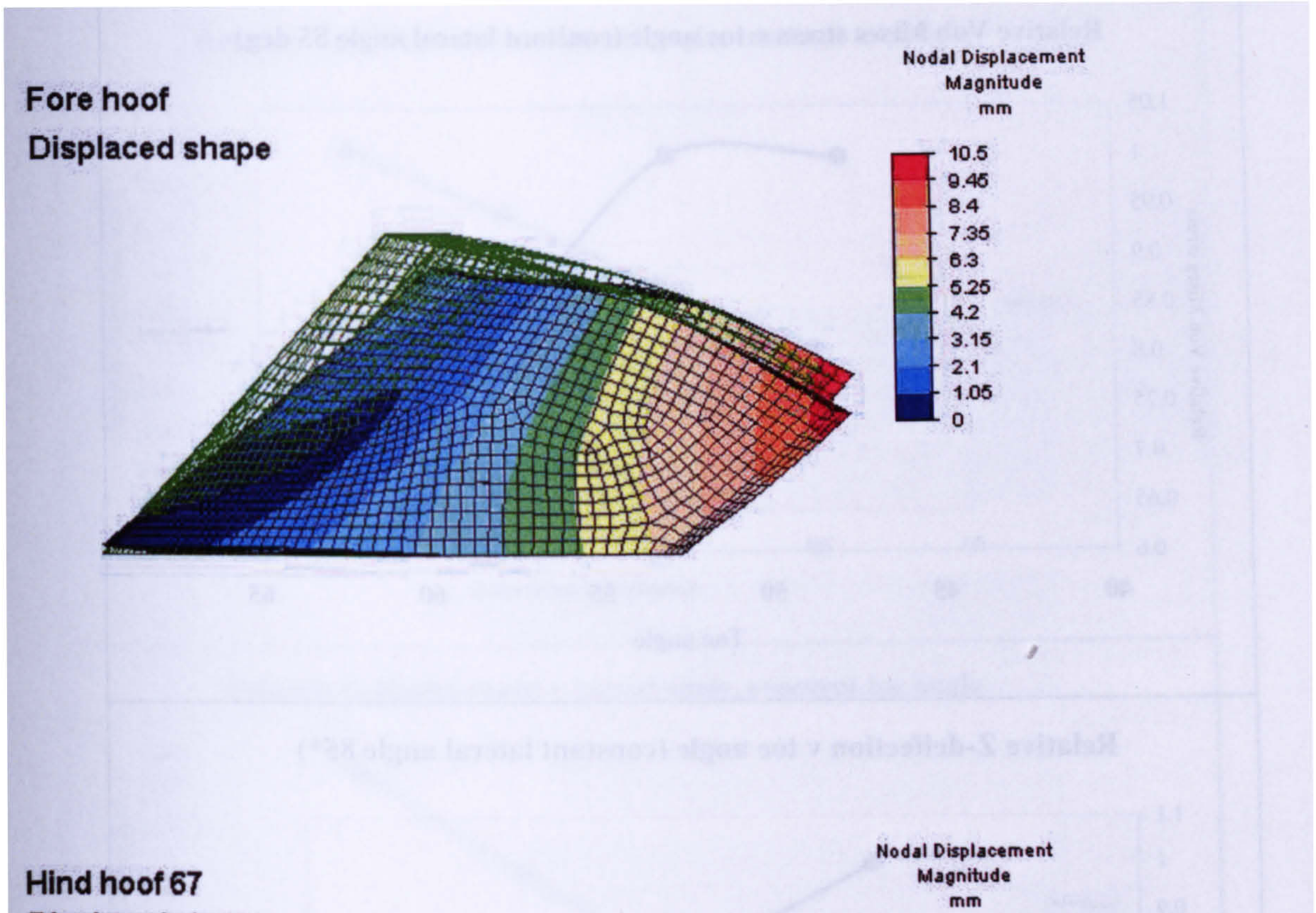
