# Response of cattle with clinical osteochondrosis to mineral supplementation

by

*Gerjan van der Veen*

A dissertation in fulfilment of the requirements of the degree Master of Science in the Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria

Date submitted:
October 2016

# TABLE OF CONTENTS

# FIGURES AND TABLES

# ACKNOWLEDGEMENTS

To my wife, Orsilla van der Veen, for the continuous support.

Prof. Leon Prozesky for fulfilling the role as supervisor.

Dr. Heinz Meissner as project co-supervisor.

Prof. Geoffrey Fosgate for the statistical analysis.

Drs. Wilco Botha, André van den Berg, Benton van den Berg and Patrick Ntsibande for assisting with the data collection.

Mr. Jan van Zyl and family for the determination to solve the problem of osteochondrosis.

Department of Paraclinical Sciences, University of Pretoria.

"What is now proved was once only imagin'd"

William Blake

# SUMMARY

Response of cattle with clinical osteochondrosis to mineral supplementation

by

Gerjan van der Veen

Promoter: Prof. L. Prozesky
Co-Promoter: Dr. H.H. Meissner
Department: Paraclinical Sciences
Degree: MSc Veterinary Science

Since 1982 farmers and veterinarians mainly from the North West province of South Africa noticed an increase in cattle with associated lameness and osteopathy of unknown aetiology. Affected cattle presented with varying degrees of lameness as well as peri-articular swelling especially of the stifle joint. Cattle of all ages, particularly those between the ages of 6 - 18 months developed lesions. Lesions were also noticed among different breeds, production stages and different farming enterprises (commercial and stud farming). The only common factor among the affected cattle was the environment in which they were farmed. Culling of affected animals, especially calves, reduced the number of replacement stock to a level where some farmers were not able to produce sufficient replacement stock, consequently suffering tremendous financial losses. Investigation into the aetiology of the new sporadic osteopathy was deemed necessary.

Initially it was suspected that the condition was due to mineral deficiency, as affected cattle fed a supplement containing high levels of micro- and macro minerals responded positively within three weeks during a pre-trial.

The study was conducted to determine the level of micro- and macro minerals required to be included in a supplement that would improve the clinical condition of cattle clinically affected by osteochondrosis. Results indicated no significant difference between supplemetary treatments. A comparison between the pre-trial and the current supplements indicated that the supplements in the present study had negative dietary cation anion difference (DCAD) values. Since negative DCAD compositions may result in mild metabolic acidosis, as supported by literature, and the acidic urine collected in a small sample of the test

cattle, the hypothesis is advanced that it is unlikely that supplementation, regardless of its mineral concentration will improve the condition of the cattle if the DCAD value of the supplement is strongly negative. The hypothesis should be tested in forthcoming research and the following should be determined: (1) the specific effect a supplement with a negative DCAD value has when fed to cattle with clinical osteochondrosis, and (2) the optimal DCAD value of a supplement that would aid in limiting the severity of osteochondrosis in cattle.

# ABBREVIATIONS

*Ad lib* --- ad libitum

ALP --- alkaline phosphatase

AMP --- adenosine monophosphate

AMPase – adenosine monophosphoesterase

ATP --- adenosine triphosphate

ATPase --- adenosine triphosphatase

BMP 6 --- bone morphogenic protein 6

BSE – bovine spongiform encephalopathy

BSP --- bone sialoprotein

Ca --- calcium

$CaPO_4$ --- calcium orthophosphatase (calcium phosphate mineral)

$Cl^-$ --- chloride ion

Ctgf --- connective tissue growth factor

Cu -- copper

Da --- Daltons

DCAD --- dietary cation anion difference value

DM basis --- dry matter basis

DNA - deoxyrubinuclease

*et al.* --- et alia / and others

FGFR3 --- fibroblast growth factor receptor-3

g --- grams

GPOF --- Growth plate-orientation factor

Ihh – Indian hedgehog factor

*in vitro* --- studies performed outside the normal biological content

$K^+$ --- potassium ion

kg --- kilogram

L --- large

M --- medium

ME MJ/day --- metabolisable energy mega joules per day

meq/kg --- milliequivalents per kilogram

MMP 9 --- matrix metalloproteinase- 9

MMP 13 --- matrix metalloproteinase-13

MMP 14 --- matrix metalloproteinase- 14

MMPs – matrix metalloproteinases

Mn --- manganese

MT-MMP --- membrane-bound type 1 matrix metalloproteinase

n --- number of

$Na^+$ --- sodium ion

NRC --- national research council committee on animal nutrition

P --- phosphate

pH --- numeric scale used to specify the acidity or basicity

Pi --- inorganic phosphate

PPi – inorganic pyrophosphate

PPiase --- inorganic pyrophospatase

PPM --- parts per million

PTH --- parathyroid hormone

PTHrP --- parathyroid hormone-related peptide

S --- small

$S^{2-}$ --- sulphur ion

TNAP --- tissue non-specific alkaline phosphatase

TUNEL --- terminal deoxynucleotidyl transferase

VEGF --- vascular endothelial growth factor

XL --- extra large

Zn --- zinc

% --- percentage

% DM --- percentage dry matter

$1,25(OH)_2D_3$ --- calcitriol / 1,25 dihydroxycholecalciferol

< --- less than

# CHAPTER 1

# ENDOCHONDRAL OSSIFICATION

## 1.1 Introduction

The function of bone in the body is to provide a physical supporting structure to protect vital organs, serve as attachment for muscle to enable locomotion and act as a mineral reservoir (especially calcium and phosphate) that together with the endocrine system maintains mineral homeostasis.

The initial phase of skeletogenesis involves the recruitment and condensation of mesenchymal stem cells that commit to chondrogenesis. Perichondral bone formation is the process whereby a bone collar develops around the cartilage model.

Chondroblasts within the model proliferate, increasing the size of the model through interstitial growth (Gerstenfeld & Shapiro 1996). Chondrocytes secrete extracellular matrix molecules like collagen type II, IX, XI and proteoglycans (Wardale & Duance 1993), with collagen type II as the main component of the extracellular matrix and aggregan as the main proteoglycan (Alini *et al*. 1992). The formation of the primary centre of ossification is associated with the formation of a bony collar surrounding only the central portion of the cartilage model (Álvarez *et al*. 2005). Chondrocytes in the center of the cartilage model undergo hypertrophy and the extracellular matrix mineralize. Mineralization is reported to occur in areas that are high in proteoglycans, hyaluronic acid and C-propeptide of collagen type II (Alini *et al*. 1992). The hypertrophic chondrocytes undergo apoptosis and blood vessels arising from the periosteum transport osteogenic cells to the empty lacunae, formed by the atrophied chondrocytes, establishing the primary centre of ossification (Goff 2004, Lefebvre & Smits 2005). Chondroblasts on either end of the primary ossification center proliferate and establish the growth plate.

## 1.2 Primary Centre of Ossification

Ossification centres in growth cartilage are found in two distinct areas of the bone. The primary centre originates in the growth cartilage of the diaphysis and is responsible for the longitudinal growth of bone. The secondary centre of ossification originates in the articular epiphyseal growth cartilage located in the epiphysis and is responsible for the formation of the specific epiphyseal structures. A cartilage growth plate in the region of the metaphysis separates the primary and secondary centres of ossification. Both processes are independently regulated, with the primary centre of ossification initiated first followed by the establishment of the secondary centres of ossification. Chondrocytes in the metaphyseal growth cartilage are arranged in morphologically distinct zones. The first zone, furthest from the ossification centre, is the resting zone, followed by the zone of proliferation, hypertrophic zone and finally the zone of ossification (Goff 2004).

### 1.2.1 Resting zone

Chondrocytes of the resting zone are small and scattered in an irregular pattern and surrounded by cartilage matrix. It has been proposed that the stem-like cells giving rise to the chondrocyte columns found in the proliferative and hypertrophic zone are either the chondrocytes found in the resting zone or the uppermost chondrocytes from the proliferative column. According to Abad *et al.* (2002) the resting zone can regenerate to form a new proliferative and hypertrophic zone after the latter zones have been surgically removed. The resting chondrocytes also produce a morphogen called growth plate-orientation factor (GPOF). This soluble factor that is produced in the resting zone diffuses to the adjacent proliferating zone to guide the orientation of the chondrocytes (Abad *et al*. 2002).

### 1.2.2 Proliferative zone

Chondrocytes of the proliferating zone increase in size and form rows and columns that are longitudinal to the long axis of the developing bone. Cells at the top of the column divide through mitosis, adding cells to the column. A layer of extracellular matrix separates the chondrocytes of the column. Perichondral cells and early proliferating chondrocytes express a parathyroid hormone-related peptide (PTHrP) that maintains chondrocytes in a proliferative state and prevents hypertrophy. Chondrocytes that cease to proliferate and move into the prehypertrophic phase express the most receptors for PTH/PTHrP and express the Indian hedgehog (Ihh) factor that stimulates chondrocyte proliferation and inhibits hypertrophy.

PTHrP thus limits the number of cells able to express Ihh, creating a negative feedback loop regulating its own expression and rate of chondrocyte differentiation. Chondrocytes that escape the PTHrP control undergo prehypertrophy and express Ihh, which in turn will stimulate chondrocyte proliferation and production of PTHrP (Mackie *et al*. 2008).

### 1.2.3 Hypertrophic zone

Chondrocytes at the end of the proliferative zone mature and move into the hypertrophic zone where they will undergo phenotypic changes and apoptosis before vascular invasion in the ossification zone. Hypertrophic chondrocytes swell by as much as 20 times (Goldring *et al*. 2006) as a result of oncosis and simultaneous chondroptosis. Chondrocytes in the terminal phase of the hypertrophic zone shrink and undergo nuclear condensation (Anderson *et al*. 2005) while the integrity of the plasma membrane is maintained (Ohyama *et al*. 1997).
Apoptotic changes can be detected by end-labelling of chondrocyte DNA using the terminal deoxynucleotidyl transferase (TUNEL) procedure. Strong positive TUNEL staining was seen in prehypertrophic chondrocytes indicating that the process of apoptosis commenced in mature postmitotic chondrocytes (Ohyama *et al.* 1997). Ohyama *et al.* (1997) found a dramatic decrease of intracellular nucleotides, reductive reserves and an energy charge ratio of postmitotic chondrocytes.

Prehypertrophic chondrocytes express bone morphogenic protein 1, 2, 3, 4, and 6, Ihh, Runx2 and activate fibroblast growth factor receptor-3 (FGFR3) (Abad *et al.* 2002, Anderson *et al*. 2005, Goldring *et al*. 2006). Hypertrophic chondrocytes express vitamin D receptors, BMP 6, metalloproteinase-13 (MMP13), Runx2, type II C-propeptide, synthesize type X collagen, down-regulate synthesis of type II collagen, secrete osteonectin, osteopontin, bone sialoprotein, osteocalcin have increased activity of plasma membrane alkaline phosphatase and release of matrix vesicles (Anderson 2003, Goldring *et al*. 2006, Ohyama *et al.* 1997).

Matrix vesicles are extra-cellular membrane-invested vesicles generated by polarized budding from the lateral surface of growth plate chondrocytes. Matrix vesicles are released and undergo mineralization during the early stages of chondrocyte apoptosis and are incorporated into the extracellular matrix of the upper hypertrophic zone (Anderson *et al*. 2005, Anderson 2003).

Vesicles have a lipid bilayer membrane containing plasma membrane phospholipids like cholesterol and sphingomyelin and an unusual amount of acidic phospholipids like phospatidylserine and phosphatidic acid, which may serve as a calcium trap. The enzyme alkaline phosphatase (ALP) is concentrated on the outside of the vesicles and is essential for calcium deposition. The activity of ALP increases as the vesicles approach the zone of calcification in the growth plate. ALP is capable of hydrolysing both AMP (adenosine monophosphate) and PPi (inorganic pyrophosphate) and releases Pi (inorganic phosphate) to be incorporated into the calcium phosphate mineral. The matrix vesicle(s) also has an increased concentration of adenosine monophosphoesterase (AMPase), inorganic pyrophosphatase (PPiase) and adenosine triphosphatase (ATPase) hydrolysing AMP, PPi and ATP respectively to produce Pi. PPiases not only hydrolyses PPi but also counteracts the inhibitory effect of PPi on mineralization. PPi's levels of < 1 mM stimulate mineralization as most of the PPi is hydrolysed to Pi to be incorporated into $CaPO_4$ (calcium orthophosphatase). Concentrations between 1-2 mM exceed the hydrolysing capacity of PPiase, which has an inhibitory effect on mineralization.

Other non-phosphatase proteins that function in the role of mineralization are annexin II, annexin V, annexin VI, bone sialoprotein (BSP), osteonectin, osteocalcin and sodium-dependent phosphate transporter (Anderson *et al*. 2005, Anderson 2003). Annexin V is concentrated beneath the membrane of matrix vesicles, where it functions as an inwardly directed calcium canal. The sodium-dependent phosphate transporter increases Pi concentration during the early stages of mineral initiation (Anderson *et al*. 2005).

Mineral formation and mineralization of the extracellular matrix is believed to be a biphasic process. During phase 1 the calcium concentration of the matrix vesicles increases through the action of the calcium binding acidic membrane phospholipids and the transport capacity of annexin V. Phosphate concentration is increased through the action of the sodium-dependent phosphate transporter, ALP, PPiase, AMPase and ATPase. The sodium-dependent phosphate transporter reaches its peak activity early in the mineralization process and it has been proposed that the sodium-dependent phosphate transporter is responsible for the initial phosphate accumulation that is later followed by the activity of ALP (Anderson *et al*. 2005, Anderson 2003).

When the absorption of calcium and phosphate in the matrix vesicle exceeds the solubility factor, calcium phosphate mineral ($CaPO_4$) will precipitate (Anderson *et al*. 2005, Anderson 2003). The mineral produced is of a non-crystalline form and is thought to convert to octacalcium phosphate crystals, which is then transformed into highly insoluble hydroxyapatite (Anderson *et al*. 2005).

Phase 2 of mineralization is initiated when the hydroxyapatite crystals penetrate the membrane of the matrix vesicles with the support of phospolipases and proteases within the matrix vesicles. Calcification-inhibiting acidic proteoglycans are digested by matrix metalloproteinases (MMPs) released from the matrix vesicles (Anderson *et al*. 2005, Anderson 2003). Matrix metalloproteinase 9 (MMP 9) and MMP 13 are a gelatinase and a collagenase respectively with MMP 9 most concentrated proximal to the chondro-osseous junction (Ortega *et al*. 2004).

Mineralization is initiated when the hydroxyapatite crystals are released into the extracellular matrix. Continued formation of new hydroxyapatite crystals on pre-existing hydroxyapatite crystals requires optimally maintained levels of calcium and phosphate in the extracellular matrix (Anderson 2003). Factors such as unregulated levels of calcium and phosphate ions, pH and specific regulatory molecules at the site of mineralization can have an inhibitory effect on the mineralization process (Anderson *et al*. 2005). Chondrocytes of the hypertrophic zone secrete collagen type X and down-regulate the synthesis of collagen type II (Ohyama *et al.* 1997). The proposed hexagonal lattice structure of collagen type X combined with its affinity for proteoglycans, collagen fibre and matrix vesicles ensures that the correct matrix components stay in the hypertrophic zone for normal mineralization to occur (Shen 2005). It has been proved that matrix vesicles are associated with collagen type II and X, proteoglycan link protein and the hyaluronic acid-binding region (Kirsch & Wuthier 1994).

Kirsch & Wuthier (1994) proved that the interaction between collagen type II and X with matrix vesicles regulates the uptake of calcium through annexin V, establishing the nucleation core within the vesicle. Studies indicate that collagen alone is not the nucleator of hydroxyapatite mineralization, but merely a template on which the process will occur. Non-collagenous proteins synthesized by chondrocytes of the hypertrophic zone e.g. bone sialoprotein, osteopontin and osteocalcin are morphologically, structurally and functionally

connected to collagen fibres and induce hydroxyapatite mineralization on the collagen fibre surface (Gerstenfeld & Shapiro 1996, Wiesmann *et al*. 2004).

An avascular structure, cartilage, is converted into one of the body's most vascularized structures, bone, at the lower hypertrophic and ossification zone.
Differentiation and expansion of hypertrophic chondrocytes and invasion of blood vessels from the ossification zone require degradation of cartilage matrix surrounding the chondrocytes and the transverse septa between the chondrocytes stacked in the column respectively (Abad *et al*. 2002). Chondrocytes of the lower hypertrophic zone (Ortega *et al*. 2004), express a high concentration of several vascular endothelial growth factor (VEGF) isoforms, several fibroblast growth factors, type X collagen and MMP13 (Kanczler & Oreffo 2008), with the latter able to degrade both fibrillar collagen (Type II collagen) and aggregan (Abad *et al*. 2002). Matrix metalloproteinase 9 is the primary matrix metalloprotease involved in endochondral ossification acting as a gelatinase cleaving collagen type IV, V, XI, elastin, proteoglycans and laminins (Vu *et al*. 1998). Matrix metalloproteinase 9 is highly expressed in monocytes, preosteoclasts, osteoclasts and chondroclastic cells of unknown origin and is concentrated in the lower hypertrophic zone proximal to where vascular invasion occurs along the trabeculae (Ortega *et al*. 2004).

Angiogenesis of the hypertrophic zone is under the influence of VEGF, produced by terminal hypertrophic chondrocytes and regulated by Runx2. Individual studies with MMP9, Runx2 and Ctgf (connective tissue growth factor) knockout mice indicated reduced vascularization, lack of blood vessel invasion and a reduction of angiogenesis and matrix erosion of the ossification zone respectively (Zelzer & Olsen 2004), thereby proving the relationship between the former factors and that of VEGF in angiogenesis. Inhibition of VEGF signalling leads to reduced angiogenesis, loss of metaphyseal blood vessels, stimulation of trabecular resorption (Kanczler & Oreffo 2008) and reduced numbers of chondroclasts and osteoblasts in the growth plate (Gerber & Ferrara 2000). Vascular endothelial growth factor functions by inducing haemopoietic stem cell recruitment, monocyte chemo-attraction, bone formation and neuronal protection. The presence of VEGF and MMP9 is critical for early bone development (Kanczler & Oreffo 2008).
Matrix metalloproteinase 9 and MMP13 act synergistically to stimulate growth and differentiation of chondrocytes, degrade cartilage matrix (Anderson *et al*. 2005) and degrade unmineralised septa of hypertrophic chondrocytes (Ortega *et al*. 2004) with MMP9 releasing

VEGF from hypertrophic chondrocytes, promoting vascular invasion (Kanczler & Oreffo 2008). Blood vessels fill the empty lacunae before the arrival of osteoclasts, indicating that the transverse septa have to be degraded for vascular invasion of the growth plate to occur (Abad *et al*. 2002).

Osteoblasts deposit unmineralized bone matrix (osteoid) on the mineralized cartilage trabeculae. Only 40 % of calcified cartilage trabeculae will develop into primary bone trabeculae, with the remaining 60 % resorbed by osteoclasts at the ossification zone. Osteoid is calcified from the centre outwards. Bone trabeculae with a calcified cartilage centre are collectively known as the primary spongiosa. The cartilage core of the primary spongiosa is replaced by bone through the process of remodelling. The trabecular bone without a cartilage core is known as secondary spongiosa (Fazzalari *et al.* 1997).

## 1.3 Secondary Centres of Ossification

Chondrocytes in the epiphysis of long bones will undergo the same maturation process as those in the diaphysis for the establishment of the secondary and primary centres of ossification respectively (Lefebvre & Smits 2005). Both ossification centres involve degradation of cartilage, mineralization, angiogenesis and bone matrix deposition by osteoblasts, but ossification of the secondary centres differ in that vascularization occurs before the formation of a bony collar or hypertrophy and mineralization of the chondrocytes (Álvarez *et al*. 2005, Blumer *et al*. 2008).

Mesenchymal cells located in the perichondrium penetrate the avascular chondro-epiphyses and never originate along the surface of the articular cartilage (Burkus *et al*. 1993). Studies indicate that structural characteristics of vascular canals are very similar among vertebrates (Blumer *et al*. 2008). Differentiation of the mesenchymal cells gives rise to loose connective tissue, osteogenic cells and endothelial cells. The endothelial cells form the vascular structure within the canal, consisting of an arteriole and venule ending as a glomerulus type structure at the end of the canal (Blumer *et al*. 2008, Burkus *et al*. 1993). Osteogenic cells include cells like preosteoblasts, osteoblasts and osteocytes. These cells are responsible for the production of collagen type I that lines the cartilage canal and secondary centers of ossification. Tissue non-specific alkaline phosphatase (TNAP) plays an important role in the mineralization of collagen type I and is found in the matrix vesicles of the osteoblast (Blumer *et al*. 2008).

For the cartilage canal to advance it must have the ability to degrade cartilage components like collagen type II and aggregan, resorb the degraded products of the cartilage and synthesize angiogenic factors to stimulate growth (Blumer *et al*. 2008). It has been proposed that vascularization of the articulo-epiphyseal growth cartilage in mammals (mouse, rat and rabbit) follows two distinct processes i.e. quiescent and reactive angiogenesis.

### 1.3.1 Quiescent angiogenesis (with special reference to murine species):

Quiescent angiogenesis involves the generation of a network of cartilage canals with the aid of specific matrix metalloproteinases (MMP) i.e. MMP9, MMP13 and MMP14 (membrane-bound type1 matrix metalloproteinase; MT1-MMP), coupled with marrow cavity formation. Reactive angiogenesis, however, is VEGF dependent and is responsible for the active vascularization of hypertrophic chondrocytes with expansion of the marrow cavity (Blumer *et al*. 2008).

Developing cartilage canals move towards the centre of the epiphysis where hypertrophy of chondrocytes is initiated. The canals contain three populations of MMP expressing cells with cells co-expressing MMP13 and MT1-MMP, cells co-expressing MMP9 and MT1-MMP and cells expressing MT1-MMP alone (Álvarez *et al*. 2005, Blumer *et al*. 2008). Alvarez *et al*. (2005) localized the expression of MMP during canal formation. Matrix metalloproteinase 9 was expressed by a few perivascular cells located at the margin of the cartilage canal with both MMP13 and MT1-MMP expressed by perivascular cells at the end of the cartilage canal located at the interface with resting chondrocytes. The cells expressing MMP13 were clustered in small foci at the end of the canal. Membrane-bound type1 matrix metalloproteinase has a more extensive pattern when compared to that of either MMP9 or MMP13. Chondrocytes surrounding developing cartilage canals express type II collagen while cells inside the canal express low levels of type I collagen (Álvarez *et al*. 2005).

### 1.3.2 Reactive angiogenesis:

Cartilage canals that reach the centre of the epiphysis spread transversely and fuse to form a central cavity. Primary marrow cavity formation is associated with extended mineralization of the cartilage matrix and increased expression of MMP9, MMP13, and MT1-MMP from cells, with the majority of the cells located between the border of the cavity and the surrounding hypertrophic cartilage. The hypertrophic chondrocytes express both collagen

type X and VEGF. Multinucleated cells with osteoclast-like structures and aggregations of 5 – 10 small mononuclear cells are found at the cartilage-osseous junction and next to the terminal hypertrophic chondrocytes respectively. The small mononuclear cells possess cytoplasmic processes that are associated with resorption pits of the hypertrophic chondrocytes. Small mononuclear cells that are close to the hypertrophic chondrocytes stain positive for MMP13. Multinucleated cells and small mononuclear cells at the chondro-osseous junction stain positive for both MMP9 and MT1-MMP (Álvarez *et al*. 2005).

### 1.3.3 Articular cartilage and subchondral bone interface.

Chondroblasts that line the joint cavities differentiate into articular chondroblasts and then into articular chondrocytes. The articular chondrocytes differentiate rarely and never hypertrophy except in the tidemark. The tidemark is an area at the base of the mature articular cartilage that contains swollen chondrocytes that express collagen type X and induce mineralization (Hoemann *et al*. 2012, Lefebvre & Smits 2005). Blood vessels of the epiphyseal marrow cavity are not able to penetrate the tidemark but the tidemark is semipermeable to small (<500 Da) molecules. Penetration of the tidemark by blood vessels has been associated with osteo-arthritis. Vascular canals, however, vascularize the tidemark while the articular cartilage above the tidemark remains avascular (Hoemann *et al*. 2012).

## 1.4 Conclusion

The function of bone in the body is to provide a physical supporting structure to protect vital organs, serve as attachment for muscle to enable locomotion and act as a mineral reservoir (especially calcium and phosphate) that together with the endocrine system, maintain mineral homeostasis. Bone formation, from the recruitment of mesenchymal cells to mineralization of the apoptotic chondrocytes, is strictly regulated by molecular signalling pathways. Many of these pathways are not fully understood yet, leaving the process of bone formation still unknown. It is, however, important to note the differences between the primary and secondary centres of ossification. Ossification of the secondary centre is not preceded by perichondral bone formation or hypertrophy and mineralization of the chondrocytes as in the growth plate. Cartilage canal formation of the distal femur and proximal tibia of the bovine has not yet been described. Without the former information one can only refer to the process as described in studied species as well as to the pathology of bone that is as a result of factors negatively influencing bone formation or homeostasis.

## 1.5 References

Abad, V., Meyers, J.L., Weise, M., Gafni, R.I., Barnes, K.M., Nilsson, O., Bacher, J.D. & Baron, J., 2002, 'The role of the resting zone in growth plate chondrogenesis', *Endocrinology* vol. 143, no. 5, pp. 1851-1857.

Alini, M., Matsui, Y., Dodge, G.R. & Poole, A.R., 1992, 'The extracellular matrix of cartilage in the growth plate before and during calcification: changes in composition and degradation of type II collagen', *Calcified tissue international* vol. 50, no. 4, pp. 327-335.

Álvarez, J., Costales, L., Serra, R., Balbín, M. & López, J.M., 2005, 'Expression patterns of matrix metalloproteinases and vascular endothelial growth factor during epiphyseal ossification', *Journal of bone and mineral research* vol. 20, no. 6, pp. 1011-1021.

Anderson, H.C., 2003, 'Matrix vesicles and calcification', *Current rheumatology reports* vol. 5, no. 3, pp. 222-226.

