
# THE IMPORTANCE OF MINERALS ON THE PREVALENCE OF OSTEOCHONDROSIS IN CATTLE IN SOUTHERN AFRICA

By

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In the

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| SUPERVISOR: | Dr Heinz H. Meissner |
| CO-SUPERVISOR: | Professor Leon Prozesky |

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# ETHICAL CONSIDERATIONS

Cattle were used in trials since 2004 for the Osteochondrosis Project, which were given approval by the Ethical Committee of the Faculty of Veterinary Science for the Department of Paraclinical Sciences under the supervision of Prof. Leon Prozesky. Animal Ethics Committee registration code : V073-13.

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# Declaration

I, Frederick Kenneth Botha, declare that this dissertation for the degree Master of Science in Veterinary Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:..... Date: 2018 – 10 - 30

FK Botha (13412320)

Pretoria, 2018

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# DISSERTATION NOTE

This thesis contains different research projects and were therefor written in an article format. Each chapter focusses on a specific hypothesis, all to resolve the prevalence of osteochondrosis in cattle. Essential information is covered in each article in order to position each hypothesis, albeit a repeat of some universally important information.

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| | |
|---|---|
| AFMA | Animal Feed Manufacturing Association |
| ANOVA | analysis of variance |
| ARC | Agricultural Research Council |
| AC | absorption coefficient |
| BW | body weight |
| BWG | body weight gain |
| CEFIC | European Chemical Industry Council |
| C | carbon |
| CP | crude protein |
| CSP | calcium sodium phosphate |
| DCP | dicalcium phosphate |
| DCAD | dietary cation anion difference |
| DFP | defluorinated phosphate |
| DM | dry matter |
| DMI | dry matter intake |
| DOM | digestible organic matter |
| DOMI | digestible organic matter intake |
| DOR | diagnostic odds ratio |
| FPLSD | Fisher's protected least significant different test |
| GLM | generalized linear model |
| HE | harvesting efficiency |
| LR+ | likelihood of a positive test |
| LR- | likelihood of a negative test |
| MCP | mono-calcium phoshate |
| MDCP | mono-dicalcium phosphate |
| Meq/kg | milli equivalents per kg |
| MgO | magnesium oxide |
| MSP | monosodium phosphate |
| N | nitrogen |
| NDF | neutral detergent fibre |
| NPN | non-protein nitrogen |
| NPV | negative predictive value |
| NRC | National Research Council |
| OCD | osteochondritis dissecans |

| | |
|---|---|
| OM | organic matter |
| OP | Onderstepoort Faculty of Veterinary Science |
| OPOLS | Onderstepoort Osteochondrosis Lesion Scoring system |
| $P_i$ | inorganic phosphorous |
| pp | pages |
| PPV | positive predictive value |
| PTH | parathyriod hormone |
| R | rand (South African currency) |
| SAMIC | South African Meat Industry Company |
| SEM | standard error of the mean |
| Sn | Sensitivity |
| Sp | specificity |
| µg | microgram |
| UIP | Undegradable intake protein |
| $W^{0.75}$ | metabolic weight |

# DISSERTATION SUMMARY

## THE IMPORTANCE OF MINERALS ON THE PREVALENCE OF OSTEOCHONDROSIS IN CATTLE IN SOUTHERN AFRICA

By

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Local veterinarians and farmers in the Vryburg area in the North West Province in South Africa started to notice a disease or condition on a few show animals (cattle) during 1982 (*Prozesky et al*. 2010). This was later diagnosed as a primary effusion of the stifle joint (femoro-tibial joint) and later named arthrosis and now called osteochondrosis. The animals developed severe lameness and they lost condition which resulted in being culled or slaughtered. This new phenomenon was found in commercial and non-commercial (stock breeders) herds in female and male cattle groups and is therefore not breed or age related. Many musculo-skeletal problems since the late 1880's were related to poor supplementation in these phosphorous poor areas, and therefore osteochondrosis was thought to be also related to nutrition related problems.

Osteochondrosis is a poorly understood disease and condition that affects both human and animal species. Osteochondrosis is characterised by abnormalities of the growth cartilage and is recognised in cattle, pigs, sheep, dogs and horses. Osteochondrosis is defined as a focal disturbance of endochondral ossification and difficult to understand due to the multifactorial aetiology which may include growth rate, heredity, gene expression, trauma, nutritional factors, anatomic characteristics, and a defect in vascular supply to the epiphyseal cartilage.

A discussion panel came together which led to the "Arthrosis" project and many trials were discussed. No data was available on exactly what and how much supplements were fed to individual animals and therefore no conclusion could be made as to why as many as 40 % of the animals on

some farms were culled due to osteochondrosis. A team, called the "Arthrosis Research Team" was created to investigate the problem and to give guidance into research projects to resolve the problem and to better understand the aetiology of the health problem within cattle in this affected area.

The first step for the team was to develop a method to visually diagnose osteochondrosis in beef cattle in practice. Understanding the accuracy and the usefulness of a specific test in discriminating between several diagnostic possibilities, and osteochondrosis positive and negative animals, requires a diagnostic test to estimate the disease probability and minimize diagnostic uncertainty when used on-farm when establishing the prevalence of the disease. The OPOLS (Onderstepoort Osteochondrosis Lesion Scoring) method was developed and compared with the X-ray and post-mortem data in a trial. The results from this study indicated a highly satisfactory agreement between the on-farm OPOLS method and the pathology found with post-mortems and X-rays, and was therefore regarded as a reliable method to visually diagnose osteochondrosis in beef cattle.

The next step was to evaluate the mineral profile of beef cattle with and without osteochondrosis and the response of osteochondrosis affected animals to a short term mineral supplement. Thirty-three cattle were used in a trial to compare the mineral status of rib bones and livers from osteochondrosis affected animals (group O), osteochondrosis affected animals fed for three months with a mineral supplement (group S), and healthy animals (group C). The results showed that by feeding osteochondrosis affected cattle with a mineral supplement for three months in addition to their roughage and water intake, decreased the dry fat-free ash rib bone calcium, dry fat free rib bone zinc, bone density, and the ratio of calcium to magnesium, compared to the levels of affected animals not supplemented. A decrease in liver zinc and lead were also seen in the osteochondrosis affected cattle (group S) compared to the affected animals not supplemented. It can be concluded that osteochondrosis-affected cattle differ from healthy animals in terms of liver and bone mineral status, and that a shift in mineral status occurs when sick animals are supplemented with a mineral supplement for three months. The mean P per volume ($cm^3$) of fresh rib bone was not different for groups O, S or C, but group O had a high prevalence of animals with low P per volume.

The trial data raised the question of how osteochondrosis relates to P quality and intake. Hence, a trial was done comparing the effect of feeding four different phosphorous sources in supplements to beef cattle on the prevalence of osteochondrosis. The four different sources used were similar to the sources used in the area since the mid-1980's and known to have different P bio-availabilities. Three di-calcium phosphate (DCP) sources were compared to a mono-calcium phosphate (MCP) source and fed to steers on natural pastures for 433 days. The P source in this trial did not affect the prevalence of osteochondrosis, as only one animal in the DCP group developed osteochondrosis. Results from this trial indicated that no rib bone mineral differences occurred by feeding different P sources, although the

dry fat-free liver P, Mg, K, S and Zn levels were lower in MCP fed steers. Different P sources affected the daily growth of cattle, the total P intake per live weight gain as well as the total P intake per kg metabolic weight gain. Results from this study therefore indicated a significant relationship between the phosphorous source and growth of steers, with the highest correlation where the P source was from a well controlled continuous manufacturing production process with a higher bioavailable P.

The results found in the two mineral trials were then used to formulate a specialized mineral supplement strategy for commercial farmers and were tested on six North West Province farms, well-known for its high prevalence of osteochondrosis. A total of 24 294 cattle from different breeds, age and gender groups, were fed from August 2008 to August 2011 with the specialized mineral supplement and frequently evaluated with the OPOLS method for the prevalence of osteochondrosis. Hind legs in a total of 11 939 females, 2 913 bulls and 699 oxen were evaluated and the other 8743 animals were only evaluated as healthy or osteochondrosis affected animals. The evaluated animals received a specialized mineral pack, known as Arthrocure, which was formulated by the Onderstepoort Arthrosis research team, and added to the different summer, winter, maintenance or production supplements fed according to the production stage of the animals and the time of year. The Arthrocure product contained highly bio-available magnesium, manganese, iron, zinc, copper, cobalt, iodine, and selenium, and were fed with additional highly bio-available phosphorus. These mineral bio-availability values were based on published work and were not determined during these trials. Results from this study indicated a strong decreasing trend in the prevalence of osteochondrosis in cattle when the Arthrocure mineral supplement plus a highly bio-available P source were fed on commercial farms. Unfortunately, no commercial farm was prepared to feed a supplement without any phosphorus, due to the known fact that the area is known for its low plant P, and therefore no conclusion could be made if the results were related to either the minerals within the Arthrocure products or the supplemented P.

The results of the phosphorous trial, the bone mineral comparisons between healthy and osteochondrosis affected cattle, and the commercial trial when cattle were supplemented with specialized minerals, supported the hypothesis that osteochondrosis is related to the diet of the animals. The "Arthrosis" research team evaluated many different farm management and feeding systems, in the North West Province, since the early 2000's to get answers related to the high prevalence of osteochondrosis within this province. Feed recipes, raw material differences within the fed diets, forage sources and supplemental intakes were evaluated and compared between farms and even between camps on the same farm.

One very important finding, that was brought under the attention of the industry as well as the legislators, is the low minimum trace mineral requirements for all registered cattle supplements as

published in the South African legislation through Act 36 of 1947. This act regulates the minimum and maximum levels of some nutrients and is used by the Animal Feeds industry to register products for commercial usage, for example cattle supplements. Some important shortcomings of the minimum trace mineral levels for cattle supplements in the regulations of Act 36 were presented by Mr. Botha and Dr. Meissner at an Animal Feed Manufacturing Association (AFMA) Technical Committee meeting. Senior management members of Act 36 are also AFMA Technical Committee members and were therefore present during this meeting and were very interested in some of the shortcomings that were summarized by the Onderstepoort Arthrosis Research Team. Supplying nutrients to cattle based on the minimum nutrient values published in Act 36 (1947) created an industry where many animals receive sub-optimal mineral levels.

The calculations by the Onderstepoort Arthrosis Research Team and the high incidence of osteochondrosis in cattle on farms in the North West province created an urgent need to change the criteria used by Act 36 for registering supplements as presented. The results and in-depth focus into the supply of minerals from this Onderstepoort research team were fundamental in creating this awareness with the legislating authorities and the AFMA Technical committee decided to immediately start a process to evaluate all nutrient requirements, as published by Act 36 of 1947, with the aim to implement new regulations to meet the requirement of all animals and therefore improving the health of animals. This process was started, not only for beef cattle, but also for all the other species as one of the main projects within AFMA and as an important part of the new Animal's Feeds Bill. All the different recommendations by the seven different "species committees" will be completed in 2018 for approval by the industry.

# Chapter 1

## Introduction

Many beef animals in the North Western part of South Africa were negatively influenced since 1882 due to mineral imbalances. Some of these imbalances resulted in the prevalence of Aphosphorosis, Vryburg hepatosis and also a new health disorder, called arthrosis. Typical manifestations in these animals were rickets and osteomalacia during 1882 and 1912, followed by the Vryburg hepatosis in young calves reared on manganese-rich soils, and since 1982 also visible lesions caused by synovial fluid effusing from the stifle bone into the surrounding tissue which creates a "ball or plaque" looking lesion and called arthrosis (Elsenbrook & Neser 2002; Prozesky *et al*. 2010; Prozesky *et al*. 2016; Theiler 1912).

This disease has affected as much as 40% of the cattle on some farms and was initially called arthrosis but was later changed to osteochondrosis since more pathology became available on the affected animals (done by Dr. J Neser, Department of Paraclinical Sciences, University of Pretoria).

Osteochondrosis is a poorly understood disease and a condition that affects both the human and animal species. This disease is characterized by abnormalities of the growth cartilage and is recognized in cattle, pigs, sheep, dogs, horses and game species. Osteochondrosis is defined as a focal disturbance of endochondral ossification, in the epiphyseal or metaphyseal growth plate, and difficult to understand due to the multifactorial aetiology, which may include the animal's growth rate, heredity, gene expression, trauma, nutritional factors, anatomical characteristics, and a defect in vascular supply to the epiphyseal cartilage (Hill *et al*. 1998). There is also a new hypothesis in cattle that blood pH may play a role in the phosphorous balance, which may influence the mineral balance in the bone structure (Van der Veen *et al*. 2017).

Cattle with osteochondrosis develop joint effusions in the weight-bearing joints such as the femoro-tibial joint, which would eventually lead to lameness. This will affect the animal's ability to walk which will further influence their water and feed intake with negative effects on the performance of animals or even increase their mortality rates. Huge financial losses have been experienced at many feedlots and farms as a result of lower body condition, lower milk production, lower reproduction efficiency, decreased mating ability in bulls, higher culling rates, lower daily growth rates in growing

animals, poorer feed conversion, and increased mortalities (Persson *et al*. 2007; Prozesky *et al*. 2010; Tryon & Ferrow 1999).

Osteochondrosis in cattle has been identified in several areas around the world and as in South Africa this syndrome is prevalent in most breeds, in all age and sex groups, commercial, non-commercial and even in herds in the communal areas (Prozesky *et al*. 2010). In South Africa this phenomenon was originally diagnosed in a few show animals in the Northern Cape and North West regions, but there is more evidence that osteochondrosis is prevalent in many areas in Southern Africa and more frequently since the mid 1990's.

Commercial farmers have brought the negative impact of osteochondrosis under the attention of many agricultural institutions since 2003.These include the Department of Agriculture, Conservation and Environment of the North West Province, the Red Meat Producers Organization, breed societies, feed and feed manufacturing companies, SAMIC, and many more. This was done in an effort to research this phenomenon and to find answers as to why it prevails as well as how to prevent or limit the occurrence of the syndrome. At the time of the project, the only solution to this health problem was to slaughter the affected animals, and therefore many farmers were desperate to get information on how to prevent or treat osteochondrosis in their cattle.

Farmers, feedlots and veterinarians do not always report data from animals with clinical signs associated with osteochondrosis and therefore it is difficult to quantify the total loss in revenue due to this disease in South Africa.

Several research projects were completed since 2004 on the Arthrosis project within the Department of Paraclinical Sciences, Onderstepoort, University of Pretoria, with the aim to gain more knowledge about osteochondrosis in order to decrease the financial losses suffered by these farmers. It was also important to develop some on-farm evaluation tools to enable professionals to diagnose the health problem with a quick and practical method on-farm.

This thesis presents a scientific report of the results obtained in these studies and discusses various approaches and possible future research to prevent, or at least decrease, the occurrence of osteochondrosis. (Or something along these lines.)

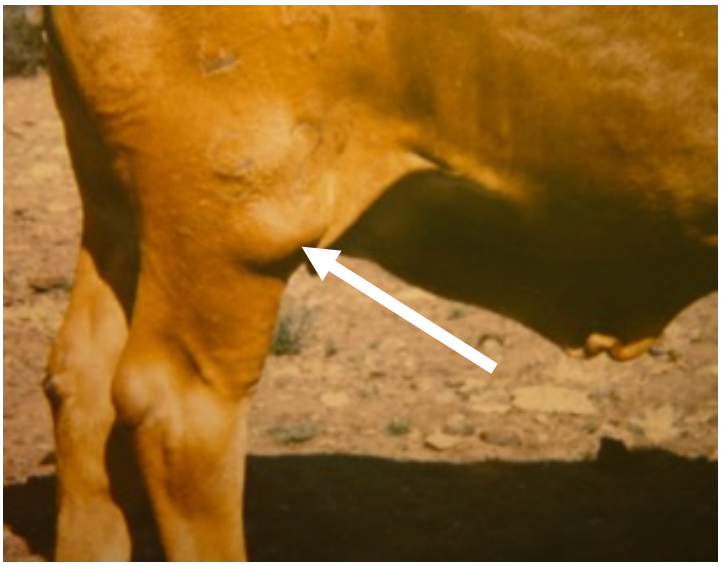

**Figure 1** Osteochondrosis affected cow (photo supplied by Prof. Leon Prozesky)

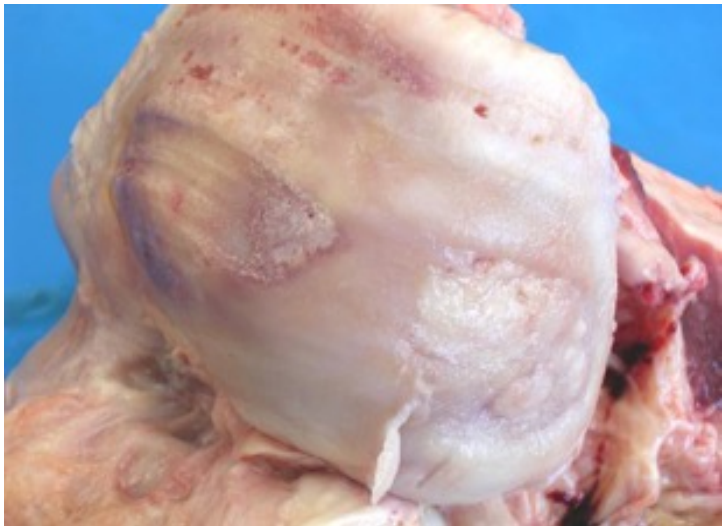

**Figure 2** Osteochondrosis affected cattle joint with severe cartilage (photo supplied by Prof. Leon Prozesky)

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# Chapter 2

# LITERATURE REVIEW

## 2.1. Osteochondrosis

Osteochondrosis is a bone joint disease characterised by the localised failure of endochondrial ossification, which can involve the growth cartilage of the articular-epiphyseal cartilage complex and the physis in a number of animal species, particularly humans, pigs, horses, dogs and cattle (Hill *et al*. 1998). According to Woodard (1997) the term osteochondrosis includes both chondrogenesis and osteogenesis, which occur in the bone growth area, that was previously normal. Systemic diseases such as metabolic bone diseases and osteochondra-dysplasia are therefore excluded from osteochondrosis. The lesions in pigs are slightly different in that there is no evidence of necrosis of the cartilage, but rather a focal failure of mineralisation of hypertrophic cartilage cells (Prozesky *et al*. 2010; Woodard 1997).

Osteochondrosis has also been defined in human medicine as an idiopathic condition characterized by disorderliness of endochondral ossification (Siffert 1981). Although the formation of fragile cartilage, failure of chondrocyte differentiation, subchondral bone necrosis, and failure of blood supply to the growth cartilage, have been proposed as an initial step in the pathogenesis of this disease, more recent publications strongly support failure of blood supply to the growth cartilage as being the most likely (Ytrehus *et al*. 2004; Ytrehus, Carlson & Ekman 2007). Localised failure of the endochrondral ossification ensues, which typically results in necrosis of affected cartilage.

Osteochondrosis was previously called osteochondritis, but later replaced by osteochondrosis by many researchers, because it is generally agreed that inflammation is not a characteristic feature of the primary lesions (Ytrehus *et al*. 2007). It has been regarded as the most important cause of leg weaknesses in pigs and is often seen in lame horses and dogs (Harari 1998; Jørgensen, Arnbjerg & Aaslyng 1995; Mcllwraith 2002).

In veterinary medicine, the terms osteochondritis and osteochondrosis were not used until the 1960's (Leighton 1998) when researchers started with studies in this field, which mainly focused on lesions in the canine shoulder, but the term "chronic deforming arthrosis" was introduced as the appropriate name for the prevalent lesions associated with leg weakness in pigs (Ytrehus et al. 2007).

Ytrehus *et al*. (2007) concurred that osteochondrosis is a better general term for this condition than osteochondritis, with modifiers (latens, manifesta, dissecans) included to designate the stage of the disease process. They also consider osteochondrosis, literally meaning bone-cartilage condition, to be an acceptable designation for a disease affecting the growth cartilage and the transformation of the tissue into bone in focal areas. Three sub-categories of osteochondrosis were described with the formation of loose bodies in the joint as latens, manifesta and dissecans. Osteochondrosis latens was the term used when the focal area of cartilage necrosis is confined to the epiphyseal cartilage. Osteochondrosis manifesta is when necrosis is macroscopically and radiologically visible (Van der Veen et al. 2017). According to Trostle *et al*. (1998) the term osteochondritis dissecans (OCD) is used to identify lesions of osteochondrosis that undergo dissection along a defective osteochondral junction and form flaps of partially detached articular cartilage.

### 2.2. Endochondral ossification

Endochondral ossification is an essential process during the rudimentary formation of the long bones, the growth of the length of long bones, and the natural healing of bone fractures. This is the process in which hyaline cartilage is replaced by bone, particularly where cartilage is replaced by bone from both sides through a sequential process of cell proliferation, extra-cellular matrix synthesis, cellular hypertrophy, matrix mineralization, and vascular invasion (Prozesky *et al*. 2010). There are two areas of specialized growth cartilage that are present at the end of long bones during the periods of skeletal growth and development (Ytrehus *et al*. 2007). The physis (growth plate) is present on either side of the primary centre of ossification and is responsible for longitudinal growth. The second area is the epiphyseal cartilage which is present between the secondary centres of ossification and the articular-epiphyseal cartilage complex and is responsible for producing the shape of the long bone ends. The growth cartilage in both these regions is replaced by bone through a sequential process of cell proliferation, extracellular matrix synthesis, cellular hypertrophy, matrix mineralization, and vascular invasion and defined as endochondral ossification (Lefebvre & Smits 2005). The advantage of this process is also described by this research group to be that growth and elongation is achieved by continuous addition of cartilage and subsequent replacement by bone in such a way that the animal is able to bear weight while still growing.

Lesions of osteochondrosis occur in three fundamental areas of abnormal endochondral ossification, non-articular epiphysis, articular cartilage of the epiphysis and the growth plate (Woodard 1997). These lesions appear similar in the different locations, but a different mechanism exists, which leads to failure of proper endochondral ossification of the cartilage. The clinically affected animals show visible joint effusions, particularly at the stifle joint (Prozesky *et al*. 2010).

### 2.3 Aetiology of osteochondrosis

According to Farnum & Wilsman (1986), Woodard (1997) and Ytrehus *et al*. (2007), the pathophysiology of this disease remains unknown and might be multifactorial with no single factor accounting for all aspects of the disease. The interaction between all these factors may also be confusing and any factor that degrades the overall quality of the subchondral bone and cartilage can contribute to the development of osteochondrosis (Trostle *et al*. 1998). There are also theories that the prevalence of osteochondrosis may be due to ischemia, trauma as well as constitutional disease (Woodard 1997). These causes are not necessarily mutually exclusive, but may play a pathogenic role together with other factors.

Woodard (1997) also mentioned that osteochondrosis is more prevalent in larger breed animals, for example "giant" breed dogs than small dogs, and it is rarely seen in wild animals, although veterinarians in the South African wildlife industry diagnosed several wildlife animals with osteochondrosis in different areas around South Africa over the past 15 years. The lesions in pigs are slightly different in that there is no evidence of cartilage necrosis, but rather a focal failure of mineralisation of the hypertrophic cartilage cells (Woodard 1997).

### 2.4 Predisposing factors

Several aetiologies and predisposing factors have been proposed, such as high levels of concentrate feeding, high growth levels, genetics, ischaemia, excess calcium feeding, hormonal influences, and trauma (Trostel, McLaughlin & Pool 2002; Woodard 1997). According to Hill *et al*. (1998) the occurrence of osteochondosis in pigs has a strong relationship with the inheritance factor, although in cattle no strong relationship has been reported. Trostel *et al*. (2002) also described that osteochondrosis lesions in dogs are typically bilateral with mild trauma to the necrotic cartilage that may result in cleft formation of the affected cartilage with the release of cartilage degeneration products into the joint, causing a synovitis clinically evident as joint effusion and pain with lameness.

Various predisposing factors may be associated with osteochondrosis in cattle, but it is difficult to evaluate them separately, due to their apparent interaction with each other (Trostle *et al*. 1998). Some of these factors include rapid growth, nutrition, trauma, heredity, anatomical characteristics and a flawed vascular supply to the epiphyseal cartilage (Hill *et al*. 1998; Trostle *et al*. 1998; Ytrehus *et al*. 2007).

Osteochodrosis has been described in calves and feedlot cattle reared under intensive conditions where rapid growth rate and subsequent susceptibility to trauma have been thought to be the contributing factors, although clinical signs in cattle associated with osteochondrosis were rarely reported (Jensen *et al*. 1981; Wegener & Heje 1992; White, Rowland & Whitlock 1984). There have, however, been isolated cases of osteochondrosis in beef cattle in extensive farming systems in Australia and in South Africa (Prozesky *et al*. 2010). It was surmised that the Australian cases were as a result of a genetically inherited disease (Hill *et al*. 1998).

### 2.4.1 Nutrition

There is a strong correlation between the occurrence of osteochondral lesions in horses, pigs, and poultry and their growth rate due to a high energy ration (Hurtig *et al*. 1993; Olsson & Reiland 1978; Prozesky *et al*. 2010).

Several research groups found a relationship between copper deficiency and the prevalence of osteochondrosis in sheep, cattle, pigs, horses and red deer (Audigé *et al*.1995; Bridges *et al*. 1984; Bridges & Moffit 1990; Brooks 1984; Gunson *et al*. 1982; Pitt, Fraser & Thurley 1980; Pond, Krook & Klevay 1990; Smith *et al*. 1975; Suttle & Angus 1978; Thompson 1993). These lesions were termed articular osteochondrosis and are caused either by primary copper deficiency or by exposure to factors that inhibit copper absorption or metabolism (Hurtig *et al*. 1993). This research group suggested a relationship between low copper intake in fast-growing horses, inferior collagen quality, biomechanically weak cartilage and bone, and lesions of osteochondrosis dissecans. This relationship was also reported in young Wapiti cross red deer hybrids in New Zeeland (Thompson *et al*. 1994; Wilson & Grace 2001) and by Audigé *et al*. (1995) in red deer. These authors noticed clinical and epidemiological observations of an outbreak of osteochondrosis, poor skeletal development and enzootic ataxia in red deer on a severely copper deficient farm, which was monitored as part of a two-year observational health and production study.

Reiland *et al*. (1978) found a positive effect in cattle between osteochondrosis and their dietary intake. During this study with 48 bulls, all of the rapidly growing bulls that were given a high-energy diet *ad libitum,* developed signs of osteochondrosis in multiple joints compared to only 50% of the bulls on the limited intake of the slow-growing diet.

The outbreak of osteochondrosis in bulls was described by Davies and Munro (1999) when these bulls were fed on diets that undersupplied dietary requirements of minerals and vitamins. Analysis of the metacarpal bone from two bulls revealed adequate magnesium, phosphorous and bone

ash, but a slightly low calcium and vitamin A concentration. The analysis suggested inadequate Ca, Na, and Cu intake and a mild deficiency of vitamin A, D and E. A gradual clinical improvement was seen in the majority of the animals, and after two to three weeks the farmer noted that the growth rate and coat quality had improved significantly after these animals were fed a balanced mineral and vitamin supplement to meet the requirements of these animals. This outbreak provides evidence that mineral and vitamin imbalances are a likely contributing factor to the development of osteochondrosis in growing cattle.