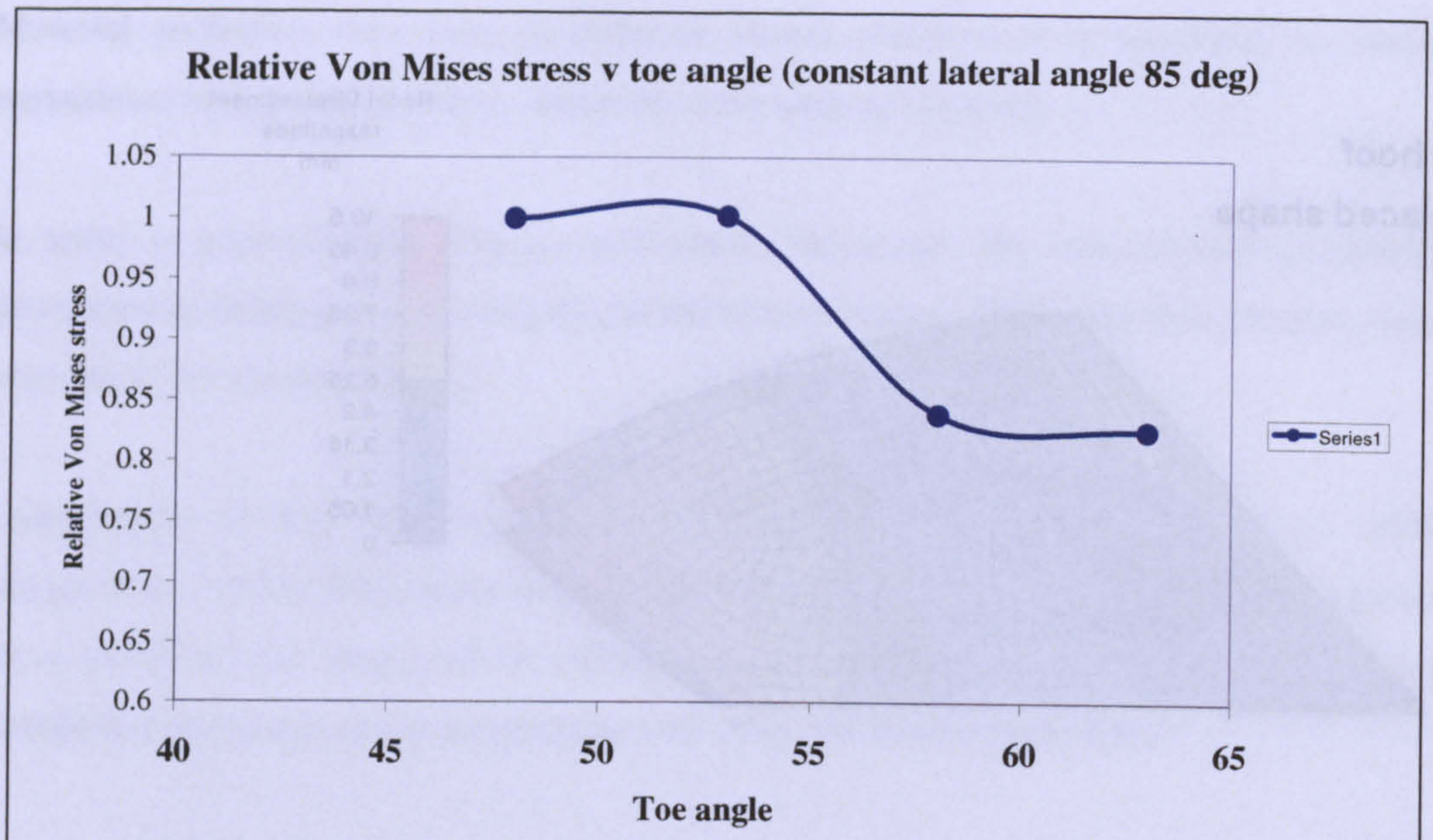