Anderson, H.C., Garimella, R. & Tague, S.E., 2005, 'The role of matrix vesicles in growth plate development and biomineralization', *Front Biosci* vol. 10, no. 1, pp. 822-837.

Blumer, M.J., Longato, S. & Fritsch, H., 2008, 'Structure, formation and role of cartilage canals in the developing bone', *Annals of Anatomy-Anatomischer Anzeiger* vol. 190, no. 4, pp. 305-315.

Burkus, J.K., Ganey, T.M. & Ogden, J.A., 1993, 'Development of the cartilage canals and the secondary center of ossification in the distal chondroepiphysis of the prenatal human femur', *The Yale journal of biology and medicine* vol. 66, no. 3, pp. 193-202.

Fazzalari, N., Moore, A., Byers, S. & Byard, R., 1997, 'Quantitative analysis of trabecular morphogenesis in the human costochondral junction during the postnatal period in normal subjects', *The Anatomical Record* vol. 248, no. 1, pp. 1-12.

Gerber, H. & Ferrara, N., 2000, 'Angiogenesis and bone growth', *Trends in cardiovascular medicine* vol. 10, no. 5, pp. 223-228.

Gerstenfeld, L. & Shapiro, F., 1996, Expression of bone-specific genes by hypertrophic chondrocytes: Implications of the complex functions of the hypertrophic chondrocyte during endochondral bone development', *Journal of cellular biochemistry* vol. 62, no. 1, pp. 1-9.

Goff, J.P. 2004, 'Minerals, Bones, and Joints', in *Duke's Physiology of Domestic Animals* ed. R.O. Reece, 13th edn, Wiley Blackwell, pp. 593.

Goldring, M.B., Tsuchimochi, K. & Ijiri, K., 2006, 'The control of chondrogenesis', *Journal of cellular biochemistry* vol. 97, no. 1, pp. 33-44.

Hoemann, C.D., Lafantaisie-Favreau, C., Lascau-Coman, V., Chen, G. & Guzmán-Morales, J., 2012, 'The cartilage-bone interface', *Journal of Knee Surgery* vol. 25, no. 2, pp. 085.

Kanczler, J. & Oreffo, R., 2008, 'Osteogenesis and angiogenesis: the potential for engineering bone', *Eur Cell Mater* vol. 15, no. 2, pp. 100-114.

Kirsch, T. & Wuthier, R.E., 1994, 'Stimulation of calcification of growth plate cartilage matrix vesicles by binding to type II and X collagens', *The Journal of biological chemistry* vol. 269, no. 15, pp. 11462-11469.

Lefebvre, V. & Smits, P., 2005, 'Transcriptional control of chondrocyte fate and differentiation', *Birth Defects Research Part C: Embryo Today: Reviews* vol. 75, no. 3, pp. 200-212.

Mackie, E., Ahmed, Y., Tatarczuch, L., Chen, K. & Mirams, M., 2008, 'Endochondral ossification: how cartilage is converted into bone in the developing skeleton', *The international journal of biochemistry & cell biology* vol. 40, no. 1, pp. 46-62.

Ohyama, K., Farquharson, C., Whitehead, C.C. & Shapiro, I.M., 1997, 'Further observations on programmed cell death in the epiphyseal growth plate: comparison of normal and dyschondroplastic epiphyses', *Journal of Bone and Mineral Research* vol. 12, no. 10, pp. 1647-1656.

Ortega, N., Behonick, D.J. & Werb, Z., 2004, 'Matrix remodeling during endochondral ossification', *Trends in cell biology* vol. 14, no. 2, pp. 86-93.

Shen, G., 2005, 'The role of type X collagen in facilitating and regulating endochondral ossification of articular cartilage', *Orthodontics & craniofacial research* vol. 8, no. 1, pp. 11-17.

Vu, T.H., Shipley, J.M., Bergers, G., Berger, J.E., Helms, J.A., Hanahan, D., Shapiro, S.D., Senior, R.M. & Werb, Z., 1998, 'MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes', *Cell* vol. 93, no. 3, pp. 411-422.

Wardale, R.J. & Duance, V.C., 1993, 'Quantification and immunolocalisation of porcine articular and growth plate cartilage collagens', *Journal of cell science* vol. 105 ( Pt 4), no. Pt 4, pp. 975-984.

Wiesmann, H., Meyer, U., Plate, U. & Höhling, H., 2004, 'Aspects of collagen mineralization in hard tissue formation', *International review of cytology* vol. 242, pp. 121-156.

Zelzer, E. & Olsen, B.R., 2004, 'Multiple roles of vascular endothelial growth factor (VEGF) in skeletal development, growth, and repair', *Current topics in developmental biology* vol. 65, pp. 169-187.

# CHAPTER 2

# HISTORY OF SOUTH AFRICAN MINERAL DEFICIENCIES

## 2.1 Introduction

Only 15% of South Africa is potentially suitable for arable farming, leaving 85% to be potentially utilized as pasture for livestock production. The different vegetation types can be divided into seven different biomes: Savanna, Grassland, Forest, Fynbos, Nama Karoo, Succulent Karoo, Thicket and Desert, with the first five regarded as grazable lands. The distribution of most grazable lands coincides with the summer rainfall areas. Low erratic rainfall during summer and cold temperatures during winter affect the quantity and quality of the vegetation respectively (Tainton 1999).

Plants only take up a certain fraction of minerals available in the soil. Most mineral deficiencies are area specific as soil characteristics are directly related to certain mineral deficiencies. The type of soil, species, yield, stage of maturity, climate and management all influence the mineral concentrations of plants (McDowell 1996). Hutcheon investigated lamsiekte and stiff sickness and found that both diseases were more prevalent in areas where the soil is of a calcareous, light, sandy, or porous formation and especially after a drought period. The same class of animal i.e. young growing animals of both sexes, cows in calf, or lactating cows were more susceptible to develop either condition (Theiler 1912). Twenty-one minerals have been identified as being essential with regard to ruminant nutrition of which phosphorus is probably the best known deficient mineral (Tainton 1999).

Grasses show a marked drop in protein and mineral content and increased levels of fibre during the dry season (Van Niekerk & Jacobs 1985). Du Toit, Louw and Malan in 1930 conducted a countrywide analytical plant survey to determine the areas deficient in phosphates. The survey included analysis of the calcium, magnesium, potassium, sodium, chlorine, crude protein, crude fibre and soluble ash content of the vegetation. Clear differences between bushes and grasses were recorded. The difference in calsium content between bushes and grasses that grew on the same calciferous soils was frequently more than 1 % (dry basis) compared to 0.3 % respectively. Phosphate levels in grass varied from 0.12 –

0.17 % in summer to 0.05 – 0.07 % in winter. Interestingly, Karoo bush contained even and significantly higher concentrations of phosphate throughout the year than grass (Boyazoglu 1973). Theiler (1912) quoted D. T. Mitchell's finding that when cattle grazed the Karoo veld for a period of time and moved back to the grassland, they did not contract lamsiekte as soon. The protein content of grasses varied between 7 – 9 % in summer and 3.3 – 4 % in winter. Karoo bush of the same area had protein concentrations that varied between 7 – 10 % throughout the year (Boyazoglu 1973).

Increased animal growth rates and management practices will ensure a higher mineral demand to satisfy the need of the animal (McDowell 1996). Theiler, Green and Du Toit (1928) proved that one could improve conception from 51% to 80% and nearly double the weight of calves by supplementing phosphate to animals farmed in a phosphate deficient area (Armoedsvlakte).

Loss of livestock productivity due to mineral related deficiencies is not uncommon in South Africa. The northwestern parts of South Africa, in particular the North West and Northern Cape Provinces are well known for their mineral deficiencies and some of the diseases pertaining to mineral deficiencies will be discussed.

## 2.2 Mineral deficiency diseases in North West South Africa

### 2.2.1 Osteomalacia and Rickets

Osteomalacia, or stiff sickness is a metabolic bone disease that develops due to a nutritional mineral imbalance. Metabolic bone disease, however, can also result from toxicity or endocrine pathology (Weisbrode 2007). Osteoblasts are responsible for deposition of bone matrix (osteoid), initiating both the mineralization process of the osteoid and the resorption of this matrix by osteoclasts (Weisbrode 2007). The process of osteoid mineralization requires an adequate supply of minerals, normal circulating levels of vitamin D and functioning osteoblasts (Francis & Selby 1997).

Rickets and osteomalacia are defined as the absence or delay in the mineralization of growth cartilage and bone collagen and newly formed bone collagen and osteoid respectively (Berry *et al.* 2002). The most common cause of osteomalacia or rickets is the deficiency of phosphate or vitamin D (Weisbrode 2007). Vitamin D levels are tightly regulated and synthesis depends on various factors with parathyroid hormone (PTH), hypocalcaemia and hypophosphataemia all increasing the synthesis of vitamin D, whereas synthesis is suppressed

by hypercalcaemia, hyperphoshataemia and impaired renal function (Berry *et al.* 2002). Diets low in either calcium or phosphate lead to the development of rickets but only diets deficient in phosphate lead to the development of osteomalacia (Francis & Selby 1997). Experiments with knockout vitamin D receptor mice proved that feeding high doses of oral calcium could reverse the osteomalacia that developed as a result of no vitamin D mediated calcium absorption (Lips 2006).

The pathology of rickets includes the thickening of the growth plates resulting from decreased mineralization. The metaphyses "flare" as osteocytes cannot adhere to osteoid or cartilage, leading to a build-up of cartilage in the metaphyseal region of long bones. Cortical bone can appear normal but may deform under normal loads (Weisbrode 2007). Cattle with advanced stages of osteomalacia have enlarged marrow cavities with serous atrophy of fat and thinning of the cortical bone. The condition is painful, with most animals lying down. Standing animals present with a shifting lameness and an arched back (Theiler 1912). Thick osteoid seams cover most surfaces of the trabecular and cortical bone histologically (Lips 2006). Both rickets and osteomalacia are reversible with supplementation of the deficient mineral or vitamin D.

### 2.2.2 Botulism

Botulism in South Africa was first observed by travellers to South Africa in the late 18$^{th}$ century (Kriek & Odendaal 1994). Identification of losses dates back as far as 1805 when a commission encountered cattle suffering from lamsiekte (botulism) on fifteen different farms (Theiler 1912). Dr. Hutcheon, Colonial Veterinary Surgeon, investigated both botulism and stiffsickness and concluded that both conditions occurred in cattle that had a craving for bones and other animal matter. Both diseases were more prevalent in areas where the soil was of a calcareous, light, sandy, or porous formation especially after a drought. The same class of animal i.e. young growing animals of both sexes, cows in calf, or lactating cows are more susceptible.

Both diseases were attributed to a phosphate-deficient diet. Experiments to associate botulism with phosphate deficiencies were conducted from 1895 to 1986 and results proved that none of the cattle supplemented with bone meal manifested with clinical signs indicative of either stiff sickness or botulism (Theiler 1912). Subsequent observations and experimental investigations revealed that while stiff sickness resulted directly from phosphate deficiency,

botulism was a toxaemia that resulted from ingestion by cattle of bones or other carcass material infested with *Clostridium botulinum* type D (Kriek & Odendaal 1994; Theiler 1927). This bacterium produces a toxin that inhibits the release of acetylcholine at the neuromuscular junction so that no stimulus reaches the motor end-plate, causing flaccid paralysis. Paralysis of the respiratory tract is normally the cause of death (Kriek & Odendaal 1994). Cattle not deprived of any mineral will not touch carcass debris, but those suffering from a phosphate deficiency will express osteophagia i.e. a craving for bones (pica), to increase ingestion of phosphate (Theiler 1927). Outbreaks of botulism associated with phosphorus deficiency and osteophagia extend over long periods and resulted in massive livestock losses (Kriek & Odendaal 1994).

### 2.2.3 Vryburg hepatosis

Geophagia, another form of pica (Neser *et al*. 1997), may indicate a deficiency of minerals such as sodium, magnesium, phosphate, copper, cobalt, sulphur and manganese in the diet of ruminants (Elsenbroek & Neser 2002). Geophagia can, however, lead to toxicosis, as described by Neser *et al*. (1997). Since 1974 farmers from the Vryburg area reported an increase in the death of calves raised in specific areas where the soil is rich in manganese that derived from the weathering of superficial dolomite formations. The frequency of geophagia increased in calves aged between 7–12 days with high mortalities 7–10 days later due to severe subacute to chronic cholangiohepatitis, constipation and dehydration in untreated cases (Neser *et al*. 1997). Manganese interferes with the absorption of iron and cobalt from the gastrointestinal tract by competitively binding to the sites of absorption of the latter minerals (Elsenbroek & Neser 2002). The initial stimulus for geophagia in calves is unknown but it is possibly an instinctive mechanism to secure the necessary minerals,especially iron. Parenteral injection of iron and vitamin $B_{12}$ at 1–2 days of age and two weeks later proved to be effective as a preventative measure (Neser *et al*. 1997).

## 2.3 Discussion

Supplementation of minerals has been extensively used after the pathogenesis of botulism and osteomalacia was described. Even before that, these conditions were ascribed to ‘the absence of sufficient phosphates in the food’ (Theiler 1912). Bone meal was fed for extended periods as a phosphate supplement until the outbreak of Bovine Spongiform Encephalopathy (BSE) in the United Kingdom during 1985 (Prozesky *et al*. 2016). The outbreak of BSE lead to the banning of the use of bone meal as a phosphate supplement, forcing livestock

producers to revert to chemically manufactured phosphates as a phosphate source in supplementary feed.

Phosphorus supplementation by means of feeding bone meal improved weight gain and fertility of cattle significantly. The greatest response to phosphate supplementation was not during the winter months when the phosphate levels of vegetation were the lowest, but during the summer months (Boyazoglu 1973). Weight gain occurs most rapidly when energy and protein is readily available from the vegetation and it is during this phase that cattle require adequate minerals to support growth. Supplementation of cattle with minerals during periods of energy and protein malnutrition has no advantages or economical benefit (McDowell 1996). However, cattle farmed in phosphate deficient areas without additional supplementary phosphate during periods when adequate energy and protein levels are available, may lose as much as 25 – 30 % of their predicted final summer weight gain. Supplementing phosphate when either protein or energy levels are deficient tends to have a negative effect on feed intake and live mass (Van Niekerk & Jacobs 1985). Different formulations of feed supplements are used depending on the quality and quantity of grazeable vegetation available.

During summer, vegetation has sufficient levels of protein and energy and supplementary feed only needs to enhance the phosphate concentration. The two main ingredients of a summer supplementary lick are salt and phosphate, and the ratio depends on the amount of phosphate required by the specific group. Cattle have the ability to taste salt and a desire for it (McDowell 1996). Gordon *et al.* (1954) proved that cattle grazed on phosphate deficient pasture do not express a predilection for phosphate supplementary feed compared to a feed containing only calcium carbonate. Levels of phosphorus ingested were markedly below the level to prevent development of aphosphorosis (Gordon *et al.* 1954). Katz (1937) contradicted the observations of Theiler and Green (1932), stating that osteophagia is a subconscious irresistible desire to restore the equilibrium of especially phosphate (Gordon *et al.* 1954), but Theiler and Green (1932) admitted that there is no obvious preference between materials containing phosphate (Gordon *et al.* 1954). Cattle with aphosphorosis definitely show an increased incidence of pica. Gordon *et al.* (1954) stated that osteophagia is probably due to either a matter of chance, imitation of behaviour or experience from previous episodes of osteophagia. The function of the salt is to increase the palatability and insure adequate

intake of the phosphate supplementary feed with intake levels varying between 100--200 g per animal per day

The composition of pastures changes during the dry period. Vegetation actively stops growing, loses its green colour, and increases in fibre and lignin content (McDowell 1996). The concentration of phosphorus and protein in grasses varied between 0,12–0,17% and 7–9% in summer and 0,05–0,07% and 3,3–4% in winter respectively (Boyazoglu 1976). Supplementing 60% of cattle's protein daily requirements increased low quality roughage intake by 34,5% compared to a control group (Van Niekerk & Jacobs 1985).

The main purpose of winter supplementation is to maintain the body condition score of cattle throughout the dry period as most cows are pregnant and will calve down as the wet season commences. The main ingredients of a winter supplementary feed are protein and phosphate. Urea is included in the diet as a non-protein nitrogen source. One kg of urea contains 460 g of nitrogen, which is able to produce 2 875 g of protein (Meissner 1999). Urea is broken down to ammonia, which is used to synthesize microbial protein that will be digested and absorbed by the animal further down the gastrointestinal tract. The average consumption of a winter supplement is 350 – 500 g per animal per day. Van Niekerk and Jacobs (1985) stated that low level energy supplementation had an insignificant positive effect on roughage intake and live mass change.

Production supplements provides protein, energy and phosphate to the animal and can be used on any veld type or during any season. The main purpose of the supplementary feed is to increase the condition score of animals by raising the plane of nutrition. The feed is mostly intended for growing and lactating animals.

## 2.4 Conclusion

Loss of livestock productivity due to mineral related deficiencies is not uncommon in South Africa. Of these, phosphorus is the most widespread and most researched mineral deficiency. Phosphate supplementation significantly reduces the incidence of botulism and osteomalacia and also significantly improves fertility of cows and growth rates of calves in cattle farmed on phosphate deficient land. However, supplementing phosphate when either protein or energy levels are deficient tends to have a negative effect on feed intake and live mass. Supplementary feeds are used extensively to improve productivity of livestock. Different formulations are used depending on the quantity and quality of the natural pasture. Supplementary feeds do not only provide animals with energy and protein during periods when levels are deficient, but provide the necessary minerals especially phosphate. Mineral requirements increase as productivity and management increase. It is, however, important to note that mineral supplementation will only be beneficial when protein and energy levels are adequate.

## 2.5 References

Berry, J.L., Davies, M. & Mee, A.P., 2002, 'Vitamin D metabolism, rickets, and osteomalacia,' *Seminars in musculoskeletal radiology* 6 (3), 173-182.

Boyazoglu, P., 1973, 'Mineral imbalances of ruminants in southern Africa' *South African journal of animal science*.Suid-Afrikaanse tydskrif vir veekunde.

Boyazoglu, P.A., 1976, 'A review of mineral imbalances of grazing animals in southern Africa', *Journal of the South African Veterinary Association* 47 (2), 129-132.

Elsenbroek, J. & Neser, J., 2002, 'An environmental application of regional geochemical mapping in understanding enzootic geophagia of calves in the Reivilo area, South Africa', *Environmental Geochemistry and Health* 24 (2), 159-181.

Francis, R.M. & Selby, P.L., 1997, 'Osteomalacia', *Baillière's clinical endocrinology and metabolism* 11 (1), 145-163.

Gordon, J., Tribe, D. & Graham, T., 1954, 'The feeding behaviour of phosphorus-deficient cattle and sheep', *The British Journal of Animal Behaviour* 2 (2), 72-74.

Kriek, N. & Odendaal, M., 1994, 'Botulism', *Infectious Diseases of Livestock*.Oxford Press, Cape Town., 1354-1371.

Lips, P., 2006, 'Vitamin D physiology', *Progress in biophysics and molecular biology* 92 (1), 4-8.

McDowell, L.R., 1996, 'Feeding minerals to cattle on pasture', *Animal Feed Science and Technology* 60 (3), 247-271.

Meissner, H., 1999, 'Nutrient supplementation of the grazing animal' First ed. In Tainton, N. ed. *Veld Management in South Africa*. Pietermaritzburg: University of Natal Press. 334 p.

Neser, J., De Vries, M., De Vries, M., Van der Merwe, A., Loock, A., Smith, H., van der Vyver, F.H., Elsenbroek, J.H.& Delport, R., 1997, 'The possible role of manganese poisoning in enzootic geophagia and hepatitis of calves and lambs: to the editor,' *Journal of the South African Veterinary Association* 68 (1), p. 4-6.

Prozesky, L., Neser, J., Meissner, H., Botha, K., Jacobs, L., Shepstone, C., Viljoen, H., Köster, H., de Brouwer, C., van Zyl, J. and van der Veen, G., 2016, 'Preliminary report on osteochondrosis in cattle in the north-western parts of South Africa', *Onderstepoort Journal of Veterinary Research*, *83*(1), a1083.

Tainton, N.M.ed., 1999, 'Veld Management in South Africa,' First ed. Pietermaritzburg: University of Natal.

Theiler, A., 1927, 'Lamsiekte (Parabotulism) in cattle in South Africa: cause of the disease and experiments upon its production in the lamsiekte area'.

Theiler, A., 1912, 'Facts and theories about stijfziekte and lamziekte', *Second Report of the Director of Veterinary Research, Union of South Africa*, 7-78.

Theiler, A., Green, H.H. & Du Toit, P.J., 1928, 'Studies in mineral metabolism. III. Breeding of cattle on phosphorus deficient pasture', *The Journal of Agricultural Science 18*(03), pp.369-371.

Van Niekerk, B. & Jacobs, G., 1985, 'Protein, energy and phosphorus supplementation of cattle fed low-quality forage', *South African Journal of Animal Science* 15 133-136.

Weisbrode, S., 2007, 'Bone and Joints', Fourth edition ed. In McGavin, M.& Zachary, J. eds. Pathologic basis of veterinary disease. St. Louis, Missouri: Mosby Elsevier. 1041 p.

***Figure 1**: Tollie suffering from botulism (attempting to stand) (Theiler 1912).*

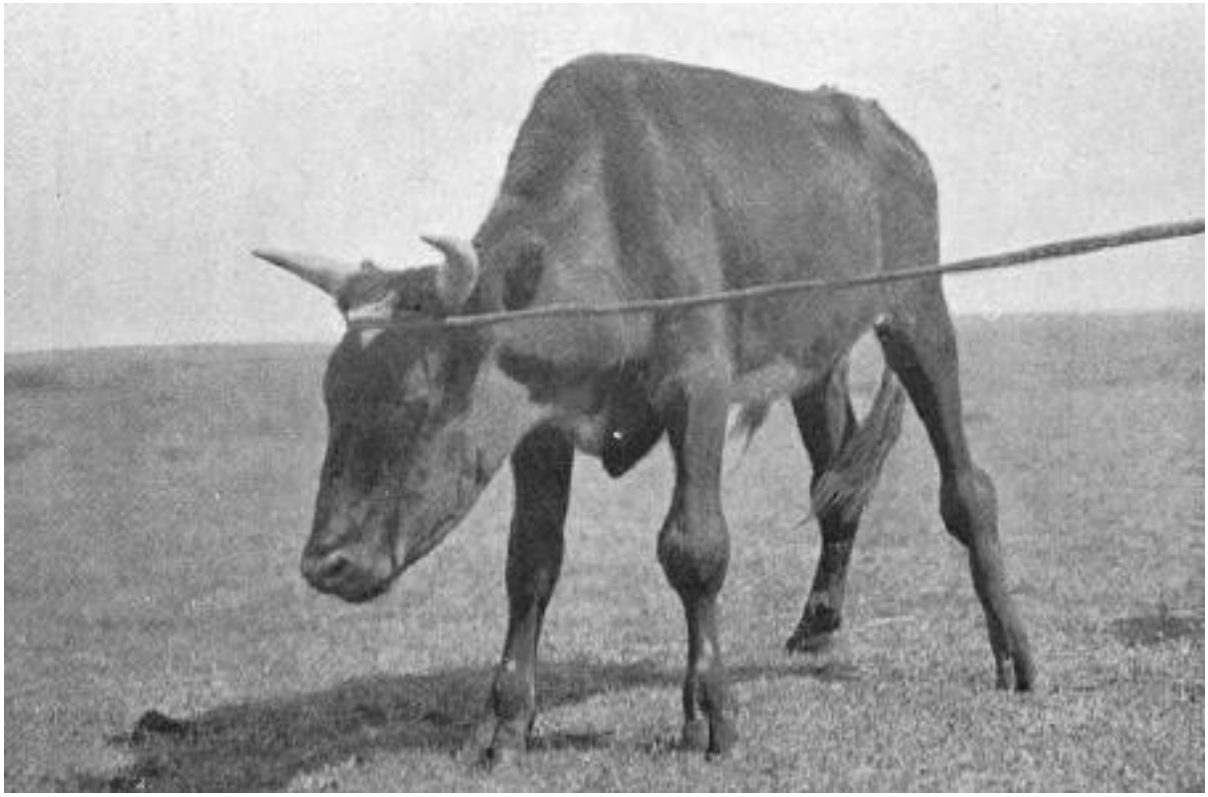


***Figure 2**: Animal suffering from osteomalacia (Theiler 1912).*

# CHAPTER 3

# OSTEOCHONDROSIS

## 3.1 Introduction

Osteochondrosis is a broad term pertaining to a group of lesions associated with the persistence of growth cartilage in the epiphyseal or physeal growth plates as a result of failure of endochondral ossification (Laverty & Girard 2013). König first introduced the term in 1888 to describe a pathological condition of the articular cartilage that leads to the formation of loose bodies in the joint (Ytrehus, Carlson & Ekman 2007). Ytrehus *et al.* (2007) introduced three subdivisions of osteochondrosis: (1) A focal area of cartilage necrosis confined to the epiphyseal cartilage is termed osteochondrosis latens. (2) If the area of necrosis is visible both macroscopically and radiologically it is designated as osteochondrosis manifesta. (3) Fissure formation of the necrotic cartilage extending through the articular cartilage is termed osteochondrosis dissecans. Different aetiological components of osteochondrosis have been proposed. The incidence of osteochondrosis is most prevalent during the period of rapid growth and especially in those animals where rapid growth occurs (Ekman & Carlson 1998). Grøndalen (1974) proposed that if the osteochondrotic lesion does not affect the surface of the joint cartilage, the lesion might heal or undergo repair after the period of growth is over. If the osteochondrotic lesion penetrates or disrupts the joint cartilage it will develop as a progressive arthrosis.

## 3.2 Proposed aetiologies of osteochondrosis

### 3.2.1 Effects of rapid growth, energy and protein on osteochondrosis

Economical pressure for increased meat production places emphasis on the rate of genetic selection for increased production and increased management to accelerate increased growth rates. Leg weakness in pigs increased significantly during this phase of increased production affecting as many as 85–90% of swine (Frantz *et al*. 2008). Reducing the levels of energy and protein in the diet of pigs did not significantly reduce the prevalence and severity of osteochondrosis (Ekman & Carlson 1998, Grøndalen 1974, Knight *et al*. 1985, Nakano *et al*. 1984, Woodard, Becker & Poulos 1987). Reducing the growth rate did not significantly reduce the prevalence of osteochondrotic lesions in pigs, which is the opposite to what

occured in dogs, where rapid growth had an increased effect on the development of skeletal pathology (Ytrehus *et al.* 2007).