Calcium deficiency with non-optimal calcium to phosphorous ratios caused a severe outbreak of osteoarthritis in fattening bulls which was probably osteochondrosis (Heinola *et al*. 2006). Calcium deficiency caused more serious lesions in 5 to 8-month-old calves than older calves that were 12 to 18-month-old. Osteoarthritis lesions occurred in more than 80% of the animals with a calcium deficient diet.

Several research groups have reported osteochondrosis type lesions in feedlot cattle and bull stations where the feed given was thought to be one of the causative factors (Jensen *et al*. 1981; Reiland *et al*. 1978; Trostle *et al*. 1997; Trostle *et al*. 1998; Weisbrode *et al*. 1982).

The application of a non-calving season in cows on some farms also creates some challenges regarding the nutrient supply through the plant material as well as the supplementations given during the year. These farms do not always change the nutrient supply according to their needs, which may create a deficit in some macro and micro nutrients. Supplements to grazing extensive farms in the South African market may contain raw materials like sodium chloride, mono-ammonium phosphate, limestone, mono-calcium phosphate, mono-di-calcium phosphate, ammonium sulphate, and many more, and these sources may change the blood pH if fed at some specific quantities (DeGaris & Lean 2008). These raw materials are effective to induce aciduria or a metabolic acidosis due to the dietary cation anion difference (DCAD) value which are negative (<0 mEq/kg) in most lick supplements in these areas. The lowered plasma pH is induced by feeding anions like chloride and sulphur, which increases the responsiveness of tissue receptors to the parathyroid hormone (PTH) (Horst *et al*. 1997). The low plasma pH will increase the calcium metabolism from the bone to buffer the system, and therefore also the flow of magnesium and phosphorous as well as the animal's requirement, absorption and excretion of these minerals. This was confirmed in chickens where a metabolic acidosis increased the incidence of tibial dyschondroplacia (Whitehead 1997) but still needs to be evaluated in cattle as a precursor to osteochondrosis.

### 2.4.2 Age

Osteochondrosis is commonly considered to be a disease process of animals that have not achieved skeletal maturation (Trostle *et al*. 1998). Clinically, the condition has been reported in cattle as young as 5 to 7 months of age, but the average age at which cattle begin to demonstrate clinical signs is 18 to 24 months. In contrast, the research by Heinola *et al*. (2006) showed a high incidence of osteochondrosis in young calves that were 5 to 8 months of age. However, age is but one factor as many studies in pigs, horses and dogs indicated that osteochondrosis is not only related to age, but there are several factors that contribute (Trostle *et al*. 1998; Ytrehus *et al*. 2007).

### 2.4.3 Heredity

Osteochondrosis can be found in cattle of all breeds, commercial and non-commercial, and in all age groups (Trostle *et al*. 1997; Trostle *et al*. 1998). The only common denominator among the affected animals is the geographic area in which they live. The Afrikaner breed, however, seems to be more resistant to these conditions as explained by Trostle *et al*. (1997). Many cases of osteochondrosis have been reported in dairy and beef cattle breeds, and numerous cases were found in pure bred animals, but this may be the result of trial designs which were done to limit genetic variation. Differences in the prevalence of osteochondrosis between breeds and different genetic lines of pigs, dogs and horses also strongly indicated that there is a heritable factor involved as well (Ytrehus *et al*. 2007).

Histone deacetylases (HDACs) remove acetyl groups from ε-amino groups in lysine residues within histones and other proteins (Bradley, McGee-Lawrence & Westendorf 2011). This post-translational modification alters protein stability, protein-protein interaction, and chromatin structure. HDACs activity plays important roles in the development of all organs and tissues, including the mineralized skeleton. Endochondral ossification is a highly organized and tightly controlled development process and Hdacs play a very important role in its orchestration and this gives rise to the majority of bones in the skeleton. Given the important role of HDACs activity in bone cell function and skeletal development, it is not surprising that aberrant class II Hdacs activity is associated with skeletal diseases in humans, including osteoarthritis and osteoporosis. There are 18 genes that encode Hdacs in the human and mouse genomes, although there are greater than 1800 transcription factor genes which are important to regulate gene expression.

Hill *et al*. (1998) did a study on extensively managed Brahman, grazing native pastures, and found clinically observed osteochondrosis in several lame bulls necropsied. All these bulls shared a common ancestral sire and were fed in an environment without concentrated rations, trauma, hard

floors, or any other factor related to intensive farming, which strengthen the possible or likely role of inheritance and gender. The role of heritability in the development of osteochondrosis in cattle is therefore still unclear.

### 2.4.4 Sex

Osteochondrosis is reported almost three times more often in bulls than in other cattle (Trostle *et al*. 1997). This statistic may be deceiving due to the following reasons: steers are commonly raised in feedlots and slaughtered at an age before they develop clinical signs of osteochondrosis; steers may be underrepresented in clinical trials; and bulls may receive more attention due to their higher economic value (Trostle *et al*. 1998). Many research studies also use bulls rather than females in order to limit variation, and this might lead to the inappropriate conclusion that males are more predisposed to the development of osteochondrosis.

In a study by Persson *et al*. (2007) it was found that bulls with osteochondrosis reduced the pregnancy rate in a beef producing herd and therefore their profitability. Ninety-three percent of these bulls had a lesion on a stifle joint which created the pain to prevent them from mounting the females, despite their acceptable sperm morphology.

### 2.4.5 Environment

The development of osteochondrosis in cattle may also be predisposed by the environment and therefore it has been reported that cattle in intensive systems, with limited exercise on concrete floors, may be more susceptible (Reiland *et al*. 1978; White *et al*. 1984). These intensive practices might increase the stress on long bones of the limbs and as a result the prevalence of osteochondrosis (Trostle *et al*. 1998).

Trauma has been one of the most widely proposed causes of osteochondrosis in all species (Ekman & Carlson 1998). In support of the traumatic aetiology, predilection sites in all affected species tend to be located in areas of local biomechanical stress (Prozesky *et al*. 2010). The role of trauma in the pathogenesis of osteochondrosis may depend on the stage of the disease that is considered. Although trauma may be involved in converting a subclinical osteochondrosis lesion to an osteochondrosis dissecans lesion, the necessary severity of the trauma, is usually minimal and often includes only the forces involved in normal ambulation (Prozesky *et al*. 2010). There is, however, no clear evidence that acute macro trauma is involved in the initiation of primary lesions of osteochondrosis.

### 2.4.6 Anatomical characteristics

The anatomical form and function of a joint determine the frequency of osteochondrosis dissecans occurrence in humans (Smillie 1960). It was hypothesized that local traumatic factors which may vary among joints, based on necropsy findings, are important in the aetiology of osteochondrosis in dogs (Olsson 1987).

### 2.4.7 Primary dyschondroplasia

Osteochondrosis may begin as a generalized abnormality of chondrocyte development and maturation, leading to multifocal altered endochondral ossification (Olsson & Reiland 1978). The cause of change was not defined, but one suggestion was that it occurred secondary to ischaemia-induced necrosis of vascular channels. The authors further hypothesized that secondary cartilage necrosis may occur as a result of biomechanical stress.

### 2.4.8 Ischaemic necrosis

Ytrehus *et al.* (2007) concluded that articular osteochondrosis should be defined as a focal ischaemic necrosis of growth cartilage initiated by necrosis of cartilage canal blood vessels. Because necrotic cartilage does not undergo mineralization or vascular penetration, a focal failure of endochondral ossification occurs when the ossification front approaches the lesion (Prozesky *et al.* 2010).

## 2.5 Osteochondrosis in Southern Africa

Many research projects were done in the western parts of South Africa during the end of the 1800's on various disease syndromes in cattle, caused directly and indirectly by mineral imbalances (Prozesky *et al*. 2010; Theiler 1912; Theiler *et al.* 1927). Some of the common diseases in those areas were "stijfziekte" and "lamziekte" and it was assumed to be two different manifestations of the same disease. "Stijfziekte" or osteomalacia was associated with phosphorous deficiency and "lamziekte" was associated with the bacterial disease called botulism.

Sir Arnold Theiler continued with research, most of it on Armoedsvlakte research farm in the North West Province, into the cause and treatment of "stijfziekte" (Prozesky *et al*. 2010). He discovered in 1919 that "lamziekte" was a separate disease and not related to "stijfziekte", and that "lamziekte" manifested where cattle were intoxicated by toxins released from anaerobic saprophytic bacteria present on bones which these cattle were eating (Theiler *et al*. 1927).

Another phenomenon in this area was cattle which consumed bones (pica) and this was proved to be directly associated with aphosphorosis (McDowell 1996; Prozesky *et al*. 2010). These cravings were abolished in minutes after an intravenous infusion of sodium phosphate. Research has also proven that these bone consuming cattle were mostly attracted to older bones, and not to any other animal products (fat, meat or blood).

Another enzootic, mineral imbalance related disease, Vryburg hepatosis, occurs in the North West and Northern Cape Provinces in South Africa. This was related to high manganese, low iron and low cobalt. This disease is normally seen in calves and lambs and manifests clinically as geophagia and chronic cholangiohepatitis with resulting icterus (Prozesky *et al.* 2010). This disease causes high morbidity and mortality rates as found by Elsenbroek and Neser (2002). They also indicated that manganese interferes with iron and cobalt absorption in the digestive tract by competitive binding to the receptor sites. Farmers that were supplementing cattle with phosphorous containing supplements seemed to have resolved the musculo-skeletal abnormalities associated with mineral imbalances in the North West and Northern Cape Provinces in South Africa, Namibia and Botswana.

In 1982, farmers and local veterinarians began to notice a new health condition that affected cattle (Prozesky *et al*. 2010). These animals developed joint effusions of the weight-bearing joints in particular, such as the femoro-tibial joint, and they eventually had to be slaughtered as a result of severe lameness and loss in condition. The disease was initially seen in a few show-animals but was later noticed on a number of farms during the mid-1990's, with some herds having up to 40% of the animals affected. The disease has also been reported in other areas around Southern Africa, for example in Cradock in the Eastern Cape, Olifantshoek in the Northern Cape, Harrismith in Kwazulu-Natal, Theunissen and Boshof in the Free State, Francistown and Lobatsi in Botswana, and Gobabis in Namibia. The new disease prevailed in cattle of all ages, different breeds, all age classes, in commercial and non-commercial herds, although Afrikaner cattle seem to be more resistant to the condition (Prozesky *et al*. 2010).

In clinically affected animals the disease was characterized by visible effusions from joints, particularly the stifle joint, and it was also associated with inflammation, pain, and eventually lameness in affected animals. This affects the animal's ability to walk which will influence their water and feed intake (ability to graze) and eventually their performance or ability to live.

Histopathological examination of affected bones on some of these sick animals from the affected areas by Dr. J Neser and Prof. L Prozesky (Department of Paraclinical Sciences, University

of Pretoria) revealed lesions similar to osteochondrosis and not rickets or osteomalacia ("stiff sickness") as described by Sir Arnold Theiler (Prozesky, Neser & de Brouwer 2005; Prozesky 2006). The lesions observed were in various joints including the atlanto-axial (neck), tibio-tarsal (hock), scapulo-humeral (shoulder) and femoro-pelvic (hip joints) but were consistently most prominent in the femoro-tibial (stifle) joints, often clinically evident as joint effusion (Prozesky *et al*. 2005). This high prevalence on the stifle joint created the concept to develop a visual on-farm evaluation tool for professionals to diagnose osteochondrosis, which in the mean time was confirmed as the "new health condition" on cattle at farm level. This was not done before and therefore had to be tested by a team of researchers.

### 2.6 Problem statement

Osteochondrosis around Southern Africa increased since the 1980's and therefore it is crucial to investigate means to decrease the prevalence of osteochondrosis in cattle. According to the literature the aetiology of osteochondrosis is multifactorial. However, since many farms in the phophorous deficient areas, like Vryburg and Reivilo, experienced a high incidence of osteochondrosis in cattle, it is assumed that phosphorous may be an important predisposing factor for osteochondrosis in Southern Africa.

### 2.7 Hypothesis

($H_0$): There is no difference in the prevalence of osteochondrosis in cattle that receive mineral and vitamin supplements (with more bioavailable phosphorous) compared to cattle that do not receive any mineral and vitamin supplements (containing more bioavailable phosphorous).

($H_1$): The prevalence of osteochondrosis decreases if cattle receive mineral and vitamin supplements (containing more bioavailable phosphorous).

### 2.8 Benefits arising from the project

The anticipated benefits of the project are as follows:

2.8.1 It will be ascertained if nutrition plays a role in the prevalence of osteochondrosis and if so, will this help to narrow down the factors which are involved in the prevalence of osteochondrosis in cattle in Southern Africa;

2.8.2 This project may provide more information if a visual on-farm osteochondrosis evaluation system is possible and accurate to diagnose osteochondrosis in cattle;

2.8.3 This project may provide more information regarding phosphorous intake and bioavailability which will help to optimize the intake of this expensive nutrient;

2.8.4 This project may determine if it is possible to formulate a supplement that will reduce the percentage of osteochondrosis affected animals in commercial operations;

2.8.5 This project will help to obtain information to give guidance to the Animal Feeds Act to rectify the current low mineral levels, set as a minimum by Act 36 of 1947.

2.8.6 This may help to improve the standards of supplements in the market.

### 2.9 Aims/Objectives

To describe osteochondrosis in cattle in South Africa.

2.9.1 To compare an on-farm diagnostic test with the gold standard test, post-mortem method as well as the X-ray radiology.

2.9.2 To identify the mineral levels within liver and bone which maybe associated with osteochondrosis in cattle.

2.9.3 To compare the effect of different sources of phosphorous on the prevalence of osteochondrosis.

2.9.4 To evaluate the effect of mineral nutrition on the prevalence of osteochondrosis in clinically affected cattle.

2.9.5 To compare the bioavailability of various phosphorous sources on the prevalence of osteocondrosis in cattle.

2.9.6 To develop a formulation model to formulate a mineral supplement to minimize the prevalence of osteochondrosis in commercial herds in the Southern Africa.

2.9.7 To establish nutrition and management practises on cattle farms to reduce the prevalence of osteochondrosis.

2.9.8 To improve the mineral levels requirement that is used by Act 36 of 1947 for supplements in the cattle market.

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# Chapter 3

# DEVELOPING A METHOD TO VISUALLY DIAGNOSE OSTEOCHONDROSIS IN BEEF CATTLE IN PRACTICE

## Abstract

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Since 1982, many farmers and veterinarians have noticed an increase in the incidence of lameness that negatively affected the production of cattle in the North West Province of South Africa**,** as well as in many other areas in Southern Africa. Most of these animals developed effusions on the weight-bearing joints, such as the femoro-tibial joint, and they had to be slaughtered as a result of severe lameness and loss in condition. The disorder was later confirmed as being osteochondrosis which prevailed in all classes of commercial and non-commercial cattle – male, female, different breeds and in all age classes. Understanding the accuracy and the usefulness of a specific test in discriminating between several diagnostic possibilities, and osteochondrosis positive and osteochondrosis negative animals, requires a diagnostic test to estimate the disease probability and minimize the diagnostic uncertainty when used on-farm when establishing the prevalence of the disease. To that effect, two different groups of animals of varying ages and sexes from clinically affected herds were included in a trial to evaluate the Onderstepoort Osteochondrosis Lesion Scoring system, generally referred to as the OPOLS method. A first group of 35 animals were visually evaluated per hind leg (n=70) with the OPOLS method and the outcomes compared with post-mortem pathology after slaughter. A second group of 36 animals were then evaluated with the OPOLS method twice in one year and then compared with X-ray evaluation (n=144) on the same day. The OPOLS method showed a method sensitivity on the femoro-tibial joint on the hind legs of the cattle of 90.0%, 85.7% and 88.6% with X-rays, post-mortems, and the combined X-ray and post-mortem data respectively. The diagnostic accuracy was 79.9% (X-ray), 80.0% (post-mortem) and 79.9% (combined X-ray and post-mortem) with a prevalence odds ratio of 3.71, 8.00 and 5.09. The 95% confidence interval was 74.3 – 92.6% for sensitivity and 32.6 to 78.6% for specificity, with the best agreement between the OPOLS method and the post-mortems. Results from this study indicated a highly satisfactory agreement between the OPOLS method and the pathology found with post-mortems (“the golden standard”) and X-rays, and therefore the OPOLS method can be regarded as a reliable method to visually diagnose osteochondrosis in beef cattle.

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**Keywords:** Osteochondrosis, cattle**,** Onderstepoort osteochondrosis lesion scoring, post-mortem, X-ray.

# DEVELOPING A METHOD TO VISUALLY DIAGNOSE OSTEOCHONDROSIS IN BEEF CATTLE IN PRACTICE

## Introduction

Many farmers and veterinarians began to notice a new health condition in 1982 that affected the legs and ease of walking of cattle (Prozesky *et al*. 2010). This disease was initially seen in a few show animals, but was later noticed on many farms during the mid-1990's. Most of these animals developed joint effusions of the weight-bearing joints in particular, such as the femoro-tibial joint, and they eventually had to be slaughtered as a result of severe lameness and loss of condition. Some of the herds had as much as 40% of their animals affected by the condition, initially called arthrosis, but later confirmed as being osteochondrosis.

Osteochondrosis has since been reported in many areas around southern Africa, for example in Cradock in the Eastern Cape, Olifantshoek in the Northern Cape, Vryburg and Reveilo in North West, Harrismith in Kwazulu-Natal, Theunissen and Boshof in the Free State, Francistown and Lobatsi in Botswana, and Gobabis in Namibia (Prozesky *et al*. 2010). Osteochondrosis prevailed in cattle of all sex classes, different breeds, all age classes, in commercial and non-commercial herds, although Afrikaner cattle seem to be more resistant to the condition.

In clinically affected animals, osteochondrosis was characterized by visible effusions from joints, particularly the stifle joint, and it was also associated with inflammation, pain, and eventually lameness in affected animals. This affects the animal's ability to walk, which will influence their water and feed intake (ability to graze) and eventually their reproduction and production performance with negative consequences on profitability.

The development of an on-farm evaluation system was important to establish the prevalence of osteochondrosis in beef animals on farms. A team of scientists created an on-farm evaluation score sheet and people were trained to visually score animals in a working area, subjectively, as animals walk through the working area. The visual method was called the **O**nderste**p**oort **O**steochondrosis **L**esion **S**coring system (OPOLS). This visual method had to be tested in order to evaluate the sensitivity, specificity and prevalence of osteochondrosis in the animals evaluated with this method and then compared with pathology results when other tests (X-rays and post-mortems) are used.

Understanding the accuracy and the usefulness of a specific test in discriminating between several diagnostic possibilities, disease or disease-free, requires diagnostic test results to estimate the disease probability and minimize diagnostic uncertainty (Scott, Greenberg & Poole 2008).

A core activity of a veterinarian is to diagnose a clinical condition with the assistance of a diagnostic test to predict the clinical condition probability and to minimize diagnostic uncertainty and costs. Understanding a specific test is therefore important for making a diagnosis through the use of investigational tests. The performance characteristics and accuracy of a diagnostic test for a health disorder are expressed as the proportion of animals that has a true positive rate for the disease (sensitivity) as well as a high true negative rate for the disease (specificity).

From a statistical point of view, the effectiveness and the precision of the test method, to identify animals with or without the disease, are important and needs to be tested within the 95% confidence interval in order to diagnose, screen, estimate risk, guiding therapy and staging patients or animals in clinical trials (Scott *et al*. 2008).

This research was conducted to validate the OPOLS system with other accepted radiology test methods to enable professionals to diagnose, screen and manage osteochondrosis on farms with a practical system that is scientifically validated.

## Materials and methods

### On-farm evaluation system

The purpose of the OPOLS system development was mainly to develop an on-farm visual lesion scoring system as a diagnostic test to clinically evaluate animals as indicated in Table 3.1. All animals were individually put through a gate crash and evaluated for possible osteochondrosis related lesions at the femoro-tibial joint. These clinical diagnostic evaluations on the cattle were done by at least two trained persons simultaneously to minimize any biased decisions.

The animals in this trial were evaluated with the OPOLS system with only a “yes” or “no” for lesions in animals with or without the disease to validate the method and not the sub-categories or the size of a lesion, nor on lameness. The main reason was to compare the subjective evaluation of visible lesions with the pathology evaluation thereof through methods like post-mortems or x-rays, done on the same animals immediately after the OPOLS evaluation.

It is important for future reference and usage to describe the complete OPOLS system in detail for specialists to fully understand this practical method to do an on-farm assessment of osteochondrosis on cattle. A score sheet was developed to use on-farm to score animals for lesions (type and size) and lameness, and to gather information like animal weight, sex and age as set out in Table 3.1 to develop trends within a cattle population. The size of the lesions is very subjective and therefore not evaluated in this trial due to the risk of bias and it is probably too inaccurate to measure the size of swellings due to visible effusions from the femoro-tibial joints.

**Table 3.1** Osteochondrosis lesion scoring sheet

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **OPOLS 2008† scoring sheet** | | | | | | | | | | | | | |
| **Evaluator 1 name** | | **Date:** | **Lesions** | | | | | | | **Optional extras** | | | | |
| **Evaluator 2 name** | | | **Ball ‡** | | **Flat ‡** | | **Lameness** | | **Comments** | **Animal weight** | **Cow** | **Bull** | **Ox** | **Age, months** |
| | | **Animal number/name** | | | | | | | | | | | | |
| | | | **L** | **R** | **L** | **R** | **L** | **R** | | | | | | |
| | **1** | | | | | | | | | | | | | |
| | **2** | | | | | | | | | | | | | |
| | **3** | | | | | | | | | | | | | |
| | **4** | | | | | | | | | | | | | |
| | **5** | | | | | | | | | | | | | |

† Onderstepoort Osteochondrosis Lesion Scoring System (2008).

‡ Ball or Flat lesions are only scored as a "yes" of a "no".

1. Scoring is done by trained personnel, and a minimum of two people to limit biased errors.
2. The stifle joints are scored on both the left and right leg of the animal.
3. The degree of lameness is also scored, if it is conclusively attributed to osteochondrosis and not from other causes of lameness such as limb deformities or hoof health reasons.
4. Swelling over the stifle joint is graded as either a “yes” or “no” and include the following lesion categories:
    - **Ball category** (round and raised lesions which appears like a subcutaneous ball; the fluid in the lateral compartment of the femoro-tibial joint capsule appears to be localized, mainly distal to the tendon of insertion of the gluteobiceps muscle).
    - **Plaque category** (lesions where the synovial joint effusions create a flattened, roughly oval swelling that extends above the tendon of insertion of the gluteobiceps muscle. This often creates a double swelling, and often also into the femoro-patellar joint capsule, which extends for about 7 cm beneath the

quadriceps muscle as described by Bezuidenhout *et al*. (2000). These flatter swellings appear to be the more chronic lesions, where the fluid has stretched the joint capsule and now some of that fluid has been absorbed or the joint capsule has thickened due to fibrosis, giving the flatter appearance).

5. A degree of lameness as described by Sprecher, Hostetler and Kaneene (1997) has been developed to determine the incidence and severity of lameness in cattle (Appendix 5).
    - Grade 1 (score 1)
      The animal stands and walks normally and the rear hooves are placed with confidence where the front hooves were placed in a normal stride with a level back (normal).
    - Grade 2 (score 2)
      Marginal asymmetry in gait, such as a shortened stride and with the head slightly held lower and extended from the body. The back is flat when the animal stands, but arches when it walks (mildly lame)
    - Grade 3 (score 3)
      Lameness is more obvious. The animal uses a short stride on the affected side. There will be a slight sinking of the dew-claws in the limb opposite the affected limb and the animal stands and walks with an arched back (moderately lame).
    - Grade 4 (score 4)
      The animal has a shortened stride on the affected side and upright hocks. The animal favours one or more limbs, but can still bear some weight on them while walking. It shows obvious pain and the animal stands and walks with an arched back (lame).
    - Grade 5 (score 5)
      The animal is lame and displays severe pain. It is unable to put weight on the affected limb. The quadriceps and gluteal muscles of the affected limb are often atrophied. The animal shows pronounced arching of the back (severely lame).

**Experimental animals**

Two groups of animals of varying ages and sexes from OPOLS evaluated herds were included in a trial to validate the OPOLS method for sensitivity, specificity and the prevalence of the osteochondrosis. These test populations were selected from farms, well-known for their higher incidence rates of osteochondrosis and where the frequency of osteochondrosis in the cattle increased since the mid-1980's. The animals were individually validated against either post-mortem pathology

or radiology (X-rays) interpretation as the controls, within 48 hours after the visual OPOLS evaluation.

The first group of 35 animals (trial A) were evaluated with the OPOLS system and then compared with their post-mortem pathology (method P) results done at the Faculty of Veterinary Science (Prof L. Prozesky, Department of Paraclinical Sciences) at the University of Pretoria.

A second group of 36 animals (trial B) were also evaluated with the OPOLS system and then compared with their radiology results done by the Faculty of Veterinary Science (Dr. G. van der Veen, Department of Paraclinical Sciences) at the University of Pretoria. These animals were OPOLS scored twice over a period of one year and X-rayed (method X) and then used as comparison to the specific period only. Data from the second period evaluations was therefore not used in the first period evaluation, or *vice versa*, and therefore only compared to the evaluation of the specific day.

The stifle bone evaluation on the first group (trial A) of animals was done on a sample number of 70 (35 animals x 2 legs; method P) and the second group (trial B) on a sample number of 144 (36 animals x 2 legs x 2 time periods; method X).

**Statistical design and evaluation**

**3.1. Data analysis**

Animals were not compared in a randomly designed study, but in a group analysis and then compared with the post-mortem and radiology (X-rayed) reports from specialists from the Department of Paraclinical Sciences (Faculty of Veterinary Science) at the University of Pretoria.

In practice, the performance of a diagnosis test is often misjudged due to the narrow range between sick or healthy animals in the test (Scott *et al*. 2008) and therefore animals in this trial were selected from herds with a high incidence of osteochondrosis. However, the study group also contained the prospective cohort of consecutive animals with a diagnostic uncertainty with diseased animals with a wide range of severity as well as non-diseased animals showing clinical signs of the disease.

Data in most trials are examined in relationship to a statistical “null” hypothesis with the belief to aim at obtaining “statistical significance”, but Garner and Altman (1986) recommended that medical trials should be evaluated differently to evaluate the magnitude of some factor(s) of interest.

The results of many medical studies are published with the aim of how much a disease modifies the physiology and or anatomy in the animal, or on how a new treatment altered the prognosis, rather than only the level of the statistical significance. The use of a "hypothesis testing" is therefore limited in some veterinary studies, because there is not always a simple "yes" or "no" fundamental answer (significant or not significant) in these studies, similar to the typical hypothesis testing methods.

Using confidence intervals in medical or veterinary trials are therefore acceptable due to the fact that small differences with no real interest can be statistically significant within a large sample size, whereas clinically important effects may be ignored due to a statically non-significant number if the statistical calculations were affected by factors, for example a small sample size. The confidence interval is advantageous in studies where a population with a health problem, like osteochondrosis, are small or where the variability of osteochondrosis between different populations is a reality. The confidence interval is different from a single value estimate, for example a population mean value, and rather a range of values that are considered more accurate for the sample population.