Figure 6.4.1.i The effect of shape on the displacement of the hoof wall.

To further clarify the effects of either toe angle or quarter angles, the toe angle was first altered to 58° from 48° on the front foot but the quarter angle remained constant at 85° ; secondly the toe angle was kept constant at 48°and the quarter angles were altered from 75°to 85° .

The following results were obtained

- When the toe angle was increased from 48°to 58° deg, the Von Mises stress at the coronary band (maximum in hoof)/MDC decreased by 19% and the vertical deflection at that point decreased by 81%, figure 6.2.9.ii



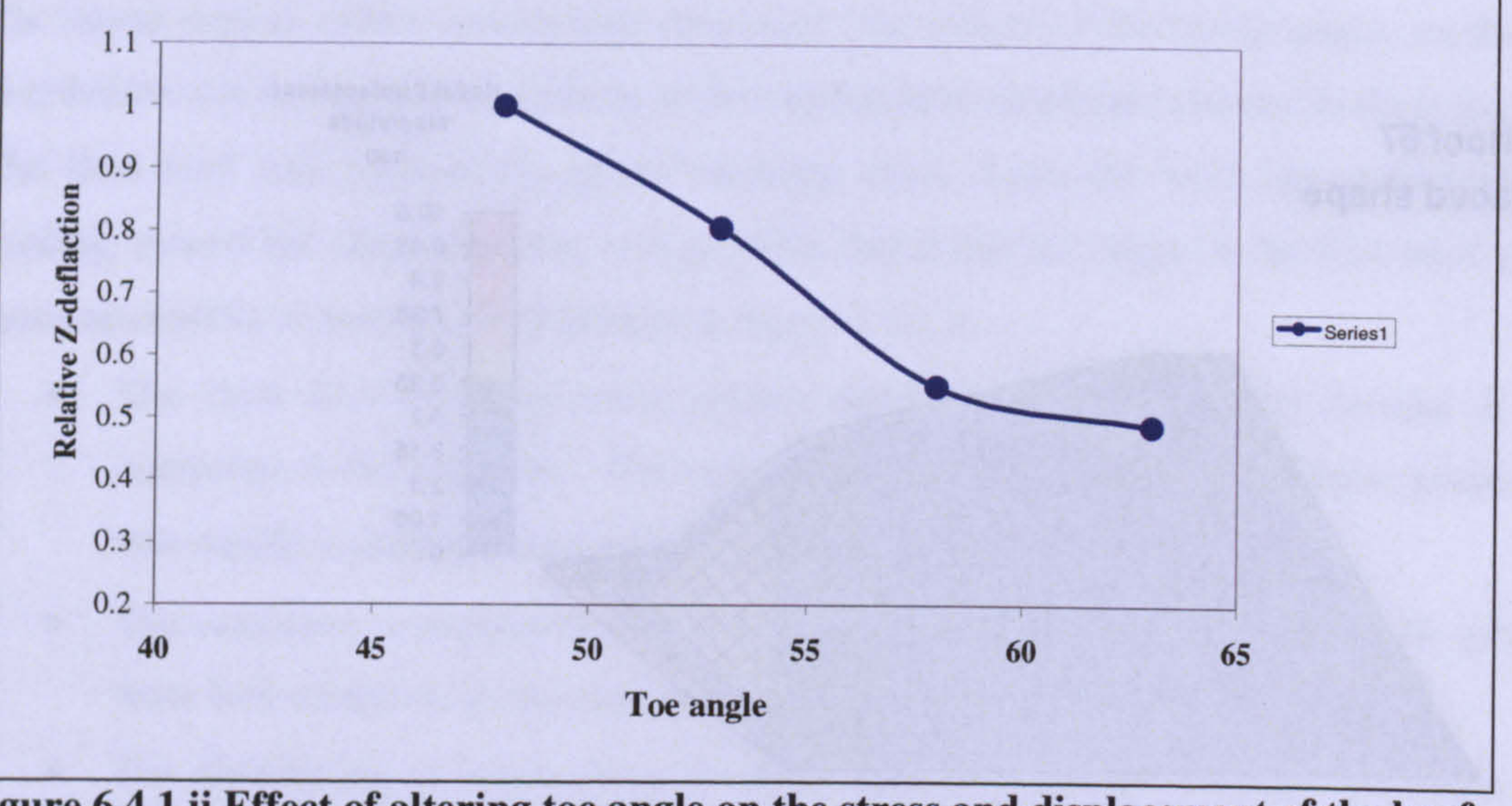


Figure 6.4.1.ii Effect of altering toe angle on the stress and displacement of the hoof capsule

- When the lateral angles were increased from 75° to 85° the Von Mises stress at the coronary band (maximum in hoof)/MDC decreased by 16% and the vertical deflection at that point decreased by 9%.