The negative effect of high–energy intake during the growth period has been described in dogs especially in the large breeds (Richardson, Schoenherr & Zicker 1997). Above recommended energy intake does not only increase the rate of bone growth but also affects the endocrine regulatory factors like growth hormone, insulin-like growth factor-1, triiodothyronine, thyroxine and insulin in the body. *Ad lib* feeding results in increased circulating concentrations of thyroxine and triiodothyronine in dogs. The thyroid hormones increase the rate of bone formation and degradation and are an important factor in the capillary penetration of degenerating cartilage cells. Increased bone growth can weaken the already less strong epiphyseal spongiosa of large breed dogs when compared to the spongiosa of small breeds, increasing the risk for the development of osteochondrotic lesions (Richardson & Zentek 1998).

Investigation of the prevalence of osteochondrosis in foals has led to the conclusion that foals fed a diet with high levels of digestible energy are predisposed to develop osteochondrosis (Glade & Belling 1986, Savage, McCarthy & Jeffcott 1993a, 1993b). Increased growth rate *per se* does not seem to be responsible for the development of osteochondrosis in foals. Feeding of a diet high in crude protein (126% NRC) only produced a low incidence of both micro- and macroscopic osteochondrosis compared to a diet high in digestible energy (129% NRC) that led to a significantly high incidence of osteochondrosis (Savage *et al.* 1993a). Glade *et al.* (1986) reported a reduction of hexosamine and hydroxyproline and an increase of the DNA contents of both the articular and growth plate cartilages when foals were fed a diet with high levels of digestible energy and protein (130% NRC). High-energy diets (129% NRC) do not affect bone quality of horses, as they had no significant effect on the cancellous or cortical bone, while feeding of excessive phosphate (388% NRC) led to the development of porous cortical and cancellous bone (Savage *et al.* 1993b).

Reiland *et al.* (1978) researched the incidence of osteochondrosis in 48 young bulls with an average weight of 100 kg. The bulls were fed either a high intensity feed *ad lib* (75.96 ME MJ/day) or low intensity feed, 80% of the *ad lib* feed (64.86 ME MJ/day). Protein levels did not influence the incidence or severity of osteochondrosis but all of the 23 bulls on the high intensity feed had a lesion in one or several joints and/or growth plates compared to only 13 out of 25 bulls fed the low intensity feed. Sixteen bulls all belonging to the high intensity group had growth plate lesions. The prevalence and intensity of osteochondrosis were more

pronounced in the high-intensity feeding group, indicating a possible correlation between the increased prevalence of osteochondrosis with rapid growth.

Dutra *et al.* (1999) reported a 60.8% and 80.4% incidence of osteochondrosis in the articular epiphyseal cartilage complex and physeal growth plate respectively in 46 12-month-old bulls of various breeds. The bulls received a diet containing 13% crude protein, 12 MJ / kg metabolizable energy, 0.7 - 0.8 % calcium and 0.42 % phosphorus (ratio 1.8:1) for a period of six months. The number of lesions per bull was significantly correlated with daily weight gain, carcass weight-, and the width of the proximal tibial epiphysis.

Jensen *et al*. (1981) investigated the lameness of cattle in feedlots and reported that 8.5% and 3.8% had characteristic osteochondrotic lesions of the stifle and atlanto-occipital joint respectively. They proposed that high-energy diets and rapid growth might have been the contributing factors that led to the development of osteochondrosis in feedlot cattle.

The high energy level theory may hold water as foals (Glade & Belling 1986, Savage *et al.* 1993a, Savage *et al.* 1993b), dogs (Richardson *et al.* 1997) and cattle (Jensen *et al*. 1981, Reiland *et al*. 1978) have an increased incidence of osteochondrosis when fed a high-energy diet. Reducing the levels of energy and protein in the diet of pigs did not significantly reduce the prevalence and severity of osteochondrosis (Ekman & Carlson 1998, Grøndalen 1974, Knight *et al.* 1985, Nakano *et al.* 1984, Woodard *et al.* 1987).

### 3.2.2 Heredity and anatomical characteristics

Osteochondrosis has been described in pigs, dogs, lambs, poultry, horses, cattle, cats and rats (Laverty & Girard 2013, Ytrehus *et al.* 2007) with different prevalences recorded between breeds of dogs, pigs-, and horses highlighting the heritable component thereof (Ytrehus *et al.* 2007). Several studies of dogs and horses indicated that osteochondrosis is inherited as a polygenetic trait (Ytrehus *et al.* 2007) but no information concerning heritability of osteochondrosis in cattle is available (Hill, Sutton & Thompson 1998).

The genetic influence of boars with high breeding values for osteochondrosis emphasized the focus on the anatomical confirmation of the offspring. It is proposed that the right combination of joint characteristics would result in local overloading of the joint predisposing to osteochondrosis, which in essence relates back to the genetic traits of the breed (Ytrehus *et al.* 2007). Selection for better back and hind leg conformation implemented for breeding animals resulted in a decrease in osteochondrotic lesions from 6.7 % in 1970 to 1.5 % in 1980 of slaughter pigs in Norway (Grøndalen 1981). De Koning *et al.* (2012) looked at the

association between osteochondrosis, conformation and locomotive characteristics of pigs. They found that pigs with swaying hindquarters had a high degree of osteochondrotic lesions in the stifle joint while pigs with a smaller inner claw (when compared to the outer claw) of the front leg had a lower degree of osteochondrotic lesions in the stifle joint.

Van Grevenhof *et al.* (2009) found that there is a low heritability for osteochondrotic lesions of the stifle joint and moderate heritability for the tarsocrural joint of horses. These results coincide with a physiological study conducted by Dik *et al.* (1999). Osteochondrotic lesions of the femoropatellar joint were rarely seen at birth and only developed between the third and eighth month of age, indicating that it is rather due to an environmental factor than genetics. Osteochondrotic lesions of the tarsocrural joint are most likely due to genetics as they were more commonly seen at birth and recovered between the second and fifth month of age (Van Grevenhof *et al*. 2009).

Anatomic and genetic factors are important in the aetiology and development of osteochondrosis of animals, but to determine the extent of the effects of these factors is difficult.

### 3.2.3 Trauma

Trauma is the most widely proposed aetiology for the development of osteochondrosis, as lesions most commonly develop in areas with increased biomechanical stress. Pool (1993) made use of the human osteochondrosis classification scheme used for comparable lesions in humans. Primary articular osteochondrosis includes both the primary defect present in the epiphyseal physis of the developing joint as a result of trauma as well as the lesion that can develop as a result of traumatic insult to the osteogenic capillary bed of the subchondral bone that would invade the cartilage model in joint development. Chronic microtrauma of mature racehorses results in articular cartilage damage resembling osteochondrosis, but in fact it should rather be classified as chronic arthrosis as development of the lesion is not directly related to osteochondrosis. Continuous microtrauma can lead to microfractures of the subchondral bone, enabling synovial fluid from the joint to enter the subchondral bone via the microfracture. The synovial fluid increases the intraosseous pressure and negatively affects the microcirculation. Diminished microcirculation leads to bone necrosis and possible collapse of the subchondral bone and subchondral cyst formation (Madry, van Dijk & Mueller-Gerbl 2010).

Healing of an acute osteochondral fracture of skeletally immature horses can produce the same lesion as what is classified as osteochondrosis of the caudal aspect of the proximal articular surface of the humerus, lesions on the medial condyle of the humerus, and lesions located on the weight bearing surfaces of the lateral trochlear ridges of the femur and talus. With healing of the fracture the deep layers of the epiphyseal cartilage die and disrupt the subchondral capillary bed, forming fibrous granulation tissue. Viable superficial layers of the epiphyseal physis keep on producing growth cartilage, increasing the thickness of the deep articular cartilage layer.

The medial condyle of the femur is in direct contact with the articulating surface of the tibia, undergoing direct loading during locomotion in the horse (Pool 1993) and in the pig (Ekman & Carlson 1998). Hill *et al.* (1998) reported more severe osteochondrotic lesions on the medial condyle of the femur compared to that of the lateral condyle, fitting the distribution pattern of weight within the stifle joint. Pool (1993) posed the question whether osteochondrosis develops as a result of abnormal mechanical loads exerted on normal developing joint surfaces or as a result of normal mechanical loads exerted on abnormal developing joint cartilage.

## 3.2.4 Dietary Mineral Factors

### 3.2.4.1 Calcium and Phosphate

It is well established that extra calcium and phosphorus are necessary for optimal mineralization of bone over and above that required for optimal growth.

Grøndalen (1974), Brennan & Aherne (1986), and Kornegay *et al.* (1990) all proved that the frequency and severity of osteochondrotic lesions in pigs were not influenced by whether a diet below or exceeding the calcium and phosphate recommendations was fed to growing pigs. Diets with calcium and phosphate levels exceeding recommendations (NAS-NRC 1979) did, however, increase the ash level of bone (Brennan & Aherne 1986) and produced a more optimal spongious bone structure (Grøndalen 1974). Although these diets increased mineralization of bone, they had no significant effect on the incidence and severity of joint lesions (Brennan & Aherne 1986). Pigs with induced hypophosphataemia had generalized disturbance of the endochondral ossification process. Lesions reproduced were similar to those of rickets, with impaired vascular penetration and excessive deposition of osteoid in the primary spongiosa microscopically. Necrosis of the primary spongia resulted in focal cartilage retentions. Focal degenerative cartilage changes in the normophosphataemic control

group developed as a result of impaired vascular penetration (Reiland *et al.* 1991). Reiland *et al.* (1991) concluded that hypophosphataemia is not an aetiological factor in the development of osteochondrosis in pigs.

Calcium supplementation of horses has been extensively studied and produced different results that vary from a low calcium deposition in skeletal ash of ponies to greater amounts of lamellar bone with a diet containing 2% calcium. Calcium supplementation above the recommended levels did not increase the incidence of osteochondrosis unless it was combined with excess dietary energy (Savage *et al.* 1993a). Supplementation of phosphate produced the most significant result with regard to osteochondrosis. Excessive dietary supplementation of phosphate (388% NRC 1989) increased the incidence of osteochondrosis in unexercised foals. Knight *et al.* (1985) reported that farms in the states of Ohio and Kentucky feeding twice the recommended NRC (1978) level of calcium, phosphate and zinc and three to four times the recommended NRC (1978) level of copper had the lowest incidence of metabolic bone disease.

Musculoskeletal disorders affect about 22% of dogs under the age of 1 year and in 20% of the dogs they have a nutrition-related aetiology (Richardson *et al.*1997). As in other species, fast growing, large breed animals are more predisposed to the development of osteochondrosis (Knight *et al.* 1985, Nakano *et al.* 1987, Olsson & Reiland 1978, Richardson & Zentek 1998) . The most commonly affected joints in dogs are the shoulder, stifle, hock and elbow (Richardson & Zentek 1998, Trostel *et al.* 2002). Nutritional excesses, especially of calcium and energy, predispose to the development of osteochondrosis in large breed dogs (Richardson *et al.* 1997). Great Dane puppies fed a diet with excess calcium (3.3% dry matter) with normal phosphate (0.9% dry matter) or high phosphate (3% dry matter) had a significant increase in the frequency and severity of osteochondrosis (Goedegebuure & Hazewinkel 1986). The high calcium diet had the same calcium absorption coefficient as the control group diet (Goedegebuure & Hazewinkel 1986) that resulted in increased calcium absorption and retention (Hazewinkel *et al*. 1991). Inadequate phosphate intake is uncommon with the feeding of commercial dog food. Increased levels of $1,25(OH)_2D_3$ and the up-regulation of NaPi-IIb protein modulates increased phosphate absorption from the gastrointestinal tract with feeding diets deficient in phosphate for an extended period of time (Marks *et al.* 2010). Efficiency of calcium and phosphate absorption from the gastrointestinal

tract is increased by 30–40% and 80% respectively in the presence of $1,25(OH)_2D_3$ (Holick 2007).

Excessive supplementation of phosphate can lead to the development of hyperphosphataemia as a result of increased gastrointestinal absorption influencing parathyroid hormone (PTH) synthesis and secretion (Slatopolsky *et al.* 2001). Intermittent exposure to PTH stimulates osteoblasts to increase bone formation; however, chronic exposure to PTH activates osteoclasts indirectly through osteoblasts (Bergwitz & Jüppner 2010). Increased osteoclastic activity and decreased osteoblastic activity would lead to increased bone resorption and decreased bone formation respectively.

Heinola *et al.* (2006) reported an outbreak of osteochondrosis in fattening bulls. Animals were mistakenly fed 60–70% calcium and twice the recommended levels of phosphate. Animals aged between 5–12 months had a higher incidence of osteochondrosis. 80% Of the calcium-deficient group compared to 30% of the animals in the control group developed osteochondrosis.

Davies & Munro (1999) reported the development of osteochondrosis in bulls after the feeding of a protein mix in which the mineral, vitamin and avoparcin additive was not included. The age of the bulls varied between four and 14 months, of which the four- to seven-month-old bulls were most severely affected. Calcium and phosphorus levels in the mineral deficient feed measured 0.31% and 1% respectively compared to the proposed formulated levels of 3.3% calcium and 1% phosphate. Dietary analysis also indicated that the diet was deficient in sodium, copper, vitamin A, vitamin D and vitamin E. General clinical improvement was seen after the feeding of a balanced diet for two to three weeks.

Prozesky *et al.* (in press) reported the clinical improvement of especially the young cattle suffering from osteochondrosis after they were supplemented with a commercial mineral supplementary feed containing high levels of bio-available micro-and macro minerals for a period of three months. On post mortem examination of the cattle that improved clinically, it was noted that especially in young cattle the damaged hyaline articular cartilage was replaced by irregular fibro-cartilage in the affected joints.

#### 3.2.4.2 Copper

Copper is one of the 22 elements present in the body that is necessary for normal skeletal development and maintenance (Hidiroglou 1980). Copper is an essential cofactor in formation by lysyl oxidase of pyridinoline cross-links between adjacent collagen fibres and bone (Hurtig *et al*. 1993). The structural integrity of the collagen depends on the cross-linkages. Copper deficiency leads to a condition similar to rickets in calves and osteomalacia in older cattle. Calves fed a diet low in copper develop a stilted gait, a knock-kneed appearance and swelling of the metacarpophalangeal and carpometacarpal joints after 160 days. Histological evaluation includes widening of the growth plate with sections of uncalcified cartilage and delayed or impaired provisional calcification (Hidiroglou 1980).

Copper deficiency in foals produced lesions throughout the body, which is not typical of osteochondrosis. The lesions appeared to be more similar to those in copper deficient cattle than osteochondrosis in horses. Histology of the growth plates and metaphyseal bone of affected foals revealed that fractures altered the normal architecture of the growth plate, affecting bone formation. The foals had fewer newly formed trabeculae in the primary spongiosa and distal metaphysis that resulted in less support to the articular cartilage. Fracture of the subchondral bone can lead to the development of osteochondral flaps (Hurtig *et al*. 1993). Zinc has been proven to reduce the level of copper absorption. Zinc stimulates the formation of metallothionein that binds copper in the intestinal epithelium. The intestinal epithelium desquamates into the lumen of the intestine and is excreted without any copper being absorbed (Bridges & Harris 1988).

## 3.3 Models of Pathogenesis

Osteochondral lesions involve both the articular cartilage and the subchondral bone. The subchondral bone plate is the lamellar bone that is immediately below the calcified articular cartilage zone, separating the articular cartilage from the bone marrow and trabecular bone. The hyaline articular cartilage consists of a superficial non-mineralized cartilage zone with predominantly type II collagen. The calcified cartilage zone is situated beneath the non-mineralized cartilage zone and separated from the latter by the “tidemark”. Collagen type X dominates the calcified zone and the collagen fibres of both the non-mineralized and calcified zones are continuous, ensuring a strong connection between the respective zones. The subchondral bone plate is situated beneath the calcified cartilage zone and separated by a cement line. The type I collagen fibres of the subchondral bone plate are not continuous with

the collagen type X fibres of the calcified articular cartilage zone and this division creates an area of weakness.

Blood vessels and nerves of the vascular channels are connected at the osteochondral junction via small perforations located in the subchondral bone plate. These perforations also act as a direct communication between the uncalcified articular cartilage and the marrow cavity. Areas of the joint experiencing the greatest level of stress have the highest concentration of perforations and these areas have a particularly rich blood supply. Nutrients in the former regions reach the calcified cartilage zone by means of diffusion from both the blood vessels and the synovial fluid. Areas of articular cartilage with a limited number of blood vessels are dependent on diffusion of minerals from the synovial fluid as a source of nutrition (Madry *et al.* 2010).

Subchondral bone thickness, strength and density vary according to the shape as well as the loading distribution of the joint. Convex articular surfaces have a much thinner but more uniform distribution of bone than that of the complementary component. The subchondral bone of the human tibial plateau is thicker in the centre than at the periphery and the medial condyle stronger than the lateral condyle. Strength measurements of the tibial plateau can be divided into a strong weight-bearing area (medial and lateral condyles), a weak central zone and intermediate paramedial and paralateral zones. Studies also showed that one could determine the density and strength of the subchondral bone by measuring the mineralization pattern of the bone, as there is a significantly high correlation between strength and mineralization (Madry *et al.* 2010).

### 3.3.1 Fragility of cartilage and/or bone

Bone size and shape and tissue quality of both the mineral and collagen, rate of bone turnover and architecture of the trabecular bone, influence the fragility of bone. Bone fragility includes factors like strength, brittleness and failure to work. The mineral component provides the stiffness and the collagen fibres the toughness of bone (Viguet-Carrin *et al.* 2006). Metabolic bone diseases like osteoporosis and osteomalacia both lead to an increase in bone fragility but differ in the pathogenesis thereof. Reduced bone mass and the increased frequency of microcracks result in brittle bone seen during osteoporosis. Osteoporotic bone can only absorb a limited amount of energy before it fractures. Failure of mineralization leads to the development of rickets and osteomalacia in young animals and adult animals respectively. Osteomalacia is a disease of bone only, while rickets involve bone as well as the cartilage

that undergoes endochondral ossification. Bone becomes soft and deforms with weight bearing. Histological sections of both the cortical and trabecular bone have increased seams of unmineralized osteoid (Weisbrode 2007).

Mechanical properties of cancellous bone depend on the apparent density of the material and the arrangement of the trabeculae rather than the relationship between the water, organic and mineral components (Currey 2003). Collagen fibrils are stabilized by crosslinks that form through enzymatic and nonenzymatic processes. Lysyl oxidase, a copper-dependent enzyme, converts telopeptidyl lysine and hydroxylysine into the aldehydes, allysine and hydroxyallysine respectively. Lysyl oxidase deaminates allysine and hydroxyallysine through oxidation of the hydroxyline residue after a series of nonenzymatic processes that occurr in the crosslinking sites of the structural proteins. The structural proteins at the crosslinking sites are transformed to hydroxyallysine, which through the process of condensation with the lysyl or hydroxylysyl side chain form crosslinks between collagen fibrils. These crosslinks are still immature and unstable. Bone turnover rate affects the amount of mature and immature crosslinks but not the process of conversion from immature to mature crosslinks. Copper deficiency leads to a reduction of crosslinks as a result of inhibition of the enzyme lysyl oxidase. Bone strength decreases due to the reduction of collagen crosslinks (Knott & Bailey 1998, Viguet-Carrin *et al.* 2006).

Dammrich (1991) recorded with his experiments in Great Dane puppies that over-nutrition leads to rapid bone and muscle growth. Both the cancellous and cortical bone had lower bone density and subsequently a lower resistance to biomechanical stressors. Some dogs develop joint lesions below the growth plate, as the reduced density of the spongiosa could not support the normal biomechanical loads.

### 3.3.2 Primary dyschondroplasia

Dyschondroplasia is the retention of unvascularised, unmineralised cartilage of the growth plate that extends distally into the metaphysis (Farquharson *et al.* 1995), disrupting the process of endochondral ossification (Jeffcott & Henson 1998). The retained cartilage may predispose the joint to secondary damage, leading to the development of osteochondrosis (Jeffcott & Henson 1998). Ytrehus *et al.* (2007), however, disagree with the statement of Jeffcott & Henson (1998) that osteochondrosis develops as a result of dyschondroplasia. It is generally accepted that dyschondroplasia is due to the inability of prehypertrophic chondrocytes to undergo terminal differentiation (Farquharson *et al.* 1995, Farquharson &

Jefferies 2000), a process during which cartilage will undergo vascularization, mineralization and resorption. During the differentiation, production of type II collagen is down-regulated, production of type X collagen up-regulated and an increase in production of ALP is noticed (Farquharson *et al.* 1995). Another proposed hypothesis for the development of dyschondroplasia is the failure of metaphyseal blood vessels to invade the hypertrophic chondrocyte zone due to a defect in the metaphyseal vasculature structure and the production of abnormal cartilage matrix (Farquharson & Jefferies 2000).

Known dietary factors that lead to an increase in tibial dyschondroplasia in chickens are an altered calcium phosphate ratio, cysteine, homocysteine, salicylic acid, copper, mycotoxins and commercial fungicides like thiurum and disalfiram. Vitamin D metabolites, especially that of 1,25-dihydroxycholecalciferol, are the only known dietary factors that can prevent tibial dyschondroplasia in chickens (Farquharson & Jefferies 2000). Development of metabolic acidosis also increases the incidence of tibial dyschondroplasia in chickens. The suggested pathophysiology is reduced conversion of 25-hydroxycholicalciferol to 1,25-dihydroxycholecalciferol in the kidneys (Orth &Cook 1994). Chondrocytes are essential for the degradation of the matrix and the invasion of the metaphyseal blood vessels (Farquharson *et al.* 1995, Orth & Cook 1994). Death of prehypertrophic chondrocytes would lead to the accumulation of immature cartilage that cannot be invaded by metaphyseal blood vessels because, as indicated in *in vitro* studies, dyschondroplastic cartilage is more resistant to vascular penetration than normal mineralized cartilage. Angiographic studies of turkeys with tibial dyschondroplasia indicated that there were normal numbers of epiphyseal and metaphyseal blood vessels except in the dyschondroplastic lesion (Orth & Cook 1994).

### 3.3.3 Necrosis of epiphyseal cartilage and subchondral bone.

Osteochondrosis is a broad term pertaining to a group of lesions associated with the persistence of growth cartilage in the epiphyseal or physeal growth plate as a result of failure of endochondral ossification (Laverty & Girard 2013). Early articular-epiphyseal cartilage lesions are confined to the growth cartilage and do not involve either the articular cartilage or subchondral bone. Local ischaemia and chondronecrosis are the proposed pathogenesis, as these lesions have been associated with abnormal cartilage canals in pigs and horses. It is only after the ossification front reaches the defect that it protrudes into the subchondral bone (Ekman & Carlson 1998). The chemical properties of the necrotic epiphyseal cartilage change to those of articular cartilage, a substance that does not undergo vascularization. The

cartilage core remains as endochondral ossification advances, incorporating the cartilage core into the subchondral bone. Subchondral bone adjacent to the retained cartilage may adapt and undergo myelofibrosis and trabecular remodelling. Subchondral bone rarely undergoes necrosis (Ekman & Carlson 1998). Retained cartilage can also extend into the articular cartilage, predisposing to the formation of an osteochondral or cartilaginous fragment (Laverty & Girard 2013).

The vascular supply of the growth cartilage is bilaterally symmetrical and both species and site specific. Blood vessels located in close proximity to the ossification zone anastomose with blood vessels that originate from the subchondral bone, replacing the source of the blood supply from the perichondrium with that of the subchondral bone (Laverty & Girard 2013). In pigs the area around the anastomoses between blood vessels of the growth cartilage and those of the subchondral bone develop a small accessory centre of ossification or extension of the ossification zone (Ytrehus *et al.* 2004). Olstad *et al.* (2008) indicated through vascularization studies of the equine tarsus that cartilage canals can be divided into three sections, proximal-, mid- and distal terminus. As the ossification front advances it incorporates the mid terminus and cartilage canal vessels anastomose with the subchondral vessels. The anastomosis shifts the main arterial blood supply of the distal terminus from the perichondrium to that of the subchondral vessels and these blood vessels transverse the ossification front. The arterial supply of the proximal terminus cartilage canals still derive from the perichondrium. Canals undergo chondrification at an earlier stage than those of the distal terminus (Olstad *et al.* 2008). Chondrification is the process whereby the cartilage canal regresses and in the pig and horse it has been proven to be age- and joint dependent (Laverty & Girard 2013, Ytrehus *et al.* 2004). Blood vessels, nerves and stromal cells are replaced by cartilage, as the articular cartilage becomes thinner and the animal ages (Ytrehus *et al.* 2004).

Olstad *et al.* (2008) indicated through their studies that cartilage canals that anastomose and receive their arterial supply from the subchondral bone, crossing the ossification front, are much more vulnerable to damage than cartilage canals that retain their arterial supply from the perichondrium (Olstad *et al.* 2008). Likewise, studies in pigs indicated that necrosis of growth cartilage of the femoral condyles corresponds to the regression pattern of the cartilage canals. Lesions are first noted on the axial aspect of the condyle, progressing abaxially as the animal ages and increases in weight (Ekman & Carlson 1998). The ossification zone is the most bioactive and presumably most unstable zone as cartilage converts to bone, both

expressing different mechanical properties. Any vessel passing through the zone is particularly vulnerable to damage, leading to ischaemic necrosis of cartilage (Olstad *et al.* 2008). Ischaemic lesions are permanent, as there are no anastomoses between the cartilage canals of the growth cartilage (Laverty & Girard 2013).

Increased biomechanical loads have been proposed to damage the cartilage canals, leading to chondronecrosis. For this theory to be correct, increased biomechanical loads would have to damage a single cartilage canal, leaving the surrounding canals intact. This is, however, unlikely but plausible, as studies indicated that the ossification front advances quickly, obscuring any defects (Olstad *et al.* 2015).