The above mentioned veterinary studies publish the data with either a 99%, 95% or a 90% confidence interval range and rarely on lower values (Altman *et al.* 1983; Gardner & Altman 1986). The width of the confidence interval is affected by the sample size due to the size effect on the standard error. Small trials with low numbers will therefore affect the precision and increase the width of the confidence interval. Many researchers in medical trials use the 95% confidence interval methodology similar to researchers who use the 5% level of statistical significance to describe results from trials. Altman *et al*. (1983) indicated a close relationship between a confidence interval and the results of a test of significance: if the difference between treatments are within a 5% significance level ($P<0.05$) then the zero difference is excluded from the associated 95% confidence interval. Confidence interval cannot control or deal with non-sampling errors such as biases in design, conduct, or analysis of trials, but conveys the effects of sampling variation on the precision of the estimated statistics and therefore includes the lowest and highest true effect likely to be compatible with the sample observations. Both the *P* value and the confidence interval are helpful and can be presented, but in medical and veterinary trials, if one has to be excluded, it should be the *P* value (Gardner & Altman 1986).

Many researchers are concerned with estimating quantity values, for example the mean difference between groups or a relative risk value, and therefore it is desirable to calculate the confidence interval in these trials (Altman *et al.* 1983). This gives the researcher a 95% confidence that the range of values will include the true value, for example in a 95% confidence interval.

Investigators in health studies are usually more interested to determine the size of difference between sick and healthy groups, rather than a simple indication of whether or not it is statistically significant (Gardner & Altman 1986). They prefer the usage of confidence intervals to present the results in a trial directly on the scale of data measurement.

The results with the OPOLS system were then also compared with the results with the post-mortem and X-rayed methods using the following statistical methods:

I. Kappa analysis

The Kappa coefficient is an indication of the degree of agreement for nominal or categorical data and by using clinical interpretation of studies of diagnostic tests as described by Scott *et al*. (2008). A test for bias was implemented by using the McNemar's chi-square test. Where applicable, a 95% confidence interval was used to reveal the agreement for osteochondrosis between the OPOLS method and the post-mortem or X-rayed methods within the trial groups.

II. Binomial tests on GenStat release 17.1

The binomial test in a population is an exact test of the statistical significance of deviation from a theoretically expected distribution of observations into two possible outcomes. Where applicable, $P \leq 0.05$ was accepted as being significant.

### 3.2. Hypothesis

The typical "hypothesis testing" in some veterinary studies, like this osteochondrosis study, was more complex and therefore more statistical methods were used to analyse the data, although, statistical significance was tested in the data within the following *hypothesis:*

($H_0$): There is no agreement between the visual on-farm OPOLS method and the X-ray or post-mortem methods to diagnose the prevalence of osteochondrosis in cattle.

($H_1$): The diagnosis for the prevalence of osteochondrosis with the visual on-farm OPOLS method agree with diagnosis results from the X-ray or post-mortem methods in cattle.

Table 3.2 describes the performance characteristics of the diagnostic test as described by Scott *et al*. (2008).

**Table 3.2** Performance characteristics in a diagnostic test (adapted from Scott *et al*. 2008)

| Test characteristic | Alternative name | Question addressed |
|---|---|---|
| Prevalence | Pre-test probability | How common is the disease in the test population? |
| Sensitivity (Sn) | True positive rate | What is the proportion of animals with the disease which have a positive test? |
| Specificity (Sp) | True negative rate | What is the proportion of animals without the disease which have a negative test? |
| Positive predictive value (PPV) | Post-test probability of disease with a positive test | What is the probability of the disease if an animal has a positive test? |
| Negative predictive value (NPV) | Post-test probability of no disease with a negative test | What is the probability of not having the disease if an animal has a negative test? |
| Diagnostic accuracy | | What proportion of all tests gave the correct results? True positives plus true negatives as a proportion of all the tests. |
| Likelihood ratio of a positive test (LR+) | | How much more likely is a positive test to be found in an animal with the disease than in an animal without the disease? Ratio = true possitive rate / false positive rate. |
| Likelihood ratio of a negative test (LR-) | | How much more likely is a negative test to be found in an animal with the disease than in an animal without the disease? Ratio = false negative rate / true negative rate. |
| Diagnostic odds ratio (DOR) | | How much more likely will the test make the correct diagnosis than an incorrect diagnosis in animals with the disease? Ratio = LR+ / LR- |

## Results

The OPOLS on-farm method was validated by comparing the visual observations of each animal within a group with the specific animal's pathology, either done by X-ray radiology or post-mortem pathology and the results are summarized in Tables 3.3, 3.4 and Appendix 4. The visual observations and the X-ray and post-mortem examinations in these trials were only done with either a "yes" if the animal was scored or examined as a positive animal or a "no" if the animal was negative for osteochondrosis and the results were then compared to the pathology of the same animal and leg.

The OPOLS visual observations on the stifle-bone were statistically validated in three different combinations with the different legs on individual animals;

i. X-ray pathology (method X)
ii. Post-mortem pathology (method P)
iii. X-ray plus post-mortem pathology combined in one data set (method XP)

The OPOLS visual observations for a specific group were then compared with the X-ray examinations to validate the animal's pathology with YESs and NOs between the two examination methods from the same leg and animal. This was also done with the method P where the OPOLS examinations were compared to the results found with the post-mortem examinations. The OPOLS observations were also compared to the pathology data, when both X-ray and post-mortem method examinations were combined into one data set and then compared with the combined OPOLS evaluations.

**Table 3.3** A summary of the osteochondrosis OPOLS on-farm observation results on the stifle bones of test cattle compared to the X-ray, post-mortem, and combined X-ray and post-mortem examinations

| **Test characteristic** ‡ | **X-ray data: Summary of data on the two hind legs (stifle bones) with two observation times.** | **Post-mortem data: Summary of data on the two hind legs (stifle bones).** | **Post-mortem + X-ray combined data: Summary of data on the two hind legs (stifle bones)** |
|---|---|---|---|
| | **Method X** | **Method P** | **Method XP†** |
| Sensitivity | 90.0 | 85.7 | 88.6 |
| Specificity | 29.2 | 57.1 | 39.5 |
| 95% confidence interval for Sensitivity | (83.3, 94.2) | (74.3, 92.6) | (83.1, 92.5) |
| 95% confidence interval for Specificity | (14.9, 49.2) | (32.6, 78.6) | (25.6, 55.3) |
| True prevalence | 83.3 | 80.00 | 82.2 |
| Positive predictive value PV+ | 86.4 | 88.9 | 87.2 |
| Negative predictive value PV- | 36.8 | 50.0 | 42.9 |
| Odds ratio or Prevalence ratio | 3.71 | 8.00 | 5.09 |
| Odds ratio for diagnosis on right leg | 8.30 | 10.5 | 12.1 |
| Odds ratio for diagnosis on left leg | 2.30 | 6.80 | 3.15 |
| likelihood ratio of a positive test result LR+ | 1.27 | 2.00 | 1.46 |
| likelihood ratio of a negative test result LR- | 0.34 | 0.25 | 0.29 |
| Observed agreement/Diagnostic accuracy | 79.9 | 80.0 | 79.9 |
| Kappa coefficient | 0.21 | 0.41 | 0.29 |
| Total observations, n | 144 | 70 | 214 |
| Number needed to diagnose 10 positive tests(NND) | 52 | 23 | 36 |

† This is the combined data of groups for methods X and P. Animals were examined either by X-ray or post-mortem method, and not by both methods on the same animal; Sensitivity = true positive rate; Specificity = true negative rate; Odds ratio = how much more likely will the test make a correct diagnosis than an incorrect diagnosis in animals with osteochondrosis; NND = the number of animals needed to give 10 positive tests.

The prevalence of osteochondrosis in these trial groups, for method X, method P and method XP were 83.3%, 80% and 82.2% respectively, with 144 legs evaluated with X-ray radiology and 70

legs evaluated with the post-mortem method. The study revealed a strong agreement on the osteochondrosis true positive animals between the visual OPOLS on-farm diagnostic test method, the X-ray and the post-mortem test method. The test method sensitivity (true positive rate) between the OPOLS system was 90% comparing to method X, 85.7% to method P and 88.6% to method XP. This is a strong indication that the visual OPOLS developed system has a high sensitivity for animals with osteochondrosis. The 95% confidence interval for the true positive animals (sensitivity) was also high with a result of 83.3 – 94.2% when the OPOLS true positive animals were compared to the true positive animals with method X, 74.3 – 92.6% for method P, and 83.1 – 92.5% if the X-ray and post-mortem data are combined (method XP). Not only is the sensitivity high (> 80%) but, the range of values were also high and narrow, which means that the true positive test compares well between the OPOLS, the X-ray and the post-mortem methods. If method P is the gold standard, then the 95% confidence interval with the OPOLS method in these trials indicated that evaluators will score animals with osteochondrosis correctly 74.3 to 92.6 times out of a 100 animals. This is a strong indication that the examinations with the visual OPOLS system is highly comparable with the examinations with the post-mortem method. The trial results further indicated a high probability of osteochondrosis in animals, where animals with osteochondrosis were positive diagnosed with the OPOLS system and then validated against the pathology results of groups X, P and XP with “positive test probability” results of 86.4%, 88.9% and 87.2%, respectively.

The specificity on the other hand, was expected to be lower due to some lesions seen on femoro-tibial joints with the X-ray and post-mortem methods, but still too small to see with the OPOLS method. These small lesions were lesions which were in a degenerated stage or which were still in the early developmental stage, and therefore still too small to visually observe on the stifle-bone. The proportion of healthy animals in these trials which scored negative for osteochondrosis (true negative test) with the OPOLS system, were respectively 29.2%, 57.1% and 39.5% when validated against the results from the methods X, P and XP. The 95% confidence interval for the test method specificity was 14.9 – 49.2% when the OPOLS scored animals was compared to method X, 32.6 – 78.6% to method P, and 25.6 – 55.3% compared to method XP. This was less accurate than the true positive test, but expected. The X-ray specificity results were the lowest due to this method’s inaccuracy to measure non-bone fibrous connective lesions or swellings, and much lower than the gold standard post-mortem examination results (57.1%) when the results were validated against the OPOLS method.

The research data was also analysed with the Cohen’s KAPPA methodology to evaluate the agreement between the two integers for qualitative (categorical) items as explained by Smeeton (1985). This was done to evaluate the agreement between osteochondrosis using the OPOLS test and

the X-ray, or post-mortem or the combined X-ray and post-mortem methods, with an alternative test often used by professionals to test a treatment method in "sick animals". The results of the Kappa coefficient were respectively 0.21 (fair agreement), 0.41 (moderate agreement), and 0.29 (fair agreement) for methods X, P and XP. The low number of legs evaluated (n=214) together with the low negative true values (specificity) for osteochondrosis in these trials, may be the main reasons for the low KAPPA coefficient value. However, the Kappa coefficient for the method P data was still the highest, which is an indication that some lesions might be missed with X-rays. The gold standard to diagnose Osteochondrosis in animals is either by post-mortem pathology or by examining the animal in theatre, but both too expensive to be used in commercial animal production systems.

Results from these trials have also shown that the OPOLS system is more likely to make the correct rather than the incorrect diagnosis in cattle with osteochondrosis. The diagnostic odds ratio in this research was 3.71 for method X, 8.00 for method P and 5.09 for method XP, which further supports the high agreement between the post-mortem (gold standard) and the OPOLS methods. This research also found an odds ratio for osteochondrosis on the right leg of 8.3 with method X, 10.5 with method P, and 12.1 with method XP. The odds ratio for these methods for osteochondrosis on the left leg was respectively, 2.30, 6.80 and 3.15. Examination data from both legs have therefore shown that it is highly likely that osteochondrosis is prevalent in cattle once the evaluator diagnose osteochondrosis positively with the OPOLS system.

The likelihood ratio of a positive test (LR+) was also tested, which is defined as how much more likely is a positive osteochondrosis to be found in cattle with osteochondrosis than in cattle without osteochondrosis (ratio = true positive rate/false positive rate)( Scott *et al*. 2008). The results from these trial groups indicated the LR+ to be 2.0, 1.27, and 1.46, when the OPOLS method was validated with the method P, method X, and method XP respectively. With the "odds" ratio of 8.0 and a "likelihood" ratio of 2.0, this research test indicate a strong agreement between the OPOLS method and the pathology with method P, in cattle with and without osteochondrosis. The likelihood ratio of a negative test (LR-) was again low and expected, and defined as how much more likely is a negative test to be found in cattle with osteochondrosis than in cattle without osteochondrosis (ratio = false negative rate/true negative rate). The LR- results with the OPOLS method validation were 0.25, 0.34, and 0.29, respectively with methods X, P and XP. This also indicate that the method will have a low false negative rate in cattle populations with or without osteochondrosis, which is an indication of 34.3% false negatives per 100 true positive animals. These results together with the low confidence interval for specificity values are a strong indication that the OPOLS method is less accurate to identify true negative animals in comparison to true positive animals.

The data was also statistically evaluated with the two-times binomial test to evaluate if there is any statistical significance on the prevalence of osteochondrosis in the stifle-joint between the left and the right leg of cattle for the two animal groups evaluated in these trials. Table 3.4 include the results from two trials;

- 70 stifle-bone OPOLS examinations compared with post-mortem pathology.
- 144 stifle-bones OPOLS examinations compared with X-ray radiology pathology.

**Table 3.4** A summary of the two-times binomial test results to evaluate the OPOLS examination data for each hind leg compared with the pathology when the X-ray and post-mortem methods were used to diagnose osteochondrosis in beef cattle

| | **Sample** | **Sample number** | **Successes** | **Proportion, %** | **Difference, %** | **Standard Error between proportions** | **Probability, *P*** |
|---|---|---|---|---|---|---|---|
| X-ray pathology data | Right leg | 72 | 67 | 93.1 | 19.1 | 0.06 | 0.002 * |
| | Left leg | 72 | 53 | 73.6 | | | |
| Visual observation with OPOLS | Right leg | 72 | 65 | 90.3 | 7.0 | 0.06 | 0.218 |
| | Left leg | 72 | 60 | 83.3 | | | |
| | **Sample** | **Sample number** | **Successes** | **Proportion, %** | **Difference, %** | **Standard Error between proportions** | **Probability, *P*** |
| Post-mortem pathology data | Right leg | 35 | 27 | 77.1 | 5.8 | 0.095 | 0.55 |
| | Left leg | 35 | 29 | 82.9 | | | |
| Visual observation with OPOLS | Right leg | 35 | 23 | 65.7 | 22.9 | 0.097 | 0.023 * |
| | Left leg | 35 | 31 | 88.6 | | | |

*$P \leq 0.05$ was accepted as being significant.

The cattle group used in the X-ray method validation did show a statistical difference between the right and the left leg, with 93.1% of the positive tested osteochondrosis on the right leg in comparison to only 73.6% on the left leg ($P$ =0.002; SE=0.06). The cattle group did not show the same statistical difference when the same legs were compared to the OPOLS system with 90.3% of the positive scores on the right leg and 83.3% on the left leg*),* and therefore not different (*p=0.218; SE=0.056).*

Another cattle group were evaluated to test the trend in the different legs when the OPOLS examinations were compared to the post-mortem pathology results on the same leg. The results from this evaluation did show a significant difference between the positive tested osteochondrosis animals favouring the left leg (88.6%) in comparison to the right leg (65.7%) when examined with the OPOLS

system (*p=0.023; SE=0.097).* On the other side, no difference per leg was found with the post-mortem method (gold standard) on the same group of animals when the stifle-bones with osteochondrosis were compared, with 82.9% on the left leg compared to the 77.1% on the right leg (*p=0.55; SE=0.095).*

The prevalence of osteochondrosis for the different legs was inconsistent between the different animal groups and non-specific to only one side as seen with the different animal groups in Table 3.4 and this is probably also an indication that osteochondrosis are influenced by external factors. The trend with the different legs, when the gold standard post-mortem results were evaluated, also indicated no difference as seen in Table 3.4. Furthermore, the trend of positive osteochondrosis in animals in the method X group on the right leg indicated that animals tend to have osteochondrosis on the right when it was evaluated with the two-times binomial test, but when it was evaluated with alternative statistical models (Table 3.3), one could see that the specificity (true negative rate) with the X-ray methodology was only 14.9 – 49.2%. This confirms the view that X-ray examination will miss some fibrous connective tissue as well as the synovial fluid and may not be used as the gold standard for osteochondrosis.

The diagnostic accuracy (observed agreement) of a test can also be evaluated as the proportion of all tests with the correct results, the true osteochondrosis (true positive tests) and true non-osteochondrosis (true negative tests) on all the tests that were done. The diagnostic accuracy when the OPOLS system in this research was validated, were 79.9% against method X, 80% against method P, and 79.9% against method XP. This indicates a good relationship between the OPOLS, the post-mortem and the X-ray methods, although most results tend to show a slightly better comparison between the OPOLS method and the gold standard post-mortem method.

The number of animals needed to diagnose 10 animals with a positive test for osteochondrosis was 23 if one compares data from the OPOLS system with the post-mortem data, but the number increased to 52 if the X-ray method is used. This low number of animals needed to diagnose 10 positive animals with osteochondrosis with the OPOLS system, when it was compared to the post-mortem method, was another indication that it compared well with the “gold-standard”.

## Discussion

The trials were conducted to determine if the newly developed on-farm visual evaluation method, called the Onderstepoort Osteochondrosis Lesion Scoring System (OPOLS), was accurate to diagnose the prevalence of osteochondrosis in beef cattle to improve animal performance and to

decrease mortalities on farms that are affected by osteochondrosis. Peri-articular swellings of the stifle bone, acute or chronic, are evaluated per leg as an indication of excessive synovial fluid due to inflammation in the joint (Trostle *et al*. 1997, Trostle *et al*. 1998). The unique OPOLS visual system was developed by a group of professionals at the Department of Paraclinical Sciences, University of Pretoria, to enable professionals in the field to not only diagnose osteochondrosis, but also follow trends on a farm or in a group of cattle. No such system was in use or has been validated to use as an on-farm tool other than the methods like radiology, post-mortem or theatre procedures, all very expensive and not practical for commercial farms. The peri-articular swellings are classified with the OPOLS system as either a ball or a plaque swelling (as described by Trostle *et al*. 1998; Bezuidenhout *et al*. 2000).

The system also had to be practical and easy to use, even with animals on extensive farms, due to the limited time in which the evaluator has to score these animals. Some cattle on extensive farms are wild and ferocious when handled in a confined space or crash-pen, which further increased the importance to develop a practical and quick system to evaluate cattle for the prevalence of osteochondrosis.

The purpose of these trials was to validate the OPOLS method against methods like X-rays and post-mortems, used by professionals to diagnose osteochondrosis in cattle. The aim of this study was not to validate the sub-categories of the OPOLS system, but rather to focus on the accuracy to diagnose osteochondrosis with a quick visual on-farm system. The examinations were therefore purely based on “yes” or “no” for animals with or without osteochondrosis on the stifle-bone. However, some professionals do use the OPOLS system with sub-categories and even with categories within the sub-categories as follows:

a. Ball lesions
    i. “Fifty cents” (score 1) 3 - 4 $cm^2$ x 1.5 cm
    (This should not be confused with a pronounced gluteobicep tendon in calves)
    ii. “Golf ball” (score 2) 4 – 5 $cm^2$ x 3 cm
    (Usually this is more readily seen in the flexion of the stifle)
    iii. “Tennis ball” (score 3) 6 - 7 $cm^2$ x 5 cm
    (Usually this is more readily seen in the flexion or extension of the stifle)
    iv. “Large round ” (score 4) Extensive oval to round swelling
    (This almost fills the entire stifle area)

b. Plaque lesions
    i. “Small flat” (score 1) 3 x 2 x 1.5 cm

(This is an oval, flattened/plaque-like swelling)

ii. "Medium flat" (score 2) 8 x 6 x 2 cm

(This is an oval swelling, extending to above the tendon of insertion of the gluteobiceps muscle)

iii. "Large flat" (score 3) 15 x 9 x 3 cm

(This is an oval swelling, extending to above the tendon of insertion of the gluteobiceps muscle)

iv. "Very Large flat" (score 4) > 20 x 12 x 4 cm

(Similar to the large round except that it has a flatter appearance)

These categories with their sub-categories have never been scientifically tested or evaluated for accuracy and therefore cannot be recommended until such time. The main purpose for the sub-categories, when these evaluators use the OPOLS system with the sub-categories, is to follow the size of the peri-articular swelling and the degree of lameness within an animal from one evaluation date to another date, in order to follow the development or degeneration of the specific lesion. This could be helpful, but until these sub-categories are in future scientifically validated, this cannot be supported to be accurate nor recommended. The OPOLS system can therefore only be used as a method based on "yes" or "no" for animals with or without osteochondrosis on the stifle-bone by professionals.

Acute peri-articular swellings are associated with ball shaped lesions, where chronic peri-articular swellings are mostly associated with plaque lesions (Trostle *et al*. 1997, Trostle *et al*. 1998). Lameness is often associated with osteochondrosis (Prozesky *et al*. 2016) and therefore also scored, with the standard one to five locomotion evaluation system (Sprecher *et al*. 1997), as animals walk through the OPOLS evaluation area. This locomotion system (also referred to as laminitis scoring system) classify the cattle's walking ability from normal (score one) to lame (score five) and this information is then also used to manage the health status from one evaluation date to another. All the data on the OPOLS scoring sheet is then analysed and used as management information to adjust the environment, nutrition or treatment strategies for the specific farm or a specific group of animals within a specific cattle herd on a farm.

Animals from herds in the North West province (South Africa) were selected where the prevalence of osteochondrosis in cattle were more common and higher than the norm for Southern African cattle farms since the mid 1980's. The main reason for this was to validate the OPOLS method within a population with more true positives to improve the validation with less cost implications. Using a population with low to no osteochondrosis on the femoro-tibial joint, would have resulted in many more animals being slaughtered to do post-mortems, or X-rayed, and therefore

more dependable on professional resources to be done. The prevalence of osteochondrosis in both cattle trial groups for the X-ray group (method X), the post-mortem group (method P), and the combined X-ray and post-mortem data (method XP) were 83.3%, 80% and 82.2% respectively, with 144 legs validated against the X-ray method and 70 legs validated against the post-mortem method (Table 3.3). This high prevalence rate on these farms confirmed the high mortality and morbidity rates due to osteochondrosis as mentioned by farmers and professionals from that area.

The results of many veterinary studies are published with the aim to establish how much a sickness modifies the physiology and or anatomy in the animal, or how a new treatment altered the prognosis, rather than only the level of the statistical significance (Garner & Altman 1986). The confidence interval is advantageous in studies where a population with a health problem, like osteochondrosis, is small or where the variability of osteochondrosis between different populations is a reality.

The results from this OPOLS validation research indicated a strong agreement between the OPOLS, post-mortem and the X-ray methods. The true-positive animals (with osteochondrosis) with the OPOLS method and then examined with the X-ray, post-mortem, and with the combined data (X-ray data plus the post-mortem data) had a respective agreement of 90%, 85.7% and 88,6%. The 95% confidence interval for the true positive cattle in these trials was high with 83.3 – 94.2%, 74.3 – 92.6% and 83.1 to 92.5% respectively, when the positive identified animals with the OPOLS data were validated against methods X, P and XP. These values are high (Altman *et al*. 1983; Garner & Altman 1986) and indicative that one can use the OPOLS method to identify cattle with osteochondrosis. The highest positive predicted value for animals scored with the OPOLS system was found to be with the post-mortem method with a value of 88.9% in addition to the highest odds ratio of 8.00 for animals with osteochondrosis. This also supports the accuracy of animals found to have osteochondrosis with the OPOLS system when it validates well with the gold standard post-mortem method. The positive predicted values and the odds ratio validated against the X-ray and the combined data were 86.4% and 3.71, and 87.2 and 5.09, respectively and also high.

It was expected to have a lower specificity value in cattle without osteochondrosis due to lesions that were too small to visually identify with a method like OPOLS. This was confirmed with the lower 95% confidence interval for specificity as 14.9 – 49.2%, 32.6 – 78.6%, and 25.6 – 55.3%, for methods X, P, and XP respectively. The amount of accumulated synovial fluid within the stifle-joint are not related to the extent of the osteo-arthritic damage, but it is the reason for the acute peri-articular or chronic peri-articular swelling (unpublished data: Van der Veen) and therefore also a contributory factor to the lower specificity with the OPOLS system against the X-ray method. This

may be overcome with shorter OPOLS examination intervals on farms where mortalities due to osteochondrosis are a problem, to follow the swellings more frequently in order to see if the lesion degenerated or if a lesion developed or appeared since the previous examination.

The prevalence of osteochondrosis for the different legs was also found to be inconsistent between different animal groups and non-specific to one leg side, as seen with the different animal trials in Table 3.4 and this is probably also an indication that osteochondrosis are influenced by external factors. The trial results for the X-ray examined group indicated that animals tend to have osteochondrosis more on the right leg when it was evaluated with the two-times binomial test, but when it was evaluated with alternative statistical models (Table 3.3) one could see that the specificity (true negative rate) with the X-ray methodology was only 14.9 – 49.2%. This confirms the view that X-ray examinations will miss some fibrous connective tissue as well as the accumulated synovial fluid and may not be used as the gold standard for osteochondrosis. Therefore one may argue that the statistical difference in the legs may not be true, using the X-ray method's statistical difference results in this trial. The results from the post-mortem examination indicated no difference between the prevalence of osteochondrosis in the stifle-bone between the left or right leg, and therefore more probable.

The diagnostic accuracy (an indication of the likelihood to make the correct diagnosis) of the OPOLS method was also validated against the other methods and found to be 80 % against post-mortems, 79.9% against X-rays, and 79.9% when the post-mortem and X-ray data sets were combined. These values also indicate a very strong agreement between the OPOLS system and the post-mortem diagnosis, which are the opposite when methods have a poor agreement and closer to the zero value.

The results from this research show a high confidence interval for animals with osteochondrosis that were diagnosed positive with the visual on-farm OPOLS method as validated against the post-mortem and X-ray methods, with the highest observed agreement with the post-mortem diagnosis. This diagnostic accuracy agreement was only 80% and with a 95% confidence interval for sensitivity of 74.3% - 92.6%, professionals may misdiagnose 20% of cattle with osteochondrosis, but the OPOLS method is still within acceptable accuracy according to Garner and Altman (1986) for medical related studies.

The number of animals needed to diagnose 10 animals with a positive test for osteochondrosis was 23 if one compares data from the OPOLS system with the post-mortem data, but this low number

of cattle was probably due to the fact that herds with a high prevalence of osteochondrosis were used. The equation to calculate the number of animals needed to diagnose is:

Number of animals needed to get 10 correct positives = (((1 / ((sensitivity – (1 - specificity))) x 10 = (((1 / ((true positive rate) - (1 – false positive rate))) x 10

One would expect this number of cattle, needed to be evaluated with the OPOLS system, to be much higher when a normal herd (with a low prevalence of osteochondrosis) is evaluated.