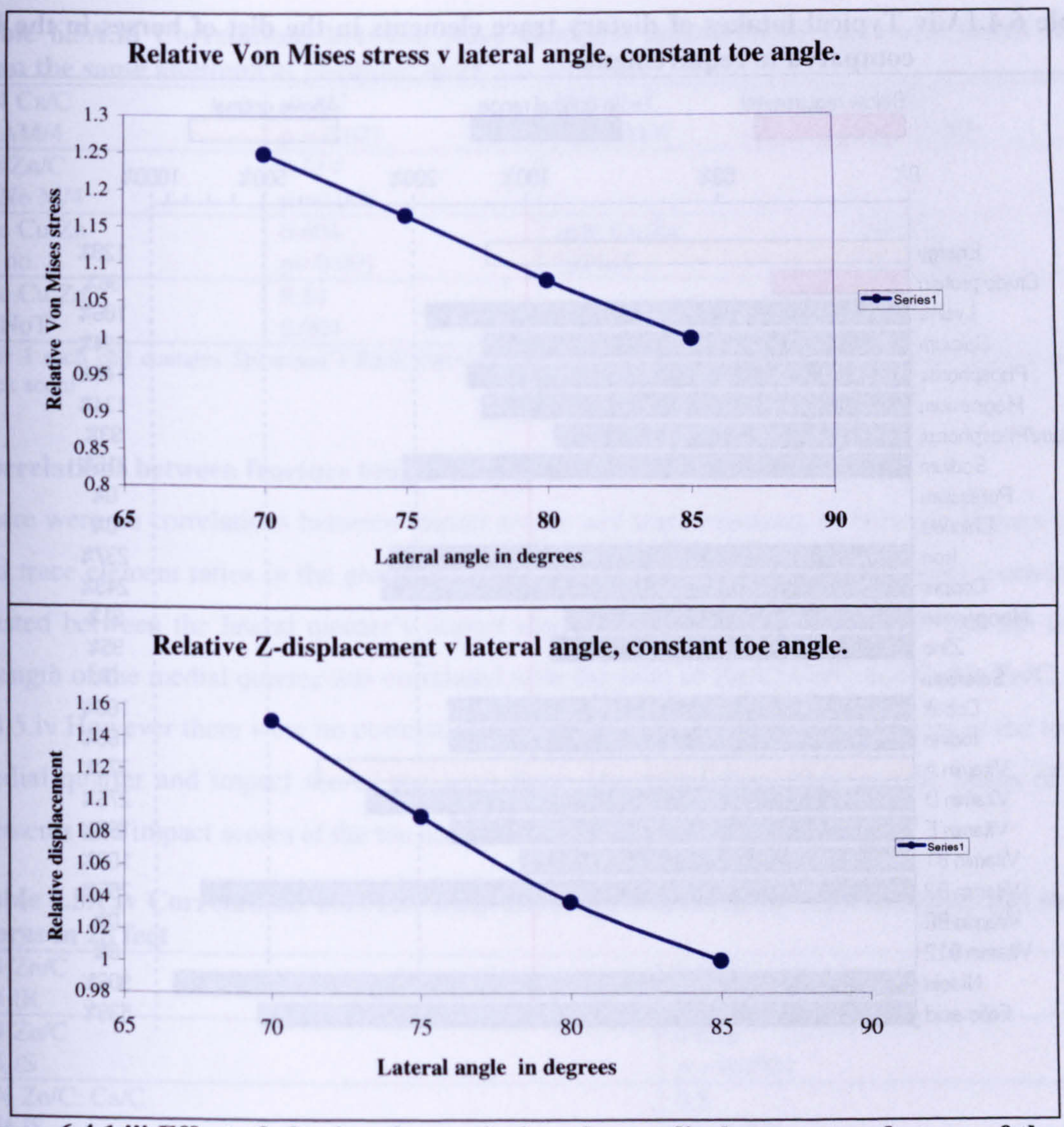