## 3.4 Healing of Osteochondral Defects

Successful healing of an osteochondral defect depends on the size of the lesion, as small lesions are more likely to heal than large defects. Studies conducted to evaluate the healing of large osteochondral lesions on the medial femoral condyle of goats revealed that the lesion did not heal after one year. Osteochondral defects are filled with a blood clot if the lesion communicates with the bone marrow. Pleuripotent cells in the blood clot differentiate into chondrocytes and osteoblasts. These cells are responsible for the synthesis of extracellular matrix, cartilaginous repair tissue and subchondral bone. Two weeks after the initial insult the bone defect is lined by bone cells that differentiated from mesenchymal cells. The cartilage section is lined by spindle shaped cells representing the cartilaginous repair cells. The hyaline cartilage adjacent to the lesion does not partake in the repair process but in fact dies off and becomes acellular. The new tissue does not fully integrate with the existing cartilage matrix. After a few months fibro-cartilaginous tissue is formed, functioning to protect the hyaline cartilage at the edges of the defect but is not able to withstand mechanical load over a period of time (Madry *et al.* 2010).

## 3.5 References

Bergwitz, C. & Jüppner, H., 2010 'Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23', *Annual Review of Medicine* 61, 91-104.

Brennan, J. & Aherne, F., 1986, 'Effect of dietary calcium and phosphorus levels on performance, bone bending moment and the severity of osteochondrosis and lameness in boars and gilts slaughtered at 100 or 130 kg body weight', *Canadian Journal of Animal Science* 66(3), 777-790.

Bridges, C.H. & Harris, E.D., 1988, 'Experimentally induced cartilaginous fractures (osteochondritis dissecans) in foals fed low-copper diets', *Journal of the American Veterinary Medical Association* 193(2), 215-221.

Currey, J.D., 2003., 'Role of collagen and other organics in the mechanical properties of bone', *Osteoporosis International* 14(5), 29-36.

Dammrich, K., 1991, 'Relationship between nutrition and bone growth in large and giant dogs', *The Journal of nutrition* 121 (11 Suppl), S114-21.

Davies, I.H. & Munro, R., 1999, 'Osteochondrosis in bull beef cattle following lack of dietary mineral and vitamin supplementation', *The Veterinary record* 145(8), 232-233.

de Koning, D., van Grevenhof, E., Laurenssen, B., Ducro, B., Heuven, H., de Groot, P.N., Hazeleger, W. & Kemp, B., 2012, 'Associations between osteochondrosis and conformation and locomotive characteristics in pigs', *Journal of animal science* 90(13), 4752-4763.

Dik, K.J., Enzerink, E. & Weeren, P., 1999, 'Radiographic development of osteochondral abnormalities, in the hock and stifle of Dutch Warmblood foals, from age 1 to 11 months', *Equine Veterinary Journal* 31(S31), pp.9-15.

Dutra, F., Carlsten, J. & Ekman, S., 1999, 'Hind Limb Skeletal Lesions in 12-Month-Old Bulls of Beef Breeds', *Journal of Veterinary Medicine Series A* 46 (8), 489-508.

Ekman, S. & Carlson, C.S., 1998, 'The pathophysiology of osteochondrosis', *Veterinary Clinics of North America: Small Animal Practice* 28(1), 17-32.

Farquharson, C., Berry, J., Mawer, E., Seawright, E. & Whitehead, C., 1995, 'Regulators of chondrocyte differentiation in tibial dyschondroplasia: an in vivo and in vitro study', *Bone* 17(3), 279-286.

Farquharson, C. & Jefferies, D., 2000, 'Chondrocytes and longitudinal bone growth: the development of tibial dyschondroplasia', *Poultry science* 79(7), 994-1004.

Frantz, N.Z., Andrews, G.A., Tokach, M.D., Nelssen, J.L., Goodband, R.D., DeRouchey, J.M., & Dritz, S.S., 2008, 'Effect of dietary nutrients on osteochondrosis lesions and cartilage properties in pigs', *American Journal of Veterinary Research* 69(5), 617-624.

Glade, M. & Belling, T., 1986, 'A dietary etiology for osteochondrotic cartilage', *Journal of equine veterinary science* 6(3), 151-155.

Goedegebuure, S.A. & Hazewinkel, H.A., 1986, 'Morphological findings in young dogs chronically fed a diet containing excess calcium', *Veterinary pathology* 23(5), 594-605.

Grøndalen, T., 1974, 'Osteochondrosis and arthrosis in pigs, 6: Relationship to feed level and calcium, phosphorus and protein levels in the ration', *Acta Veterinaria Scandinavica (Denmark)*.

Grøndalen, T., 1981, 'Osteochondrosis and arthrosis in Norwegian slaughter-pigs in 1980 compared to 1970', *Nordisk veterinaermedicin* 33(9-11), 417-422.

Grøndalen, T., 1974, 'Osteochondrosis and arthrosis in pigs. II. Incidence in breeding animals', *Acta Veterinaria Scandinavica* 15(1), 26-42.

Hazewinkel, H.A., Van Den Brom, Walter E, van't Klooster, A.T., Voorhout, G. & Van Wees, A., 1991, 'Calcium metabolism in Great Dane dogs fed diets with various calcium and phosphorus levels', *J Nutr* 121(11 Suppl), 99-106.

Heinola, T., Jukola, E., Nakki, P. & Sukura, A., 2006, 'Consequences of hazardous dietary calcium deficiency for fattening bulls', *Acta Veterinaria Scandinavica* 48 25.

Hidiroglou, M., 1980, 'Zinc, copper and manganese deficiencies and the ruminant skeleton: A review', *Canadian Journal of Animal Science* 60(3), 579-590.

Hill, B., Sutton, R. & Thompson, H., 1998, 'Investigation of osteochondrosis in grazing beef cattle', *Australian Veterinary Journal* 76(3), 171-175.

Holick, M.F., 2007, 'Vitamin D deficiency', *New England Journal of Medicine* 357(3), 266-281.

Hurtig, M., Green, S.L., Dobson, H., Mikuni-Takagaki, Y. & Choi, J., 1993, 'Correlative study of defective cartilage and bone growth in foals fed a low-copper diet', *Equine veterinary journal* 25(S16), 66-73.

Jeffcott, L. & Henson, F., 1998, 'Studies on growth cartilage in the horse and their application to aetiopathogenesis of dyschondroplasia (osteochondrosis)', *The Veterinary Journal* 156(3), 177-192.

Jensen, R., Park, R.D., Lauerman, L.H., Braddy, P.M., Horton, D.P., Flack, D.E., Cox, M.F., Einertson, N., Miller, G.K. & Rehfeld, C.E., 1981, 'Osteochondrosis in feedlot cattle', *Veterinary pathology* 18(4), 529-535.

Knight, D., Gabel, A., Reed, S., Embertson, R., Tyznik, W. & Bramlage, L., 1985, 'Correlation of dietary mineral to incidence and severity of metabolic bone disease in Ohio and Kentucky', *Paper presented at the Proceedings of the annual convention of the American Association of Equine Practitioners (USA)*.

Knott, L. & Bailey, A.J., 1998, 'Collagen cross-links in mineralizing tissues: a review of their chemistry, function, and clinical relevance', *Bone* 22(3), 181-187.

Kornegay, E., Combs, N., Veit, H. & Lindemann, M., 1990, 'Articular cartilage condition score of distal humerus and femur of swine as influenced by dietary Ca-P levels, sex and age', *Canadian Journal of Animal Science* 70(1), 255-258.

Laverty, S. & Girard, C., 2013, 'Pathogenesis of epiphyseal osteochondrosis', *The Veterinary Journal* 197(1), 3-12.

Madry, H., van Dijk, C.N. & Mueller-Gerbl, M., 2010, 'The basic science of the subchondral bone', *Knee surgery, sports traumatology, arthroscopy* 18(4), 419-433.

Marks, J., Debnam, E.S. & Unwin, R.J., 2010, 'Phosphate homeostasis and the renal-gastrointestinal axis', *American journal of physiology.Renal physiology* 299(2), F285-96.

Nakano, T., Aherne, F., Brennan, J. & Thompson, J., 1984, 'Effect of growth rate on the incidence of osteochondrosis in growing swine', *Canadian Journal of Animal Science* 64(1), 139-146.

Nakano, T., Brennan, J. & Aherne, F., 1987, 'Leg weakness and osteochondrosis in swine: a review', *Canadian Journal of Animal Science* 67(4), 883-901.

Olsson, S.E. & Reiland, S., 1978, 'The nature of osteochondrosis in animals. Summary and conclusions with comparative aspects on osteochondritis dissecans in man', *Acta radiologica.Supplementum* 358 299-306.

Olstad, K., Ytrehus, B., Ekman, S., Carlson, C. & Dolvik, N., 2008, 'Epiphyseal cartilage canal blood supply to the tarsus of foals and relationship to osteochondrosis', *Equine veterinary journal* 40(1), 30-39.

Olstad, K., Ekman, S. & Carlson, C.S., 2015, 'An Update on the Pathogenesis of Osteochondrosis', *Veterinary pathology* 52(5), 785-802.

Orth, M.W. & Cook, M.E., 1994, 'Avian tibial dyschondroplasia: a morphological and biochemical review of the growth plate lesion and its causes', *Veterinary pathology* 31(4), 403-404.

Pool, R., 1993, 'Difficulties in definition of equine osteochondrosis; differentiation of developmental and acquired lesions', *Equine veterinary journal* 25(S16), 5-12.

Prozesky, L., Neser, J., Meissner, H., Botha, F.K., Jacobs, L., Shepstone, C., Viljoen, H.J., Köster, H.H., de Brouwer, C., van Zyl, J. & van der Veen, G., 'Preliminary report on osteochondrosis in cattle in the North-Western parts of South Africa', *Onderstepoort Journal of Veterinary Research*, In press.

Reiland, S., Håglin, L. & Sjöberg, H, 1991, 'Experimental hypophosphataemia in growing pigs: Effects on endochondral ossification in comparison to osteochondrosis', *Journal of comparative pathology* 105(3), 247-254.

Reiland, S., Stromberg, B., Olsson, S.E., Dreimanis, I. & Olsson, I.G., 1978, 'Osteochondrosis in growing bulls. Pathology, frequency and severity on different feedings', *Acta radiologica.Supplementum* 358 179-196.

Richardson, D.C., Schoenherr, W.D. & Zicker, S.C., 1997, 'Nutritional management of osteoarthritis', *Veterinary Clinics of North America: Small Animal Practice* 27(4), 883-911.

Richardson, D.C. & Zentek, J., 1998, 'Nutrition and osteochondrosis', *Veterinary Clinics of North America: Small Animal Practice* 28(1), 115-135.

Savage, C., McCarthy, R. & Jeffcott, L., 1993a, 'Effects of dietary phosphorus and calcium on induction of dyschondroplasia in foals', *Equine veterinary journal* 25(S16), 80-83.

Savage, C., McCarthy, R. & Jeffcott, L., 1993b, 'Histomorphometric assessment of bone biopsies from foals fed diets high in phosphorus and digestible energy', *Equine veterinary journal* 25(S16), 89-93.

Slatopolsky, E., Brown, A. & Dusso, A., 2001, 'Role of phosphorus in the pathogenesis of secondary hyperparathyroidism', *American journal of kidney diseases* 37(1), S54-S57.

Trostel, C.T., McLaughlin, R.M. & Pool, R.R., 2002, 'Canine lameness caused by developmental orthopedic diseases: Osteochondrosis', *Compendium* 24 836-854.

Van Grevenhof, E., Schurink, A., Ducro, B., Van Weeren, P., Van Tartwijk, J., Bijma, P. & Van Arendank, J.A.M., 2009, 'Genetic variables of various manifestations of osteochondrosis and their correlations between and within joints in Dutch warmblood horses', *Journal of animal science* 87(6), 1906-1912.

Viguet-Carrin, S., Garnero, P. & Delmas, P, 2006, 'The role of collagen in bone strength', *Osteoporosis International* 17(3), 319-336.

Weisbrode, S., 2007, 'Bone and Joints' Fourth edition ed. In McGavin, M.& Zachary, J. eds. Pathologic basis of veterinary disease. St. Louis, Missouri: Mosby Elsevier. 1041 p.

Woodard, J.C., Becker, H.N. & Poulos, P.W., 1987, 'Effect of diet on longitudinal bone growth and osteochondrosis in swine', *Veterinary pathology* 24(2), 109-117.

Ytrehus, B., Ekman, S., Carlson, C.S., Teige, J. & Reinholt, F.P., 2004, 'Focal changes in blood supply during normal epiphyseal growth are central in the pathogenesis of osteochondrosis in pigs', *Bone* 35(6), 1294-1306.

Ytrehus, B., Carlson, C.S. & Ekman, S., 2007, 'Etiology and pathogenesis of osteochondrosis', *Veterinary pathology* 44(4), 429-448.

# CHAPTER 4

# MATERIALS AND METHODS

## 4.1 Materials and methods

### 4.1.1 Experimental animals

Forty-three clinically affected Brahman cattle were included in the feeding trial. Genetic variation as study variable was limited by selecting cattle from a single extensive farm situated in the Vryburg district. See Table 1 for starting weight, sex and age.

Cattle were farmed extensively on the natural sweet veld, with both stud and commercial animals selected for the trial. Farm management practice include an all year round calving season with weaning of calves at 6 - 8 months of age or approximately 230 kg live weight. Supplementary feed is fed at the recommended daily allowance per animal throughout the year with the formulation adapted according to the season and the availability and quality of the vegetation present. Majority of the animals that developed osteochondrosis on the farm are between the ages of 6-18 months with males affected more frequently than females. An increased incidence of lameness with or without associated peri-articular swelling of the stifle joint is more prevalent in calves born during a dry summer period and raised on winter pasture with reduced availability of dry matter (personal communication: J. van Zyl, 2015). Cattle for the study were selected based on age, the degree of lameness and size of periarticular swelling.

The study animals were slaughtered at the end of the trial. No necropsies were performed on these animals.

**Table 1**: Description of experimental animals

| Animal number* | Starting weight (kg) | Sex | Age (Year born) | Age when included in sudy (years) | Supplement group |
|---|---|---|---|---|---|
| 473 | 474 | Female | 2008 | 5 | A |
| 102 | 245 | Female | 2012 | 1 | A |
| 204 | 433 | Female | 2008 | 5 | A |
| 86 | 221 | Female | 2012 | 1 | A |
| 358 | 300 | Female | 2008 | 5 | A |
| 111 | 245 | Male | 2012 | 1 | A |
| 67 | 267 | Male | 2012 | 1 | A |
| 249 | 312 | Male | 2012 | 1 | A |
| 60 | 278 | Male | 2012 | 1 | A |
| 880 | 292 | Male | 2012 | 1 | A |
| 77 | 434 | Male | 2012 | 1 | A |
| Simbra | 317 | Male | 2012 | 1 | A |
| 205 | 281 | Female | 2012 | 1 | A |
| 341 | 246 | Male | 2012 | 1 | A |
| 577 | 309 | Female | 2012 | 1 | B |
| 153 | 194 | Male | 2012 | 1 | B |
| 46 | 469 | Female | 2008 | 5 | B |
| 455 | 524 | Female | 2008 | 5 | B |
| 73 | 275 | Female | 2012 | 1 | B |
| 360 | 265 | Male | 2012 | 1 | B |
| 293 | 192 | Female | 2012 | 1 | B |
| 338 | 234 | Male | 2012 | 1 | B |
| 320 | 193 | Female | 2012 | 1 | B |
| Bull (no nr) | 300 | Male | 2012 | 1 | B |
| 179 | 29 | Female | 2012 | 1 | B |
| 171 | 327 | Male | 2012 | 1 | B |
| 135 | 296 | Male | 2012 | 1 | B |
| 304 | 198 | Female | 2012 | 1 | B |
| 104 | 197 | Female | 2012 | 1 | C |
| 170 | 215 | Male | 2012 | 1 | C |
| 118 | 211 | Female | 2012 | 1 | C |
| 840 | 325 | Male | 2012 | 1 | C |
| 350 | 496 | Female | 2008 | 5 | C |
| 139 | 222 | Male | 2012 | 1 | C |
| 416 | 155 | Female | 2012 | 1 | C |
| 254 | 283 | Male | 2012 | 1 | C |
| 305 | 216 | Female | 2012 | 1 | C |
| 252 | 267 | Male | 2012 | 1 | C |
| 284 | 263 | Female | 2012 | 1 | C |
| 950 | 259 | Male | 2012 | 1 | C |
| 854 | 300 | Male | 2012 | 1 | C |
| 161 | 363 | Male | 2012 | 1 | C |
| 322 | 243 | Female | 2012 | 1 | C |

A - contained 25 % of the recommended NRC (2001) mineral values. No additional phosphate was added to the supplement.
B - contained 25 % of the recommended NRC (2001) mineral values with the exception of added mono-ammonium phosphate (MAP) to an equivalent level to that of supplement 3.
C - contained 80% of the recommended NRC (2001) mineral levels and was used as the control diet.
Standard Deviation – (Weight; A = 79.45, B = 121.79, C = 82.34) (Age; A = 1.70, B = 1.45, C = 1.03).
Average – (Weight; A = 310.36, B = 271.79, C = 267.67) (Age; A = 1.86, B = 1.57, C = 1.26)

### 4.1.2 Visual lesion evaluation

The 43 cattle were clinically evaluated once every second week for the duration of the feeding trial. Three independent persons (two final year veterinary students and principle investigator) each individually recorded clinical signs to limit any biased decisions. Visual evaluation included grading of both the size of the peri-articular swelling and the degree of lameness. Cattle were visually inspected in the camps in an attempt to keep the stresslevels to a minimum.

Peri-articular swelling and lameness can occur simultaneously or independently of each other (Trostle *et al.* 1997, 1998). The size of the peri-articular stifle joint swelling does not correlate with the extent of the osteo-arthritic lesion (Unpublished data from radiographic analysis: G. van der Veen, 2014), but is merely an indication of excessive synovial fluid produced as a result of joint inflammation (Trostle *et al*. 1998). Acute stifle joint swelling has a pronounced bulging appearance while chronic swelling has a more flattened appearance. The deposition of fibrous connective tissue in the stifle joint capsule depresses the swelling, producing the flattened appearance seen with chronic joint swellings (Trostle *et al.* 1998). No research has been conducted to date to determine the time period for the stifle joint to deposit additional connective tissue in the capsule. It is of importance to note that the degree of swelling does not relate to the extent of osteo-arthritic damage but merely to the amount of synovial fluid that accumulates within the affected joint (Unpublished data: G. van der Veen, 2015). The stifle joint swellings were recorded as either acute or chronic, left or right, and the size thereof as outlined in Table 2.

**Table 2**: Visual lesion evaluation form.

| Lesion scoring 2013: Onderstepoort study 2013 | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date: | Acute peri-articular stifle swelling | | | | | | | | Chronic lesion (fibrosis of joint capsule) stifle joint | | | | | | | | Degree of lameness (hind leg) | | | | | | | | | |
| Evaluator: | S | | M | | L | | XL | | S | | M | | L | | XL | | 0 | | 1 | | 2 | | 3 | | 4 | |
| Animal number: | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R |
| | | | | | | | | | | | | | | | | | | | | | | | | | | |

S = small (approx. diameter 2.5 cm)
M = medium (approx. diameter 6 cm)
L = large (approx. diameter 10 cm)
XL= extra-large (approx. diameter >10 cm)

L = left hind
R = right hind

Lameness was graded on a scale of 0 - 4 with 4 being non-weight bearing (Table 3).

**Table 3**: Grading score for lameness.

| Lameness score of hind leg | Score definition |
|---|---|
| 0 | No clinical signs of lameness observed. |
| 1 | Conformational changes noticed and slight lameness observed. |
| 2 | Moderate lameness observed. |
| 3 | Severely affected but still weight bearing |
| 4 | Severely affected with very little to non-weight bearing. |

The summarised data collected from post mortem evaluations and radiographs of cattle included in osteochondrosis trials were compared to the external evaluation findings of the same animals to determine the sensitivity and specificity of the external evaluation method developed to grade the degree of osteochondrosis. The external evaluation included the recording of the size of the articular swelling as well as the degree of lameness.

These results indicate a highly significant correlation between the accuracy of the external evaluation method and that of either post mortem and/or radiographic evaluation to confirm osteochondrosis (Unpublished data: K. Botha, 2015).

### 4.1.3 Trial groups

The study consisted of two feeding trials with three supplements tested with each trial. A total of forty-three clinically affected cattle were randomly divided for the feeding trials. Each trial tested three supplement groups (respective supplement groups were the same for trial 1 and 2). Each trial was performed at a different time to keep cattle numbers low to prevent overcrowding of camps and to avoid any possible feed trough bullying that could affect the outcome of the trial. Cattle of respective supplement groups had separated camps and each camp was equipped with a water trough (municipal water), hayrack and two Taltec feed troughs that provided sufficient feeding space. The number and sex of cattle included in each trial group is provided in Table 4.

**Table 4**: Number and sex of cattle per trial and group.

| Trial | Group | Number of cattle per group | Sex | |
|---|---|---|---|---|
| | | | Male | Female |
| 1 | A (fed Supplement 1) | 6 | 1 | 5 |
| | B (fed Supplement 2) | 7 | 2 | 5 |
| | C (fed Supplement 3) | 7 | 3 | 4 |
| 2 | A (fed Supplement 1) | 8 | 7 | 1 |
| | B (fed Supplement 2) | 7 | 4 | 3 |
| | C (fed Supplement 3) | 8 | 5 | 3 |
| | Total | 43 | 22 | 21 |

### 4.1.4 Trial feeds

The experimental lick supplements were formulated to have the same base ingredients and to only vary in micro- and macro mineral concentrations (See Table 5, Appendix 1, 2 and 3). The three supplements were:

- Supplement 1 contained 25 % of the recommended NRC (2001) mineral values. No additional phosphate was added to the supplement.
- Supplement 2 contained 25 % of the recommended NRC (2001) mineral values with the exception of added mono-ammonium phosphate (MAP) to an equivalent level to that of supplement 3.
- Supplement 3 contained 80% of the recommended NRC (2001) mineral levels and was used as the control diet.

For each supplement the same mineral premix base, Arthrocure B (ANH, personal communication), was included to ensure comparable standard and quality of minerals. This premix contains 80 % of the recommended NRC (2001) mineral values. The bioavailability of both micro- and macro minerals is of high quality (composition not shown due to confidentiality implications). The absorption percentage according to the NRC (2001) of MAP is 80 %. Ingredients and amounts used to formulate the trial supplementary feeds are presented in Table 5.

Cattle had *ad lib* access to baled *Eragrostis teff* hay for the total duration of the trial.

Supplementary feed was collected and weighed on a weekly basis to determine the average individual consumption (grams) per day. The cattle were fed for a total period of 12 weeks.

**Table 5**: List of ingredients and amounts used to formulate the respective trial supplements.

| **Ingredients** | **Supplement 1** | | **Supplement 2** | | **Supplement 3** | |
|---|---|---|---|---|---|---|
| | As Is % | Mix (kg) | As Is % | Mix (kg) | As Is % | Mix (kg) |
| Salt | 25 | 250 | 25 | 250 | 25 | 250 |
| Bran 15% | 15 | 150 | 12.5 | 125 | 12.5 | 125 |
| Hominy Chop | 13 | 130 | 12.4 | 124 | 12.4 | 124 |
| Peanut shells | 12.70 | 127 | 7.8 | 78 | 7 | 70 |
| Urea | 12 | 120 | 11 | 110 | 11 | 110 |
| Molasses | 12 | 120 | 12 | 120 | 11.95 | 119.5 |
| Sunflower O/C 38% | 6 | 60 | 6 | 60 | 6 | 60 |
| Ammonium sulphate | 3 | 30 | 3 | 30 | 3 | 30 |
| Limestone | 1 | 10 | 5 | 50 | 5 | 50 |
| Mono-ammonium phosphate (MAP) | 0 | 0 | 5 | 50 | 5 | 50 |
| DSM Arthrocure B | 0.3 | 3 | 0.3 | 3 | 1.15 | 11.5 |
| **Total** | 100 | 1000 | 100 | 1000 | 100 | 1000 |

### 4.1.5 Feeding period

The feeding period of trial 1 commenced on 2013/05/13 and ended on 2013/08/05, a feeding period of 12 weeks. The feeeding period of trial 1 and 2 overlapped for a period of three weeks. The feeding period of trial 2 commenced on 2013/07/15 and ended on 2013/08/12, afeeding period of four weeks. The testing of supplements during the second trial was halted before the schedueled end period due to ethical considerations. The study was conducted at the Onderstepoort Veterinary Faculty, University of Pretoria, South Africa. The study was approved by the ethics research committee of the University of Pretoria (V073/13).

### 4.1.6 Feed analysis

Trial supplementary feeds were analysed by Cumberland Valley Analytical Services (1415 Industry Drive, Hagertown, MD 21742). The dry matter percentage, dietary cation anion difference, estimated metabolisable energy, protein, phosphate and calcium were calculated with values based on analysis on raw material matrix (ave in RSA) and not on analysis per lick mix. See Appendix 1, 2, 3 and 4 (December 2012 supplement).

### 4.1.7 Urinalysis

Urine was collected from cattle that urinated at free will in the crush. Observers used a self-made pole with a cup attached at the end to collect the free flow urine sample. Collected urine samples were analysed immediately with the Combur$^{9}$Test® (Roche) urine dipstick. Urine samples were collected at the end of the feeding period as investigation measure.

### 4.1.8 Statistical analysis

An overall disability score was calculated as the sum of the scores for each clinical category (lameness, acute and fibrosis) for both hind legs (left and right). The change in each clinical category score was calculated by subtracting the baseline from all subsequent values (forming "delta" scores). Delta scores were assessed for normality by calculating descriptive statistics, plotting histograms and performing the Anderson-Darling test from available software (MINITAB Statistical Software, Release 13.32, Minitab Inc, State College, PA, USA). Categorical data were described using proportions and 95% mid-P exact confidence intervals (CI) and comparing among diets using chi-square tests. Categorical data analysis was performed using available freeware (Epi Info, version 6.04, CDC, Atlanta, GA, USA). Delta scores were descriptively presented as medians and ranges and transformed by ranking prior to statistical analysis. Delta scores were compared among diet groups using linear mixed models that included animal as a random effect and diet as a fixed effect. Unless stated otherwise, all statistical analyses were performed using commercially available software (IBM SPSS Statistics Version 22, International Business Machines Corp., Armonk, NY, USA) and results interpreted at the 5% level of significance.