## Conclusion

In clinically affected animals, the osteochondrosis was characterized by visible effusions from joints, particularly the stifle joint, and it was also associated with inflammation, pain, and eventually lameness in affected animals. An on-farm method to evaluate the prevalence of osteochondrosis in cattle was necessary to evaluate animals on-farm with a practical and quick method which had to be accurate to diagnose cattle with osteochondrosis.

Understanding the accuracy and the usefulness of such a test in discriminating between several diagnostic possibilities, disease or disease-free, requires diagnostic test results to estimate the disease probability and minimize diagnostic uncertainty (Scott *et al*. 2008).

Such an on-farm evaluation system was developed by a team of scientists of Onderstepoort Faculty of Veterinary Science (University of Pretoria) and called the Onderstepoort Osteochondrosis Lesion Scoring system (OPOLS) which in this study was validated against an X-ray and post-mortem method.

The results from this study indicated that the OPOLS system is very sensitive for cattle with osteochondrosis and correlates well with the pathology of clinically affected animals when a post-mortem or X-ray is done and can therefore be used as an on-farm evaluation tool to diagnose osteochondrosis. The OPOLS system can be used by trained professionals to evaluate cattle on an individual base and for comparisons between examination periods to follow trends or to adjust specific farm protocols with the aim to decrease the prevalence of osteochondrosis. The accuracy of the OPOLS system was also high for animals with osteochondrosis and it can be used as an evaluation tool in further research on animals to better understand the aetiology of osteochondrosis.

Research on osteochondrosis with the assistance of the OPOLS on-farm practical evaluation system needs to be expanded to test some new hypotheses regarding the effect of mineral imbalances, nutritional supplements, phosphorous sources, gender and age, on the prevalence of osteochondrosis. Using the data from this test in trials will help to better understand the high prevalence of osteochondrosis on some farms and to decrease the losses of these farms due to this disease. Further research to validate the accuracy of the sub-categories, within the OPOLS system, may also add value to professionals to evaluate the size of lesions from one evaluation period to another, although one may expect more inaccuracy when more sub-categories are added.

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# Chapter 4

# EVALUATING THE MINERAL PROFILE OF BEEF CATTLE WITH AND WITHOUT OSTEOCHONDROSIS AND THE RESPONSE OF OSTEOCHONDROSIS AFFECTED ANIMALS TO A SHORT TERM MINERAL SUPPLEMENT.

## Abstract

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Since 1882, the production of beef cattle in the North Western part of South Africa was negatively influenced by mineral imbalances. Some of these imbalances resulted in Aphosphorosis and Vryburg hepatosis and since 1982 also osteochondrosis, which prevail in all classes of commercial and non-commercial cattle - male, female, different breeds and in all age classes - with huge financial implications. To study the success of mineral supplementation to rectify osteochondrotic cases, 33 cattle were used in a trial to compare the mineral status of rib bones and livers from osteochondrosis affected animals (n=18, group O), osteochondrosis affected animals fed for three months with a mineral supplement (containing also protein and energy) (n=5, group S), and healthy animals (n=10, group C). Group O were fed on good quality hay and necropsied within 14 days after arrival at Onderstepoort. Group S was randomly selected from the 23 osteochondrosis affected cattle (identified with the OPOLS method) from farms in the North West Province, and fed for 90 days with a energy-protein-mineral supplement at 1500 g per animal per day as well as *ad libitum* with *Eragrostis curvula* hay The supplement added 15 g P, 38.4 g Ca, 4.9 g Mg, 360 mg Zn, 98 mg Cu, 3.3 mg Co, 3.3 mg I, 225 mg Mn, 1.13 mg Se, and 143 mg Fe) to their daily diet. Group S also received an additional phosphorous supplement during the first 30 days of the trial which supplied 4.8 g P and 8 g Ca per animal per day. Group O cattle were lower in dry fat free bone ash Ca ($p=0.001$) and P (p=0.043) as well as dry liver Mn (p=0.001) compared to healthy animals. Group O cattle also had higher dry rib bone Cu, Mn and Hg, as well as dry liver Cu, Zn and Pb than group C ($p<0.001$). The mean P per volume ($cm^3$) fresh rib bone was not different for groups O, S or C, but group O had a high prevalence of animals with low P per volume. The results showed that by feeding osteochondrosis affected cattle with a mineral supplement for three months additional to their roughage and water intake, decreased the dry fat-free ash rib bone calcium, dry fat free rib bone zinc, bone density, the ratio of calcium to magnesium, and the liver zinc and lead, compared to the levels of affected animals not supplemented. It can be concluded that osteochondrosis affected cattle differ in liver and bone mineral status from healthy animals, and that a shift in mineral status occur when sick animals are supplemented with a mineral supplement for 3 months.

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# EVALUATING THE MINERAL PROFILE OF BEEF CATTLE WITH AND WITHOUT OSTEOCHONDROSIS AND THE RESPONSE OF OSTEOCHONDROSIS AFFECTED ANIMALS TO A SHORT TERM MINERAL SUPPLEMENT.

## Introduction

Many beef animals in the North Western part of South Africa were negatively influenced since 1882 due to mainly mineral imbalances. Some of these imbalances resulted in the prevalence of Aphosphorosis, Vryburg hepatosis and since 1982 also osteochondrosis. Typical manifestations in animals were rickets and osteomalacia during 1882 and 1912, followed by the Vryburg hepatosis in young calves reared on manganese-rich soils, and since 1982 also visible lesions caused by synovial fluid effusing from the stifle bone into the surrounding tissue which creates a "ball or plaque" looking lesion and after many post-mortems, called osteochondrosis (Elsenbrook & Neser 2002; Neser *et al*. 1997; Prozesky *et al*. 2010; Theiler 1912).

In South Africa this illness was originally diagnosed in a few show animals in the Northern Cape and North West regions, but there is more evidence that osteochondrosis is prevalent in many areas in Southern Africa and more frequently since the mid 1990's in all classes of commercial and non-commercial cattle (Prozesky *et al*. 2010). Commercial farmers from the North West province have brought the negative impact of osteochondrosis under the attention of many agricultural institutions since 2003, in an effort to get more research done on this new phenomenon and to find answers as to why this prevail and how to prevent or limit the occurrence of the syndrome.

Many farmers were desperate to get more information on how to prevent or treat osteochondrosis in order to decrease their financial losses, because the only solution to this health problem at the time of the project was to slaughter the sick animals. These losses were not only due to higher animal mortalities or lowered production performances, but it also included long-term losses, because many high merit genetic animals were also slaughtered. Farmers, feedlots and veterinarians do not always report data from osteochondrosis affected animals or farms, and therefore no history or facts could be used to assist the research group to identify possibilities associated with the prevalence of osteochondrosis.

This disease is difficult to understand due to the multifactorial aetiology, which may include growth rate, heredity, gene expression, trauma, nutritional factors, anatomical characteristics, and a

defect in vascular supply to the epiphyseal cartilage (Corbellini *et al.* 1991; Ekman & Carlson 1998; Grøndalen 1974; Hill *et al*. 1998; Ollson 1987; Trostel *et al*. 2002; Woodard 1997; Ytrehus *et al*. 2007).

The initial thought was that the manifestation of osteochondrosis on the stifle joint was probably associated with all the mineral imbalances in the North West region, due to all the various bone dystrophies and other syndromes in cattle from this area. Another important aspect in the area was the low phosphorous levels in the soil and plant material and the question was if this plays a role as initiating factor for the disease in the cattle. Some farmers also believed that the health disorder was only associated with Bonsmara (breed) bulls in intensive feeding programs, and that high energy rations together with specific genes and male hormones created the lesions. This was later found to be purely speculative after animals from other breeds, gender and farming systems were also diagnosed with osteochondrosis at various ages since 1982 (Prozesky *et al*. 2010).

The nutrient and mineral supplementation by farmers in South Africa were also inconsistent and together with the genetic selection for higher production and the changes in farm calving practices, like all year calving instead of seasonal calving, created an inadequate supply or intake of bio-available minerals to the animals. The higher growth rates of the calves as well as the higher milk production of the cows, changed the nutritional requirements of animals, but these new requirements were not always fulfilled with changed supplementation strategies or pasture management practices.

Forages in natural extensive pasture grazing systems in Southern Africa do not always meet the requirement of animals and their mineral requirements can only be met through extra supplementation practices. Minerals that are generally deficient in these pastures on Ca-rich soils are phosphorous (P), magnesium (Mg), potassium (K), sodium (Na), cobalt (Co), selenium (Se), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), and iodine (I)(Engels 1981; NRC 2016). Engels (1981) also described differences in animal performance reponses to P supplementation in Ca-rich soils. The level of mineral deficiencies is geographical specific and the level of minerals and vitamins in the roughages changes across and within seasons, which affect the animals according to their specific requirement for the production stage. Summer pastures in South Africa are generally deficient in P and Na, and natural pastures in areas like the North West province are specifically well-known for their low P levels. These mineral deficiencies created many pathologic conditions in beef cattle in the North West province of South Africa, like rickets, osteomalacia, Vryburg hepatosis, and Aphosphorosis (Prozesky *et al*. 2010).

Supplements in Southern Africa are formulated by professional animal nutritionists to meet the cattle requirements according to the age of the animal, their production and reproduction stage, the quality and quantity of the pastures, and the production and management system. The daily requirement of animals is then met by formulating a supplement with raw materials based on their bio-available nutrient levels, and on the animal's total daily pasture and water intake during a specific season. The minerals in the water of a specific camp do play an important role and are therefore analyzed and then used in the total supply and requirement calculation to meet the animal's daily requirement. The challenge with supplements in natural pasture systems is the variability in daily supplement intakes between animals and different farms, which are influenced by many pasture related factors like, nutrients levels, quality, palatability, season and many more. The supplement also contains some supplement related properties, which will affect the animal's daily supplement intakes, such as the nutrient level, the type and chemical form of raw materials used, the palatability of raw materials, salt level, and the bio-availability of the raw material sources. The type of raw materials in these supplements also have an effect on the animal's pasture intakes (substitution rate) and rumen function (Köster *et al*. 1996; Le Roux *et al*. 1999). All these mentioned factors make it more complex to calculate a diet due to the animal, the diet and the pasture's effect on nutrient intake and rumen function and its effect on animal performance and health.

The lack of information associated with this new health disorder, osteochondrosis in the cattle, from affected farms and professionals involved working on these farms in Southern Africa, initiated the research process to evaluate the mineral levels in these cattle in a trial to compare the mineral status of animals with or without osteochondrosis. This trial was used as a "pilot" project to start the long term project with the aim to treat clinical cases or to prevent osteochondrosis in cattle.

The financial losses due to osteochondrosis steadily increased since the mid 1990's and in 2004 farmers from the North West province decided to donate 24 clinically affected (osteochondrosis) cattle from different age and gender groups and a trial was started and managed by the Veterinary Faculty of the University of Pretoria on the farm, Kaalplaats. Another 10 unaffected animals were donated as the control group, 5 cattle from the North West Province Department of Agriculture and 5 cattle from Kaalplaats, nearby Onderstepoort (Pretoria, South Africa).

## Materials and methods

### Experimental design and animals

Twenty-three osteochondrosis affected animals were used in this trial together with the 10 unaffected animals as the control animals to do mineral analysis on their liver and rib bone samples, to compare the mineral profiles of animals with and without osteochondrosis (for details regarding the methodology on tissue and bone analysis see Appendix 1). The 24 osteochondrosis affected animals were donated by farmers from herds known for their high prevalence of osteochondrosis, of which only 23 were used in the trial within the affected groups.

The 33 animals were divided into 3 groups, namely:

- The **affected group** consisting of 18 osteochondrosis affected animals (**group O**).
- A **feeding group** consisting of 5 osteochondrosis affected animals were fed a mineral supplement for three months. These animals were randomly selected from the 23 osteochondrosis affected animals (**group S**). The main aim in this trial was to compare the mineral status of osteochondrosis affected animals with the mineral status of healthy animals, and to also see if supplementation plays any role in the affected animals. This was done with only 5 affected animals as a preliminary trial for the Arthrosis team's trials to follow.
- The **control group** consisting of 10 unaffected healthy animals (**group C**).

The 18 affected animals were fed a good quality hay (Eragrostis curvula) and were then necropsied over a two-week period, after arriving at Onderstepoort. Fluid samples from the joints of all the animals were tested for bacteria and mycoplasmas to ensure that animals, with or without osteochondrosis, were not negatively affected in their joints due to any bacterial or mycoplasma infection. The animals were all evaluated by Pathologists from Onderstepoort for lesions associated with musculo-skeletal abnormalities to ensure that only animals diagnosed with osteochondrosis were used as affected animals in the affected and feeding groups. The necropsies were done by Dr. J. Neser and Prof. L. Prozesky, both from the Department of Paraclinical Sciences at the Faculty of Veterinary Science at the University of Pretoria, South Africa.

The feeding group of five animals where fed on a research farm, Kaalplaats, close to Onderstepoort (Pretoria, South Africa) for the three-month period (3 August until 8 November), using the following diet:

- A winter supplement (high in minerals and vitamins and manufactured by a commercial feed manufacturer as per Table 4.1) was daily fed at 1.5 kg per animal per day plus *E. curvula*, fed on an *ad lib* basis. The supplement supplied 15 g P, 38.4 g Ca and 4.9 g Mg per animal per day additional to the grass and water supplied nutrients.

- The five animals also received 80 grams per day of an additional P6 phosphorous supplement (4.8 g P and 8 g Ca / animal / day) during the first 30 days of the trial.
- Both supplements were daily fed to animals on a group basis in feed troughs and therefore not individually to simulate typical on-farm practices.

Table 4.1 contains the detail of the winter supplement that was fed to the feeding group of animals at 1500 g per animal per day with the *E. curvula. E. curvula* is a typical roughage source on many beef farms in South Africa and were therefore used in this trial. The *E. curvula* bales used in the trial were not analysed, but a typical chemical analysis (from a commercial feed analysis laboratory) of this perennial grass of the Central Highveld area is shown in Table 4.2. The *E.curvula* used in this trial was sourced from the Central Highveld are and not from the P depleted North West province.

**Table 4.1** A summary of the supplement mix used in the feeding group with the nutrient levels of the supplement

| **Commercial winter lick/supplement** | |
|---|---|
| **Raw materials in product** | **kg / ton of feed** |
| Processed sunflower oil cake meal† | 85 |
| Sunflower oilcake meal, >36% CP | 260 |
| Defatted maize germ meal | 75 |
| Soya hulls | 86 |
| Wheaten bran, 16% CP | 20 |
| Molasses, sugarcane, >78% Brix | 110 |
| Urea (287% CP) | 41 |
| Ammonium sulphate | 30 |
| Limestone | 67 |
| Mono ammonium phosphate | 24 |
| Sodium chloride, coarse | 200 |
| Winter mineral and vitamin premix ‡ | 2 |
| **Total, kg** | **1000** |
| **Analysis (on "as fed" basis)** | **%** |
| Dry matter | 90.5 |
| Crude protein | 32 |
| Non-protein nitrogen, % of crude protein | 53.8 |
| UIP as % of CP (NRC) | 20.3 |
| Crude fat | 1.2 |
| Crude fibre | 8.5 |
| Calcium | 2.56 |
| Phosphorous | 1 |
| Magnesium | 0.33 |
| Sulphur | 1.19 |

† Processed with bypass enhancing technology to increase the raw material's UIP level to minimum 75 % of crude protein. ‡The premix contained only inorganic minerals (g mineral per premix pack) at the following level per 2kg pack: Zn = 240 g, Copper = 65 g, Cobalt = 0.65 g, Iodine = 2.2 g, Manganese = 150 g, Selenium = 0.75 g, Iron = 95 g ; CP = crude protein; UIP = rumen undegradable protein. Fed at 1500 g per animal per day with *ad lib E.curvula* hay

**Table 4.2** The analysis of *Eragrostis curvula*, grown in the Central Highveld area where the trial was done

| ***Eragrostis curvula*, typical chemical analysis** | |
|---|---|
| **Nutrients** | **g/kg dry matter basis †** |
| Dry matter | 88 |
| Crude protein | 74 |
| Acid detergent fibre | 485 |
| Neutral detergent fibre | 723 |
| Lignin | 64 |
| Sugar | 17 |
| Ash | 71 |
| Calcium | 2.3 |
| Phosphorous | 1.1 |
| Potassium | 13 |

† Results summary from the FeedFirst (Pretoria, South Africa) laboratory data base (2010 - 2016) of *E. curvula*

**Measurement summary:**

- The feeding group received their measured intake of 1 500 g of winter supplement daily with *ad libitum E. curvula*.
- Minerals were analysed at the Nutrilab of the University of Pretoria on liver and rib bone samples of all the animals.
    - 18 osteochondrosis affected animals were sampled at necropsy within the first 2 weeks after they arrived at Onderstepoort from the different farms that donated animals.
    - The 5 osteochondrosis affected animals on the trial feed were sampled at the end of the 3 month trial period.
    - The 10 healthy control animals were sampled at necropsy.

- Bone and liver samples for micro- and macro-mineral analysis.
    - Rib bone samples have been collected from all the animals and these samples have been analysed for the following minerals: Ca, P, Mg, Bone ash, Bone ash Ca, Bone ash P, Cu, Zn, Mn, Hg, F, and Fe. Phosphorous density tests were also done to compare the level of P between the different treatments. These analyses were used to compare the 18 affected animals with the 10 non-affected control animals and the 5 animals that were supplemented for the three months.

- Some heavy metals analysis were included in this project to evaluate the possibility of heavy metal contamination due to the mining activities in the area where the first incidences of osteochondrosis in cattle were observed.
- The 15cm rib bone samples were collected from the last rib on the left hand side of all the animals
- Liver samples have been collected from all the animals and these samples have been analysed for the following minerals: Cu, Mn, Fe, Zn and Pb. These analyses were used to compare the 18 affected animals with the 10 non-affected control animals and the 5 osteochondrosis affected animals that were supplemented for three months. (for details regarding the methodology on tissue and bone analysis see Appendix 1).

- Fluids from the joints were analysed for bacteria and mycoplasma at necropsy.

**Pathological examination of donated and control animals**

In both the affected and control groups, the same clinical and analytical observations were made on all the animals (n=34). This was done by two pathologists, Prof. L Prozesky and Dr. J Neser and all musculo-skeletal abnormalities were reported. The data used in this experiment was only from animals which tested negative for bacteria and mycoplasma during necropsy. One animal was removed from the trial due to a positive test for bacteria and mycoplasma and another animal with abnormal high mineral values in the rib bone and liver samples. Only 5 of the 10 control animals and 9 of the 18 osteochondrosis affected animals were tested for liver Pb to evaluate Pb toxicity.

**Statistical design and analysis**

**4.1. Data analyses**

The animals were not compared in a randomly designed study, but rather in a group analysis. The Generalized Linear Model (GLM) on GenStat release 17.1 was used to test the statistical significance of deviation from the theoretically expected distribution of observations into two possible outcomes. Fitting data from all the distributions, apart from the “Normal”, is complicated by the fact that their variances change according to their means. The GLM extend the usual regression analysis to cater for a non-normal distribution, like in this trial, in comparison to the ANOVA regression analysis which caters for trials with a “normal” distribution. GLM also incorporates a link function that defines the transformation required to make the model linear (Payne 2014). This model was used for some of the analyses, such as the liver and bone data that did not agree with the assumptions for normality and

homogeneous variances to test for differences between the three treatment effects. The treatments were separated using the Fisher's protected least significant different test (FPLSD) at a 5% significance level (Snedecor & Cochran 1980). Where applicable, $P \leq 0.05$ was accepted as being significant.

### 4.2. Hypothesis

($H_0$): There is no difference in the liver and rib bone mineral status in cattle with osteochondrosis or ostechondrosis affected animals that receive short term mineral and vitamin supplements compared to healthy cattle.

($H_1$): The mineral status of osteochondrosis affected animals differ from healthy animals or osteochondrosis affected cattle which receives a short-term mineral and vitamin supplement.

## Results and discussion

The 33 cattle were divided into the three groups and will be discussed as the **affected group (O)**, the **feeding group (S)**, and the **control group (C)** with all the results summarized in Tables 4.3, 4.4 and 4.5.

### Mineral levels in the rib bone samples

The average mineral concentrations from the cattle in these trials are summarized in Table 4.3 for the rib bone samples within the different groups. The density and the concentration of phosphorous per volume rib bone for the different cattle groups are summarized in Table 4.4.

**Table 4.3** The mineral analysis of the rib bone samples of cattle with osteochondrosis (affected), healthy animals (control) and osteochondrosis affected animals that were fed a vitamin and mineral supplement mix for three months with *E. curvula ad lib*

| Response variate, bone samples | Animal treatment groups | Mean | SEM | n | Control, *p value* | Affected, p value | F pr test per variate |
|---|---|---|---|---|---|---|---|
| Dry fat-free Ca, % | Control | 24 [a] | 0.28 | 8 | | | |
| | Affected | 23 [b] | 0.19 | 17 | 0.007 | | F pr = <0.001 |
| | Feeding | 21 [c] | 0.31 | 5 | < 0.001 | < 0.001 | |
| Dry fat-free P, % | Control | 11.2 [a] | 0.14 | 8 | | | |
| | Affected | 10.7 [b] | 0.09 | 17 | 0.016 | | F pr = 0.016 |
| | Feeding | 10.5 [b] | 0.17 | 5 | 0.009 | 0.321 | |
| Dry fat-free Mg, % | Control | 0.25 [b] | 0.01 | 8 | | | |
| | Affected | 0.25 [b] | 0.01 | 17 | 0.861 | | F pr = 0.030 |
| | Feeding | 0.30 [a] | 0.02 | 5 | 0.028 | 0.008 | |
| Dry fat-free Ca : Mg | Control | 96.5 [a] | 4.99 | 8 | | | |
| | Affected | 94.6 [a] | 3.35 | 17 | 0.751 | | F pr = 0.001 |
| | Feeding | 69.9 [b] | 4.57 | 5 | 0.001 | 0.001 | |
| Dry fat-free bone ash, % | Control | 63.28 | 0.75 | 8 | | | |
| | Affected | 63.01 | 0.51 | 17 | 0.774 | | F pr = 0.079 |
| | Feeding | 60.70 | 0.91 | 5 | 0.04 | 0.039 | |
| Dry fat-free bone ash Ca, % | Control | 37.9 [a] | 0.30 | 8 | | | |
| | Affected | 36.5 [b] | 0.20 | 17 | 0.001 | | F pr = 0.001 |
| | Feeding | 34.5 [c] | 0.35 | 5 | <0.001 | <0.001 | |
| Dry fat-free bone ash P, % | Control | 17.7 [a] | 0.19 | 8 | | | |
| | Affected | 17.1 [b] | 0.13 | 17 | 0.013 | | F pr = 0.043 |
| | Feeding | 17.3 [b] | 0.24 | 5 | 0.318 | 0.289 | |
| Dry fat-free bone ash Mg, mg Mg/g bone ash | Control | 4.01 [b] | 0.21 | 8 | | | |
| | Affected | 3.95 [b] | 0.15 | 17 | 0.83 | | F pr = 0.018 |
| | Feeding | 4.94 [a] | 0.33 | 5 | 0.02 | 0.005 | |
| Dry fat-free Cu, mg/kg | Control | 2.10 [b] | 0.15 | 5 | | | F pr = < 0.001 |
| | Affected | 3.81 [a] | 0.15 | 17 | <0.001 | | |
| Dry fat-free Zn, mg/kg | Affected | 101 [a] | 7.18 | 17 | | | F pr = <0.001 |
| | Feeding | 51.0 [b] | 6.71 | 5 | | 0.002 | |
| Dry fat-free Mn, mg/kg | Control | 1.91 [c] | 0.16 | 8 | | | |
| | Affected | 3.41 [b] | 0.20 | 17 | <0.001 | | F pr = <0.001 |
| | Feeding | 12.6 [a] | 1.35 | 5 | <0.001 | <0.001 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Dry fat-free Hg, µg/kg | Control | 0.40 [b] | 0.02 | 3 | | | F pr = <0.001 |
| | Affected | 16.8 [a] | 0.37 | 16 | <0.001 | | |
| Dry fat-free F, g/kg | Control | 0.92 | 0.28 | 8 | | | F pr = 0.084 |
| | Affected | 0.59 | 0.12 | 17 | 0.209 | | |
| | Feeding | 0.28 | 0.11 | 5 | 0.088 | 0.196 | |

SEM = standard error of the mean; n = number of animals used; DM = dry matter. [abc] Different superscript in the same variate means a difference if $p < 0.05$, then F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used). Control = healthy animals **(C**), Affected = osteochondrosis affected animals **(O**), Feeding = osteochondrosis affected animals fed for 3 months with a supplement **(S)**

The osteochondrosis affected animals (group O) in this research project have shown significantly lower rib bone mineral levels compared to the healthy cattle (group C) for dry fat-free Ca (23 vs. 24%, $p$=0.007*)*, dry fat-free P (10.7 vs. 11.2%, $p$=0.016*)*, dry fat-free bone ash Ca (36.5 vs. 37.9%, $p$=0.001) and dry fat-free bone ash P (17.1 vs. 17.7%, $p$=0.013). The mean values of group C and O tested within the published "normal" ranges for dry fat free rib bone ash Ca in cattle within 36 to 39.6%, but the group S tested lower and significantly different to groups C and O (Field 2000; McDowell 1997; Pfeffer & Hristov 2005; Underwood & Suttle 1999). All three groups tested within the published normal range of 17 to 18% for dry fat-free bone ash P as summarized in Table 4.6. The results from this trial also indicated a lower dry fat-free Ca percentage (21 vs. 24 vs. 23%) as well as a lower dry fat-free bone ash Ca percentage (34.5 vs. 37.9 vs. 36.5%) for group S in comparison to groups C and O respectively.

Group O and C did not differ ($P > 0.05$*)* in dry fat-free Mg (0.25 vs. 0.25%), dry fat-free bone ash (63.0 vs. 63.3%), dry fat-free bone ash Mg (0.40 vs. 0.40%), or dry fat-free F (0.59 vs. 0.92 g/kg). The mean values for both groups C and O were similar, but different to group S, although all (C = 0.40; O = 0.40; S = 0.49) were lower than the standard published values of 1 to 1.2% (McDowell 1997; Underwood & Suttle 1999).

Suttle (2010) mentioned a dry fat-free bone Ca to Mg ratio of under 50:1 as an indication that Mg is within the animals required "normal" range. The Ca to Mg ratio within groups O, C and S were higher than the 50:1 ratio with values of 94.6, 96.5 and 69.9 respectively, which indicated a Mg deficiency if based on only dry fat-free bone analyses. Groups O and C were not statistically different for the mentioned ratio, but both were different to group S, which tested lower. The healthy and osteochondrosis affected animals were not different in mean dry fat-free bone ash Mg percentage, but both were significantly lower and different to the supplemented group. Most publications indicate minerals to be more accurate when measured on a dry-fat free ash basis as well as per mg mineral per cubic cm fresh rib bone (Field 2000; McDowell 1997; Pfeffer & Hristov 2005; Underwood & Suttle 1999). Based on the percentage Mg on a dry fat-free ash basis, the results indicated that all groups were low in Mg and therefore it was concluded that the animals were deficient in Mg.