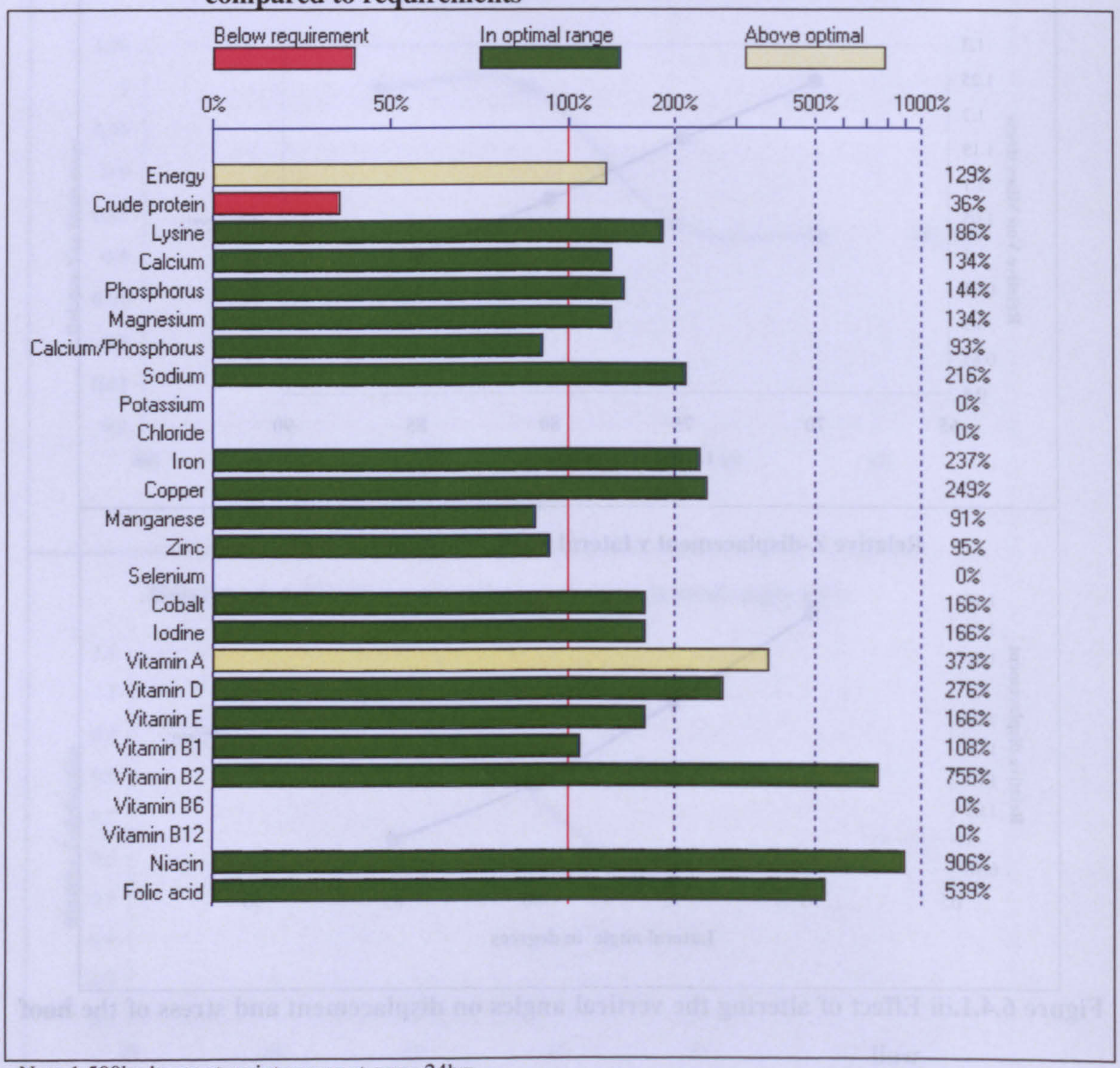