Supplementary statistics see appendix 5.

# CHAPTER 5

# DISCUSSION AND CONCLUSION

## 5.1 Introduction

Farmers investigated several possibilities with the hope of stumbling across an explanation. Initial thoughts was that osteochondrosis developed as a result of mineral deficiencies, specifically that of phosphate. Before the aetiology of osteomalacia (stiff sickness) and/or botulism (lamsiekte) was known, Hutcheon stated in his 1886 report that: 'the predisposing and primary cause (of lamsiekte and stiff sickness) is undoubtedly due to the absence of sufficient phosphates in the food' (Theiler 1912). The North West province is known for its deficiency of minerals especially that of phosphate. Early phosphate supplementation studies conducted at Armoedsvlakte proved that additional phosphate significantly reduced both the incidence of botulism and osteomalacia. The supplementation of phosphate significantly increased the fertility of cows and the average weaning weight of calves. Phosphate, *per se*, made farming a much more sustainable enterprise on phosphate deficient land (Theiler, Green & Du Toit 1928). Bone meal was fed as a source of phosphate for extended periods until the outbreak of Bovine Spongiform Encephalopathy (BSE) in the United Kingdom during 1985 (Prozesky *et al.* 2016). Inclusion of ruminant products (bone meal) into livestock feed was banned after the outbreak of BSE. The sanction forced livestock producers to make use of chemically manufactured phosphates as a supplementation source. Farmers across South Africa religiously supplement phosphate and other essential minerals to improve production and prevent any mineral related pathology.

A study of clinically affected heifers was done in 2003 at the Faculty of Veterinary science, Onderstepoort. The investigation concluded that the condition was that of osteochondrosis and not primary arthritis as previously suspected. Mineral analysis indicated a marginal calcium deficiency and normal hepatic trace mineral levels (Cu, Zn and Mn) (Prozesky *et al*. 2016).

Farmers donated another 28 clinically affected cows to the University of Pretoria during June 2004. Detailed post mortem examinations were done on 21 of the clinically affected animals. The post mortem included inspection of all the joints of the legs, several intervertebral joints, collection of bone samples for histopathological examination and liver and bone samples for mineral analysis.

The 7 remaining animals were included into a feeding trial to determine if there was any beneficial effect pertaining to the degree of lameness and size of the peri-articular swelling when additional minerals were supplemented. The cattle were fed for a period of three months. The animals had *ad lib* access to *Eragrostis teff* hay and were fed 1.5 kg of a commercial production lick per animal per day. Eighty grams (80g) of a mineral premix (Arthrocure) was added to the commercial production lick for the first month of the feeding period. The premix was formulated to include high levels of micro-and macro minerals and 6% phosphate [formulation not available (intellectual property)].

Results from the three-month feeding period indicated that all the cattle, especially the young animals, had reduced visible peri-articular swelling of the affected joints and improvement in the degree of lameness.

The University of Pretoria acquired more clinically affected cattle from the geographically important area (North-West Province, South Africa) for an additional feeding trial during December 2012. Cattle had *ad lib* access to *Eragrostis teff* hay and were fed restricted amounts of supplementary feed that contained high levels of bio-available micro- and macro minerals. All the cattle showed significantly improvements in the degree of lameness with reduced peri-articular swelling of the affected joints (G van der Veen & L Prozesky unpublished data, 2012).

Results of both the 2004 and 2012 pre-trials validated the need to investigate the level of mineral supplementation necessary to aid in limiting the severity of osteochondrosis in cattle i.e. decreased degree of lameness and the size of the peri-articular swelling.

The scoring system used to classify the severity of and chronicity of osteochondrosis in cattle was developed and used by farmers who originally investigated the problem of osteochondrosis in cattle themselves (personal communication: J van Zyl, 2012). The farmers only made use of external features like the size and shape of the peri-articular swelling and the degree of lameness to grade the severity and chronicity of the osteochondrosis. The scoring system used was not until recently proved to be a reliable method for classifying cattle with osteochondrosis (Unpublished data: K. Botha, 2015)

## 5.2 Results

Data of trial 1 and 2 were combined for each supplement group to simplify explanation. Hereafter, any value represented by a supplement group is the average value for trial 1 and 2 for that specific supplement group, unless otherwise specified.

### 5.2.1 Initial weight, supplement and phosphate intake

Statistical analysis indicated that there was no significant difference between the starting weights of the respective groups of both trial. The average weight of group A, B and C were 310 kg, 290 kg and 267 kg respectively. Supplement intake varied among the groups with the average collective daily supplement intakes for groups A, B and C of being 370 g, 255 g, and 292g respectively. Daily supplement intakes calculated as grams consumed per kg live weight were 1.19g, 0.88g and 0.91g per kg live weight respectively for group A, B and C.
The calculated average daily phosphate intake per animal differed between the groups.
Consumed values depicted in table 6.

Cattle of trial 1 were fed for the total period of 12 weeks. The cattle of trial 2 were, however, only fed for a period of 4 weeks. Feeding was suspended due to ethical considerations as the majority of cattle fed during trial 2 became severely lame, surpassing the ethical cut-off point. Enough data could be collected from cattle of trial 2 to be included in the statistical analysis.

### 5.2.2 Lameness evaluation

Recording the size of the peri-articular swelling and the degree of lameness of the trial cattle was used as a method to determine the effect of the supplements. Visual grading of lesions has been statistically proved to be a valid method (Unpublished data: K. Botha, 2015). Analysis of the initial values for all the cattle indicated that there were no significant differences pertaining to the sex, age, weight, lameness score, acute score, fibrosis score and the overall disability score (Table 7).
Study results (Tables 8 and 9) indicated no significant difference between the degree of lameness, chronic peri-articular swelling and total disability score between the groups of both trial 1 and 2, and the acute peri-articular swelling score of trial 2. There was, a significant difference in the acute peri-articular swelling between the groups of trial 1 (see Discussion).

### 5.2.3 Urinalysis

A few cattle of each group were randomly selected for a detailed clinical examination. Urinalysis was done as part of the examination. The urine test strip used (Combur$^{9}$Test®; Roche) indicated a urinary pH of less than 6 (normal urine pH = 8) for all the cattle were urine could be collected from, in total four. Possible factors that could lead to the development of aciduria were investigated.

**Table 6**: Calculated average daily phosphate intake of groups for trial 1 and 2 combined.

| **Supplement group** | **Average starting weight of the cattle (kg)** | **Average supplement consumption (g/day/animal)** | **Supplement P measured value (%DM)** | **Average phosphate intake (g/day/animal)** | **% P consumed less than cattle fed control supplement** |
|---|---|---|---|---|---|
| Supplement 1 (Group A) | 310kg | 370g | 0.94 | 3.48g | -47.7% |
| Supplement 2 (Group B) | 290kg | 255g | 1.59 | 4.05g | -16% |
| Supplement 3 (Group C) | 267kg | 292g | 1.79 | 5.14g | - |

**Table 7**: Comparison of experimental animal baseline values.

| Variable* | Supplement 1 | Supplement 2 | Supplement 3 | P value† |
|---|---|---|---|---|
| **Trial 1** | | | | |
| Sex | | | | 0.774 |
| Male | 0.17 (0.01, 0.59) | 0.29 (0.05, 0.67) | 0.33 (0.09, 0.67) | |
| Female | 0.83 (0.41, 0.99) | 0.71 (0.33, 0.95) | 0.67 (0.33, 0.91) | |
| Age (years) | 3.5 (1.5, 5.5) | 1.5 (1.5, 5.5) | 1.5 (1.5, 17.5) | 0.725 |
| Initial weight (kg) | 273 (221, 474) | 275 (192, 524) | 215 (155, 496) | 0.454 |
| Lameness score | 1.5 (1, 3) | 2 (0, 3) | 1 (0, 4) | 0.567 |
| Acute peri-articular swelling | 4 (2, 5) | 2 (0, 5) | 2 (0, 5) | 0.111 |
| Chronic peri-articular swelling | 0 (0, 0) | 0 (0, 0) | 0 (0, 0) | 1.0 |
| Overall disability score | 5.5 (3, 8) | 4 (0, 8) | 3 (0, 8) | 0.328 |
| **Trial 2** | | | | |
| Sex | | | | 0.384 |
| Male | 0.88 (0.52, 0.99) | 0.57 (0.22, 0.88) | 0.63 (0.28, 0.89) | |
| Female | 0.13 (0.01, 0.48) | 0.43 (0.12, 0.78) | 0.38 (0.11, 0.72) | |
| Age (years) | 1.5 (1.5, 2.5) | 1.5 (1.5, 1.5) | 1.5 (0.5, 2.5) | 0.742 |
| Initial weight (kg) | 287 (246, 434) | 290 (193, 327) | 265 (216, 363) | 0.452 |
| Lameness score | 0 (0, 3) | 0 (0, 2) | 0 (0, 2) | 0.976 |
| Acute peri-articular swelling | 3 (0, 8) | 0 (0, 6) | 0 (0, 8) | 0.343 |
| Chronic peri-articular swelling | 0 (0, 4) | 3 (0, 6) | 2.5 (0, 6) | 0.141 |
| Overall disability score | 4 (3, 11) | 5 (3, 6) | 5.5 (2, 8) | 0.730 |

*Categorical data presented as proportion and 95% mid-P exact confidence intervals and quantitative data presented as median and range.
†Based on chi-square tests for categorical variables and Kruskal-Wallis tests for quantitative data.

**Table 8**: Median (range) disability scores and comparison among diet groups for animals enrolled in Trial 1.

| | | Time period | | | | | | | P |
|---|---|---|---|---|---|---|---|---|---|
| **Outcome** | **Supplement** | **Baseline** | **2 weeks** | **4 weeks** | **6 weeks** | **8 weeks** | **10 weeks** | **12 weeks** | **value*** |
| **Lameness** | **1** | 1.5 (1, 3) | 1 (1, 4) | 1.5 (1, 4) | 1.5 (1, 4) | 2 (1, 3) | 1.5 (0, 4) | 1.5 (0, 4) | |
| | **2** | 2 (0, 3) | 1 (0, 3) | 1 (0, 3) | 1 (0, 4) | 1 (0, 3) | 2 (0, 3) | 2 (0, 4) | |
| | **3** | 1 (0, 4) | 1 (0, 4) | 1 (0, 4) | 1 (1, 4) | 1 (0, 2) | 1 (0, 2) | 2 (0, 2) | |
| | | | | | | | | | 0.122 |
| **Acute peri-articular swelling** | **1[a]** | 4 (2, 5) | 4 (3, 6) | 0 (0, 4) | 0 (0, 0) | 0 (0, 4) | 0 (0, 0) | 0 (0, 0) | |
| | **2[a,b]** | 2 (0, 5) | 3 (0, 5) | 0 (0, 5) | 0 (0, 6) | 0 (0, 5) | 0 (0, 0) | 0 (0, 0) | |
| | **3[b]** | 2 (0, 5) | 5 (0, 7) | 0 (0, 0) | 2 (0, 5) | 0 (0, 0) | 0 (0, 0) | 0 (0, 0) | |
| | | | | | | | | | 0.034 |
| **Chronic peri-articular swelling** | **1** | 0 (0, 0) | 0 (0, 0) | 3.5 (2, 6) | 4 (2, 6) | 4 (0, 8) | 4.5 (2, 7) | 5.5 (4, 7) | |
| | **2** | 0 (0, 0) | 0 (0, 0) | 3 (0, 6) | 3 (0, 4) | 2 (0, 4) | 3 (0, 6) | 5 (2, 6) | |
| | **3** | 0 (0, 0) | 0 (0, 0) | 2 (2, 6) | 3 (0, 6) | 2 (2, 6) | 3 (0, 6) | 4 (4, 6) | |
| | | | | | | | | | 0.189 |
| **Left stifle lesion (total)** | **1** | 2.5 (1, 7) | 3 (1, 8) | 3.5 (1, 8) | 2.5 (0, 8) | 3 (0, 7) | 3 (0, 8) | 3 (2, 8) | |
| | **2** | 1 (0, 4) | 1 (0, 6) | 3 (0, 5) | 2 (0, 4) | 2 (0, 3) | 2 (0, 4) | 3 (2, 4) | |
| | **3** | 0 (0, 8) | 2 (0, 8) | 0 (0, 5) | 4 (1, 5) | 2 (1, 4) | 3 (0, 5) | 2 (2, 6) | |
| | | | | | | | | | 0.182 |
| **Right stifle lesion (total)** | **1** | 2.5 (1, 5) | 2 (2, 3) | 3 (0, 5) | 3 (0, 4) | 3 (0, 4) | 3 (0, 5) | 3 (2, 4) | |
| | **2** | 2 (0, 6) | 2 (0, 6) | 2 (0, 6) | 2 (0, 6) | 3 (0, 7) | 3 (0, 6) | 4 (0, 8) | |
| | **3** | 2 (0, 8) | 2 (0, 8) | 3 (0, 5) | 2 (1, 5) | 2 (0, 4) | 1 (0, 4) | 4 (2, 5) | |
| | | | | | | | | | 0.825 |
| **Total disability** | **1** | 5.5 (3, 8) | 5 (4, 10) | 7 (4, 10) | 5 (4, 10) | 6 (3, 10) | 6 (4, 11) | 7 (5, 11) | |
| | **2** | 4 (0, 8) | 4 (0, 8) | 4 (4, 7) | 4 (0, 8) | 5 (0, 7) | 5 (0, 8) | 6 (3, 10) | |
| | **3** | 3 (0, 8) | 6 (0, 11) | 3 (2, 10) | 6 (4, 10) | 3 (2, 8) | 4 (0, 7) | 6 (4, 8) | |
| | | | | | | | | | 0.093 |

*Comparison between diets based on mixed-effects linear model analysing the change in variables from baseline (after rank transformation of scores) and adjusting for repeated measures by the addition of a random effect for animal. Diets without superscripts in common are significantly different after Bonferroni correction of post-hoc P values.

**Table 9**: Median (range) disability scores and comparison between different supplement groups for animals enrolled in Trial 2.

| | | Time period | | | |
|---|---|---|---|---|---|
| **Outcome** | **Supplement** | **Baseline** | **2 weeks** | **4 weeks** | **P value*** |
| **Lameness** | **1** | 0 (0, 3) | 1 (0, 6) | 1 (1, 2) | |
| | **2** | 0 (0, 2) | 0 (0, 4) | 1 (0, 1) | |
| | **3** | 0 (0, 2) | 1.5 (0, 2) | 1 (0, 2) | |
| | | | | | 0.084 |
| **Acute peri-articular swelling** | **1** | 3 (0, 8) | 0 (0, 8) | 0 (0, 4) | |
| | **2** | 0 (0, 6) | 0 (0, 3) | 0 (0, 0) | |
| | **3** | 0 (0, 8) | 0 (0, 0) | 0 (0, 0) | |
| | | | | | 0.247 |
| **Chronic peri-articular swelling** | **1** | 0 (0, 4) | 3 (0, 6) | 5 (0, 6) | |
| | **2** | 3 (0, 6) | 4 (1, 5) | 5 (4, 6) | |
| | **3** | 2.5 (0, 6) | 5.5 (4, 7) | 5.5 (3, 7) | |
| | | | | | 0.331 |
| **Left stifle lesion (total)** | **1** | 1 (0, 7) | 1.5 (0, 7) | 2.5 (0, 4) | |
| | **2** | 2 (0, 3) | 3 (0, 4) | 3 (1, 4) | |
| | **3** | 3 (2, 4) | 3 (2, 6) | 3 (2, 4) | |
| | | | | | 0.716 |
| **Right stifle lesion (total)** | **1** | 3.5 (2, 4) | 4 (1, 7) | 4 (2, 5) | |
| | **2** | 3 (1, 4) | 2 (1, 4) | 3 (2, 4) | |
| | **3** | 3 (0, 4) | 3 (2, 5) | 3.5 (1, 6) | |
| | | | | | 0.629 |
| **Total disability** | **1** | 4 (3, 11) | 5.5 (4, 14) | 6 (5, 8) | |
| | **2** | 5 (3, 6) | 5 (1, 8) | 6 (5, 7) | |
| | **3** | 5.5 (2, 8) | 6.5 (4, 9) | 6.5 (4, 9) | |
| | | | | | 0.261 |

*Comparison among diets based on mixed-effects linear model analysing the change in variables from baseline (after rank transformation of scores) and adjusting for repeated measures by the addition of a random effect for animal.

## 5.3 Discussion

The trial was conducted to determine the level of micro- and macro minerals required to be included in a supplement that would improve the clinical condition of cattle clinically suffering from osteochondrosis.

The three supplements contained the same base ingredients and only varied in mineral concentrations. The concentration of different minerals was altered with addition of either phosphate and/or the mineral premix Arthrocure B. Supplement 1 and 2 were formulated to contain only 25 % of the recommended NRC (2001) mineral values with additional phosphate added to supplement 2. Supplement 3 (control supplement) contained the highest level of minerals and was used as the control diet. The mineral levels of supplement 3 were based on that of the 2012 pre-trial supplement that had a significant positive effect when fed to cattle with clinical osteochondrosis.

The calculated daily average supplement intake per animal varied between groups A, B and C consuming 370g, 255g and 292g per animal per day respectively. Differing average starting weights (See table 6) and *ad lib* access to high quality *Eragrostis teff* hay would both have influenced the average daily consumption of supplements. *Eragrostis teff* hay is a highly palatable and nutritious roughage that would contribute a large proportion of the nutritional daily requirements of the cattle. The most important factor was to calculate the mineral intake especially that of phosphorus as it was thought that higher phosphorus intake played a key role in the recovery and prevention of osteochondrotic lesions. Calculated average daily phosphate intake per animal differed between the respective groups. Group C had an average phosphate intake of 5.14 g per animal per day. Cattle of group A and B consumed 47.7% (3.47g) and 16% (4.05g) less phosphate on a daily basis respectively when compared to the phosphate intake of group C. It was expected that especially the cattle of group C would have responded positively to the higher micro-and macro mineral intake with group A responding the least to the supplementation.

Baseline data (Table 7) for both trial 1 and 2 indicated no significant difference between the three groups with regard to lameness, acute peri-articular swelling, chronic peri-articular swelling and total disability.

Analysis of the final study results (Tables 8 and 9) indicated that there were no significant difference between the three groups for both trial 1 and 2 pertaining to lameness, chronic peri-articular swelling, total disability and the acute peri-articular swelling. The data did, however, indicate a significant difference among the three groups of trial 1 pertaining to the acute peri-articular swelling. It is important to note that all the cattle included in the study had clinical osteochondrotic lesions with the affected period of each animal unknown to the investigators. The cattle included in trial 1 most likely had early acute peri-articular swellings and the chronicity of the lesions developed into chronic peri-articular swellings around the fourth week of the feeding period, which lead to the significant finding. The importance of this finding is low as there was no significant difference between the chronic peri-articular swelling scores of the respective groups at the end of the study. This indicates that none of the cattle included in trial 1 responded to the supplementation but instead developed chronic peri-articular lesions.

It was expected that the cattle in this study fed supplement 3 would have responded positively in a similar fashion to the cattle of the 2012 pre-trial did as the mineral levels included in supplement 3 resembled that of the pre-trial 2012 supplement (Prozesky *et al*. 2016).
The fact that none of the study cattle responded positively to any of the supplementations, regardless of different micro-and macro mineral level intake between sub-groups, warrants further investigation. The results obtained in this study are contradictory to those observed during the 2012 pre-trial where cattle fed a supplement containing high levels of both micro- and macro minerals responded positively with a marked decrease in the severity of lameness and size of the peri-articular swelling.

A few cattle included in trial 1 and 2 of this study were randomly selected for a detailed clinical examination. Urinalysis was done as part of the examination. The urine test strip used (Combur$^{9}$Test$^{®}$; Roche) indicated a urinary pH of less than 6 for all the cattle from which urine could be collected, in total four. Possible factors that could lead to the development of aciduria were investigated. It is known from studies in dairy cows that a dietary cation anion difference (DCAD) value of < -200 mEq/kg is effective in inducing a sufficient metabolic acidosis and aciduria (average pH 7) (DeGaris & Lean 2008).
The DCAD value for the respective trial supplements as well as the 2012 pre-trial supplement was calculated by subtracting the anion value from the cation value. The most common equation used is: $[(Na^{+}+ K^{+})- (Cl^{-} + S^{2-})]$ (DeGaris & Lean 2008). There was a significant

difference between the DCAD value of supplements 1, 2 and 3 compared to that of the 2012 pre-trial supplement. Calculated DCAD values are depicted in table 10.

**Table 10**: Calculated DCAD values of respective supplements.

| Supplement | Calculated DCAD value (mEq/kg) |
|---|---|
| Supplement 1 | -411 |
| Supplement 2 | -466 |
| Supplement 3 | -467 |
| 2012 pre-trial supplement | +19.87 |

Further investigation into the composition of the trial supplements revealed that all three supplements contained the anionic salt ammonium sulphate, whereas the pre-trial supplement did not contain an anionic salt.

A possible explanation why the elevated mineral intakes from supplements 2 and 3 did not result in recovery from osteochondrosis is presented as follows:

The plasma pH is regulated by four factors of which the strong ion difference is one. Intestinal absorption concentration of strong anions (Chloride and Sulphate) is more than that of strong cations (Calcium, Magnesium and Ammonium) when salts like ammonium sulphate $(NH_4)_2SO_4$ are fed. The increased anion level in the plasma, in this case $SO_4^{2-}$, reduces the strong ion difference, inducing a strong ion metabolic acidosis (DeGaris & Lean 2008). Metabolic acidosis increases the responsiveness of tissue receptors to parathyroid hormone (PTH) (Horst *et al.* 1997:1269-1280). Bone responds to PTH by activation of osteocytes as well as the osteoclasts (La Perle & Capen 2006) with osteoclastic activity increasing proportionally as the plasma becomes more acidic (Arnette 2003). Calcium can be mobilized from bone either in conjunction with or independently of PTH (DeGaris & Lean 2008). Bone acts as a buffer during acute metabolic acidosis by binding hydrogen ions to carbonate and releasing the cation salts ($Na^+$, $K^+$, $Ca^{2+}$) associated with the carbonate into the extracellular fluid (Engelking 2011, Horst *et al.* 1997). This process and as the activation of osteoclasts through PTH function as a buffer during chronic metabolic acidosis (Green & Kleeman 1991).

Renal function responds to PTH through reduced reabsorption of phosphate (phosphaturia) and increased reabsorption of calcium (La Perle & Capen 2006) from the glomerular filtrate. Calcium is still excreted at elevated levels during metabolic acidosis despite the increased

reabsorption action of PTH. The charge equivalence of albumin is altered during metabolic acidosis, which leads to the release of plasma protein bound calcium (up to 40% of the total calcium) increasing the amount filtered through the glomerulus of the kidney and ultimately excreted leading to increased mineral loss (Engelking 2011). The excess dietary anions in the plasma are filtered and excreted through the kidneys. The urine produced is acidic (aciduria) in nature due to the increased concentration of excreted anions (Spanghero 2004). A urinary pH <5.5 indicates severe metabolic acidosis and should be avoided at all costs (Horst *et al.* 1997).

The majority of minerals reabsorbed from the skeleton of rats with metabolic acidosis occurred from the epiphysis, resulting in lower total bone volume of the metaphysis (Kraut *et al.* 1986). Mongin and Sauveur (1977) (q. Whitehead 1997) proposed that the anionic balance of a diet influenced the incidence rate of tibial dyschondroplasia (osteochondrosis) in chickens. Several studies followed that confirmed the proposal made by Mongin and Sauveur that metabolic acidosis increased the incidence of tibial dyschondroplasia in chickens (Whitehead 1997).

A new hypothesis was formulated based on the observations made during the trial. We hypothesize that chronic mild metabolic acidosis (urinalysis of four animals) weakens the developing calcareous bone, consequently exposing particularly fast growing cattle to traumatic fracture of the subchondral bone and articular cartilage, leading to the development of lesions associated with osteochondrosis. This hypothesis also suggests that chronic metabolic acidosis will exacerbate sub-clinical/clinical osteochondrosis. These hypotheses require further investigation. It is import to consider not only the direct dietary cause of metabolic acidosis, but to have a holistic approach which includes signalment, husbandry and regular analysis of both feed and water sources. These hypotheses require further investigation.

## 5.4 Conclusion

Investigation of cattle clinically affected by osteochondrosis in the Vryburg area on a herd basis revealed that bulls were more prone and severely affected than females, with young animals more frequently affected than adult animals (Prozesky *et al*. 2016). The higher frequency among young animals is in accordance with the definition, as osteochondrosis is a term that pertains to a group of lesions associated with the persistence of growth cartilage in the epiphyseal or physeal growth plates as a result of failure of endochondral ossification

Prozesky *et al.* (2016). The diagnosis was also supported by data from the pre-trial indicating that cattle clinically suffering from osteochondrosis responded positively when fed a supplement containing high levels of bio-available micro- and macro minerals.

The results from this study indicated that cattle with clinical osteochondrosis do not respond positively to a supplement with a low DCAD value, regardless of its micro-and macro mineral concentration. The new hypothesis suggests that cattle supplemented for an extended period of time with a low DCAD feed are predisposed to the development of osteochondrosis and/or that the low DCAD feed exacerbates sub-clinical/clinical osteochondrosis. This hypothesis needs to further investigated.

## 5.5 References

Arnett, T., 2003, 'Regulation of bone cell function by acid–base balance', *Proceedings of the Nutrition Society 62*(02), 511-520.

DeGaris, P.J. & Lean, I.J., 2008, 'Milk fever in dairy cows: A review of pathophysiology and control principles'. *The Veterinary Journal*, 176(1), 58-69.

Engelking, L., 2011, *Textbook of veterinary physiological chemistry*, 2nd rev. edn., 489-495, Elsevier, Oxford.

Green, J. & Kleeman, C.R., 1991, 'Role of bone in regulation of systemic acid-base balance', *Kidney International* 39(1), 9-26.

Horst, R.L., Goff, J.P., Reinhardt, T.A. & Buxton, D.R., 1997, 'Strategies for Preventing Milk Fever in Dairy Cattle 1, 2', *Journal of Dairy Science 80*(7), 1269-1280.