This study also revealed a difference in sick and healthy cattle, with higher mean values for the osteochondrosis affected cattle for dry fat-free Cu (3,81 vs. 2.10 mg/kg), dry fat-free Mn (3.41 vs. 1.91 mg/kg), and dry fat-free Hg (16.8 vs. 0.40 µg/kg) in their rib bone samples.

Calcium and phosphorous are stored in the bone within the animals when the dietary supply is more than the animal's calcium and phosphorous requirement (De Waal & Koekemoer 1997; Read, Engels & Smith 1986; Spangenberg 1997). Cattle also store magnesium, zinc and lead in bones when these minerals are in excess from their feed and water intake. Cattle can only utilize the bone stored magnesium and zinc when they mobilize calcium and phosphorous from the bone tissue. The accumulated bone lead will also be mobilized during the mentioned Ca and P mobilization, which may increase the risk of intoxication and therefore a decrease in normal metal-dependent enzyme functions (NRC 2001). High levels of lead causes for example derangements in porphyrin and heme synthesis, stippling of erythrocytes, microcytic hypochromic anemia, impaired neurologic functions, intestinal pain and colic, abortion and may also interfere with protein synthesis. According to Allcroft and Blaxter 1950, cattle died from lead poisoning if liver lead levels are over 20 mg/kg fresh liver, which are much higher than the levels seen in this trial.

Feeding the osteochondrosis affected animals in this trial with a protein, energy and mineral supplement, with an additional 19.8 g P, 46.4 g Ca and 4.9 g Mg for the first 30 days and 15 g P, 38.4 g Ca and 4.9 g Mg per animal per day additional to the grass and water supplied nutrients for the next 60 days decreased the dry fat-free rib bone Ca, dry fat free Ca to Mg ratio, dry fat-free bone ash Ca, and dry fat free Zn. The supplementation improved their rib bone dry fat-free Mg, dry fat-free bone ash Mg, dry fat-free Mn and Hg, when compared with the mean values of the sick animals in group O. The supplement was formulated based on information from research groups and therefore used as a prototype trial related to previous P related shortages in the province or even micro mineral deficiencies as indicated in other osteochondrosis affected species (De Waal & Koekemoer 1997; Read *et al.* 1986; Spangenberg 1997).

**Table 4.4** The density and the concentration of phosphorous per volume rib bone of cattle with osteochondrosis (affected), healthy animals (control) and osteochondrosis affected animals that were fed a vitamin and mineral supplement mix for three months (feeding)

| | | | | | *p*-value | | |
|---|---|---|---|---|---|---|---|
| **Response variate, Bone samples** | **Animal treatment groups** | **Mean** | **SEM** | **n** | **Control** | **Affected** | **F pr test per variate** |
| P per volume bone, wet mass g | Control | 60.9 [b] | 7.25 | 8 | | | F pr = 0.050 |
| | Affected | 87.9 [a] | 7.17 | 17 | 0.028 | | |
| | Feeding | 69.7 [ab] | 10.5 | 5 | 0.483 | 0.218 | |
| P per volume bone, dry mass g | Control | 50.7 | 6.15 | 8 | | | F pr = 0.089 |
| | Affected | 71.3 | 5.92 | 17 | 0.041 | | |
| | Feeding | 60.3 | 9.25 | 5 | 0.374 | 0.370 | |
| P per volume bone, DM % | Control | 82.2 | 2.42 | 8 | | | F pr = 0.143 |
| | Affected | 79.6 | 1.61 | 17 | 0.373 | | |
| | Feeding | 86.7 | 3.22 | 5 | 0.276 | 0.051 | |
| Volume bone per volume replaced, ml | Control | 43.6 | 5.20 | 8 | | | F pr = 0.127 |
| | Affected | 59.4 | 4.86 | 17 | 0.057 | | |
| | Feeding | 57.0 | 8.60 | 5 | 0.169 | 0.813 | |
| Bone density g/ml | Control | 1.41 [a] | 0.05 | 8 | | | F pr = 0.002 |
| | Affected | 1.49 [a] | 0.04 | 17 | 0.174 | | |
| | Feeding | 1.22 [b] | 0.05 | 5 | 0.017 | 0.001 | |
| % P, dry fat free bone | Control | 11.2 [a] | 0.14 | 8 | | | F pr = 0.016 |
| | Affected | 10.7 [b] | 0.09 | 17 | 0.016 | | |
| | Feeding | 10.5 [b] | 0.17 | 5 | 0.009 | 0.321 | |
| Bone mg P/cm$^3$ fresh rib bone | Control | 129 | 7.14 | 8 | | | F pr = 0.209 |
| | Affected | 128 | 4.87 | 17 | 0.936 | | |
| | Feeding | 111 | 7.81 | 5 | 0.122 | 0.103 | |

SEM = standard error of the mean; n = number of animals used; DM = dry matter. [abc] Different superscript in the same variate means a difference if $p < 0.05$, then F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used). Control = healthy animals **(C)**, Affected = osteochondrosis affected animals **(O)**, Feeding = osteochondrosis affected animals fed for 3 months with a supplement **(S)**

Phosphorous is considered the most commonly deficient mineral in cattle around the world (McDowell 1996). With this in mind and the fact that all the osteochondosis affected cattle for this trial came from an area known for its P deficiency in the pastures, the research focus was towards P and a possible relationship with osteochondrosis in these trial animals. The concentration of P was expressed per unit volume of fresh bone (mg/cm$^3$) and used as a more reliable and sensitive indicator of the P status in cattle (Little 1972; Little & Minston 1977; Read *et al.* 1986; Spangenberg 1997). The adequate level of P in the literature, expressed as mg P/cm$^3$ fresh bone is usually when the concentration of P is higher than 150 mg P per cm$^3$ (Underwoood & Suttle 1999). The mg P per cm$^3$ fresh rib bone results from this study indicated no difference between groups C, O or S with mean values of 129, 128 and 111 respectively. Although no statistical differences were found between the treatment means, the coefficient of variance were 9.06 for group C, 19.5 for group O, and 6.68% for

group S, indicating a higher variability relative to the mean for group O. This was different to the standard error data, which was due to the different sample sizes of the groups. According to Underwood and Suttle (1999), animals are deficient in P if fresh bone contains less than 120 mg P per $cm^3$. Another aspect to mention is the fact that groups O and S have shown a higher percentage of animals with deficient levels of P/$cm^3$ fresh bone. Seven out of 17 animals (41.2%) in group O, 5 out of 5 animals (100%) in group S and 1 out of 8 in group C (12.5%) had fresh rib bone P levels under 120 mg P per $cm^3$. This aspect needs to be further investigated to test the statistical significance of this probability in a homogeneous trial within a population. All groups had a P concentration similar in P per $cm^3$ fresh bone compared to the mineral content of rib bone samples published by Spangenberg (1997) and Read *et al*. (1986) for animals from the Northern Cape region.

This research also revealed no statistical differences in the rib bones of groups C, O and S for the P per volume bone (dry mass), the P per volume bone (DM %), and the volume bone per volume replaced mL. On the other hand, the healthy animals were different to the osteochondrosis affected animals for P per volume bone on a wet mass basis (group C = 60.9 vs group O = 87.9 g wet mass P/vol bone, $p$=0.028*)*. Groups C and O did not differ in bone density (1.41 vs. 1.49 g/ml) but both groups were different to the supplemented group which had a lower mean bone density of 1.22 g/ml ($p$=0.017). Group O was also lower and different to group C for the % dry fat-free bone P (10.7 vs. 11.2%; $p$=0.016).

**Mineral levels in the liver samples**

The average mineral concentrations from cattle in these trials are summarized in Table 4.5 for the livers from the animals used in the trials.

**Table 4.5** The mineral analysis of the liver samples of cattle with osteochondrosis (affected), healthy animals (control) and osteochondrosis affected animals that were fed a vitamin and mineral supplement mix for three months

| | | | | | *p*-value | | |
|---|---|---|---|---|---|---|---|
| **Response variate, liver samples** | **Animal groups** | **Mean** | **SEM** | **n** | **Control** | **Affected** | **F pr test per variate** |
| Liver Cu, mg/kg DM | Control | 125 [b] | 15.2 | 10 | | | F pr = <0.001 |
| | Affected | 283 [a] | 25.6 | 18 | < 0.001 | | |
| | Feeding | 315 [a] | 54.2 | 5 | < 0.001 | 0.675 | |
| Liver Fe, mg/kg DM | Control | 358 [a] | 59.0 | 10 | | | F pr = 0.122 |
| | Affected | 434 [a] | 53.3 | 17 | 0.307 | | |
| | Feeding | 243 [a] | 56.8 | 5 | 0.226 | 0.081 | |
| Liver Zn, mg/kg DM | Control | 114 [b] | 8.53 | 10 | | | F pr = <0.001 |
| | Affected | 145 [a] | 8.08 | 18 | 0.020 | | |
| | Feeding | 80.0 [c] | 8.48 | 5 | 0.018 | <0.001 | |
| Liver Mn, mg/kg DM | Control | 8.16 [a] | 0.79 | 10 | | | F pr = 0.001 |
| | Affected | 5.30 [b] | 0.38 | 18 | 0.001 | | |
| | Feeding | 4.62 [b] | 0.63 | 5 | 0.006 | 0.400 | |
| Liver Pb, µg/kg DM | Control | 0.10 [b] | 0.04 | 5 | | | F pr = <0.001 |
| | Affected | 278 [a] | 82.2 | 9 | 0.022 | | |
| | Feeding | 0.56 [b] | 0.22 | 5 | 0.058 | 0.023 | |

SEM = standard error of the mean; n = number of animals used; DM = dry matter. [abc] Different superscript in the same variate means a difference if $p < 0.05$. F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used); Control = healthy animals **(C)**, Affected = osteochondrosis affected animals **(O)**, Feeding = osteochondrosis affected animals fed for 3 months with a supplement **(S)**

In animals under infectious or non-infectious stress, plasma zinc and iron are normally decreased whereas the plasma copper concentration as well as the zinc levels in the liver are increased (Suttle 2010). The same trend was also seen in this research for liver Zn in the osteochondrosis affected cattle in comparison to the healthy animals, although the same trend was not seen with liver Zn in the sick supplemented cattle or with increased liver Cu levels in the two osteochondrosis affected groups. The animals in group O were significantly higher in liver Zn than groups C and S, with a mean average level of 145 mg/kg DM in comparison to the 114 mg/kg ($p$=0.020) DM for group C, and 80 mg/kg DM of group S ($p$=0.018). The mean average liver Zn for groups S were lower, also significantly different and lower than group C ($p$<0.001).

Working on a dry matter content in cattle livers of 26.7%, as found by Ludwick *et al.* (2008), the adequate level for liver Zn recommended by Puls (1994) is 94 - 375 mg Zn per kg DM. Groups C and O were within the adequate liver Zn level but on the lower end of the adequate scale, but the supplemented group was lower than the adequate level, even after feeding them with 360 mg Zn from the premix additional to the Zn in the raw materials of the supplement, roughage and water. According to Puls (1994), the adequate level of Zn and Cu gives a ratio of almost 1:1 for Cu to Zn in

the liver. Group C, O and S had a Zn to Cu ratio of 0.91, 0.51 and 0.25 respectively. The low ratio of Zn to Cu in groups O and S is an indication that the animals had high Cu levels in the liver in relation to the Zn levels, but this must be further investigated to evaluate the utilization and excretion of Zn in cattle with osteochondrosis.

Another mineral ratio of importance in the liver, is the Zn to Fe ratio. The normal ratio published for the Zn to Fe ratio is 0.94 to 2.5 in cattle (Underwood & Suttle 1999). The Zn to Fe ratio of groups C, O and S in this trial was 0.32, 0.33 and 0.33 respectively, and therefore much lower than the "normal". Iron itself in the liver was not statistically different between the groups, but the levels were higher than the "normal" levels as summarized in Table 4.6. On the other hand, the liver Zn levels differed significantly, with the highest level in the osteochondrosis affected animals and the lowest in the osteochondrosis affected animals fed with a supplement for 3 months.

A small decline in zinc concentrations in most soft tissues is seen when the deficiency develops, with a marked reduction in zinc in the animal's bone, hair, wool and feathers (Underwood & Suttle 1999). Liver zinc in this trial was the highest in the osteochondrosis affected animals but this must be further investigated in animals from the same farm on the same feed and water.

Minerals in animal tissue are susceptible to trauma, fever, stress, infection, inflammation and under such conditions serum Fe declines and serum Cu is raised in cattle. The liver Cu levels in the affected animals, group O and P, did not show a low Cu level and this is therefore an indication that Cu was adequate despite the fact that all these animals were affected by osteochondrosis which normally increases serum Cu and decreases liver Cu levels. The osteochondrosis affected cattle in this trial were therefore not associated with a Cu deficiency as have been seen by other research groups in osteochondrosis affected sheep, cattle pigs, horses and red deer (Audigé et al. 1995; Bridges & Moffit 1990; Thompson 1993).

The higher liver Cu in group O (283 mg/kg DM) and S (315 mg/kg DM) did not differ, but both groups were significantly higher than the control animals (125 mg / kg DM; $p<0.001$). All three groups were within the adequate level range of 75-300 mg/kg DM in liver samples as indicated in Table 4.6 (Pfeffer & Hristov 2005; Puls 1994; Underwood & Suttle 1999). The mean values of the supplemented animals did not show any trend of increased liver copper storage over the three-month period when they were supplemented with additional copper as per Table 4.1.

Groups C, O and S did not differ in mean liver Fe in this trial with values of 358, 434 and 243 mg/kg DM respectively. All these groups were above the marginal band of 100 to 150 mg Fe/kg DM in the liver as indicated in Table 4.6 (Underwood & Suttle 1999).

The liver Mn level was the highest for group C (8.16 mg/kg DM) which was statistically different to the other two groups, O ($p$=0.001) and S ($p$=0.006), with mean values respectively on 5.3, and 4.62 mg/kg DM. Groups O and S did not differ statistically for liver Mn. The mean liver Mn for groups O and S tested lower than the marginal band (5-7 mg Mn/kg DM) for Mn according to data published by Underwood and Suttle (1999), McDowell (1997) and Puls (1994). There was also no statistical improvement in liver Mn levels for the group supplemented for 3 months, which was similar to other published research regarding no improvement in liver Mn levels when animals are supplemented with Mn (Underwood & Suttle 1999).

The mean liver Pb levels of the osteochondrosis affected animals were statistically higher than the control and the supplemented groups. The mean value of Mn in group O was 278 µg/kg DM in comparison to 0.1 µg/kg DM for the control ($p$=0.022) and 0.56 µg/kg DM for the supplemented group ($p$=0.023). Although groups C and S tested much lower than group O, all three groups were lower than the norm of 330 to 1650 µg/kg DM in liver samples and as described by Underwood & Suttle (1999). Group S was fed on different roughages and water supply which could have changed the Pb level in the liver to a similar mean value as group C. All three groups tested lower than the levels associated with toxic levels and could therefore not have been negatively influenced by any mining activity or the dispersal of sewage sludge which normally increases Pb in the soil and plant material. Table 4.6 is a summary of mineral values from the literature for rib bone and liver samples in cattle.

**Table 4.6** A summary of published mineral values in rib bone and liver samples from cattle in different publications (Field 2000; McDowell 1997; Pfeffer & Hristov 2005; Puls 1994; Suttle 2010; Underwood & Suttle 1999)

| Mineral | Standard liver and bone mineral levels according to different publications | | | |
|---|---|---|---|---|
| | Pfeffer & Hristov (2005) | Underwood & Suttle (1999) | McDowell (1997) | Other |
| Dry fat-free Mg, % | | | | Ca to Mg ratio in rib bone of > 50:1 suggests a Mg deficiency (Suttle 2010) |
| Dry fat-free Bone ash, % | > 40 | 48 – 60 | | |
| Dry fat-free Bone ash Ca, % | 37.4 - 39.6 | 36 | 36 | 37 (Field 2000) |
| Dry fat-free Bone ash P, % | 17 - 18 | 17 | 17 | |
| Dry fat-free Bone ash Ca : P | 2.2 : 1 | | | |
| Dry fat-free Bone ash Mg, % | | Marginal : < 1.2 (6 - 12 months of age); < 0.7 (36 months of age) | 1 | |
| Dry fat-free Zn, mg/kg | Marginal = 50 - 71 | Marginal bands = 50 – 70 | | |
| Dry fat-free Mn, mg/kg | | Marginal band = 1.0 - 1.4 | | |
| Dry fat-free F, g/kg | | Normal = 0.4 - 0.7 (2yr old cattle); Normal = 0.7 - 1.1 (4yr old cattle); Normal = 0.65 - 1.22 (6yr old cattle) | | |
| Bone mg P/cm$^3$ fresh rib bone | | 12th rib : Adequate = > 150; Deficient = < 120 | | |
| Liver Cu, mg/kg DM | Marginal = 63 – 191 | Marginal band = 100 - 300; Toxic = > 600 | Critical = 25 - 75 | marginal = 15 -75; Adequate = 75 - 300; Toxic = 750 - 2400 (Puls 1994) |
| Liver Fe, mg/kg DM | | Marginal band = 100 - 150; Hepatoxic effect = > 1000 | | |
| Liver Zn, mg/kg DM | | | | Adequate level = 25 - 100 mg/kg fresh weight (Puls 1994) |
| Liver Mn, mg/kg DM | | Marginal band = 5 - 7.5 | Critical level = 6 | Adequate level = 7.5 – 18 (Puls 1994) |
| Liver Pb, µg/kg DM | | Normal = 330 - 1650; High=2640 - 6600; Toxic = > 24 000 | | High = 6000 - 30000; Toxic = 15000 – 900000(Puls 1994) |

Ca = Calcium; P = Phosphorous; Mg = Magnesium, Cu = Copper, Zn = Zinc; Mn = Manganese; Hg = Mercury; F = Fluorine; Fe = Iron; Pb = Lead; Marginal bands: Mean values for a population sample below the given ranges for more than one criterion indicate probable benefits from the specified mineral supplementation in sufficient individuals to merit intervention. Values below the marginal band indicate high probability of current or future dysfunction and impairment of health or production. Values above the marginal band indicate lower probability or likelihood of supplementation being beneficial although this is mineral dependent and still lower than the toxic level; Hepatoxic effect implies chemical-driven liver damage

**Mineral levels in liver and rib samples**

Some minerals were tested in both the liver and rib bones and therefore it was important to evaluate the shift in mineral levels in both tissues to further investigate the mineral levels between groups O, C and S.

The mean liver (283 vs. 125 mg/kg DM, $p<0.001$) and rib bone (3.81 vs. 2.1 mg/kg dry fat-free mg/kg, $p<0.001$) Cu level was the highest for the osteochondrosis affected animals in comparison to the healthy animals in this research.

Group O showed the highest dry fat-free Zn level in their rib bones (101 vs. 51.0 mg/kg, $p=0.002$) as well as in their livers (145 vs. 80 mg/kg DM, $p<0.001$) in comparison to group S. The Zn level in the bone indicated adequate levels of Zn but the livers of these cattle indicated a lower than adequate Zn for group S when the data was compared to published information as set out in Table 4.6. Animals do not have a well-developed system to store Zn and many short-term studies have shown little change in Zn levels in tissue when the level in the diet changed (Underwood & Suttle 1999). The data in this trial indicated a lower level of Zn in the bone and the liver when Zn was added to the supplement and fed at 360 mg added Zn additional to the Zn in the roughage, raw materials within the supplement and water supply. The levels in this trial in the rib bone sample did not imply a shortage of Zn and therefore could not be associated with skeletal abnormalities as described by Underwood and Suttle (1999). The recommended level for adequate Zn in growing and finishing cattle is 30 mg Zn/kg dietary DM (NRC 2016), which is lower than the supplemental Zn in group S. The lower Zn levels in group S can therefore not be explained but needs to be further investigated in follow-on trials.

The liver Mn levels of group O and S were not statistically different (5.3 vs. 4.62 mg/kg DM, $p=0.400$), but these groups were lower than the healthy cattle (8.16 mg/kg DM, $p<0.01$). On the contrary, these Mn levels differ in the rib bone analysis with group S the highest (12.6 mg dry fat-free Mn/kg, then group O on 3.41 mg dry fat-free Mn/kg, and then the lowest group, group C with a mean rib bone average of 1.91 mg dry fat-free Mn/kg). Many research papers also confirmed the variability in tissue sample results with animals on different Mn concentrations in their diets (Suttle 2010; Underwood & Suttle 1999). Plasma Mn relates more to different Mn intake levels but is difficult to accurately analyze. The results from this trial indicated low liver Mn levels in the supplemented group, although supplemented with 225 mg additional Mn from the premix (additional to the Mn in the roughage sources, other raw materials in the supplement, and the Mn in the water source). The Mn levels in the rib bone samples of all three groups tested higher than cattle published to be marginal in Mn, although the liver Mn for both the osteochondrosis affected cattle and the osteochondrosis

affected cattle supplemented for three-months both tested low to deprived in Mn according to published values for cattle (McDowell 1997; Puls 1994; Underwood & Suttle 1999).

## Conclusions

Osteochondrosis affected cattle were lower in dry fat-free bone ash calcium, dry fat-free bone ash phosphorous compared to healthy animals. These sick animals were also lower in liver manganese in comparison to healthy animals. The dry fat-free rib bone copper, dry fat-free rib bone manganese and mercury, liver copper, liver zinc, and liver lead levels on the other hand were higher in the osteochondrosis affected animals compared to the healthy animals.

Feeding osteochondrosis affected cattle with a supplement for three months additional to their roughage and water intake, decreased the dry fat-free ash rib bone calcium, dry fat free rib bone zinc, bone density, the ratio of calcium to magnesium, the liver zinc and lead compared to the levels of affected animals not supplemented. The supplementation on the other hand increased the dry fat-free rib bone ash magnesium, dry fat-free rib bone manganese and dry fat-free rib bone mercury, in the osteochondrosis affected cattle compared to the sick animals before supplementation.

The mineral differences in these animals and the fact that some minerals did not change in the three-month supplementation period, may be an indication that more research is required to better understand the effect of mineral sources in the supplementation, roughage and water that these animals utilize on farms with incidences of osteochondrosis in cattle. The difference in the rib bone calcium and phosphorous, as well as the calcium to magnesium ratio is also an indication that the intake of such minerals and the form it is supplied to the animals may possibly affect osteochondrosis in these cattle. The phosphorous level per volume bone were not significantly different but the variance as well as the number of animals with low phosphorous per $cm^3$ in the osteochondrosis affected animals indicated that more research needs to be done on phosphorous related aspects. The calcium and magnesium level per volume bone were unfortunately not analysed and therefore no comparisons could be done.

The mineral levels in the rib bone, liver and supplement were used as a starting point for the follow-up trials to better understand the aetiology of osteochondrosis. Research on these minerals as well as the physiology processes involved, needs to be expanded to test new hypotheses regarding the effect of mineral imbalances, possible mineral antagonists, nutritional supplements, nutritional management systems, and phosphorous sources in order to better understand the high prevalence of osteochondrosis on some farms. The effect of supplementation of minerals in the five osteochondrosis affected animals in this trial was only done to compare mineral levels in bones and livers, without

evaluating the effect on the osteochondrosis status of the animals, and therefore no conclusions could be made on the prevalence of osteochondrosis in the supplemented cattle.

This research showed that cattle with osteochondrosis differ in mineral status in their livers and rib bones, but some of these levels as well as the shift in mineral status, especially with supplementation, could not be explained and were different to typical changes as seen in other published research for sick animals.

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# Chapter 5

## MINERAL SUPPLEMENTATION FOR GRAZING BEEF CATTLE: A REVIEW

### Introduction

Supplementing grazing animals with minerals is far more complex than many people realize. A few important factors are summarized in this chapter to better understand the concept of supplementation of additional nutrients to beef cattle while grazing on natural pastures, with specific focus on minerals, animal factors as well as supplement management. The mineral requirements of grazing beef cattle are not always met by the mineral supply of the grazing and these nutrient deficiencies are rectified through the supply of a free-choice supplement in many farming systems (McDowell 1996). The nutrients in these supplement programs are calculated on the assumption that all animals consume the same quantity of supplement and with intakes close to targeted or formulated quantity, without considering the fact that some individuals vary their intakes (Bowman & Sowell 1997). Some animals in a group will therefore consume more and others less than the expected intake, which may affect their individual production performance (De Waal, Randall & Koekemoer 1996; McDowell 1996, Spangenberg 1997).

It is important for professionals to fully understand all aspects which might affect the way in which animals respond when they receive supplements. The inconsistent intakes by cattle were summarized by McDowell (1996) as being influenced by factors like:

- Season of the year
- Soil fertility and the type of forage
- Availability and quality of the forage
- Energy and protein of the diet
- Animal requirement (production stage, production level, reproduction, and many more)
- Salt content of the water and the supplement
- Palatability of the minerals used in the supplement as influenced by source
- Availability of fresh minerals
- Physical form of minerals (organic vs. inorganic)

The main source of dietary minerals for cattle is from their roughage intake, although some minerals will be supplied from their water and soil intake (McDowell 1996). The level of minerals in plants is related to the mineral status of the soil they grow in, as well as factors like soil type, climate,

plant species, crop yields, stage of plant maturity, season and the management of the pastures (McDowell 1996, Reid & Horvath 1980). Specific regions are associated with specific mineral deficiencies in herbivores, which are directly related to the soil characteristics (McDowell 1996). Older more acid, coarse and sandy geological formations are lower in most trace minerals in comparison to young and alkaline formations which contain an abundance of trace elements. Although it is effective to elevate mineral levels in pastures through a fertilizer program, it is not practical or cost effective on extensive grazing. Intensive pasture systems on the other hand, increase grass production per hectare with the application of non-trace element fertilizer (N, P and K), but this commonly creates low levels of Cu, Co, Se, Mn and I in the pasture. Reid and Horvath (1980) indicated that Cu, Se, Zn, Mo, Fe, and Co in most circumstances decline as the plant matures due to the dilution process and the translocation of elements from the leaves to the roots.

McDowell (1996) also indicated that the predominant forage species in an area as well as the plant's leaf to stem ratio are influenced by the climate, forage yield and the pasture management system. These changes will not only change the mineral content of the forages, but it will also change the mineral status in the animals which utilize the specific pastures.

A summary of the beef cattle mineral requirements and toxicities as published in the NRC (2016) is provided in Table 5.1. These requirements are dependent on the stage and level of production and will increase on farms where cattle realize improved growth rates, reproduction and higher milk production. An example of such higher requirements was shown by Underwood (1981) with Zn and Mn in rams during spermatogenesis and testicular development in comparison to their lower requirements for these minerals for growth. Many publications over the last few decades have shown that deficiencies in some trace minerals, such as Se, Zn, Cu and Co, may change immune system components in cattle, with a more negative effect on immune responsiveness and disease resistance, and less to no effect on their growth rates (Spears 2000; Suttle & Jones 1989). Spears *et al*. (1986) also published a study to confirm the same effect where cattle required higher mineral rates for immune responsiveness and disease resistance during a study when cattle were bi-monthly injected with Se and vitamin E where no clinical deficiency was apparent (muscular dystrophy). The results from this study indicated a reduced death loss (4.2% vs 15.3%) from birth to weaning, confirming the reason to suggest that animals may need more minerals for optimal immune responsiveness and disease resistance compared to the NRC's suggested mineral requirements for growth (McDowell 1996). Another important aspect to keep in mind is that many mineral studies were done with controlled rations in dry lots which may not be indicative of cattle's requirements under grazing conditions.