Figure 6.4.1.iii Effect of altering the vertical angles on displacement and stress of the hoof wall

Table 6.4.1A.iv Typical intakes of dietary trace elements in the diet of horses in the UK compared to requirements



Note 1 500kg horse at maintenance at grass 24hrs

Correlations between trace elements in 28 left fores, cracks and fracture toughness

In the data set of 28 left fores, there were no correlations between crack scores and trace elements at the toe or the lateral quarter. However there were correlations between Ca/C at the medial quarter and the geometric area and number scores of the medial quarter as well as a correlation between medial Zn/C and medial geometric number score. The Cu/C: Zn/C ratio at the toe was correlated with the geometric and severity number scores at the toe.

Table 6.5A.iii Correlations between trace elements measured by laser and crack scores from the same anatomical positions in 28 left fore feet

M/4 Ca/C GSAM/4	-0.437 p = 0.023	M/4 Ca/C GSNoM/4	-0.52 p = 0.005
M/4Zn/C GSNo M/4	0.514 p =0.006		
mdc Cu:Zn toe no	0.604 p= 0.001	mdc Cu:Zn SSNoT	0.601 p = 0.001
mdc Cu:Zn GSNoT	0.54 0.004		

Note 1 each cell contains Spearman's Rank correlation coefficient and p value between the trace element and the crack score

Correlations between fracture toughness and trace elements

There were no correlations between impact scores and trace elements or between impact scores and trace element ratios in the group of mixed feet. In the data set of 28 left fores, correlations existed between the lateral quarter's impact strength and resistance and Zn/C and the impact strength of the medial quarter was correlated with the ratio of Zn/C: Ca/C and Ca/C: Zn/C, table 6.3.5.iv However there were no correlations between trace element concentrations at the toe and medial quarter and impact scores nor were there any correlations between the ratios of trace elements and impact scores at the toe and lateral quarter.

Table 6.5A.iv Correlations between trace elements measured by laser ablation and impact scores in 28 feet

L/4 Zn/C L/4 IR	0.568 p= 0.002
L/4 Zn/C L/4 IS	0.654 p = 0.0001
M/4 Zn/C: Ca/C M/4 IS	0.5 p = 0.008
M/4 Ca/C : Zn/C M/4 IS	0.43 p= 0.025

Correlations between trace elements, crack scores and fracture toughness within different shaped hooves

In the data set of 28 left fores, there were correlations between fracture toughness and area and number scores in hooves with round bases and upright dorsal inclination,(RU) and between toe fracture toughness and crack number scores in hooves with a round base and wide dorsal inclination (RW).

Correlations in fracture toughness and crack scores in hooves with different shaped bases or different vertical inclination or different toe angles

In the data set of 28 left fore feet, medial impact and crack scores were correlated most strongly in hooves with upright angles; when correlations existed they were inversely correlated. Correlations at the toe were most frequent in hooves with normal toe angles. Correlations at the lateral quarters were found in hooves with regular vertical angles.

Correlations between trace elements and fracture toughness in hooves of different shapes or with different dorsal inclination or toe angles

In the data set of 28 left fores, the round regular shaped hooves had the most correlations between medial trace elements and fracture toughness,(table 6.3.6A.iii), but few shapes had any correlations between trace elements and fracture toughness at their lateral quarters and toes.

Table 6.4.1A.v 2ANOVA[1] block and treatment design to compare scores within and between anatomical positions and between score types

Anatomical positions / Crack score	Whole	L/4	R/4	Toe
TCNo	[4c]11	21	31	41
SSNo	12[4r]	22	32	42
GSNo	13	23	33	43
TCA	14	24	34	44
SSA	15	25	35	45
GSA	16	26	36	46

Note 1. General linear model

Note 2. Sig. $p<0.05$

Note 3. Tukey multiple comparisons

Note 4. [c]Column ≡ block, [r]Row ≡ treatment