Kraut, J.A., Mishler, D.R., Singer, F.R. & Goodman, W.G., 1986, 'The effects of metabolic acidosis on bone formation and bone resorption in the rat', *Kidney International* 30(5), 694-700.

La Perle, K.M.D. & Capen, C.C, 2006, 'Endocrine system', in M.D. McGavin & J.F. Zachary (eds.), *Pathologic basis of Veterinary Disease*, 4th edn., 693-742, Mosby Elsevier, Missouri.

Mongin, P. & Sauveur, B., 1977, 'Interrelationships between mineral nutrition, acid-base balance, growth and cartilage abnormalities', *Growth and poultry meat production*, 235-237.

National Research Council., 2001, 'Nutrient requirements of dairy cattle', 7th rev. ed., *National Academy of Sciences*., Washington, DC.

Prozesky, L., Neser, J., Meissner, H., Botha, F.K., Jacobs, L., Shepstone, C., Viljoen, H.J., Köster, H.H., de Brouwer, C., van Zyl, J. & van der Veen, G., ´Preliminary report on osteochondrosis in cattle in the North-Western parts of South Africa´, *Onderstepoort Journal of Veterinary Research*, In press.

Spanghero, M., 2004, 'Prediction of urinary and blood pH in non-lactating dairy cows fed anionic diets', *Animal feed science and technology 116*(1), 83-92.

Theiler, A., 1912, 'Facts and theories about stijfziekte and lamziekte', *Second Report of the Director of Veterinary Research, Union of South Africa*, 7-78.

Theiler, A., Green, H.H. & Du Toit, P.J., 1928, 'Studies in mineral metabolism. III. Breeding of cattle on phosphorus deficient pasture', *The Journal of Agricultural Science 18*(03), 369-371.

Trostle, S.S., Nicoll, R.G., Forrest, L.J. & Markel, M.D., 1997, 'Clinical and radiographic findings, treatment, and outcome in cattle with osteochondrosis: 29 cases (1986-1996) ', *Journal of the American Veterinary Medical Association 211*(12), 1566-1570.

Trostle, S.S., Nicoll, R.G., Forrest, L.J., Markel, M. & Nordlund, K., 1998, 'Bovine osteochondrosis', *The Compendium on continuing education for the practicing veterinarian* 20(7), 856-863.

Whitehead, C.C., 1997, 'Dyschondroplasia in poultry', *Proceedings of the Nutrition Society 56*(03), 957-966.


***Figure 3**: The affected hind leg loses its normal anatomical conformation and adapt a straight conformation.*


***Figure 4**: Clinically affected cattle adapt a saw-horse stance in an attempt to reduce the weight bearing on affected joints.*


***Figure 5**: Hindquarter muscle atrophy with visible peri-articular swelling of the stifle joint.*


***Figure 6**: Chronic medium sized periarticular swelling.*

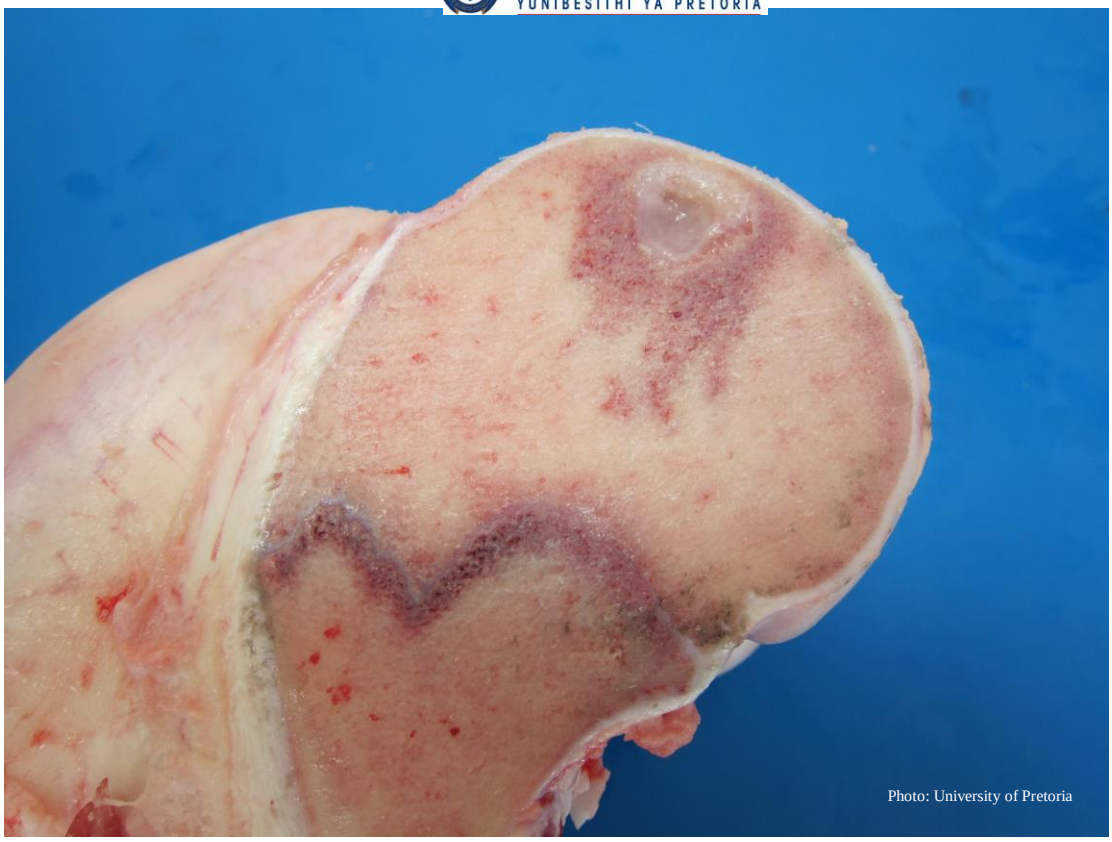



**Figure 7**: Normal bovine femoral condyles (articulating cartilage surface).

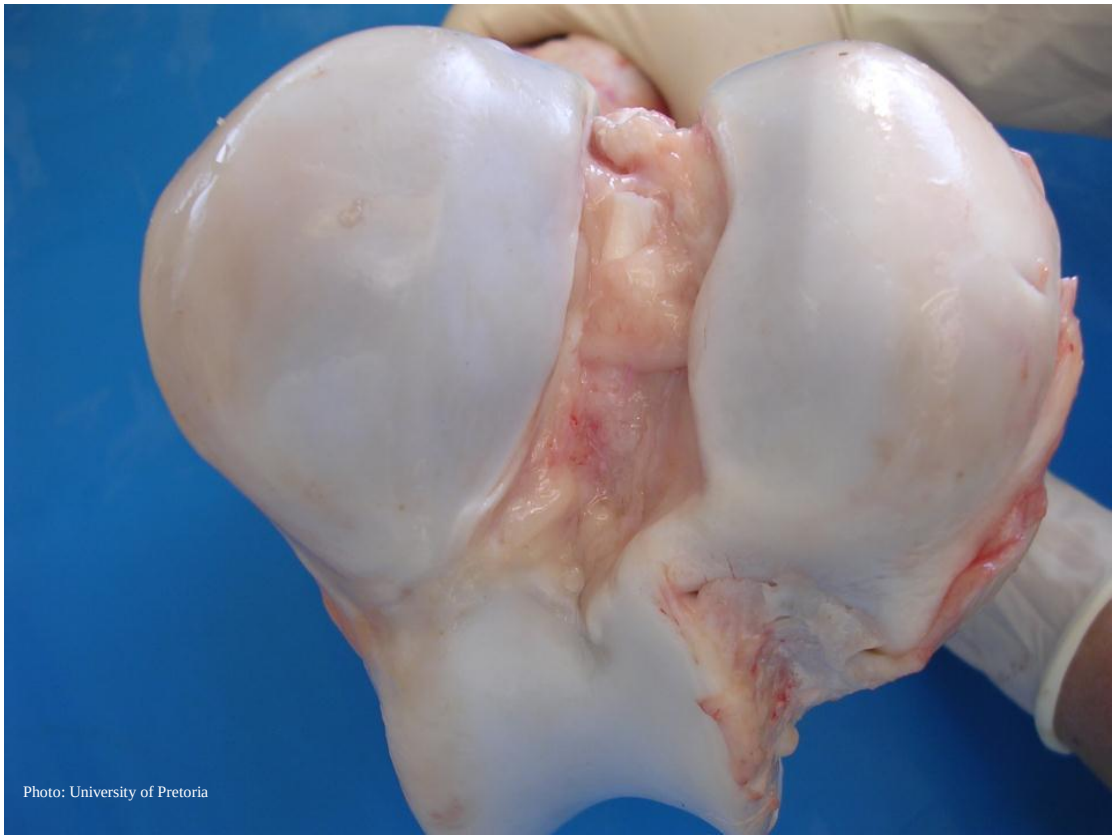



**Figure 8**: Transverse cut surface of affected femoral condyle exposing a subchondral bone cyst

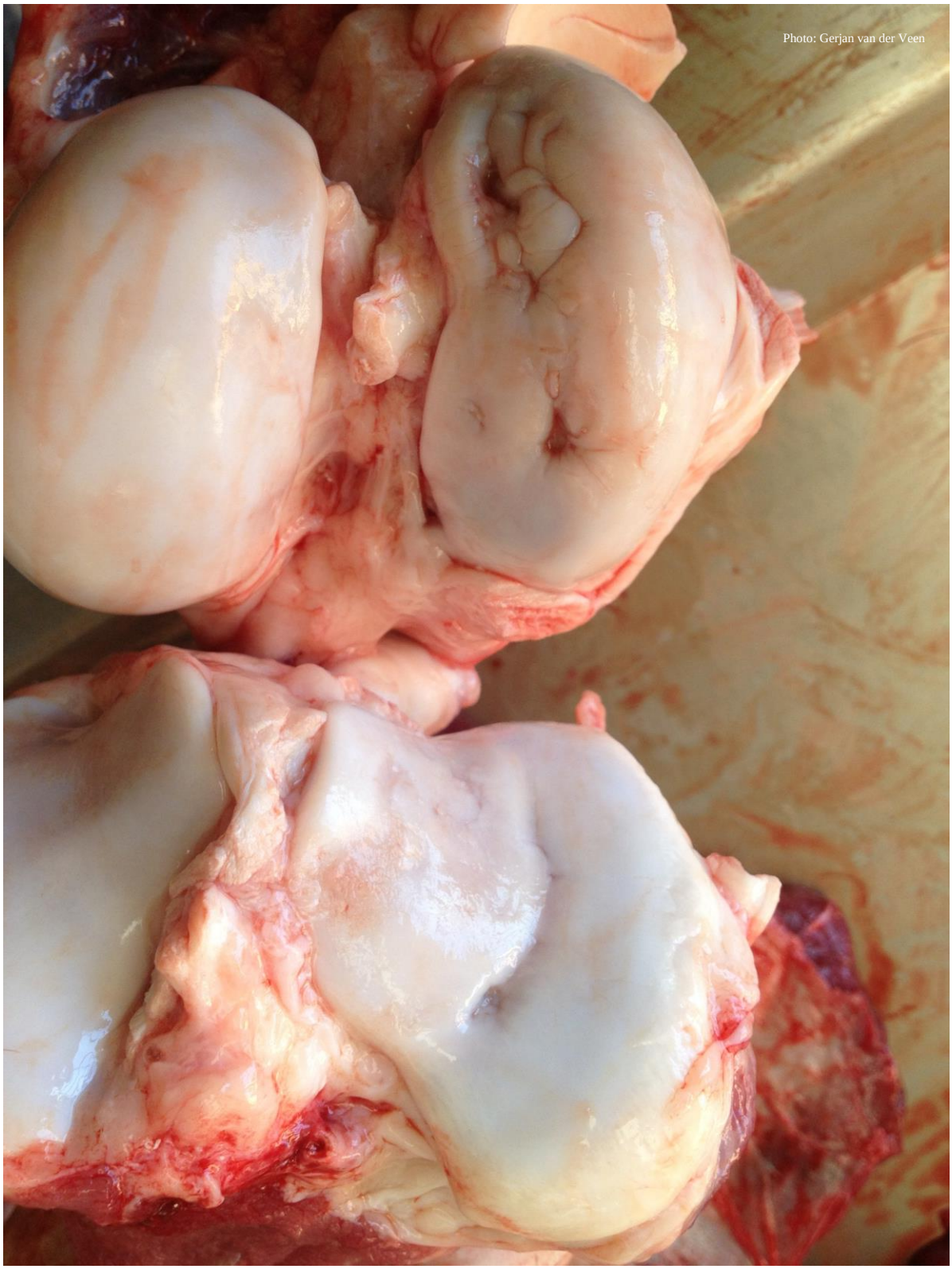



**Figure 9**: Right hind stifle joint: Osteochondrotic lesions on the articulating cartilage surface of the lateral femoral condyl and lateral tibeal platue.

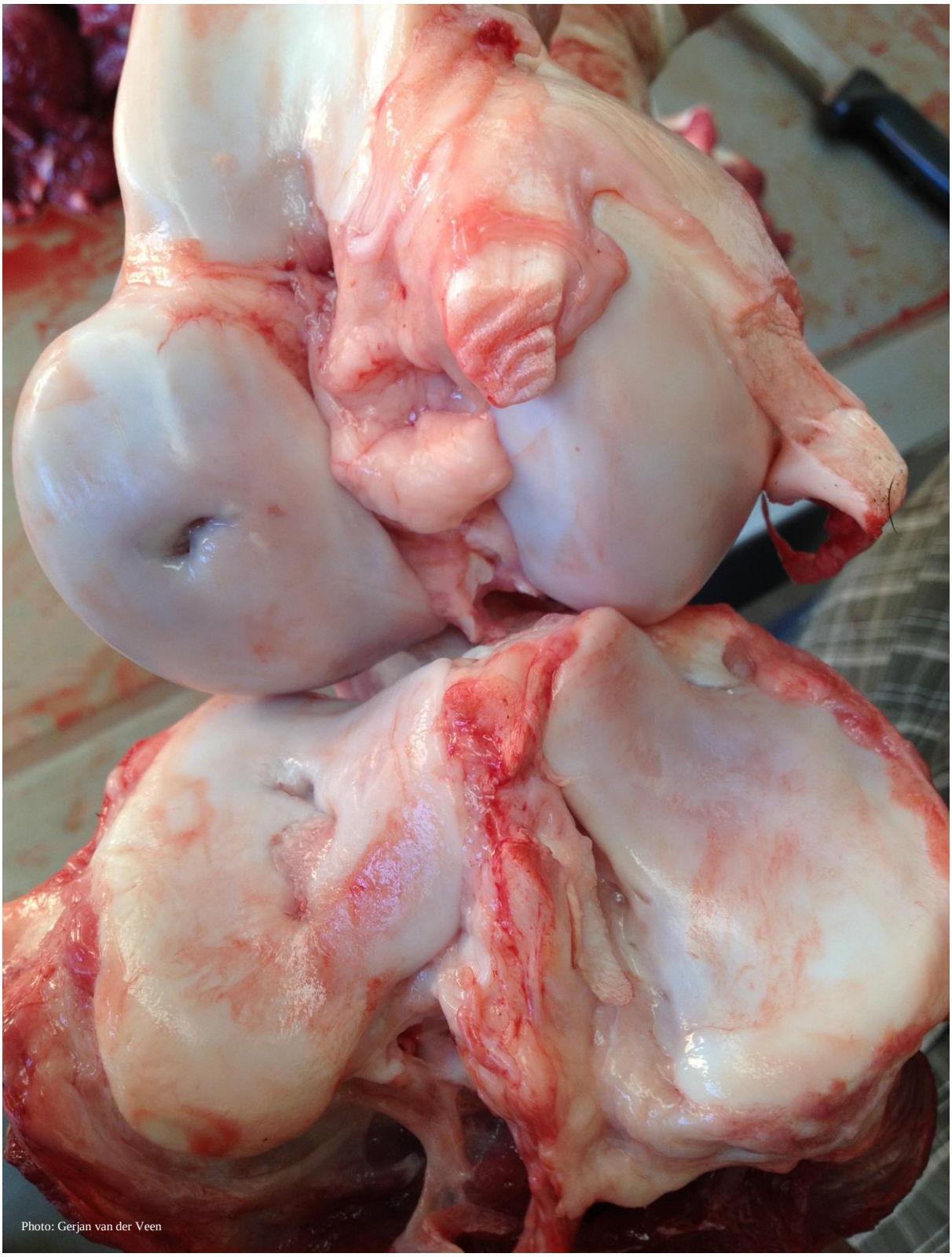



**Figure 10**: Left hind stifle joint: Osteochondrotic lesions on the articulating cartilage surface of the lateral femoral condyl and lateral tibeal platue

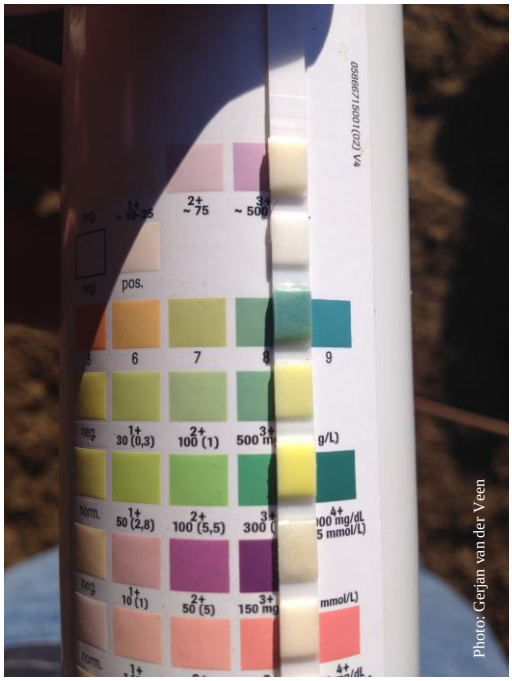



***Figure 12:*** *Urinary dipstick indicating normal bovine urine pH of 8.5.*

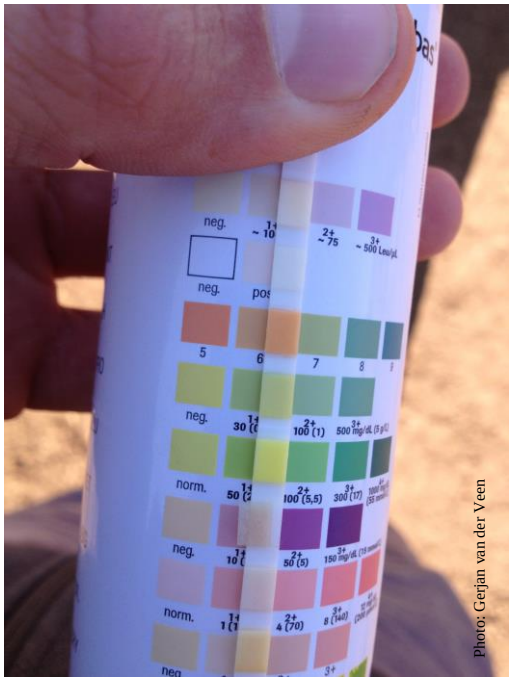



***Figure 11:*** *Urinary dipstick indicating aciduria with a pH of 6 (as measured in sub-group A).*

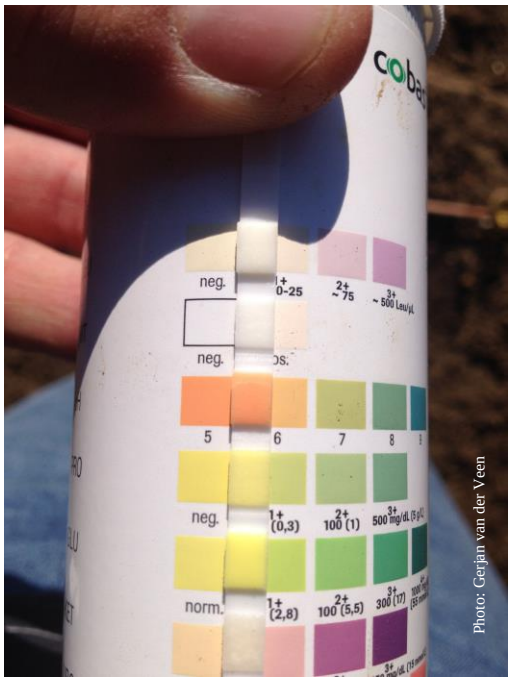



***Figure 13:*** *Urinary dipstick indicating aciduria with a pH of 5 (as measured in sub-group B).*

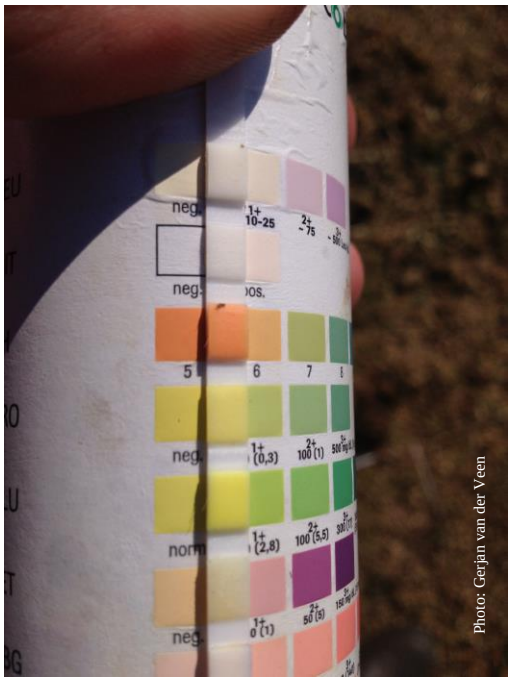



***Figure 14:*** *Urinary dipstick indicating aciduria with a pH of 5 (as measured in sub-group C).*

# Appendix 1

Analysis of supplement 1

## CUMBERLAND VALLEY ANALYTICAL SERVICES

Laboratory services for agriculture ... from the field to the feed bunk.

**Type:** BYPRODUCT, MISC
**Farm:** OP
**Desc:** LICK 1
DU TOIT JACOLENE
FEED FIRST

**Copies to:**

**Regression:** OH

Lab ID: **14907 391**
Sampled:
Arrived: **07/01/2013**
Completed: **07/07/2013**
Reported: **07/07/2013**

***LICK 1***

**SAMPLE INFORMATION**

| | | | |
|---|---|---|---|
| Lab ID: | 14907 391 | Series: | |
| Crop Year: | 2013 | Version: | 1.0 |
| Cutting#: | | | |
| Feed Type: | BYPRODUCT, MISC | | |

| CHEMISTRY ANALYSIS RESULTS | |
|---|---|
| Moisture | 11.3 |
| Dry Matter | 88.7 |

| PROTEINS | % SP | % CP | % DM |
|---|---|---|---|
| Crude Protein | | | 33.7 |
| Adjusted Protein | | | 33.7 |
| Soluble Protein | | | |
| Ammonia (NPN) | | | |
| ADF Protein (ADICP) | | | |
| NDF Protein (NDICP) | | | |
| NDR Protein (NDRCP) | | | |
| Rumen Degr. Protein | | | |
| Rumen Deg. CP (Strep.G) | | | |

| FIBER | % NDF | % DM |
|---|---|---|
| ADF | | |
| NDF | | |
| aNDFom | | |
| NDR (NDF w/o sulfite) | | |
| peNDF | | |
| Crude Fiber | | |
| Lignin | | |
| NDF Digestibility (12 hr) | | |
| NDF Digestibility (24 hr) | | |
| NDF Digestibility (30 hr) | | |
| NDF Digestibility (48 hr) | | |
| NDF Digestibility (240 hr) | | |
| Indigestible NDF | | |

| MINERALS | |
|---|---|
| Ash (%DM) | 35.23 |
| Calcium (%DM) | 2.05 |
| Phosphorus (%DM) | 0.94 |
| Magnesium (%DM) | 0.39 |
| Potassium (%DM) | 1.76 |
| Sulfur (%DM) | |
| Sodium (%DM) | 10.306 |
| Chloride (%DM) | |
| Iron (PPM) | 480 |
| Manganese (PPM) | 492 |
| Zinc (PPM) | 496 |
| Copper (PPM) | 229 |
| Molybdenum (PPM) | |
| Selenium (PPM) | |
| Nitrate Ion (%DM) | |
| Selenium | |

| FERMENTATION |
|---|
| pH |
| Total VFA |
| Lactic Acid (%DM) |
| Lactic as % of Total VFA |
| Acetic Acid (%DM) |
| Propionic Acid (%DM) |
| Butyric Acid (%DM) |
| Isobutyric Acid (%DM) |
| Titratable Acidity (meq/100gm) |
| 1, 2 Propanediol (%DM) |

| QUALITATIVE |
|---|
| Mold Count (col/gm) |
| Yeast Count (col/gm) |
| Corn Silage Processing Score |
| Particle Size ( Penn State ) |
| - Particles greater than 0.75" |
| - Particles from 0.31" to 0.75" |
| - Particles less than 0.31" |

| CARBOHYDRATES | % Starch | % NFC | % DM |
|---|---|---|---|
| Silage Acids | | | |
| Ethanol Soluble CHO (Sugar) | | | |
| Water soluble CHO (Sugar) | | | |
| Starch | | | |
| Soluble Fiber | | | |
| Starch Digestibility (7 hr) | | | |
| Fatty Acids, Total (%DM) | | | |
| Crude Fat | | | |
| Acid Hydrolysis Fat | | | |

| ENERGY & INDEX CALCULATIONS |
|---|
| TDN (%DM) |
| Net Energy Lactation (mcal/lb) |
| Net Energy Maintenance (mcal/lb) |
| Net Energy Gain (mcal/lb) |
| NDF Dig. Rate (Kd, %HR, Van Amburgh) |
| NDF Dig. Rate (Kd, %HR, Mertens) |
| Starch Dig. Rate (Kd, %HR, Mertens) |
| Relative Feed Value (RFV) |
| Relative Feed Quality (RFQ) |
| Milk per Ton (lbs/ton) |
| Dig. Organic Matter Index (lbs/ton) |
| Non Fiber Carbohydrates (%DM) |
| Non Structural Carbohydrates (%DM) |
| DCAD (meq/100gdm) |

*Powered by Cumberland Valley Analytical Services*

14515 Industry Drive, Hagerstown, MD 21742
www.foragelab.com | mail@foragelab.com | 301-790-1980 | 800-CVAS-LAB

# Appendix 2

Analysis of supplement 2

## CUMBERLAND VALLEY ANALYTICAL SERVICES

Laboratory services for agriculture ... from the field to the feed bunk.