Breed also plays a role in mineral metabolism due to the difference in efficiency of mineral absorption between breeds which has been reported to be 5 – 35% for Mg, 40 – 80% for P, and 2 – 10% for Cu (McDowell 1996). Du, Hemkin and Harmon (1996) also confirmed breed differences in trace mineral metabolism for Cu, Fe and Zn in Jerseys in comparison to Holsteins. Ward, Spears and Gengelbach (1995) showed higher plasma copper concentrations, apparent absorption and retention of Cu in Angus steers in comparison to Simmental steers and an indication of breed differences in gastrointestinal copper absorption. Gooneratne *et al*. (1994) reported that these differences in copper metabolism between Simmental and Angus cattle occur at both the absorptive and post-absorptive level, which resulted in much higher biliary copper concentrations in the Simmental. Littledike, Wittum and Jenkins (1995) compared the copper, zinc and iron status of Angus, Braunvieh, Charolais, Gelbvieh, Hereford, Limousin, Red Poll, Pinzgauer, and Simmental breeds utilizing the same ration and found liver copper higher in Limousin cattle in comparison to the other breeds, except for the Angus. Very little differences were observed in Zn and Fe, except for lower liver Zn concentrations in the Pinzgauer when compared to the Limousin. No breed effects were seen for serum zinc and copper concentrations in this trial.

**Table 5.1** Recommended mineral requirements and toxicities for beef cattle (NRC 2016)

| **Mineral requirements ‡ and maximum tolerable concentrations (dry matter basis) †** | | | | | |
|---|---|---|---|---|---|
| **Nutrient** | **Unit** | **Growing and finishing cattle †** | **Gestating cows †** | **Early lactating cows†** | **Maximum Tolerable concentration†** |
| Calcium | % | as per table 6.1 | as per table 6.1 | as per table 6.1 | (0.02 x DMI) ++ |
| Chromium | mg/kg | | | | 1000 § |
| Cobalt | mg/kg | 0.15 | 0.15 | 0.15 | 25 |
| Copper | mg/kg | 10 | 10 | 10 | 40 ¶ |
| Iodine | mg/kg | 0.5 | 0.5 | 0.5 | 50 |
| Iron | mg/kg | 50 | 50 | 50 | 500 |
| Magnesium | % | 0.10 | 0.12 | 0.20 | 0.40 |
| Manganese | mg/kg | 20 | 40 | 40 | 1000 |
| Molybdenum | mg/kg | | | | 5 |
| Nickel | mg/kg | | | | 50 |
| Phosphorous | % | as per table 6.1 | as per table 6.1 | as per table 6.1 | (0.007 x DMI) ++ |
| Potassium | % | 0.60 | 0.60 | 0.70 | 2 |
| Selenium | mg/kg | 0.10 | 0.10 | 0.10 | 5 |
| Sodium | % | 0.06 - 0.08 | 0.06 - 0.08 | 0.10 | |
| Sulfur | % | 0.15 | 0.15 | 0.15 | 0.30 - 0.50 ¶ |
| Zinc | mg/kg | 30 | 30 | 30 | 500 |
| Aluminum | mg/kg | | | | 1000 |
| Arsenic | mg/kg | | | | 50 (100 from organic forms) |
| Bromide | mg/kg | | | | 200 |
| Cadmium | mg/kg | | | | 0.50 |
| Fluorine | mg/kg | | | | 40 - 100 |
| Lead | mg/kg | | | | 30 |
| Mercury | mg/kg | | | | 2 |
| Strontium | mg/kg | | | | 2000 |

† Adapted from the NRC (2016)
‡ Dietary mineral requirements can be influenced by dietary antagonists
§ Highly dependent on source
¶ Dependant on animal age, diet type, and dietary antagonists. See Mineral Tolerances of Animals (NRC 2005)
++ Is the maximum tolerable concentration of the mineral as recommended by the NRC (2016)

Table 6.1 shows the equations to calculate the Ca and P requirements of beef cattle as recommended by the NRC (2016). These macro mineral requirements are influenced by many factors and this is therefore the main reason for not showing Ca and P values in Table 5.1.

Mineral supplementation is only important if the dietary energy and protein supply to cattle are adequate to support their production stage and productivity. Cattle on pastures will have a lower forage intake if the grazing contains factors like low protein (< 7%) or a high degree of lignification, which will lead to lower mineral consumption and therefore nutrient inadequacies (McDowell 1996).

A strong relationship was also found by Moore and Kunkle (1995) between voluntary forage intake with 58 dried grasses and straws, and the crude protein of the forages, when the crude protein of the forage was less than 7 percent (DM). This relationship was also confirmed in many other research groups (Kartchner 1980; Köster *et al*. 1996; McCollum 1997; Minson 1990).

Some pastures also contain low mineral levels or even mineral antagonists, which will result in a lower supply of absorbable minerals to the cattle utilizing these pastures. Marston and Lusby (1995) have shown that animals eating roughages with less than 65% DM digestibility will decrease their dry matter intake due to the animal's rumen fill factor, therefore space is occupied in the rumen. To supply mineral supplements, when the energy or protein supply is lacking, is not cost effective and will have no purpose and might even have a negative effect, when given to animals that are losing weight (Van Niekerk & Jacobs 1985). It is therefore critical to first optimize the energy, protein and forage intake to grazing cattle before a mineral supplement is fed in order to optimize the performance and health of the animals.

**Supplementation methods**

There are two ways to provide mineral supplements to cattle, the indirect and the direct method. The optimum system to provide supplemental minerals to grazing cattle is to add the deficient minerals to a concentrate containing the other palatable nutrients (e.g. protein, energy) to ensure adequate intakes, but it is not always practical or cost-effective to use. Using mineral-containing fertilizers on pastures or altering the soil pH to encourage the growth of specific pasture species are some of the indirect methods to supply minerals through pasture to cattle. High soil pH changes mineral uptake, like Se and Mo in plants which potentially cause deficiencies like Cu and Co in animals and increases the risk of Se and Mo toxicity (McDowell 1996). These indirect methods are complex soil-plant-climate-mineral interrelationship systems which are not always cost-effective or practical in extensive grazing systems, although very efficient in some intensive grazing systems.

Supplementing minerals to cattle in the drinking water, free-choice supplements/licks, concentrates, oral dosing/drenches, rumen mineral boluses, or injectable products are more cost-effective with some benefits and disadvantages to the animal and the environment. Feeding minerals to beef cattle in soluble forms in the drinking water is very effective, but expensive and therefore not used in extensive farming. The use of oral dosing or the drenching of minerals to cattle are very accurate but too labour intensive. It also creates more stress, due to the increase in handling frequency of the animals. This method has benefits with minerals like Cu and Se which can be stored in the liver, but cannot rectify mineral deficiencies like Co, if the dosing interval is more than 7 to 14 days apart (McDowell 1996). Injecting trace minerals intramuscular have been highly successful to rectify

Cu, Se, I and Zn deficiencies when the injectable sources which are used are slowly absorbed into the tissue. This method of supplementation is very expensive and not always cost-effective in extensive feeding operations. Another disadvantage of injectable minerals is that animals cannot down-regulate their mineral uptake from these muscle-injected minerals and therefore may experience toxicity if they already have an abundance thereof (for example copper).

Lee and Marston (1969) have shown good results in Australia by effectively supplementing Co for more than 5 years to animals with rumen preparations, which stay in the reticulo-rumen, from where the heavy pellets or boluses slowly release the minerals into the gastro-intestinal tract to be absorbed. Other minerals that are effectively supplied through boluses, are Se, Zn, Cu and Mg, with some of the slow release soluble glass-mineral pellet preparations lasting for up to 18 months in the reticulo-rumen (McDowell 1996). This technology is a more expensive form of supplementation, but a more accurate supply of minerals in comparison to the free-choice method, where individual animal intakes are variable and inconsistent.

Cattle in South Africa have been supplemented for years with minerals, as in many other regions in the world, through inexpensive free-choice mineral mixtures. These free-choice mixtures are often also called free-access licks and are mixtures or individual minerals that are supplied to animals on a voluntary intake basis. There is also a perception that animals can choose a specific mineral from a cafeteria system (minerals in individual feeders per mineral) when they are deficient in the specific mineral. McDowell (1996) summarized many publications which have shown that cattle do not have the ability to know which minerals and how much thereof are needed, and therefore they do not have the ability to select “cafeteria-style” supplements (Arnold 1964; Coppock, Everett & Merrill 1972; Gordon, Tribe & Graham 1954; Hutjens & Young 1976; Maller 1967; Muller et al. 1977; Schmidt-Nielsen 1994).

Studies by Theiler, Green and du Toit (1924), Becker, Neal and Shealy (1933), and Bohstedt (1957), on the other hand clearly indicated that P deficient cattle and lambs have the ability to increase their intake of P, by chewing on bones to meet their P requirement. Bone consumption was related to a phosphorous deficiency, as bone intake was abolished after an intravenous infusion of buffered sodium phosphate (Denton *et al*. 1986). Cattle with mineral shortages, like P and Mg, will sometimes have depraved appetites (pica), but with the compulsive craving to eat low P materials like soil, gravel, dirt, and tree bark. In cattle, the craving for P is generally satisfied when they consume aged bones, although the etiology for pica in animals may be multi-factorial and currently not well understood (Firyal 2007). Schmidt-Nielsen (1994) indicated that bone meal consumption is related to olfactory stimuli and that phosphate-deficient cattle did not consume a mixture which contained

unattractive raw materials such as meat, blood and fat. Ashed bones as well as bone meal heated to $500^0$C are also not attractive to P deficient cattle due to its loss of organic attractant.

Cattle will rather select a palatable less nutritive diet above an unpalatable diet containing all the required nutrients, even if that means death (Arnold 1964). The same trend was shown in a study by Gordon *et al.* (1954) where they saw a preference for Ca-carbonate alone, compared to a combined Ca-carbonate and dicalcium phosphate supplement in P-deficient cattle and sheep. The animals in this study developed aphosphorosis due to their P deficiency as a result of their preference to calcium carbonate above the combined P containing mixture.

Coppock *et al*. (1972) has seen the same trend in low Ca and P intake lactating dairy cows which did not consume enough free choice dicalcium phosphate to rectify their deficiencies, as an indication that these cows have no or very limited appetite for Ca or P. Similar results were also seen with dairy cows fed minerals in a cafeteria style, which did not consume the minerals to meet their requirements (Hutjens & Young 1976; Muller *et al*. 1977).

**Supplementation intake variation**

Tait *et al*. (1992) monitored free-choice mixed supplement intakes with an innovative computer system to evaluate individual animal consumption, time and the duration of feed trough visits of Holstein steers (350 kg average) in a 3.25 ha pasture camp. Daily supplement intakes in this trial varied between 60 to 330 g per steer, with 65% of the steers consuming 100 to 250 g per day. On average the steers visited the feed troughs three times per day, with the preferred visit time being late evenings between 20:00 and 23:00. Cockwill *et al.* (2000) reported average daily intakes of 445 g per day of a mineral and molasses supplement, with a range of 0 to 1650 g per day when individual intakes of cows, heifers and calves were evaluated. Bowman and Sowell (1997) reported the percentage of ruminants that does not consume supplements according to feeding method to be 14% for blocks, 23% for liquids, and 15% for dry supplements with an average coefficient of variation for individual supplement intake of 79%, 23% and 41%, respectively. McDowell (1996) on the other hand also indicated 10% lower intakes in cattle when blocks were fed in comparison to dry licks, and therefore the physical structure of a block must be monitored to ensure that cattle are not under- or overfed on blocks. Lower individual intake variation (cv= 31%) was seen in grazing heifers when they were offered a grain-protein cubed supplement in troughs in comparison to the intake when they were given the same quantity of feed in a molasses- urea block (cv = 57%; Kendall, Ducker & Hemingway 1980a). The steers in the same feeding system had an intake variance of 55% for the cubed supplement in comparison to the molasses-urea block with a very high intake variance of 82%.

Medicated molasses blocks gave a coefficient of variation in individual supplement intake of 249% in steers, with 9% non-feeders, and 27% of the cattle that consumed less than the targeted amount (Graham, Pern & Linehan 1977). Seventeen percent of heifers on natural pastures did not consume measurable quantities of a urea-molasses liquid supplement (Nolan *et al.* 1974). This intake variation in grazing sheep has been shown to be as high as 50% for blocks, 33% for dry supplements, and 49% for liquid feeds by Bowman and Sowell (1997). Wagnon (1966) has shown a positive relationship between non-feeding cattle and forage availability, which is important when the grazing system is planned. The high variation in individual supplement consumption and the large proportion of non-feeders under specific conditions, are factors which explain the variation in cattle performance on the same supplements.

Mineral supplement consumption will increase when cattle are on overgrazed or low-quality pastures in comparison to cattle on high quality pastures (McDowell 1996). The increase in consumption will also happen during the winter season (summer rainfall regions) when the forage quality is low. Drought periods will also increase supplement consumption due to low forage quality and poor palatability, resulting in lower forage mineral intakes and a higher mineral supply from the supplement.

Cattle that were left without mineral supplements over long periods of time, will over-consume their daily mineral requirement two- to ten-fold, when they receive supplements after the long no-supplement period, until their appetite is satisfied which may create toxicity risks if this is not managed (McDowell 1996). Mineral supplements may also be exposed to moisture (rain) which increases the risk of mold growth, but this may be prevented with the inclusion of salt in a supplement at a level of 20 to 40%. Livestock often exhibit neophobia when they are exposed to new feeds, which is not always related to palatability (Bowman & Sowell 1997). This is characterized by a low intake period, which increases over a period of time until animals reach a stable intake. This phenomenon plays less of a role if animals were previously supplemented in comparison to inexperienced cattle. This may last up to 14 days in feedlot cattle according to Hicks *et al*. (1990).

Placing the supplement in feeders close to water troughs in their shaded resting areas, and in more than one area in big camps, will improve supplement consumption. Another challenge of supplementation is to keep the supplement feeders filled and not to allow any feeder to become empty, which is a challenge on many big farms. Frequent filling of the feeders will keep the supplement fresh and it will improve the consistency in daily intake. Wagnon (1966) recommended 91 cm feed trough space per cow for grazing beef cows to decrease fighting and dominance/submissive behaviour around the supplement feeder in comparison to animals that were fed with 180cm trough space. The smaller trough space did not allow cows to fight without backing

away from the feeders due to the smaller space, and therefore allowed less cows to be pushed away. Bigger trough space allowed more time for fighting instead of time spend on eating.

Group strategies are also important in providing supplements which are specifically formulated to supply the required nutrients to the animals. More farms are changing their calving season to an "all year calving strategy" without changing their supplements, which then create the opportunity to undersupply or oversupply minerals to some of the animals within a group.

Dew, Stoddard and Bateman (1954) have shown that cattle will consume eight-fold more steamed bone meal when mixed with salt. It is therefore important to include raw materials in the supplement mixture to increase consumption in cattle. Minerals in general (excluding salt) are highly unpalatable and therefore to achieve the formulated consumption, supplements must contain raw materials which improve palatability. Mono-sodium phosphate is as palatable as bone meal, and di-calcium phosphate is more palatable than defluorinated phosphate (Coppock *et al.* 1972; McDowell 1996). Coppock *et al*. (1972) concluded that cattle preferred a low pH supplement (pH 3.5 like di-calcium phosphate) above an alkaline supplement (pH 8.5 like defluorinated phosphate). MgO is highly unpalatable and must be mixed with more palatable ingredients to be consumed at higher levels in Mg deficient cattle, to prevent health disorders like grass tetany as an example (McDowell 1996).

**Supplementation influence grazing behaviour**

Optimal livestock production is influenced by how grazing cattle adjust their grazing behaviour according to the environmental and forage changes on a farm, which is paramount to understand when professionals develop management strategies for supplementation (Krysl & Hess 2011). Grazing behaviour in cattle is influenced by many environmental factors like air temperature, wind velocity, humidity, walking distance, as well as vegetative characteristics within a camp. Cattle and sheep predominantly graze during the day, but when day temperatures are > 25°C, night grazing can increase up to 70% of the total daily grazing time in comparison to minimal night grazing when daily temperatures are below 15°C (Arnold 1981).

The disruption of grazing activities by supplementation regimens could also affect the forage intakes and therefore the animal's performance (Krysl & Hess 2011). In one study by Adams (1985) the results have shown a 11.3% forage intake decrease in the maize-supplemented steers compared to the unsupplemented group grazing Russian wild rye. The supplemented steers differ in total grazing time per 24-h period, despite the fact that they did not graze for 2 to 4 hrs after supplementation. There was also a trend in this study for steers to graze 102 min longer per 24-h period when they were fed at 7:30am in comparison to the steers that were fed at 13:30pm. This study, furthermore, indicated

that steers increased their daily grazing distance from 3.9 km/d to 4.6 km/d (fed am) and 4.2 km/d (fed pm) when they received an energy-containing supplement. Sarker and Holmes (1974) have seen a daily grazing time decrease of 1.5h/d when cows on perennial ryegrass were supplemented with increased levels of concentrates (from 2 kg/d to 8 kg/d). Dunn, Havstad and Ayers (1988) indicated that previous grazing experience can influence grazing behaviour and daily travel distance, with older 5- and 7-yr-old cows walking less (4.6 and 4.2 km/d) than the low grazing experience 3-yr-old cows (5.6 km/d). Caton and Dhuyvetter (1997) on the other hand has shown an increased utilization of grazed roughage when lower levels of energy were supplemented.

Cattle grazing on low-quality forages increased their harvesting efficiency (HE; grams of forage OM intake per kg of body weight per minute spent grazing) from 8 to 60% when they were fed a protein supplement (Barton *et al*. 1992; Hess *et al.* 1994). Köster *et al.* (1996) has shown improved total digestible organic matter intake when a protein supplement was provided to beef cattle on low quality pastures. They also saw an increase in low-quality forage intake when ruminally available N was fed to cattle, but it reached a plateau where after the forage intake declined. The results from this study indicated that 4g total digestible intake protein/kg $BW^{0.75}$ will maximise the total digestible organic matter intake from low-quality prairie hay. This illustrated the importance of a specific winter period strategy to improve the utilization of low quality forages to optimize the production efficiency of cattle. Minson (1990) noted that NPN and true protein sources stimulated forage intake, although the relative response of daily intake was higher with true protein sources compared to NPN sources in the data sets he analyzed.

High starch supplements on the other hand decreased the harvesting efficiency (HE) of cattle as indicated in the study by Adams (1985). Le Roux *et al*. (1999) fed cattle with three different energy supplements on low-quality *Cymbopogon-Themeda* winter veld grass and found the same negative intake response when cattle were fed a starch containing supplement. They also found the highest digestible organic matter intake for cattle when they were supplemented with sucrose in comparison to animals receiving starch, and also somewhat higher with cellulose in comparison to starch (sucrose, cellulose, starch; 36.9, 34.2, 30.8 $g/kg^{0.75}$ DOMI of hay consecutively; $P$=0.09). Merrill and Klopfenstein (1984) have shown that cattle which received energy supplements containing soybean hulls (fibre type) gained weight more efficiently in comparison to cattle which received an energy supplement containing maize (starch type).

Grazing time and forage intake were consistent when forage availability was over 3000 kg/ha, but the rate of consumption decreased four-fold and the grazing time increased two-fold when the forage availability decreased to 500 kg/ha (Allden & Whittaker 1970). The same trend was also reported by Scarnecchia, Nastis and Malechek (1985) who showed that daily grazing time increased

from 380 to 656 min when the forage availability decreased from 919 to 144 kg/ha. Using a rotational grazing system will decrease the cattle's grazing time due to the change in behavioural response with the animal's anticipation of being moved to the new pasture (Walker, Heitschmidt & Dowhower 1989). The cattle in this trial increased their grazing time during the 1st day in the new camp because of exploratory activity which can last from 24 to 72 h (Krysl & Hess 2011).

**Dietary salt affects intakes**

The consumption of mineral supplements in cattle decreases when their drinking water contains high levels of salt (Berger & Rasby 2011). Ruminants have a natural craving for salt, but if their water already contains high levels of salt, then their consumption of the free-choice mineral mixture (containing salt) will decrease due to the fulfilment of salt from their water intake. Supplements in the high salt containing water areas can be reformulated to exclude salt in the supplement with the aim to stimulate supplement intakes through the usage of intake enhancing raw materials like molasses (McDowell 1996).

The craving for salt makes salt a good carrier for other minerals in a cattle supplement on pastures, but regulating supplement intake with salt is not precise, and therefore it is suggested to adjust the salt content throughout the feeding period to achieve the desired feed intake (Berger & Rasby 2011). The voluntary intake of salt in cattle usually exceeds the requirement for Na and Cl due to the salt craving.

Using salt to manage free-choice supplement intake is a management tool on many farms to easily manage the supply of nutrients to ruminants in a "more controlled" system. However, it is very important to have abundant clean water available when salt is used in a supplement to control intake, because it can increase the animal's water intake by 50 to 75 percent. Cattle are very tolerant to high salt intake and therefore salt toxicity is seldom seen. Cattle have the ability to excrete the surplus salt through the kidneys into their urine, but water is necessary for this process. The daily voluntary intake of salt in most cattle classes is approximately 100g per 100 kg body weight (Berger & Rasby 2011).

As a rule of thumb, cattle that are fed salt-containing supplements will increase their water intake with an additional 4.16 liters per 100 g of salt intake (Berger & Rasby 2011). If cattle graze in an area where the water contains high levels of salt (> 5000 ppm total dissolved solids), then one may expect a decrease in supplement intake or a possibility for salt toxicity.

Cattle get accustomed to salt in mixed supplements, and therefore one must adjust the salt levels accordingly, to keep supplement intakes at constant levels. As forage quality changes within a

season, it is not uncommon to change the salt level within a supplement four to seven times during the grazing season. The adjustment of salt in a supplement is influenced by forage intake, the water's salt content, the palatability of the ingredients within the supplement, and the animal's adaptation and craving for salt. Table 5.2 is a summary of salt quantity estimates to limit feed intake in cattle according to their body weights and which can be used with Table 5.3 to formulate salt limited supplements.

**Table 5.2** Estimated salt intake of cattle on a salt limited supplement (adapted from Berger & Rasby 2011)

| Body weight, kg | Salt consumption †, kg / day | | |
|---|---|---|---|
| | Low | Average | High |
| 136 | 0.136 | 0.227 | 0.272 |
| 227 | 0.227 | 0.272 | 0.318 |
| 318 | 0.272 | 0.318 | 0.409 |
| 409 | 0.318 | 0.409 | 0.499 |
| 499 | 0.363 | 0.499 | 0.590 |
| 590 | 0.409 | 0.590 | 0.681 |
| 681 | 0.454 | 0.681 | 0.726 |

† Assumes drinking water is low in total dissolved solids. Based on coarse salt

Calculating the salt inclusion in the supplement for a free-choice supplement for cattle can be done as described by Berger and Rasby (2011) as follows:

a. The first step is to formulate the supplement for the specific group of animals to establish the specific intake of the supplement, excluding the salt. If the mean body weight for the group of animals is for example 499 kg, then the average salt intake is as indicated in Table 5.2, 0.499 kg per animal per day.
b. The second step is to use the salt intake column in Table 5.3 and to go down to the 0.499 kg salt intake value for this example.
c. Salt inclusion will be determined by the formulated intake per animal per day. For example, if a supplement (non-salt feed) was formulated to be fed at 910 g in the above mentioned example, then one moves across the 0.499 salt intake line until one gets to the "non-salt feed" value closest to the calculated supplement intake.
d. The recommended salt inclusion will be established by the "salt inclusion" in the heading, which is 35% for this example.
e. The recommended supplement intake, including the salt, is as per Table 5.3, then the value indicated by "total feed". For this example a supplement with 35 % salt should be fed at a total daily rate of 1.41 kg per animal in the free-choice system.

**Table 5.3** Estimated salt level to include in the mixture for desired intake of non-salt feed for cattle (adapted from Berger & Rasby 2011)

| Salt intake, kg/day | | % Salt in the supplement † | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **6** | **8** | **10** | **12** | **14** | **16** | **18** | **20** | **25** | **30** | **35** | **40** | **50** |
| **0.14** | Total feed | 2.27 | 1.68 | 1.36 | 1.13 | 0.95 | 0.86 | 0.77 | 0.68 | 0.54 | 0.45 | 0.41 | 0.32 | 0.27 |
| | Non-salt feed | 2.13 | 1.54 | 1.23 | 1.00 | 0.82 | 0.73 | 0.64 | 0.54 | 0.41 | 0.32 | 0.27 | 0.18 | 0.14 |
| **0.18** | Total feed | 3.04 | 2.27 | 1.82 | 1.50 | 1.32 | 1.13 | 1.00 | 0,91 | 0.73 | 0.59 | 0.50 | 0.45 | 0.36 |
| | Non-salt feed | 2.86 | 2.09 | 1.63 | 1.32 | 1.13 | 0.95 | 0.82 | 0,73 | 0.54 | 0.41 | 0.32 | 0.27 | 0.18 |
| **0.23** | Total feed | 3.77 | 2.81 | 2.27 | 1.91 | 1.63 | 1.41 | 1.27 | 1.13 | 0.91 | 0.77 | 0.64 | 0.54 | 0.45 |
| | Non-salt feed | 3.54 | 2.59 | 2.04 | 1.68 | 1.41 | 1.18 | 1.04 | 0.91 | 0.68 | 0.54 | 0.41 | 0.32 | 0.23 |
| **0.27** | Total feed | 4.54 | 3.40 | 2.72 | 2.27 | 1.95 | 1.72 | 1.50 | 1.36 | 1.09 | 0.91 | 0.77 | 0.68 | 0.54 |
| | Non-salt feed | 4.27 | 3.13 | 2.45 | 2.00 | 1.68 | 1.45 | 1.23 | 1.09 | 0.82 | 0.64 | 0.50 | 0.41 | 0.27 |
| **0.32** | Total feed | 5.31 | 3.95 | 3.18 | 2.63 | 2.27 | 2.00 | 1.77 | 1.59 | 1.27 | 1.04 | 0.91 | 0.82 | 0.64 |
| | Non-salt feed | 4.99 | 3.63 | 2.86 | 2.32 | 1.95 | 1.68 | 1.45 | 1.27 | 0.95 | 0.73 | 0.59 | 0.50 | 0.32 |
| **0.36** | Total feed | 6.04 | 4.54 | 3.63 | 3.04 | 2.59 | 2.27 | 2.00 | 1.82 | 1.45 | 1.23 | 1.04 | 0.91 | 0.73 |
| | Non-salt feed | 5.67 | 4.18 | 3.27 | 2.68 | 2.22 | 1.91 | 1.63 | 1.45 | 1.09 | 0.86 | 0.68 | 0.54 | 0.36 |
| **0.41** | Total feed | 6.81 | 5.08 | 4.09 | 3.40 | 2.91 | 2.54 | 2.27 | 2.04 | 1.63 | 1.36 | 1.18 | 1.00 | 0.82 |
| | Non-salt feed | 6.40 | 4.68 | 3.68 | 3.00 | 2.50 | 2.13 | 1.86 | 1.63 | 1.23 | 0.95 | 0.77 | 0.59 | 0.41 |
| **0.45** | Total feed | 7.58 | 5.67 | 4.54 | 3.77 | 3.22 | 2.81 | 2.50 | 2.27 | 1.82 | 1.50 | 1.32 | 1.13 | 0.91 |
| | Non-salt feed | 7.13 | 5.22 | 4.09 | 3.31 | 2.77 | 2.36 | 2.04 | 1.82 | 1.36 | 1.04 | 0.86 | 0.68 | 0.45 |
| **0.50** | Total feed | 8.31 | 6.22 | 4.99 | 4.18 | 3.59 | 3.13 | 2.77 | 2.50 | 2.00 | 1.68 | 1.41 | 1.23 | 1.00 |
| | Non-salt feed | 7.81 | 5.72 | 4.49 | 3.68 | 3.09 | 2.63 | 2.27 | 2.00 | 1.50 | 1.18 | 0.91 | 0.73 | 0.50 |
| **0.54** | Total feed | 9.08 | 6.81 | 5.45 | 4.54 | 3.90 | 3.40 | 3.04 | 2.72 | 2.18 | 1.82 | 1.54 | 1.36 | 1.09 |
| | Non-salt feed | 8.53 | 6.26 | 4.90 | 3.99 | 3.36 | 2.86 | 2.50 | 2.18 | 1.63 | 1.27 | 1.00 | 0.82 | 0.54 |
| **0.59** | Total feed | 9.85 | 7.35 | 5.90 | 4.90 | 4.22 | 3.68 | 3.27 | 2.95 | 2.36 | 1.95 | 1.68 | 1.45 | 1.18 |
| | Non-salt feed | 9.26 | 6.76 | 5.31 | 4.31 | 3.63 | 3.09 | 2.68 | 2.36 | 1.77 | 1.36 | 1.09 | 0.86 | 0.59 |
| **0.64** | Total feed | 10.58 | 7.94 | 6.35 | 5.31 | 4.54 | 3.95 | 3.54 | 3.18 | 2.54 | 2.09 | 1.82 | 1.59 | 1.27 |
| | Non-salt feed | 9.94 | 7.31 | 5.72 | 4.68 | 3.90 | 3.31 | 2.91 | 2.54 | 1.91 | 1.45 | 1.18 | 0.95 | 0.64 |
| **0.68** | Total feed | 11.35 | 8.49 | 6.81 | 5.67 | 4.86 | 4.27 | 3.77 | 3.40 | 2.72 | 2.27 | 1.95 | 1.68 | 1.36 |
| | Non-salt feed | 10.67 | 7.81 | 6.13 | 4.99 | 4.18 | 3.59 | 3.09 | 2.72 | 2.04 | 1.59 | 1.27 | 1.00 | 0.68 |

† Assumes drinking water is low in total dissolved solids. Based on the usage of coarse salt

**Dietary cation-anion difference within supplements**

Mineral supplements contain different levels of $Na^+$, $K^+$, $Cl^-$ and $S^{2-}$, which are used to calculate the dietary cation-anion difference (DCAD) of the total diet with the following equation (DeGaris & Lean 2008);

$DCAD = (Na^+ + K^+) – (Cl^- + S^{2-})$ in mEq/kg DM

Low DCAD cattle diets can induce aciduria and metabolic acidosis as reported in many studies on dry pre-calving dairy cows NRC (2001) These cows are fed diets which are low negative DCAD (5 to -100 mEq/kg DM) to enhance Ca status around calving due to the effect of DCAD on the animal's acid-base homeostasis (NRC 2001, Goff & Horst 1997). The primary goal with these late lactation low DCAD diets in late lactation cows is to minimize the risk of hypocalcemia and clinical milk fever around parturition.

The strong ion difference is one of the four factors that regulates blood plasma pH (Van der Veen *et al*. 2017). Strong anions, like Cl and S, can be absorbed in the intestinal tract in higher concentrations compared to strong cations, like Ca, Mg, when salts like ammonium sulfate or high sulfur containing raw materials are fed (DeGaris & Lean 2008; Van der Veen *et al*. 2017). The resulting metabolic acidosis then increases the responsiveness of tissue (for example in bone) receptors to the parathyroid hormone (PTH), which will activate the osteocytes as well as the osteoclasts through osteoclastic activity proportionally as the plasma pH lowers (La Perle & Capen 2006; Arnett 2003). This process increases the mobilization of Ca from bones either in conjunction or apart from PTH, to act as a buffer during acute or chronic metabolic acidosis through the binding of hydrogen ions to carbonate and release the cation salts into the extracellular fluid (Engelking 2011; Green & Kleeman 1991; Horst *et al*. 1997; Van der Veen *et al*. 2017).

Research with low DCAD diets, which created metabolic acidosis, have shown an increase in the prevalence of osteochondrosis in chickens, and in rats it was found that most minerals were reabsorbed from the epiphysis resulting in lower total bone volume in the metaphysis (Kraut *et al*. 1986; Mongin & Sauver 1977; Whitehead 1997). Another very important factor with metabolic acidosis, is the lower reabsorption of phosphorous and an increased reabsorption of calcium in the kidneys as a result of the renal function's response to PTH (La Perle & Capen 2006).

The duration and the severity of metabolic acidosis are not well documented in grazing cattle when supplementation is done with low DCAD supplements. In Southern Africa, the usage of ammonium sulfate as a cost-effective nitrogen source for cattle, the usage of high S containing DCP and MCP, and the additional inclusion of flower of sulphur, increase the total S in the supplements and decrease the DCAD values of supplements. Metabolic acidosis may therefore play a role on farms

where cattle eat large portions of a low DCAD supplement. Water in some areas in South Africa contains high S levels and with supplement feed troughs close to the water troughs, it is not known how the combined effect of a low DCAD and the high S containing water will affect the cattle's plasma pH. This can be evaluated in future supplementation research with cattle.

**Mineral sources and their bioavailability**

Different mineral sources have different bioavailabilities and therefore any value of an element in a feed is of little value to the animal, if the bioavailability of the source is not known. The bioavailability of a mineral is an indication of the mineral portion that an animal utilizes to meet its requirement and must be considered when mineral supplements are formulated (McDowell 1996). The cost-effectiveness of any mineral source in a supplement must therefore be formulated on the basis of bioavailable elements per kg, together with the other beneficial properties (for example palatability, hazards, mix ability, hygroscopic traits, and many more).

All the biochemical processes within the body of an animal require trace minerals for normal functioning and any deficiencies thereof can result in signs associated with clinical or subclinical deficiencies (Spears 2003). Minerals at toxic levels in cattle are not always clinically observed when minerals are consumed at concentrations that are too high as illustrated in Graph 5.1. This can also be true at low and deficient levels when minerals are consumed at concentrations that are too low.

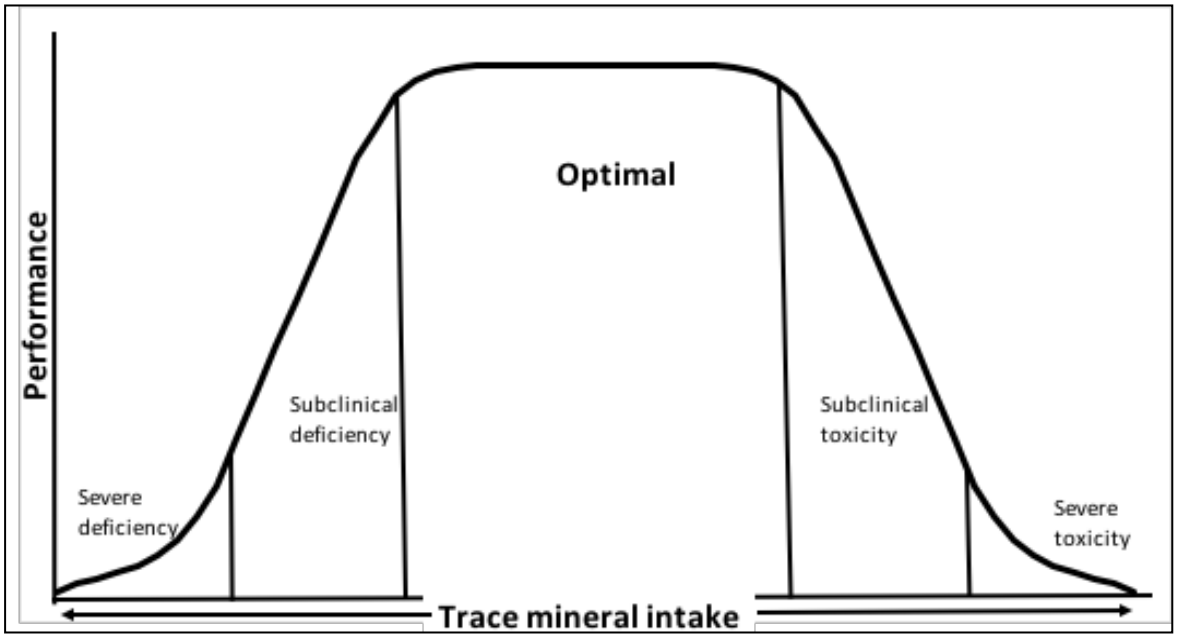


**Figure 5.1** Relationship between trace element intake and the response within beef cattle (adapted from Spears 2003)

Another very important factor to consider when a supplement is formulated, is the potential negative interaction between different minerals (also called antagonists), when they are present in high quantities in the water or the feed sources of cattle (Spears 2003). This so-called mineral antagonistic effect can affect mineral absorption, and therefore the animal's requirements of specific trace minerals. Antagonists can also occur when minerals are in competition for a "transporter" during the absorption process. Typical antagonists are sulfur which negatively impacts on copper absorption and liver copper, the molybdenum-sulfur-copper interaction which negatively impacts on copper absorption, and iron which negatively impacts on the absorption of copper, zinc and manganese (Hansen & Spears 2009; Hansen *et al*. 2010; Spears, Lloyd & Fry 2011; Suttle 2010; Suttle & Angus 1978; Van Niekerk & Jacobs 1985). Iron is in abundance in the southern African raw materials, and therefore important to evaluate when supplements are formulated. Table 5.4 contains mineral values per source as published in the NRC (2016) with an absorption coefficient (AC) of the primary element as published in the NRC (2001). The NRC (2016) published the mineral values with a relative bioavailability for the different trace mineral sources as indicated in Tables 5.5.

**Table 5.4** NRC 2016 values for beef cattle for the mineral content per source with their absorption coefficient per source as published by the NRC 2001 for dairy cattle

| Mineral Element Source | Primary Mineral Element content on dry matter basis (NRC Beef Cattle 2016) † | Absorption coefficient (AC) of primary element (NRC dairy 2001) |
|---|---|---|
| **Calcium sources †** | **Ca (%)** | **AC of Ca source** |
| Bone meal, steamed [fg] | 30.7 | 0.95 |
| Calcium carbonate, $CaCO_3$ [fg] | 39.40 | 0.75 |
| *Calcium chloride anhydrous, $CaCl_2$* [cp] ¶ | 36.1 | 0.95 |
| *Calcium chloride dihydrate, $CaCl_2.2H_2O$* [cp] ¶ | 27.5 | 0.95 |
| Calcium hydroxide, $Ca(OH)_2$ [cp] | 54.1 | 0.55 |
| *Calcium oxide, CaO* [cp] ¶ | 71.5 | 0.50 |
| Calcium phosphate, monobasic, $Ca(H_2PO_4)_2$, from defluorinated phosphoric acid [fg] | 16.4 | 0.95 |
| Calcium sulphate, dihydrate, $CaSO_4.2H_2O$ [cp] | 23.3 | 0.70 |
| Dicalcium phosphate, dibasic, $CaHPO_4$, from defluorinated phosphoric acid [fg] | 22.0 | 0.94 |
| Dolomitic Limestone(magnesium) [fg] | 22.3 | 0.60 |
| Limestone, ground [fg] | 34.0 | 0.70 |
| Magnesium oxide, MgO [fg] | 3.07 | 0.70 |
| Oystershell, flour (ground) [fg] | 38.0 | 0.75 |
| **Phosphorous sources †** | **P (%)** | **AC of P source** |
| Ammonium phosphate (monobasic), $(NH_4)\ H_2PO_4$ [fg] | 24.7 | 0.80 |
| Ammonium phosphate (dibasic), $(NH_4)\ H_2PO_4$ [fg] | 20.6 | 0.80 |
| Bone meal, steamed [fg] | 12.9 | 0.80 |
| Calcium phosphate, monobasic, $Ca(H_2PO_4)_2$, from defluorinated phosphoric acid [fg] | 21.6 | 0.80 |
| Dicalcium phosphate, dibasic, $CaHPO_4$, from defluorinated phosphoric acid [fg] | 19.3 | 0.75 |
| Phosphate, defluorinated [fg] | 18.0 | 0.65 |
| Phosphate rock [fg] | 13.0 | 0.30 |
| Phosphate rock, low-fluorine [fg] | 14.0 | 0.30 |
| *Phosphoric acid, $H_3PO_4$* [fg] ¶ | 31.6 | 0.90 |
| Sodium phosphate (monobasic) monohydrate, $NaH_2PO_4.H_2O$ [fg] | 22.5 | 0.90 |
| Sodium tripolyphosphate (meta- and pyrophosphate) $Na_5P_3O_{10}$ [fg] | 25.0 | 0.75 |
| Soft rock phosphate, colloidal clay [fg] | 9.00 | 0.30 |
| **Sodium sources §** | **Na (%)** | **AC of Na source** |
| Bone meal, steamed [fg] | 5.69 | 0.90 |
| Phosphate, defluorinated [fg] | 4.90 | 0.90 |
| Potassium chloride, KCl [fg] | 1.00 | 0.90 |
| Sodium bicarbonate, $NaHCO_3$ | 27.0 | 0.90 |
| Sodium carbonate, monohydrate, $Na_2CO_3.H2O$ [cp] | 37.1 | 0.90 |
| Sodium chloride, NaCl [fg] | 39.3 | 0.90 |

| | | |
|---|---|---|
| Sodium phosphate (monobasic) monohydrate, $NaH_2PO_4.H_2O$ [fg] | 16.7 | 0.90 |
| Sodium selenate, $Na_2SeO_4.10H_2O$ | 12.5 | 0.90 |
| Sodium selenite, $Na_2SeO_3$ | 26.6 | 0.90 |
| Sodium sesquicarbonate dihydrate, $Na_2CO_3+NaHCO_3.2H_2O$ [fg] | 30.5 | 0.90 |
| Sodium sulfate decahydrate, $Na_2SO_4.10H_2O$ [cp] | 14.3 | 0.90 |
| Sodium tripolyphosphate, meta and pyro-phosphate, $Na_5P_3O_{10}$ [fg] | 31.0 | 0.90 |
| **Chloride sources** [§] | **Cl (%)** | **AC of Cl source** |
| Ammonium chloride [cp] | 66.3 | 0.90 |
| *Calcium chloride anhydrous, $CaCl_2$* [cpg ¶] | 63.9 | 0.90 |
| *Calcium chloride dihydrate, $CaCl_2.2H_2O$* [cp ¶] | 48.2 | 0.90 |
| Cobalt dichloride hexahydrate, $CoCl_2*6H_2O$ [cp] | 29.8 | 0.90 |
| Cupric chloride dihydrate, $CuCl_2.2H_2O$ [cp] | 41.7 | 0.90 |
| Magnesium chloride hexahydrate, $MgCl_2.6H_2O$ [cp] | 34.9 | 0.90 |
| Manganese dichloride, $MnCl_2$ [cp] | 56.3 | 0.90 |
| Manganese chloride tetrahydrate, $MnCl_2.4H_2O$ [cp] | 35.8 | 0.90 |
| Potassium chloride, KCl [fg] | 47.3 | 0.90 |
| Sodium chloride, NaCl [fg] | 60.7 | 0.90 |
| Zinc chloride, $ZnCl_2$ [cp] | 52.0 | 0.90 |
| **Sulfur sources** [†] | **S (%)** | **AC of S source** |
| Ammonium phosphate (dibasic), $(NH_4)\ H_2PO_4$ [fg] | 2.16 | [‡] |
| Ammonium phosphate (monobasic), $(NH_4)\ H_2PO_4$ [fg] | 1.46 | [‡] |
| Ammonium sulfate, $(NH_4)_2SO_4$ [fg] | 24.1 | [‡] |
| Bone meal, steamed [fg] | 2.51 | [‡] |
| Calcium phosphate, monobasic, $Ca(H_2PO_4)_2$, from defluorinated phosphoric acid [fg] | 1.22 | [‡] |
| Calcium sulphate, dihydrate, $CaSO_4.2H_2O$ [cp] | 18.6 | [‡] |
| Cupric sulphate, pentahydrate, $CuSO_4.5H_2O$ | 12.8 | [‡] |
| Dicalcium phosphate, dibasic, $CaHPO_4$, from defluorinated phosphoric acid [fg] | 1.14 | [‡] |
| Ferrous sulphate heptahydrate, $FeSO_4.7H_2O$ [fg] | 12.4 | [‡] |
| Magnesium sulphate heptahydrate, $MgSO_4.7H_2O$ [fg] | 13.3 | [‡] |
| Manganese sulphate monohydrate, $MnSO_4.H_2O$ [cp] | 19.0 | [‡] |
| Manganese sulphate pentahydrate, $MnSO_4.5H_2O$ [cp] | 13.3 | [‡] |
| *Phosphoric acid, $H_3PO_4$* [fg ¶] | 1.55 | [‡] |
| Potassium sulpate, $K_2SO_4$ [fg] | 17.4 | [‡] |
| Sodium sulphate decahydrate, $Na_2SO_4.10H_2O$ [cp] | 9.95 | [‡] |
| Zinc sulphate monohydrate, $ZnSO_4.H_2O$ [fg] | 17.7 | [‡] |
| **Potassium sources** [†] | **K (%)** | **AC of K source** |
| Potassium bicarbonate, $KHCO_3$ [cp] | 39.1 | 0.90 |
| Potassium carbonate, $K_2CO_3$ [cp] | 56.6 | 0.90 |
| Potassium chloride, KCl [fg] | 50.0 | 0.90 |
| Potassium iodide, KI [fg] | 21.0 | 0.90 |

| | | |
|---|---|---|
| Potassium sulfate, $K_2SO_4$ [fg] | 41.8 | 0.90 |
| **Magnesium sources** [†] | **Mg (%)** | **AC of Mg source** |
| Dolomitic Limestone(magnesium) [fg] | 9.99 | 0.30 |
| Limestone, ground [fg] | 2.06 | 0.30 |
| Magnesium carbonate, $MgCO_3$+$Mg(OH)_2$ [fg] | 30.8 | 0.35 |
| Magnesium chloride hexahydrate, $MgCl_2.6H_2O$ [cp] | 12.0 | 0.90 |
| Magnesium hydroxide, $Mg(OH)_2$ [cp] | 41.7 | 0.70 |
| Magnesium oxide, MgO [fg] | 56.2 | 0.70 |
| Magnesium sulphate heptahydrate, $MgSO_4.7H_2O$ [fg] | 9.80 | 0.90 |

[†] NRC (2016); [fg] = Feed-grade source; [cp] = Chemically pure form; [‡] No data available; [§] NRC (2001); [¶] Use with caution when handling and mixing, can be extremely hazardous; Sources should be analyzed or manufacturer's analyses should be used when available. Element composition of a source is listed if specific element concentration is > or equal to 1.0% for macromineral elements, or > 10 000 mg/kg for micromineral elements, except for fluorine concentrations which are not listed because of potential toxicity. The mineral source listed first under each mineral element was generally the standard with which the other sources were compared to establish relative bioavailabilities. The compostions of hydrated mineral sources are shown including the waters of hydration. Mineral element compositions of feed-grade sources vary by source, processing method, site of mining and manufacturer

**Table 5.5** NRC 2016 values for trace mineral content for beef cattle with their relative bioavailabilities (%) per source

| Mineral Element Source [†] | Primary Mineral Element content on dry matter basis [†] | Relative Bioavailability, % |
|---|---|---|
| **Cobalt** | **Co (%)** | **(%)** |
| Cobalt carbonate, $CoCO_3$ | 43 – 47 | 100 |
| Cobalt sulfate, $CoSO_4.7H_2O$ | 21.0 | 100 |
| *Cobalt oxide, $Co_3O_4$* [‡] | 24.0 | 0 - 20 |
| **Copper** | **Cu (%)** | **(%)** |
| Cupric sulfate, pentahydrate, $CuSO_4.5H_2O$ | 25.2 | 100 |
| *Cupric sulfate, anhydrous, $CuSO_4$* [‡] | 39.9 | 100 |
| Cupric chloride, tribasic, $Cu_2(OH)_3Cl$ | 58.0 | 100 |
| Cupric oxide, CuO | 75.0 | 0 - 10 |
| *Cupric carbonate, monohydrate, $CuCO_3.Cu(OH)_2.H_2O$* [‡] | 50 – 55 | 60 - 100 |
| **Iron** | **Fe (%)** | **(%)** |
| Ferrous sulfate, monohydrate, $FeSO_4.H_2O$ | 30.0 | 100 |
| Ferrous sulfate, heptahydrate, $FeSO_4.7H_2O$ | 20.0 | 100 |
| Ferrous carbonate, $FeCO_3$ | 38.0 | 15 - 80 |
| *Ferric oxide, $Fe_2O_3$* [‡] | 69.9 | 0 |
| *Ferric chloride, hexahydrate, $FeCl_3.6H_2O$* [‡] | 20.7 | 40 - 100 |
| *Ferrous oxide* [‡] | 77.8 | [j] |
| **Iodine** | **I (%)** | **(%)** |
| Ethylenediamine dihydroiodide, (EDDI) $C_2H_8N_2$2HI | 79.5 | 100 |
| Calcium iodate, $Ca(IO_3)_2$ | 63.5 | 100 |
| Potassium iodide KI | 68.8 | 100 |
| *Potassium iodate, $KIO_3$* [‡] | 59.3 | [j] |
| *Cupric iodide, CuI* [‡] | 66.6 | 100 |
| **Manganese** | **Mn (%)** | **(%)** |
| Manganous sulfate, monohydrate, $MnSO_4.H_2O$ | 29.5 | 100 |
| Manganous oxide, MnO | 60.0 | 70 |
| *Manganous dioxide, $MnO_2$* [‡] | 63.1 | 35 - 95 |
| *Manganous carbonate, $MnCO_3$* [‡] | 46.4 | 30 - 100 |
| *Mangenous chloride, tetrahydrate, $MnCl_2.4H_2O$* [‡] | 27.5 | 100 |
| **Selenium** | **Se (%)** | **(%)** |
| Sodium selenite, $Na_2SeO_3$ | 45.0 | 100 |
| *Sodium selenate, $Na_2SeO_4.10H_2O$* [‡] | 21.4 | 100 |
| **Zinc** | **Zn (%)** | **(%)** |
| Zinc sulfate, monohydrate, $ZnSO_4.H_2O$ | 35.5 | 100 |
| Zinc oxide, ZnO | 72.0 | 50 - 80 |
| Zinc sulfate, heptahydrate, $ZnSO_4.7H_2O$ | 22.3 | 100 |
| *Zinc carbonate, $ZnCO_3$* [‡] | 56.0 | 100 |
| *Zinc chloride, $ZnCl_2$* [‡] | 48.0 | 100 |

[†] NRC Beef Cattle (2016); [fg] = Feed-grade source; [cp] = Chemically pure form; [j] = No data available [‡] Less commonly used sources are in italics and marked. Mineral element compositions of feed-grade sources vary by source, processing method, site of mining and manufacturer. Sources should be analyzed or manufacturer's analyses should be used when available. Element composition of a source is listed if specific element concentration is > or equal to 1.0% for macromineral elements, or > 10 000 mg/kg for micromineral elements, except for fluorine concentrations which are not listed because of

potential toxicity. The compostions of hydrated mineral sources are shown including the waters of hydration. The mineral source listed first under each mineral element was generally the standard with which the other sources were compared to establish relative bioavailabilities

Spears (2003) indicated that organic trace minerals, minerals with complexed or chelated bonds with ligands, like amino acids or polysaccharides, do have a beneficial effect on cattle. Inorganic element sources in supplements for cattle are partially replaced with these more expensive, but higher bioavailable organic trace element sources and which show a positive effect on production traits like reproduction, milk production, daily growth rates, subcutaneous fat thickness and immune response (Ballantine *et al*. 2002; Hansen *et al*. 2006a; Malcolm-Callis *et al*. 2000; Nocek & Patton 2002; Nocek, Socha & Tomlinson 2006; Spears & Kegley 2002; Stanton *et al*. 2000; Tiffany *et al*. 2003; Tiffany & Spears 2005). Another form of elements, called hydroxy-trace-minerals were introduced in the cattle market since the late 1990's. Copper chloride [$Cu_2(OH)_3Cl$], zinc hydroxyl-chloride [$Zn_5(OH)_8Cl_2$], and manganese hydroxyl-chloride [$Mn_2(OH)_3Cl$] are insoluble in water, but become soluble under acidic conditions as found in the abomasum of ruminants (Spears 2014). These hydroxy-trace-minerals have a higher ability to "bypass" the rumen in comparison to other inorganic sulfate minerals, with a higher absorption level compared to inorganic minerals in the sulfate form. Shaeffer (2006) has shown that steers which were supplemented with hydroxyl-zinc had an improved apparent absorption (19.8 vs. 9.9%) and a higher retention of zinc (77 vs. 35 mg/day) compared to the group that received zinc sulfate.

Cattle have a well developed homeostatic mechanism to alter the absorption and/or excretion of essential trace minerals in response to any change in their mineral requirement or consumption, to maintain a fairly narrow range of an element in their body tissue through different cellular transporters (Spears 2014). When trace minerals are fed at levels higher than requirements, then many of these transporters will be down-regulated or up-regulated when minerals are low or marginal. However, animals can only down-regulate to a certain degree and when these minerals are fed at extremely high levels, animals will show subclinical or clinical signs of toxicosis.

**Inorganic P sources in South Africa**

Phosphorous has been identified in Southern Africa as one of the most limiting nutrients due to the low phosphorous levels in the soil and pastures of many beef farms (De Waal *et al*. 1996; De Waal & Koekemoer 1997; Prozesky *et al*. 2016; Read *et al*. 1986; Spangenberg *et al*. 1997; Theiler 1912). Phosphorous is therefore discussed in detail due to the importance thereof in the supplement formulations and management systems and the importance of the nutrient to prevent health disorders when deficient.