**Type:** BYPRODUCT, MISC
**Farm:** OP
**Desc:** LICK 2
DU TOIT JACOLENE
FEED FIRST

**Copies to:**

**Regression:** OH

Lab ID: 14907 392
Sampled:
Arrived: 07/01/2013
Completed: 07/07/2013
Reported: 07/07/2013

***LICK 2***

**SAMPLE INFORMATION**

| | | | |
|---|---|---|---|
| Lab ID: | 14907 392 | Series: | |
| Crop Year: | 2013 | Version: | 1.0 |
| Cutting#: | | | |
| Feed Type: | BYPRODUCT, MISC | | |

| CHEMISTRY ANALYSIS RESULTS | |
|---|---|
| Moisture | 11.8 |
| Dry Matter | 88.2 |

| PROTEINS | % SP | % CP | % DM |
|---|---|---|---|
| Crude Protein | | | 35.8 |
| Adjusted Protein | | | 35.8 |
| Soluble Protein | | | |
| Ammonia (NPN) | | | |
| ADF Protein (ADICP) | | | |
| NDF Protein (NDICP) | | | |
| NDR Protein (NDRCP) | | | |
| Rumen Degr. Protein | | | |
| Rumen Deg. CP (Strep.G) | | | |

| FIBER | % NDF | % DM |
|---|---|---|
| ADF | | |
| NDF | | |
| aNDFom | | |
| NDR (NDF w/o sulfite) | | |
| peNDF | | |
| Crude Fiber | | |
| Lignin | | |
| NDF Digestibility (12 hr) | | |
| NDF Digestibility (24 hr) | | |
| NDF Digestibility (30 hr) | | |
| NDF Digestibility (48 hr) | | |
| NDF Digestibility (240 hr) | | |
| Indigestible NDF | | |

| MINERALS | |
|---|---|
| Ash (%DM) | 37.16 |
| Calcium (%DM) | 2.37 |
| Phosphorus (%DM) | 1.59 |
| Magnesium (%DM) | 0.38 |
| Potassium (%DM) | 1.57 |
| Sulfur (%DM) | |
| Sodium (%DM) | 11.568 |
| Chloride (%DM) | |
| Iron (PPM) | 460 |
| Manganese (PPM) | 494 |
| Zinc (PPM) | 452 |
| Copper (PPM) | 223 |
| Molybdenum (PPM) | |
| Selenium (PPM) | |
| Nitrate Ion (%DM) | |
| Selenium | |

| FERMENTATION |
|---|
| pH |
| Total VFA |
| Lactic Acid (%DM) |
| Lactic as % of Total VFA |
| Acetic Acid (%DM) |
| Propionic Acid (%DM) |
| Butyric Acid (%DM) |
| Isobutyric Acid (%DM) |
| Titratable Acidity (meq/100gm) |
| 1, 2 Propanediol (%DM) |

| QUALITATIVE |
|---|
| Mold Count (col/gm) |
| Yeast Count (col/gm) |
| Corn Silage Processing Score |
| Particle Size ( Penn State ) |
| - Particles greater than 0.75" |
| - Particles from 0.31" to 0.75" |
| - Particles less than 0.31" |

| CARBOHYDRATES | % Starch | % NFC | % DM |
|---|---|---|---|
| Silage Acids | | | |
| Ethanol Soluble CHO (Sugar) | | | |
| Water soluble CHO (Sugar) | | | |
| Starch | | | |
| Soluble Fiber | | | |
| Starch Digestibility (7 hr) | | | |
| Fatty Acids, Total (%DM) | | | |
| Crude Fat | | | |
| Acid Hydrolysis Fat | | | |

| ENERGY & INDEX CALCULATIONS |
|---|
| TDN (%DM) |
| Net Energy Lactation (mcal/lb) |
| Net Energy Maintenance (mcal/lb) |
| Net Energy Gain (mcal/lb) |
| NDF Dig. Rate (Kd, %HR, Van Amburgh) |
| NDF Dig. Rate (Kd, %HR, Mertens) |
| Starch Dig. Rate (Kd, %HR, Mertens) |
| Relative Feed Value (RFV) |
| Relative Feed Quality (RFQ) |
| Milk per Ton (lbs/ton) |
| Dig. Organic Matter Index (lbs/ton) |
| Non Fiber Carbohydrates (%DM) |
| Non Structural Carbohydrates (%DM) |
| DCAD (meq/100gdm) |

*Powered by Cumberland Valley Analytical Services*

14515 Industry Drive, Hagerstown, MD 21742
www.foragelab.com | mail@foragelab.com | 301-790-1980 | 800-CVAS-LAB

# Appendix 3

Analysis of supplement 3

## CUMBERLAND VALLEY ANALYTICAL SERVICES

Laboratory services for agriculture ... from the field to the feed bunk.

**Type:** BYPRODUCT, MISC
**Farm:** OP
**Desc:** LICK 3
DU TOIT JACOLENE
FEED FIRST

**Copies to:**

**Regression:** OH

Lab ID: **14907 393**
Sampled:
Arrived: **07/01/2013**
Completed: **07/07/2013**
Reported: **07/07/2013**

***LICK 3***

| SAMPLE INFORMATION | | | |
|---|---|---|---|
| Lab ID: | 14907 393 | Series: | |
| Crop Year: | 2013 | Version: | 1.0 |
| Cutting#: | | | |
| Feed Type: | BYPRODUCT, MISC | | |

| CHEMISTRY ANALYSIS RESULTS | |
|---|---|
| Moisture | 11.2 |
| Dry Matter | 88.8 |

| PROTEINS | % SP | % CP | % DM |
|---|---|---|---|
| Crude Protein | | | 36.3 |
| Adjusted Protein | | | 36.3 |
| Soluble Protein | | | |
| Ammonia (NPN) | | | |
| ADF Protein (ADICP) | | | |
| NDF Protein (NDICP) | | | |
| NDR Protein (NDRCP) | | | |
| Rumen Degr. Protein | | | |
| Rumen Deg. CP (Strep.G) | | | |

| FIBER | % NDF | % DM |
|---|---|---|
| ADF | | |
| NDF | | |
| aNDFom | | |
| NDR (NDF w/o sulfite) | | |
| peNDF | | |
| Crude Fiber | | |
| Lignin | | |
| NDF Digestibility (12 hr) | | |
| NDF Digestibility (24 hr) | | |
| NDF Digestibility (30 hr) | | |
| NDF Digestibility (48 hr) | | |
| NDF Digestibility (240 hr) | | |
| Indigestible NDF | | |

| MINERALS | |
|---|---|
| Ash (%DM) | 40.83 |
| Calcium (%DM) | 2.79 |
| Phosphorus (%DM) | 1.76 |
| Magnesium (%DM) | 0.27 |
| Potassium (%DM) | 1.40 |
| Sulfur (%DM) | |
| Sodium (%DM) | 12.709 |
| Chloride (%DM) | |
| Iron (PPM) | 385 |
| Manganese (PPM) | 399 |
| Zinc (PPM) | 85 |
| Copper (PPM) | 36 |
| Molybdenum (PPM) | |
| Selenium (PPM) | |
| Nitrate Ion (%DM) | |
| Selenium | |

| FERMENTATION |
|---|
| pH |
| Total VFA |
| Lactic Acid (%DM) |
| Lactic as % of Total VFA |
| Acetic Acid (%DM) |
| Propionic Acid (%DM) |
| Butyric Acid (%DM) |
| Isobutyric Acid (%DM) |
| Titratable Acidity (meq/100gm) |
| 1, 2 Propanediol (%DM) |

| QUALITATIVE |
|---|
| Mold Count (col/gm) |
| Yeast Count (col/gm) |
| Corn Silage Processing Score |
| Particle Size ( Penn State ) |
| - Particles greater than 0.75" |
| - Particles from 0.31" to 0.75" |
| - Particles less than 0.31" |

| CARBOHYDRATES | % Starch | % NFC | % DM |
|---|---|---|---|
| Silage Acids | | | |
| Ethanol Soluble CHO (Sugar) | | | |
| Water soluble CHO (Sugar) | | | |
| Starch | | | |
| Soluble Fiber | | | |
| Starch Digestibility (7 hr) | | | |
| Fatty Acids, Total (%DM) | | | |
| Crude Fat | | | |
| Acid Hydrolysis Fat | | | |

| ENERGY & INDEX CALCULATIONS |
|---|
| TDN (%DM) |
| Net Energy Lactation (mcal/lb) |
| Net Energy Maintenance (mcal/lb) |
| Net Energy Gain (mcal/lb) |
| NDF Dig. Rate (Kd, %HR, Van Amburgh) |
| NDF Dig. Rate (Kd, %HR, Mertens) |
| Starch Dig. Rate (Kd, %HR, Mertens) |
| Relative Feed Value (RFV) |
| Relative Feed Quality (RFQ) |
| Milk per Ton (lbs/ton) |
| Dig. Organic Matter Index (lbs/ton) |
| Non Fiber Carbohydrates (%DM) |
| Non Structural Carbohydrates (%DM) |
| DCAD (meq/100gdm) |

*Powered by Cumberland Valley Analytical Services*

14515 Industry Drive, Hagerstown, MD 21742
www.foragelab.com | mail@foragelab.com | 301-790-1980 | 800-CVAS-LAB

# Appendix 4

Analysis of supplement used in December 2012

## CUMBERLAND VALLEY ANALYTICAL SERVICES

Laboratory services for agriculture ... from the field to the feed bunk.

**Type:** BYPRODUCT, MISC
**Farm:** OP
**Desc:** LICK 4
DU TOIT JACOLENE
FEED FIRST

**Copies to:**

**Regression:** OH

Lab ID: **14907 394**
Sampled:
Arrived: **07/01/2013**
Completed: **07/07/2013**
Reported: **07/07/2013**

***LICK 4***

**SAMPLE INFORMATION**

| | | | |
|---|---|---|---|
| Lab ID: | 14907 394 | Series: | |
| Crop Year: | 2013 | Version: | 1.0 |
| Cutting#: | | | |
| Feed Type: | BYPRODUCT, MISC | | |

**CHEMISTRY ANALYSIS RESULTS**

| | |
|---|---|
| Moisture | 10.1 |
| Dry Matter | 89.9 |

| PROTEINS | % SP | % CP | % DM |
|---|---|---|---|
| Crude Protein | | | 33.1 |
| Adjusted Protein | | | 33.1 |
| Soluble Protein | | | |
| Ammonia (NPN) | | | |
| ADF Protein (ADICP) | | | |
| NDF Protein (NDICP) | | | |
| NDR Protein (NDRCP) | | | |
| Rumen Degr. Protein | | | |
| Rumen Deg. CP (Strep.G) | | | |

| FIBER | % NDF | % DM |
|---|---|---|
| ADF | | |
| NDF | | |
| aNDFom | | |
| NDR (NDF w/o sulfite) | | |
| peNDF | | |
| Crude Fiber | | |
| Lignin | | |
| NDF Digestibility (12 hr) | | |
| NDF Digestibility (24 hr) | | |
| NDF Digestibility (30 hr) | | |
| NDF Digestibility (48 hr) | | |
| NDF Digestibility (240 hr) | | |
| Indigestible NDF | | |

| MINERALS | |
|---|---|
| Ash (%DM) | 26.13 |
| Calcium (%DM) | 1.30 |
| Phosphorus (%DM) | 1.84 |
| Magnesium (%DM) | 0.37 |
| Potassium (%DM) | 1.45 |
| Sulfur (%DM) | |
| Sodium (%DM) | 7.258 |
| Chloride (%DM) | |
| Iron (PPM) | 376 |
| Manganese (PPM) | 247 |
| Zinc (PPM) | 144 |
| Copper (PPM) | 48 |
| Molybdenum (PPM) | |
| Selenium (PPM) | |
| Nitrate Ion (%DM) | |
| Selenium | |

**FERMENTATION**

| |
|---|
| pH |
| Total VFA |
| Lactic Acid (%DM) |
| Lactic as % of Total VFA |
| Acetic Acid (%DM) |
| Propionic Acid (%DM) |
| Butyric Acid (%DM) |
| Isobutyric Acid (%DM) |
| Titratable Acidity (meq/100gm) |
| 1, 2 Propanediol (%DM) |

**QUALITATIVE**

| |
|---|
| Mold Count (col/gm) |
| Yeast Count (col/gm) |
| Corn Silage Processing Score |
| Particle Size ( Penn State ) |
| - Particles greater than 0.75" |
| - Particles from 0.31" to 0.75" |
| - Particles less than 0.31" |

| CARBOHYDRATES | % Starch | % NFC | % DM |
|---|---|---|---|
| Silage Acids | | | |
| Ethanol Soluble CHO (Sugar) | | | |
| Water soluble CHO (Sugar) | | | |
| Starch | | | |
| Soluble Fiber | | | |
| Starch Digestibility (7 hr) | | | |
| Fatty Acids, Total (%DM) | | | |
| Crude Fat | | | |
| Acid Hydrolysis Fat | | | |

**ENERGY & INDEX CALCULATIONS**

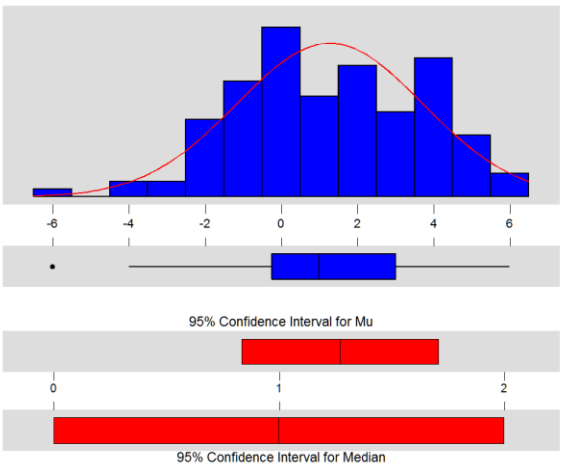
| |
|---|
| TDN (%DM) |
| Net Energy Lactation (mcal/lb) |
| Net Energy Maintenance (mcal/lb) |
| Net Energy Gain (mcal/lb) |
| NDF Dig. Rate (Kd, %HR, Van Amburgh) |
| NDF Dig. Rate (Kd, %HR, Mertens) |
| Starch Dig. Rate (Kd, %HR, Mertens) |
| Relative Feed Value (RFV) |
| Relative Feed Quality (RFQ) |
| Milk per Ton (lbs/ton) |
| Dig. Organic Matter Index (lbs/ton) |
| Non Fiber Carbohydrates (%DM) |
| Non Structural Carbohydrates (%DM) |
| DCAD (meq/100gdm) |

*Powered by Cumberland Valley Analytical Services*

14515 Industry Drive, Hagerstown, MD 21742
www.foragelab.com | mail@foragelab.com | 301-790-1980 | 800-CVAS-LAB

# Appendix 5

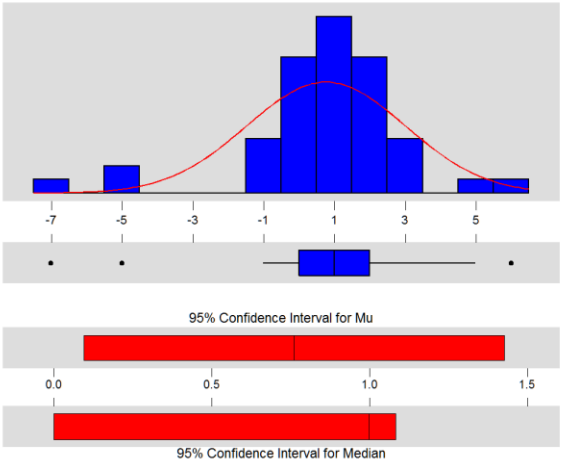
Descriptive Statistics




Variable: Delta_sum_1

| Anderson-Darling Normality Test | |
|---|---|
| A-Squared: | 1.480 |
| P-Value: | 0.001 |
| Mean | 1.27049 |
| StDev | 2.43947 |
| Variance | 5.95102 |
| Skewness | -1.4E-01 |
| Kurtosis | -4.4E-01 |
| N | 122 |
| Minimum | -6.00000 |
| 1st Quartile | -0.25000 |
| Median | 1.00000 |
| 3rd Quartile | 3.00000 |
| Maximum | 6.00000 |
| 95% Confidence Interval for Mu | |
| 0.83324 | 1.70774 |
| 95% Confidence Interval for Sigma | |
| 2.16702 | 2.79090 |
| 95% Confidence Interval for Median | |
| 0.00000 | 2.00000 |

Descriptive Statistics




Variable: Delta_sum_2

| Anderson-Darling Normality Test | |
|---|---|
| A-Squared: | 2.330 |
| P-Value: | 0.000 |
| Mean | 0.76087 |
| StDev | 2.24286 |
| Variance | 5.03043 |
| Skewness | -1.21896 |
| Kurtosis | 3.88671 |
| N | 46 |
| Minimum | -7.00000 |
| 1st Quartile | 0.00000 |
| Median | 1.00000 |
| 3rd Quartile | 2.00000 |
| Maximum | 6.00000 |
| 95% Confidence Interval for Mu | |
| 0.09482 | 1.42692 |
| 95% Confidence Interval for Sigma | |
| 1.86031 | 2.82494 |
| 95% Confidence Interval for Median | |
| 0.00000 | 1.08273 |

# Appendix 6

**Onderstepoort Journal of Veterinary Research**
ISSN: (Online) 2219-0635, (Print) 0030-2465



# Response of cattle with clinical osteochondrosis to mineral supplementation

**Authors:**
Gerjan van der Veen[1]
Geoffrey T. Fosgate[2]
Frederick K. Botha[1]
Heinz H. Meissner[1]
Lubbe Jacobs[3]
Leon Prozesky[1]

**Affiliations:**
[1]Department of Paraclinical Sciences, University of Pretoria, South Africa

[2]Department of Production Animal Studies, University of Pretoria, South Africa

[3]Lubern Animal Feeds, Hartswater, South Africa

**Corresponding author:**
Gerjan van der Veen,
gerjan@deltamune.co.za

**Dates:**
Received: 28 Sept. 2016
Accepted: 25 Nov. 2016
Published: 24 Feb. 2017

**How to cite this article:**
Van der Veen, G., Fosgate, G.T., Botha, F.K., Meissner, H.H., Jacobs, L. & Prozesky, L., 2017, 'Response of cattle with clinical osteochondrosis to mineral supplementation', *Onderstepoort Journal of Veterinary Research* 84(1), a1365. https://doi.org/10.4102/ojvr.v84i1.1365





Since 1982, farmers in the North West province and other parts of South Africa have noticed an increase in the incidence of lameness in cattle. Macro- and microscopical lesions of joints resembled osteochondrosis. Pre-trial data indicated that cattle with osteochondrotic lesions recovered almost completely when fed a supplement containing bio-available micro- and macrominerals of high quality. In the present trial, 43 clinically affected cattle of varying ages (1–5 years) and sexes were randomly divided into three groups. Each group was fed the same commercial supplement base with differing micro- and macromineral concentrations to determine the effect of mineral concentrations on the recovery from osteochondrosis. Both supplements 1 and 2 contained 25% of the recommended National Research Council (NRC) mineral values. Additional phosphate was added to supplement 2. Supplement 3, containing 80% of the NRC mineral values, was used as the control. Results from all three groups indicated no recovery from osteochondrosis. Urine pH of a small sample of the test cattle showed aciduria (pH < 6). Supplement analysis revealed addition of ammonium sulphate that contributed sulphate and nitrogen to the supplement. Supplementary dietary cation anion difference (DCAD) values were negative at -411 mEq/kg, -466 mEq/kg and -467 mEq/kg for supplements 1, 2 and 3, respectively, whereas the pre-trial supplement was calculated at +19.87 mEq/kg. It was hypothesised that feeding a low (negative) DCAD diet will predispose growing cattle to the development of osteochondrosis or exacerbate subclinical or clinical osteochondrosis in cattle.

## Introduction

'Osteochondrosis' is a broad term pertaining to a group of lesions associated with the persistence of growth cartilage in the epiphyseal or metaphyseal growth plate as a result of failure of endochondral ossification (Laverty & Girard 2013; Olsson 1987; Olsson & Reiland 1977; Ytrehus, Carlson & Ekman 2007). König first introduced the term in 1888 to describe a pathological condition of the articular cartilage that leads to the formation of loose bodies in the joint (Ytrehus et al. 2007).

Different aetiological factors for osteochondrosis have been proposed. Rapid growth has been described as one of the main contributing factors predisposing to the development of osteochondrosis (Ekman & Carlson 1998; Olsson & Reiland 1977; Reiland 1977). Osteochondrosis has been described in pigs (Grøndalen 1974), dogs (Trostel, McLaughlin & Pool 2002), newborn lambs (Corbellini et al. 1991), poultry (Whitehead 1997), turkeys (Poulos 1977), horses (Jeffcott 1991), cattle (Trostle et al. 1998), cats (Ralphs 2005) and rats (Kato & Onodera 1984).

Since 1982, farmers in the North West province and other parts of South Africa have noticed an increase in the incidence of lameness in cattle. Clinical signs included varying degrees of lameness and peri-articular swelling, especially of the stifle joints. Macro- and microscopical lesions of joints resembled osteochondrosis. The initial aetiological factor thought to predispose to the development of osteochondrosis was that of a mineral deficiency, particularly phosphate. The North West province of South Africa is known for its mineral-related pathologic conditions such as osteomalacia, botulism (Theiler, Green & Du Toit 1928) and Vryburg hepatosis (Elsenbroek & Neser 2002; Neser et al. 1997). A survey conducted in 2004 indicated that several similar factors such as the breeding season, age, sex, anatomical conformation, nutritional supplementation and management were all commonly found throughout the affected geographical region. It was concluded that osteochondrosis is not the result of a single factor, but a multifactorial problem (Prozesky et al. 2016).

The most common joints of cattle affected by osteochondrosis are the shoulder, elbow and stifle joint (Hill, Sutton & Thompson 1998; Jensen et al. 1981; Reiland et al. 1977; Trostle et al. 1997;

Weisbrode et al. 1982). Peri-articular swelling as a result of inflammation (Trostle et al. 1998) can be noticed and is most evident in the elbow and stifle joints. Osteochondrosis of the stifle joint alters the anatomical conformation of the hind leg. The normal angular conformation is lost as the affected hind leg straightens as the lesion progresses in severity (Hill et al. 1998). Severely affected cattle adapt a sawhorse stance to reduce the load on affected joints.

Several immature Brahman cattle of both sexes (exact number unknown) suffering from varying degrees of lameness and peri-articular swelling (clinical osteochondrosis) were included in a feeding trial (henceforth referred to as the pre-trial) during December 2012 at Onderstepoort. The purpose of the trial was to determine if cattle with clinical osteochondrosis would benefit from additional mineral supplementation. The supplement was fed for a period of 3 weeks and contained above normal levels of bio-available micro- and macrominerals. The clinically affected cattle responded positively with a marked decrease in the severity of lameness and size of the peri-articular swelling (unpublished data). Results of the pre-trial emphasised the importance of determining the concentration of minerals necessary to be included in a supplement that would benefit cattle suffering from clinical osteochondrosis. This study was conducted to investigate the required mineral levels that would promote clinical improvement of cattle with clinical osteochondrosis.

## Material and methods

Forty-three clinically affected Brahman cattle of varying ages and sexes, originating from the same geographical area, were included in the feeding trial. Animals were weighed and clinically evaluated at the start of the trial.

The trial supplements were formulated to have the same basic ingredients and to only vary in micro- and macro-mineral concentrations. The three supplements were as follows:

- Supplement 1 contained 25% of the recommended NRC (2001) mineral values. No additional phosphate was added to the supplement.
- Supplement 2 contained 25% of the recommended NRC (2001) mineral values with the exception of added mono-ammonium phosphate (MAP) to an equivalent level to that of supplement 3.
- Supplement 3 contained 80% of the recommended NRC (2001) mineral levels and was used as the control diet.

For each supplement, the same mineral premix base, Arthrocure B (ANH®, pers. comm., 2013), was included to ensure comparable standard and quality of minerals. The bioavailability of both micro- and macrominerals included in the Arthrocure premix is high (composition not shown because of confidentiality implications). The absorption percentage of the phosphate source used (MAP) in supplements 2 and 3 according to the National Research Council (2001) is 80%. Ingredients and amounts used to formulate the trial supplementary feeds are provided in Table 1.

Cattle had *ad lib* access to baled *Eragrostis teff* hay for the total duration of the trial. Supplementary feed was collected and weighed on a weekly basis to determine the average individual consumption (grams) per day. The cattle were fed for a total period of 12 weeks.

The cattle were evaluated clinically every second week for the total duration of the trial. Three independent observers each individually recorded clinical signs to limit any biased decisions. Visual evaluation included grading of both the size of the peri-articular swelling and the degree of lameness. Acute peri-articular swelling has a pronounced bulging appearance, whereas chronic peri-articular swelling has a more flattened appearance because of thickening of the joint capsule that depresses the swelling. The chronicity, size and location of each peri-articular swelling were recorded in a format similar to Figure 1.

Lameness was graded on a scale of severity as shown in Table 2.

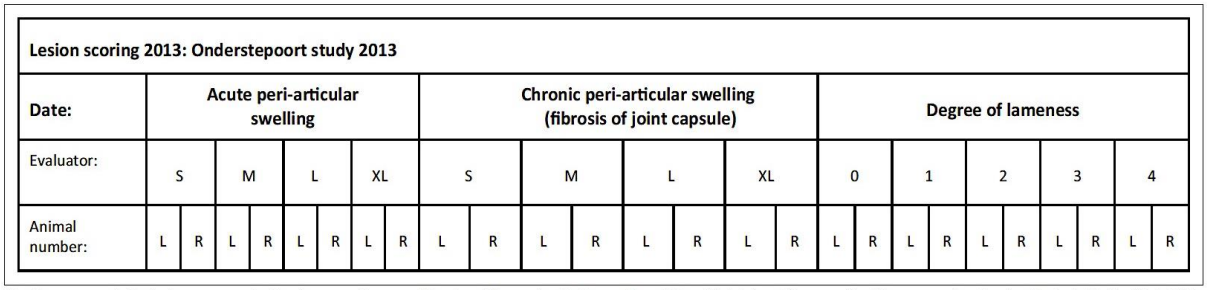
### Statistical analysis

An overall disability score was calculated as the sum of the scores for each clinical category (lameness, acute and fibrosis) over both hind legs (left and right). The change in each clinical category score was calculated by subtracting off the baseline from all subsequent values (forming 'delta' scores). Delta scores were assessed for normality by calculating descriptive statistics, plotting histograms and performing the Anderson-Darling test in available software (MINITAB Statistical Software, Release 13.32, Minitab Inc, State College, PA, USA). Categorical data were described using proportions and 95% mid-P exact confidence intervals (CI) and compared among diets using chi-square tests.

**TABLE 1:** List of ingredients and amounts used to formulate the respective trial supplements.

| Ingredients | Supplementary feed 1 | | Supplementary feed 2 | | Supplementary feed 3 | |
|---|---|---|---|---|---|---|
| | As is % | Mix (kg) | As is % | Mix (kg) | As is % | Mix (kg) |
| Salt | 25.00 | 250 | 25.0 | 250 | 25.00 | 250.0 |
| Bran 15% | 15.00 | 150 | 12.5 | 125 | 12.50 | 125.0 |
| Hominy chop | 13.00 | 130 | 12.4 | 124 | 12.40 | 124.0 |
| Peanut shells | 12.70 | 127 | 7.8 | 78 | 7.00 | 70.0 |
| Urea | 12.00 | 120 | 11.0 | 110 | 11.00 | 110.0 |
| Molasses | 12.00 | 120 | 12.0 | 120 | 11.95 | 119.5 |
| Sunflower O/C 38% | 6.00 | 60 | 6.0 | 60 | 6.00 | 60.0 |
| Ammonium sulphate | 3.00 | 30 | 3.0 | 30 | 3.00 | 30.0 |
| Limestone | 1.00 | 10 | 5.0 | 50 | 5.00 | 50.0 |
| Mono-ammonium phosphate (MAP) | 0.00 | 0 | 5.0 | 50 | 5.00 | 50.0 |
| DSM Arthrocure B | 0.30 | 3 | 0.3 | 3 | 1.15 | 11.5 |
| **Total** | **100** | **1000** | **100** | **1000** | **100** | **1000** |

This table presents the formulation of the three different supplementary feeds used during the trial. The original supplement formulation did not include ammonium sulphate, and it was only after analysis that the inclusion thereof became known. Both the as is % and kilogram (kg) amounts of the respective ingredients for each supplement are presented.



| Lesion scoring 2013: Onderstepoort study 2013 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date: | Acute peri-articular swelling | | | | | | | | Chronic peri-articular swelling (fibrosis of joint capsule) | | | | | | | | Degree of lameness | | | | | | | | | | | | |
| Evaluator: | S | | M | | L | | XL | | S | | M | | L | | XL | | 0 | | 1 | | 2 | | 3 | | 4 | | | | |
| Animal number: | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | L | R | | | |

The figure presents the lesion score evaluation form used to record the size of the peri-articular swelling of the stifle joint and the severity of lameness of each animal included in the trial. Cattle were evaluated every second week for the total duration of the trial. S, small peri-articular swelling of the stifle joint (approx. diameter 2.5 cm); M, medium peri-articular swelling of the stifle joint (approx. diameter 6 cm); L, large peri-articular swelling of the stifle joint (approx. diameter 10 cm); XL, extra-large peri-articular swelling of the stifle joint (approx. diameter >10 cm); L, left stifle joint; R, right stifle joint.

**FIGURE 1:** Visual lesion evaluation form.

**TABLE 2:** Grading score for lameness.

| Lameness score | Score definition |
|---|---|
| 0 | No clinical signs of lameness observed |
| 1 | Conformational changes noticed and slight lameness observed |
| 2 | Moderate lameness observed |
| 3 | Severely affected but still weight-bearing |
| 4 | Severely affected with very little to non-weight-bearing |

This table presents the criteria used to determine the severity of lameness. Each hind leg of the animal was scored individually.

Categorical data analysis was performed using available freeware (Epi Info, version 6.04, CDC, Atlanta, GA, USA). Delta scores were descriptively presented as medians and ranges and transformed by ranking prior to statistical analysis. Delta scores were compared among diet groups using linear mixed models that included animal as a random effect and diet as a fixed effect. Unless stated otherwise, statistical analyses were performed using commercially available software (IBM SPSS Statistics Version 22, International Business Machines Corp., Armonk, NY, USA) and results interpreted at the 5% level of significance.

Forty-three cattle with clinical osteochondrosis were randomly divided into three groups. Groups were housed in separate camps, and each camp was equipped with a water trough (municipal water), hayrack and two Taltec feed troughs that provided sufficient feeding space.

### Clinical examination and urinalysis

The clinical examination was done to investigate any possible causes for the negative results obtained during the trial. Results obtained from the clinical examination were used for differential diagnoses only. Cattle sample size was not statistically calculated as the examination was only done after the negative results were obtained and was for interest' sake only.

Urine was collected from cattle that urinated voluntarily in the crush. The same animals from which urine was collected were clinically examined. Observers used a pole with a cup attached at the end to collect the free flow urine sample. Collected urine was analysed immediately with the Combur$^{9}$Test® (Roche) urine dipstick.

## Results

### Initial weight, supplement and phosphate intake

Statistical analysis indicated that there was no significant difference between the starting weights of the respective groups. The average weight of groups 1, 2 and 3 was 310 kg, 290 kg and 267 kg, respectively. Supplement intake varied between the groups, with the average collective daily supplement intakes for groups 1, 2 and 3 being 370 g, 255 g and 292 g, respectively. Daily supplement intake calculated as grams consumed per kg live weight was 1.19 g, 0.88 g and 0.91 g per kg live weight, respectively, for groups 1, 2 and 3.

The calculated average daily phosphate intake per animal differed between the groups. Consumed values are presented in Table 3.

### Lameness evaluation

The size of the peri-articular swelling and the degree of lameness of the trial cattle were used to determine the effect of the supplements. Visual grading of lesions has been statistically proven to be a valid method (unpublished data: K. Botha, 2016). Analysis of the initial values for all the cattle indicated that there were no significant differences pertaining to the sex, age, weight, lameness score, acute peri-articular swelling score, chronic peri-articular swelling (fibrosis) score and the overall disability score. The majority of cattle included in group 1 had acute peri-articular swellings, whereas the cattle included in groups 2 and 3 had very similar numbers of acute peri-articular swelling and chronic peri-articular swelling scores. Although the degree of lameness varied between animals within a group, the average lameness score ranged between 1 and 2 for all three groups.

Study results indicated no significant difference between the degree of lameness, acute peri-articular swelling, chronic peri-articular swelling and total disability score between the respective groups.

### Clinical examination and urinalysis

Four cattle from which urine could be collected were used for the clinical examination. None of the clinical parameters

**TABLE 3:** Calculated average daily phosphate intake of respective sub-groups.

| Supplement and sub-group | Average starting weight of the cattle (kg) | Average supplement consumption (g/day/animal) | Supplement P measured value (%DM) | Average phosphate intake (g/day/animal)† | % P consumed less than cattle fed supplement 3 |
|---|---|---|---|---|---|
| Supplement 1 | 310 | 370 | 0.94 | 3.48 | -47.7 |
| Supplement 2 | 290 | 255 | 1.59 | 4.05 | -16.0 |
| Supplement 3 | 267 | 292 | 1.79 | 5.14 | - |

†, Calculated respective phosphate intake (grams per animal per day) to indicate the difference of phosphate intake between the respective groups.

were abnormal for any of the four cattle examined. The urine of the respective animals was chemically evaluated with a urine test strip (Combur⁹Test®; Roche) and indicated a urinary pH of < 6 for all the four cattle.

### Statistical analysis

Statistical analysis indicated no significant difference between the respective supplements with regard to the measured parameters, with the exception of the acute peri-articular score of group 1 (Table 4). The significance thereof is, however, not of importance as the cattle only developed the more chronic form of the osteochondrotic peri-articular swelling (chronic peri-articular swelling).

**TABLE 4:** Statistical analysis of the baseline values as well as study results.

| Parameters | Baseline values (*P*)† | | Results (*P*)‡ | |
|---|---|---|---|---|
| | Study 1 | Study 2 | Study 1 | Study 2 |
| Sex | 0.774 | 0.384 | - | - |
| Age | 0.725 | 0.742 | - | - |
| Weight | 0.454 | 0.452 | - | - |
| Lameness score | 0.567 | 0.976 | 0.122 | 0.084 |
| Acute peri-articular swelling | 0.111 | 0.343 | 0.034§ | 0.247 |
| Chronic peri-articular swelling | 1.000 | 0.141 | 0.189 | 0.331 |
| Overall disability | 0.328 | 0.730 | 0.093 | 0.261 |

Significance based on a 95% confidence interval.
†, Based on chi-square tests for categorical variables and Kruskal–Wallis tests for quantitative data; ‡, comparison among diets based on mixed-effects linear model analysing the change in variables from baseline (after rank transformation of scores) and adjusting for repeated measures by the addition of a random effect for animal; §, indicates significance.

## Discussion

The trial was conducted to determine the level of micro- and macrominerals required to be included in a supplement that would improve the clinical condition of cattle clinically affected by osteochondrosis.

The three supplements contained the same basic ingredients and only varied in mineral concentrations. The mineral levels of supplement 3 were based on those of the 2012 pre-trial supplement that had a significant positive effect when fed to cattle with clinical osteochondrosis (Prozesky et al. 2016).

The daily average supplement intake per animal between the groups varied (Table 3). Differing average starting weights (Table 3) and *ad lib* access to high quality *Eragrostis teff* hay would both have influenced the average daily consumption of supplements. *Eragrostis teff* hay is highly palatable and nutritious roughage that would contribute a large proportion of the nutritional daily requirements of the cattle. The most important factor was to calculate the mineral intake especially that of phosphate as it was thought that higher phosphate intake played a key role in the recovery and prevention of osteochondrotic lesions. Calculated average daily phosphate intake per animal differed among the respective groups. Group 3 had an average phosphate intake of 5.14 g per animal per day. Cattle of groups 1 and 2 consumed 47.7% (3.47 g) and 16% (4.05 g) less phosphate on a daily basis, respectively, when compared to the phosphate intake of group 3. It was expected that especially the cattle of group 3 would have responded positively to the higher micro- and macromineral intake, with group 1 responding the least to the supplementation.

Peri-articular swelling and lameness can occur simultaneously or independently of each other (Trostle et al. 1997, 1998). The size of the peri-articular swelling does not correlate with the extent of the osteo-arthritic lesion (unpublished data from radiographic analysis: Van der Veen), but is merely an indication of excessive synovial fluid produced as a result of joint inflammation (Trostle et al. 1998).

No work to date has been conducted to determine the time period it takes for the joint to deposit additional connective tissue in the capsule.

Baseline data and the final study results for groups 1, 2 and 3 indicated no significant difference between the lameness, acute peri-articular swelling, chronic peri-articular swelling and total disability scores.

All the cattle included in the study had clinical osteochondrotic lesions with the affected period of each animal unknown to the investigators. Several animals with acute peri-articular swellings developed chronic peri-articular swellings during the course of the study. The shift in chronicity of the peri-articular swelling is an indication that those animals did not respond to the supplementation but instead the severity of the osteochondrotic lesions remained the same or progressed.

It was expected that the cattle of this study fed supplement 3 would have responded positively in a similar fashion as did the cattle in the 2012 pre-trial, as the mineral levels included in supplement 3 resembled those of the pre-trial 2012 supplement.

The fact that none of the study cattle responded positively to any of the supplementations, regardless of different micro- and macromineral level intake between sub-groups, warranted further investigation. The results obtained in this study are contradictory to those observed during the 2012 pre-trial where cattle fed a supplement containing high levels of both micro- and macrominerals responded positively with a marked decrease in the severity of lameness and size of the peri-articular swelling (Prozesky et al. 2016).

Possible factors that could lead to the development of aciduria were investigated. It is known from studies in dairy cows that a dietary cation anion difference (DCAD) value of < -200 mEq/kg is effective in inducing a metabolic acidosis and aciduria (average pH 7) (DeGaris & Lean 2008).

The DCAD value for the respective trial supplements as well as the 2012 pre-trial supplement was calculated by subtracting the anion value from the cation value.

The most common equation used is $[(Na^+ + K^+) - (Cl^- + S^{2-})]$ (DeGaris & Lean 2008). There was a significant difference between the DCAD value of supplements 1, 2 and 3 compared to that of the 2012 pre-trial supplement. Calculated DCAD values are provided in Table 5.

Further investigation into the composition of the trial supplements revealed that all three supplements contained the anionic salt, ammonium sulphate, whereas the pre-trial supplement did not contain an anionic salt, leading to a higher DCAD value.

A possible explanation why the elevated mineral intakes from supplements 2 and 3 did not result in recovery from osteochondrosis is as follows.

The plasma pH is regulated by four factors of which the strong ion difference is one. Intestinal absorption concentration of strong anions (chloride and sulphate) is more than that of strong cations (calcium, magnesium and ammonium) when salts like ammonium sulphate $[(NH_4)_2SO_4]$ are fed. The increased anion level in the plasma, in this case $SO_4^{2-}$, reduces the strong ion difference, inducing a strong ion metabolic acidosis (DeGaris & Lean 2008). Metabolic acidosis increases the responsiveness of tissue receptors to parathyroid hormone (PTH) (Horst et al. 1997). Bone responds to PTH by the activation of osteocytes as well as the osteoclasts (La Perle & Capen 2006) with osteoclastic activity increasing proportionally as the plasma becomes more acidic (Arnett 2003). Calcium can be mobilised from bone either in conjunction with or independently of PTH (DeGaris & Lean 2008). Bone acts as a buffer during acute metabolic acidosis by binding hydrogen ions to carbonate and releasing the cation salts ($Na^+$, $K^+$, $Ca^{2+}$) associated with the carbonate into the extracellular fluid (Engelking 2011; Horst et al. 1997). This process, as well as the activation of osteoclasts through PTH, functions as a buffer during chronic metabolic acidosis (Green & Kleeman 1991).

**TABLE 5:** Calculated dietary cation anion difference values of respective supplements.

| Supplement | Calculated DCAD value (mEq/kg)† |
|---|---|
| Supplement 1 (Group1) | -411 |
| Supplement 2 (Group 2) | -466 |
| Supplement 3 (Group 3) | -467 |
| 2012 pre-trial supplement | +19.87 |

DCAD, dietary cation anion difference.
†, DCAD value calculated with the following equation: $[(Na^+ + K^+) - (Cl^- + S^{2-})]$.

Renal function responds to PTH through reduced reabsorption of phosphate (phosphaturia) and increased reabsorption of calcium (La Perle & Capen 2006) from the glomerular filtrate. Calcium is still excreted at elevated levels during metabolic acidosis despite the increased reabsorption action of PTH. The charge equivalence of albumin is altered during metabolic acidosis, which leads to the release of plasma protein bound calcium (up to 40% of the total calcium) increasing the amount filtered through the glomerulus of the kidney and ultimately excreted leading to increased mineral loss (Engelking 2011). The excess dietary anions in the plasma are filtered and excreted through the kidneys. The urine produced is acidic (aciduria) in nature because of the increased concentration of excreted anions (Spanghero 2004). A urinary pH < 5.5 indicates severe metabolic acidosis and should be avoided at all cost (Horst et al. 1997).

The majority of minerals reabsorbed from the skeleton of rats with metabolic acidosis occurred from the epiphysis, resulting in lower total bone volume of the metaphysis (Kraut et al. 1986). Mongin and Sauveur (1977) (q. Whitehead 1997) proposed that the anionic balance of a diet influenced the incidence rate of tibial dyschondroplasia (osteochondrosis) in chickens. Several studies followed that confirmed the proposal made by Mongin and Sauveur that metabolic acidosis increased the incidence of tibial dyschondroplasia in chickens (Whitehead 1997).

A new hypothesis was formulated based on the observations made during the trial. We hypothesise that chronic mild metabolic acidosis weakens the developing calcareous bone, consequently exposing particularly fast growing cattle to traumatic fracture of the subchondral bone and articular cartilage, leading to the development of lesions associated with osteochondrosis. This hypothesis also suggests that chronic metabolic acidosis will exacerbate subclinical or clinical osteochondrosis. These hypotheses require further investigation. It is important to consider not only the direct dietary cause of metabolic acidosis but to have a holistic approach which includes signalment, husbandry and regular analysis of both feed and water sources.

## Conclusion

Investigation of cattle clinically affected by osteochondrosis in the North West province on a herd basis revealed that bulls were more prone and severely affected than cows, with young animals more frequently affected than adult animals (Prozesky et al. 2016). The higher frequency among young animals is in accordance with the definition, as 'osteochondrosis' is a term that pertains to a group of lesions associated with the persistence of growth cartilage in the epiphyseal or physeal growth plates as a result of failure of endochondral ossification.

Prozesky et al. (2016) as well as data from the pre-trial (not shown) indicated that cattle clinically suffering from osteochondrosis responded positively when fed a supplement containing high levels of bio-available micro- and macrominerals.

The results from this study indicated that cattle with clinical osteochondrosis do not respond positively to a supplement with a low DCAD value, regardless of its micro- and macromineral concentration. The new hypothesis suggests that cattle supplemented for an extended period of time with a low DCAD feed are predisposed to the development of osteochondrosis or exacerbation of subclinical or clinical osteochondrosis. This hypothesis needs to be further investigated.

## Acknowledgements

The authors thank Drs. Wilco Botha, André van den Berg, Benton van den Berg and Patrick Ntsibande for assisting with the data collection.

### Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

### Authors' contributions

G.v.d.V. was the principal investigator, responsible for the experimental and project design, care of the experimental animals, data collection and interpretation thereof. G.T.F. was responsible for the statistical analysis. F.K.B. formulated the supplements and calculated the DCAD values. H.H.M. was the nutritional advisor and co-supervisor. L.J. did the mixing of the supplements. L.P. was the study supervisor.

## References

Arnett, T., 2003, 'Regulation of bone cell function by acid-base balance', *Proceedings of the Nutrition Society* 62(2), 511–520. https://doi.org/10.1079/PNS2003268

Corbellini, C.N., Krook, L., Nathanielsz, P.W. & Kallfelz, F.A., 1991, 'Osteochondrosis in fetuses of ewes overfed calcium', *Calcified Tissue International* 48(1), 37–45. https://doi.org/10.1007/BF02555794

DeGaris, P.J. & Lean, I.J., 2008, 'Milk fever in dairy cows: A review of pathophysiology and control principles', *The Veterinary Journal* 176(1), 58–69. https://doi.org/10.1016/j.tvjl.2007.12.029

Ekman, S. & Carlson, C.S., 1998, 'The pathophysiology of osteochondrosis', *Veterinary Clinics of North America: Small Animal Practice* 28(1), 17–32. https://doi.org/10.1016/S0195-5616(98)50002-2

Elsenbroek, J.H. & Neser, J.A., 2002, 'An environmental application of regional geochemical mapping in understanding enzootic geophagia of calves in the Reivilo Area, South Africa', *Environmental Geochemistry and Health* 24(2), 159–181. https://doi.org/10.1023/A:1014247108504

Engelking, L., 2011, *Textbook of veterinary physiological chemistry*, 2nd rev. edn., pp. 489–495, Elsevier, Oxford.

Green, J. & Kleeman, C.R., 1991, 'Role of bone in regulation of systemic acid-base balance', *Kidney International* 39(1), 9–26. https://doi.org/10.1038/ki.1991.2

Grøndalen, T., 1974, 'Osteochondrosis and arthrosis in pigs. I. Incidence in animals up to 120 kg live weight', *Acta Veterinaria Scandinavica* 15(1), 1–25.

Hill, B., Sutton, R. & Thompson, H., 1998, 'Investigation of osteochondrosis in grazing beef cattle', *Australian Veterinary Journal* 76(3), 171–175. https://doi.org/10.1111/j.1751-0813.1998.tb10122.x

Horst, R.L., Goff, J.P., Reinhardt, T.A. & Buxton, D.R., 1997, 'Strategies for preventing milk fever in dairy cattle 1, 2', *Journal of Dairy Science* 80(7), 1269–1280. https://doi.org/10.3168/jds.S0022-0302(97)76056-9

Jeffcott, L.B., 1991, 'Osteochondrosis in the Horse—Searching for the key to pathogenesis', *Equine Veterinary Journal* 23(5), 331–338. https://doi.org/10.1111/j.2042-3306.1991.tb03733.x

Jensen, R., Park, R.D., Lauerman, L.H., Braddy, P.M., Horton, D.P., Flack, D.E. et al., 1981, 'Osteochondrosis in feedlot cattle', *Veterinary Pathology Online* 18(4), 529–535.

Kato, M. & Onodera, T., 1984, 'Spontaneous osteochondrosis in rats', *Laboratory Animals* 18(2), 179–187. https://doi.org/10.1258/002367784780891361

Kraut, J.A., Mishler, D.R., Singer, F.R. & Goodman, W.G., 1986, 'The effects of metabolic acidosis on bone formation and bone resorption in the rat', *Kidney International* 30(5), 694–700. https://doi.org/10.1038/ki.1986.242

La Perle, K.M.D. & Capen, C.C, 2006, 'Endocrine system', in M.D. McGavin & J.F. Zachary (eds.), *Pathologic basis of veterinary disease*, 4th edn., pp. 693–742, Mosby Elsevier, St Louis, MO.

Laverty, S. & Girard, C., 2013, 'Pathogenesis of epiphyseal osteochondrosis', *The Veterinary Journal* 197(1), 3–12. https://doi.org/10.1016/j.tvjl.2013.03.035

Mongin, P. & Sauveur, B., 1977, 'Interrelationships between mineral nutrition, acid-base balance, growth and cartilage abnormalities', in *Growth and poultry meat production*, pp. 235–237, British Poultry Science, Edinburgh.

National Research Council, 2001, *Nutrient requirements of dairy cattle*, 7th rev. edn., National Academy of Sciences, Washington, DC.

Neser, J.A., De Vries, M.A., De Vries, M., Van der Merwe, A.J., Loock, A.H., Smith, H.J.C. et al., 1997, 'The possible role of manganese poisoning in enzootic geophagia and hepatitis of calves and lambs: To the editor', *Journal of the South African Veterinary Association* 68(1), 4–6. https://doi.org/10.4102/jsava.v68i1.856

Olsson, S.E. & Reiland, S., 1977, 'The nature of osteochondrosis in animals. Summary and conclusions with comparative aspects on osteochondritis dissecans in Man', *Acta radiologica. Supplementum* 358, 299–306.

Olsson, S.E., 1987, 'General and aetiologic factors in canine osteochondrosis', *Veterinary Quarterly* 9(3), 268–278. https://doi.org/10.1080/01652176.1987.9694112

Poulos, P.W., Jr., 1977, 'Tibial dyschondroplasia (osteochondrosis) in the Turkey. A morphologic investigation', *Acta radiologica. Supplementum* 358, 197–227.

Prozesky, L., Neser, J., Meissner, H., Botha, K., Jacobs, L., Shepstone, C. et al., 2016, 'Preliminary report on osteochondrosis in cattle in the North-Western Parts of South Africa', *Onderstepoort Journal of Veterinary Research* 83(1), a1083. https://doi.org/10.4102/ojvr.v83i1.1083

Ralphs, S.C., 2005, 'Bilateral stifle osteochondritis dissecans in a cat', *Journal of the American Animal Hospital Association* 41(1), 78–80. https://doi.org/10.5326/0410078

Reiland, S., 1977, 'The effect of decreased growth rate on frequency and severity of osteochondrosis in pigs', *Acta radiologica. Supplementum* 358, 107–122.

Reiland, S., Strömberg, B., Olsson, S.E., Dreimanis, I. & Olsson, I.G., 1977, 'Osteochondrosis in growing bulls. Pathology, frequency and severity on different feedings', *Acta radiologica. Supplementum* 358, 179–196.

Spanghero, M., 2004, 'Prediction of urinary and blood pH in non-lactating dairy cows fed anionic diets', *Animal Feed Science and Technology* 116(1), 83–92. https://doi.org/10.5326/0410078

Theiler, A., Green, H.H. & Du Toit, P.J., 1928, 'Studies in mineral metabolism. III. Breeding of cattle on phosphorus deficient pasture', *The Journal of Agricultural Science* 18(3), 369–371. https://doi.org/10.1017/S0021859600019365

Trostel, C.T., McLaughlin, R.M. & Pool, R.R., 2002, 'Canine lameness caused by developmental orthopedic diseases: Osteochondrosis', *Compendium on Continuing Education for the Practicing Veterinarian-North American Edition* 24(11), 836–857.

Trostle, S.S., Nicoll, R.G., Forrest, L.J. & Markel, M.D., 1997, 'Clinical and radiographic findings, treatment, and outcome in cattle with osteochondrosis: 29 Cases (1986–1996)', *Journal of the American Veterinary Medical Association* 211(12), 1566–1570.

Trostle, S.S., Nicoll, R.G., Forrest, L.J., Markel, M. & Nordlund, K., 1998, 'Bovine osteochondrosis', *The Compendium on Continuing Education for the Practicing Veterinarian* 20(7), 856–863.

Weisbrode, S.E., Monke, D.R., Dodaro, S.T. & Hull, B.L., 1982, 'Osteochondrosis, degenerative joint disease, and vertebral osteophytosis in middle-aged bulls', *Journal of the American Veterinary Medical Association* 181(7), 700–705.

Whitehead, C.C., 1997, 'Dyschondroplasia in poultry', *Proceedings of the Nutrition Society* 56(3), 957–966. https://doi.org/10.1079/PNS19970101

Ytrehus, B., Carlson, C.S. & Ekman, S., 2007, 'Etiology and pathogenesis of osteochondrosis', *Veterinary Pathology* 44(4), 429–448. https://doi.org/10.1354/vp.44-4-429