Inorganic feed phosphates are derived from natural rock phosphates which in their natural form are unsuitable for direct use in animal feeds due to unavailability of this form of P to animals. Natural rock phosphates are changed into a more bioavailable orthophosphate $(PO4)^{3-}$ form through chemically treatments. The manufacturing process of a P source does influence the proportion of P that can be absorbed or utilized by animals to meet their nutritional requirements for phosphorous (Viljoen 2001). Many studies over the past few decades have shown distinct differences between different generic products (DCP, MCP, MDCP, DFP, MSP) regarding the bioavailability of P, even between products within the same generic or descriptive P groups as;

DCP - dicalcium phosphate anhydrous

MCP - monocalcium phosphate hydrous

MDCP - mono-dicalcium phosphate

MSP - monosodium phosphate

CSP – calcium sodium phosphate

DFP – defluorinated phosphate

The results from many studies are published without detailed descriptions about the manufacturing process as well as the bioavailability of the specific P sources used in the trials. Tables 5.6, 5.7 and 5.8 show some digestibility values from different studies as an indication that differences in bioavailable P are a reality in many species.

**Table 5.6** The bioavailability of phosphorous sources for turkeys (Waibel *et al.* 1984)

| Source | Reference source | Number of samples (n) | Relative bioavailability † | |
|---|---|---|---|---|
| | | | Variation | Average |
| Mono dicalcium phosphate (MDCP) | MDCP | 8 | 76.7 - 108.5 | 95.8 |
| Dicalcium phosphate (DCP) | MDCP | 20 | 75.1 - 106.3 | 90.3 |
| Defluorinated phosphate (DFP) | MDCP | 9 | 67.6 - 85.2 | 78.6 |

† Using tibia ash

The results from both trials in Tables 5.6 and 5.7 show the same trend in ranking the relative bioavailable P for MDCP and DCP. The values in Table 5.7 were lower compared to the values in Table 5.6, but this was due to the quantitative method used.

**Table 5.7** Phosphorous availability measured with 3-week old broilers for inorganic phosphate sources by Van der Klis and Versteegh (1996)

| Source | Total P | Available P (% of total P) |
|---|---|---|
| Calcium sodium phosphate (CSP) | 18 | 59 |
| Dicalcium phosphate anhydrous (DCP) | 19.7 | 55 |
| Dicalcium phosphate hydrous (DCP) | 18.1 | 77 |
| Monocalcium phosphate (MCP) | 22.6 | 84 |
| Mono dicalcium phosphate (MDCP) | 21.3 | 79 |
| Monosodium phosphate (MSP) | 22.4 | 92 |

Table 5.7 shows a 22-percentage unit difference between DCP sources for available P for anhydrous and hydrous DCP sources (Van der Klis & Versteegh 1996). The different MDCP commercial products with different P availabilities are also due to the same reason: the chemical reaction and the factors influencing the specific reaction in the production process which dictate all the end products and mixtures of phosphate bonds and compounds. Some manufacturing processes create temperatures that are too high (uncontrolled reaction) which increase the evaporation of the crystallization water to form an anhydrate product, with a detrimental effect on P digestibility. This loss in moisture increases the concentration of P in the DCP products and this, therefore, is a good indicator of the process and the type of product. Hydrated DCP products contain more or less 18% phosphorous, while anhydrous DCP products analyze up to 22% phosphorous. P products are also evaluated with the X-ray diffraction method to evaluate the different chemical properties of a commercial product, although the most accurate method is still *in vivo* tests.

MCP sources will always contain some DCP due to the manufacturing process, which would influence the total P digestibility in the specific commercial product depending on the ratio of DCP to MCP. Products which contain more than 80% as MCP, are classified as a MCP product. On the other hand, MDCP products contain only 50 to 80% MCP. The rest of the P is then in the DCP form, which influences P digestibility, depending on the DCP process and the amount of the DCP within a product as seen in Table 5.8.

**Table 5.8** Phosphorous availability coefficients in pigs (adapted from Viljoen 2001)

| Source | Average |
|---|---|
| Monocalcium phosphate (MCP1) EU produced MCP-F | 89.3[c] |
| Monocalcium phosphate (MCP2) EU produced | 90.8[c] |
| Mono dicalcium phosphate (MDCP) South African produced (continuous production process) | 89.2[bc] |
| Mono dicalcium phosphate (MDCP) USA produced | 83.6[ab] |
| Dicalcium phosphate hydrous (DCP) South African produced (continuous production process) | 78.6[a] |
| Monosodium phosphate (MSP) | 92.3[c] |

[abc]Values with different superscripts are significantly different at $P<0.05$

The MCP to DCP ratio in a product is determined in practice through water solubility tests (CEFIC 1999 method) as well as with a Ca to P ratio evaluation (Viljoen 2001). The P in pure MCP is fully water soluble (100%) and the P in DCP is insoluble (0%), and this can help to calculate the ratio of MCP to DCP within a product. DCP also contains more Ca, 24%, in comparison to MCP's content of 16%. A product with a Ca to P ratio of 0.8 is an indication that the product contains more than 70% P from MCP. If this ratio is higher than 0.9, it is an indication that a product contains more DCP and less MCP. The manufacturing process of the DCP will then have a much higher effect on the bioavailability of the P within a product which contains high levels of DCP.

A study by Wadsworth *et al*. (1988) has shown lower liver Cu levels (<50 mg/kg) in steers when P was supplemented through mono-ammonium phosphate in comparison to the liver levels of the unsupplemented P group (> 150 mg/kg). This effect of P on liver Cu levels was also reported by Karn and Hofmann (1990) but the mechanism for this animal response was not explained, nor an explanation why no Cu deficiency was seen.

Supplements are formulated for P based on the following;

i. the bioavailability of P
ii. the chemical analyses of the product
iii. the price per unit of bioavailable P
iv. the availability of the product
v. the product's physical handling properties
vi. and the product must be free from toxic substances.

## Conclusions

Animal scientists need to formulate supplements for beef cattle to meet the animal's requirements through precision feeding systems, in order to optimize the performance of all the

animal groups on a specific farm. This can be done if the supplement is formulated and managed with the following factors taken into account; soil-plant-mineral interactions, mineral requirements of the animals, environmental influences on the animals, intake and grazing behaviour of the animal when supplemented, method of supplementation, salt level within the water and supplement, dietary cation-anion difference, and the bioavailability and palatability of the mineral sources used.

Cattle will not respond to the minerals supplied by a supplement if all the nutrients, including protein and energy, in the diet are not well balanced with the correct sources. Southern Africa is phosphorous deficient and therefore it is one of the most important factors to consider when a supplement is formulated, to ensure that all cattle are supplied with a bioavailable P source to optimize their production. All cattle do not consume free-choice supplements, and therefore it is very important to optimize the supplement supply to the different groups with different management and supply systems to minimize the number of cattle not eating the supplement.

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# Chapter 6

# COMPARING FOUR DIFFERENT PHOSPHOROUS SOURCES IN SUPPLEMENTS FED TO BEEF CATTLE ON THE PREVALENCE OF OSTEOCHONDROSIS IN THE NORTH WEST PROVINCE

## Abstract

F.K. Botha[1*], H.H. Meissner[1], L. Prozesky[1], C.A. Shepstone[1] and C.de Brouwer[2]

The prevalence of osteochondrosis is related to mineral imbalances and therefore supplementation, which warrant further research. The aim of this study was to compare phosphorous sources used in supplements since it is known that the bioavailability of phosphorous differ. Thirty-two Bonsmara healthy steers (mean average 16h-fasted body weight of 214 kg ± 23.8) were randomly allocated to four different P sources in supplements to evaluate mineral differences in rib bone and tissue, differences in daily growth rates, and the prevalence of osteochondrosis. The P sources were, respectively, a di-calcium phosphate (DCP1) and a mono calcium phosphate (MCP) source from a well controlled manufacturing process, and two di-calcium phosphate sources (DCP2 and DCP3) from a "batch-to-batch" manufacturing process where the heating process was less controlled. The steers were fed for 433 days during which they were frequently rotated between camps containing low phosphorous natural pastures to minimize camp effects. All supplements were mixed in a ratio of 50% of the P source and 50% salt and then fed *ad lib*. Feeding the different P sources did not affect the prevalence of osteochondrosis, as only one animal in the DCP2 group developed osteochondrosis. Following slaughter, no differences were found in rib bone mineral levels between the different treatments, although the dry fat-free liver P ($p=0.001$), Mg ($p$=0.001), K ($p$=0.029), S ($p$=0.032), and Zn ($p$=0.001) were lower in the MCP fed steers. The DCP3 and DCP1 treatments had the highest dry fat free liver P (0.378 and 0.363%), Mg (0.195 and 0.182%), K (0.339 and 0.333%) and S (0.236 and 0.238%) levels. The DCP1, DCP2 and DCP3 treatments had higher dry fat-free liver Zn levels compared to the MCP group (30.3, 31.7, 32.6 vs. 26.9 mg/kg). The mean 16h-fasted body weights were higher for the MCP (353.6 kg), DCP1 (357.8 kg) and DCP3 (353.1 kg) treatments than the DCP2 group (347 kg, $p<0.001$), whereas the DCP1 treatment had the highest average daily gain and the DCP2 treatment the lowest (0.655 vs. 0.566 kg/day, $p=0.014$). Total supplement intake over the 433-day period was 27.8, 41.3, 37.1, and 33.0 kg respectively for treatments DCP1, DCP2, DCP3 and MCP, with the total mean P intake of 2812, 4086, 4056 and 3526 g per steer. The total P intake per kg live weight gain were 10.1, 17.0, 15.5 and 12.8 g for treatments DCP1, DCP2, DCP3 and MCP, respectively, and 57.8, 95.4, 88.0 and 74.1 g for total P intake per kg metabolic weight gain. Results from this study indicated a significant relationship between phosphorous source and growth of the steers, with the highest correlation where the P source was from a well controlled continuous manufacturing production process with a higher bioavailable P.

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**Keywords:** Osteochondrosis, cattle**,** rib bone, liver, minerals, phosphorous, MCP, DCP, daily gains.

# COMPARING FOUR DIFFERENT PHOSPHOROUS SOURCES IN SUPPLEMENTS FED TO BEEF CATTLE ON THE PREVALENCE OF OSTEOCHONDROSIS IN THE NORTH WEST PROVINCE

## Introduction

Mineral imbalances have been reported around the world and the minerals most likely to be lacking in cattle on pastures are Ca, P, Na, Mg, K, Fe, Mn, Co, Cu, I, Se and Zn (McDowell 1996). With all the research and results in the previous chapters regarding minerals in cattle from the Vryburg area in South Africa, as well as published research since 1927, it was decided to focus mainly on the role of different P sources in this trial.

A lot of confusion in ruminant phosphorous nutrition around the world was generated due to the degree of naturally occurring P deficiency in grazing cattle, the lack of consistent responses to P supplementation, and even the recommended P requirement for ruminants (Karn 2001). The purpose of this chapter regarding phosphorous is not to focus on all the metabolic and physiological processes of phosphorous in the animal body, but to rather focus on specific aspects regarding grazing cattle's P requirements and their P supplementation based on some research information found in the osteochodrosis trials by the Onderstepoort research group until 2005.

The mineral phosphorous in cattle is involved in many vital metabolic processes as phosphate ($H_2PO_4^-/HPO_4^{2-}$) or as phosphate-containing organic compounds, and functions:

- As a component of the DNA and RNA in cell growth
- As energy utilization and transfer as a component of adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate
- As phospholipid formation
- As hydroxyl-apatite crystals, $Ca_5(PO_4)_3OH$, in ruminants to give strength to the skeleton
- Within the maintenance of the osmotic balance as well as maintaining the acid-base within the animal's biological fluids through the inorganic phosphate form
- As phospholipids which are important in cell wall structure and integrity and also in the myelin as the sheath in nerves (Karn 2001)
- Within the ruminal microorganisms for their growth and cellular metabolism (NRC 2016; Pfeffer & Hristov 2005).

- As phytate P in ruminants which can also be hydrolyzed to yield inorganic orthophosphates and inositol or hexose through the action of endogenous phytases (Kinciad & Rodehutscord 2005)

Phosphorous is the second highest mineral in the cattle body of which 80% is found in the bones and teeth, and often discussed with calcium due to its function in bone formation. However, the ratio of calcium to phosphorous has been overemphasized in many trials over the last century and have shown no differences between animal performances between ratios of 1:1 and 7:1, provided that P intake is adequate to meet cattle requirements (Alfaro *et al*. 1988; Dowe, Matsushima & Arthaud 1957; Ricketts *et al*. 1970; Ternouth 1990; Wise, Ordoreza & Barrick 1963).

Skeletal bone consists of organic tissue (collagen fibrils), inorganic fraction (bone ash) and water. The bone mineral component consists of two to four elementary hydroxyapatite cells, each having a formula, $Ca_5(PO_4)_3(OH)$, but usually written as $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two entities, with a calcium to phosphorous ratio of 2.1:1.0. Bone ash normally contains 360 g Ca/kg, 170 g P/kg and 10 g Mg/kg and gives strength to the skeleton in vertebrates (Pfeffer, Beede & Valk 2005). The hydroxyapatite is present as cylinder shaped crystals and associated with the collagen and the fibrous protein, which anchor the crystals spatially (Ternouth 1990). The bone is an important P reservoir for resorption when the animal's P requirement exceeds the phosphorous supplied by the diet and the water consumed. Research has indicated that cattle remove up to 30% of both minerals, Ca and P, from their bones during a period of deficiency.

The loss of P in the rib bones was as high as 17 – 42% in cattle with a deficiency in P from their dietary supply (Little 1984). The concentration of Ca and P per unit of bone will be most readily reduced in bones like the vertebra and ribs (spongy), although limb bones also lose considerable amounts of minerals in the form of reduced cortical volume instead of mineral concentration (Davies & Munro 1999; Little 1984; Ternouth 1990). The variety of functions where phosphorous plays a role increases the difficulty to estimate and monitor the requirement of P in cattle and to estimate to which degree P deficiency may adversely affect the productivity of cattle (Ternouth 1990). Cattle will resorb calcium from their bones when the diet is deficient in calcium, to meet their Ca requirements for different physiological processes, resulting in health problems like osteomalacia (Prozesky *et al*. 2010). On the other hand, if there is a P deficiency, the cattle will inhibit any bone formation, which in both examples will lead to bones that are more porous.

Additional to the requirement of P in the animal's body, one also has to consider phosphorous requirement of the microbes in the rumen of cattle, to ensure optimal microbial activity. The total P content in rumen micro-organisms was reported to be 2 – 6% on dry matter base (Durand &

Kawashima 1980). Researchers reported a lower dry matter intake and digestibility when phosphorous was deficient in animals during lactation, although no differences were found during a P deficiency in the pregnancy phase (Bass *et al*. 1981; Fishwick *et al*. 1977).

Another important physiological process in cattle is the increase in saliva P secretion, when cattle are fed a low P diet (Kincaid & Rodehutscord 2005). Cattle have the ability to supply up to 50% of the P that enters the rumen through salivary P secretion which is achieved by a combination of the gland's ability to concentrate P and to increase the salivary flow rate, in order to supply the rumen with more phosphorous. Breves and Schröder (1991) found the daily supply of P through the salivary gland to be as high as 30 and 60 g per cow per day in P restricted diets. Animals with a P deficiency also experience a reduced organic matter digestibility which affects the fermentation in the rumen and in the hindgut, with a reduction in the supply of microbial protein to the duodenum (Breves & Höller 1987).

It is also very important to understand how cattle control their body P levels, in order to calculate adequate dietary P levels in grazing cattle with the understanding of possible P status indicators in various body fluids and organs for the purpose to make adjustments in the diet and/or management of animals (Karn 2001). The loss in phosphorous in cattle (herbivores) largely occurs when P is excreted through their faeces in contrast to primarily through the urine in carnivores. Researchers found urinary P in grazing steers to be 4 – 9% of the excreted P, while pen fed steers on both P depletion and P repletion diets had an urinary P (% of total excreted P) of under 1 to 4% (Betteridge & Andrewes 1986; Bortolussi, Ternouth & McMeniman 1996; 1999). Growing cattle can have faecal P levels of more than 200% of their P intake on P depletion diets, contrary to faecal P of 58 to 73% of their P intake on P repletion diets. Steers on high quality forage excreted 44 to 74% of the ingested phosphorous in their faeces according to Betteridge and Andrewes (1986). Hendricksen, Ternouth and Punter (1994) on the other hand reported a loss of 88 to 103% of ingested P in grazing steers, while Morse *et al*. (1992) reported dairy cows to excrete 68.6% of the ingested P in their faeces, 1% in their urine and 30.3% in their milk after they were fed a diet containing 0.41% P for a 4-week period.

It is generally accepted that P homeostasis in cattle is achieved through the control of faecal P losses, although it is still not clear whether P homeostasis is achieved through the control of P secretion in the saliva or the control of P absorption or both (Karn 2001). Endogenous faecal P results almost entirely from unabsorbed salivary P according to Ternouth (1990), and therefore due to salivary P volume and concentration. The volume of salivary P is related to dry matter intake and the concentration of P in the saliva is related to the concentration of P in the ruminant plasma (Karn 2001). Another area which plays a part in cattle P homeostasis, is bone resorption and urinary P

excretion. Coates and Ternouth (1992) have shown that the body's P level are probably controlled by reduced endogenous faecal P losses through a combination of reduced saliva P flow and an increased P absorption in cattle on a low P diet. The parathyroid hormone does not only increase renal calcium excretion, but it also increases the resorption of Ca and P from the bone (Karn 2001). The vitamin $D_3$ metabolite, 1,25 di-hydroxy-cholecalciferol, also increases the absorption of Ca and P from the gastro-intestinal tract.

Mineral deficiencies are not only related to the deficiency in the soils and plants where cattle graze, but it is also related to many other factors regarding supplementation as discussed in Chapter 5. The most important factors have been described to enable professionals to fully understand all aspects which might affect the performance and health challenges in grazing animals when they receive supplementation.

**Supplementing P to grazing animals**

The supply of P to animals has been guided by many publications to avoid the potential shortage of P intake rather than supplying the optimal level (Pfeffer *et al*. 2005). Many countries in the EU, South and North America have implemented legislation to decrease the excretion of P in the urine and manure of animals in order to decrease pollution. This was done by monitoring the quantity of P used on a farm, through their feed and fertilizer, and how much was excreted by the animals and removed from the specific farm by selling animal products (e.g. meat, milk, manure) or other food produced. Every farm has a so-called P quota system to control the usage of P on each farm. This created a focus to implement cattle diets formulated with P sources that are more bioavailable to the animal and therefore use less dietary P in order to excrete less P in the environment. Some of these countries manage their agriculture sectors with the objective to have a zero P balance regarding the amount of P that a farm unit imports in comparison to the amount of P exported from the same unit. This strategy or legislation is still not implemented in South Africa but may come in the future due to the more focused approach on the country's water and soil quality with more regulations to improve and protect the environment.

The recommended requirement for phosphorous in cattle for the different production cycle stages have varied a lot between research groups in the United States and the rest of the world (Karn 2001). The daily P requirement for cattle was differently calculated by the ARC (1980), the Australian Standing Committee on Agriculture (1990), and many more institutions. This creates some challenges for professionals to calculate accurate requirements in cattle and to determine whether diets are adequate in P or whether P supplements must be provided to optimize the performance of the specific group of animals. The requirement for apparent P in animals varies due to many factors such as:

- Breed specific requirements (McDowell 1996).
- The availability of P in the specific feed ingredient (Ternouth 1990).
- Whether the cattle are fed in pens or on grazing (McDowell 1996).
- The possible interactions between nutrients in the feed ingredients and water (Bortolussi et al. 1999).
- The effect of parasites and diseases (Ternouth 1990).

Table 6.1 is a summary of the equations that the USA based committee used to predict Ca and P requirements in beef cattle as published in the NRC Beef Cattle 2016.

**Table 6.1** Calcium and phosphorous requirements and maximum tolerable concentrations in beef cattle in g/d (adapted from the NRC 2016)

| | Beef cattle requirements, g/d | | | | |
|---|---|---|---|---|---|
| Mineral | Maintenance | Growing and finishing | Lactation | Pregnancy† | Maximum tolerable concentration, g/day |
| Ca | 0.0154 x SBW/0.5 | NPg x 0.071/0.5 | Yn x 1.23/0.5 | CBW x (13.7/90)/0.5 | 0.02 x DMI |
| P | 0.016 x SBW/0.68 | NPg x 0.039/0.68 | Yn x 0.95/0.68 | CBW x (7.6/90)/0.68 | 0.007 x DMI |

SBW is shrunk body weight, kg; NPg is protein requirement for gain (i.e., retained protein), g/d; Yn is milk yield, kg/d; CBW is calf birth weight, kg; DMI is dry matter intake, g/d. The digestibility for Ca is 50% and for P it is 68%. NPg = SWG x (268 – 29.4 x RE/SWG), SWG is shrunk body weight gain (kg/d), RE is retained energy, Mcal/d. RE = (kg DMI – FFM – FFL – FFP) x NEga; FFM is dry matter needed to support maintenance, kg/dag; FFL is dry matter needed to support lactation, kg/d; FFP is dry matter needed to support pregnancy, kg/d; NEga is dietary net energy available for growth in Mcal/kg; †Pregnancy is for the last 90 days of gestation

The absorption of P from the intestinal tract was observed by many research groups to be related to P intake, however some found P absorption not related to P intake and other groups found P absorption coefficients to be curvilinearly related to P intake (Karn 2001). Hendricksen et al. (1994) and his co-workers described these differences in responses to be associated with the possibility that many research papers did not publish any P intake data and many researchers found P absorption to be depressed when the P intake is below 15 mg/kg LW.

According to the NRC (2016), the true absorption value of 68% needs to be used for P to calculate the dietary requirements in cattle, except in milk fed calves where the value is higher. On the other hand, the AFRC (1991) recommended usage of P absorption coefficients of 64% and 70% for forages and concentrates, respectively.

Another method to calculate the P requirement for beef cattle was published by the Agricultural Research Council (ARC 1980) and based on the following equations;

- P requirement for maintenance is based on a fixed endogenous faecal loss of 10 mg P/kg LW. The absorption coefficients of 0.78 are used for cattle under 12 months of age (under 300 kg) and 0.58 for older cattle (over 300 kg) respectively. These maintenance values were adjusted according to Karn (2001) to a constant endogenous faecal loss of 20 mg P/kg LW with an absorption coefficient of 0.70.
- P requirement for growth = 8 g P/kg LW gain
- P requirement for foetus and uterine tissue in month 5 of gestation = 1g P/kg LW or 5 g P/kg LW gain in month 9 of gestation
- P requirement for milk production = 1 g P/L milk

- Coates and Ternouth (1992) recommended a gross dietary factor of 1.6 g P/l milk produced due to the concentration of 1.22 g P/l in milk with an absorption coefficient of 0.75 in *Bos Indicus* cattle.

Coates and Ternouth (1992) also reported P absorption values of 0.64 to 0.92 in cattle of all ages, with the lower coefficients more likely in diets high in phosphorous and well above requirements, although it was recommended to use an absorption coefficient of 0.78 for all cattle in the tropics. The same group also recommended some P absorption coefficients of 0.82 for pen fed cattle and 0.77 for un-supplemented grazing cattle (Karn 2001). Research by Bortolussi *et al.* (1996) has shown that P absorption coefficients can be decreased by low nitrogen intake when cattle received a low P intake.

A study by Call *et al.* (1978) showed no differences in rib growth, rib bone morphology and P, age at puberty, calving interval or conception rate between Hereford heifers that were fed from 165 kg live weight on diets containing either 0.14 or 0.36% P for a period of two years. Another study by Call *et al*. (1986) were conducted with heifers from weaning to fifth lactation on groups receiving a low P diet (6 to 12.1 g P/day) and compared with the control group which received 20.6 to 38.1 g P/day. Both Hereford groups received more P as they grew older. This study indicated no difference in the health status, growth rate or reproduction between animals on the low P diet and the animals on the higher P level. A group of animals in this trial was then fed a very low P diet (5.1 to 6.6 g P/day) for 12 months and animals showed clinical P deficiency within 6 months, with a negative effect on reproduction. The data from these trials were then used by the NRC (2016) committee to publish the recommendation of Call (1986) and his team to use at least 12 g P per day throughout the production cycle for a 450 kg Hereford cow.

The NRC (2016) also published the results from trials which indicated that yearling steers and calves were not adversely affected by feeding them finishing rations with low P intakes;

- As low as 14.1 g P/animal/day (0.17% P/kg DM): no negative effects vs. control yearling steers (Erickson *et al*. 1999 2002; Geisert *et al*. 2010).
- Growing steers which received 0.22 vs. 0.33% P/kg DM decreased their P intake and the quantity of P excreted with 50% and more, without any negative impact on their average daily gains or their gain to feed ratio (Greene 2000).

The raw materials within a supplement also have an effect on the P excretion or losses. Results from Vasconcelos *et al*. (2009) showed that the P excretion lowered by 20% when cattle were fed with a supplement when cottonseed meal was replaced by urea as the protein source. This replacement decreased the dietary P concentration by 29%, but the quantity of P excreted as a

percentage of P intake increased over time on the feed with 59, 74 and 88% of the intake P excreted respectively on days 30, 85 and 155. Other raw materials that may influence the percentage of P excreted as a percentage of P intake are wet distiller's grains with solubles, dry-rolled maize, steam-flaked maize, and formaldehyde treated rice bran (Buttrey *et al.* 2012; Martin-Tereso López 2010; Luebbe *et al.* 2012a; Spiehs & Varel 2009). Most of these mentioned raw materials, except for cotton seed meal, are not currently produced in South Africa and therefore not cost effective in South African cattle diets.

The bioavailability of minerals varies a lot in the literature and therefore the NRC (2016) for beef cattle made no recommendations regarding bioavailability values for different mineral sources. The endogenous faecal P in cattle is related to P intake and the plasma inorganic P with typical endogenous faecal P losses ranging from 7 to 27.5 mg/kg live weight for all cattle classes (grazing heifers, steers as well as breeding cattle) (Bortolussi *et al.* 1996; Coates & Ternouth 1992; Ternouth *et al.* 1996). According to Hendricksen *et al.* (1994), endogenous faecal P can be estimated (although it is not a constant value) by the following equation:

EFP = 4.8(±0.96) + 0.497(±0.040)PI

Where EFP is endogenous faecal phosphorous, and PI is phosphorous intake.

Ternouth *et al.* (1996) indicated that the obligatory endogenous P loss ranges from 9 to 17 mg P/kg LW in growing beef cattle which was related to a dry matter intake range of 10 to 25 g DM/kg LW. Geisert *et al.* (2010) on the other hand published a linear total P excretion equation ($R^2 = 0.49$) that was used by the NRC (2016) beef cattle committee:

P excretion (g/d) = 0.82 + 0.57*(total g P intake)

This group published the equation where the total dietary P intake in beef cattle increased from 8 to about 55 g P /animal/day.

Soil is formed from rocks, the major reservoir of phosphates, which forms orthophosphates on the surface of various adsorbents as precipitates with several inorganic cations or as organically bound phosphate (Pfeffer & Hristov 2005). Plants and soil organisms utilize this ionized phosphate which is then later utilized by animals grazing these plants. Some areas in South Africa are well known for their low phosphorous soils, as found in the North West region, where many farmers complained about health related mortalities like osteochondrosis. The P content of veld pastures in many areas in South Africa was found to be insufficient to meet the requirement of productive cattle during all seasons (Du Toit *et al.* 1940). Many studies were conducted over the past century in the P poor soil areas in the North West region in South Africa, with the results that grazing cattle's maintenance,

reproduction and calf weaning weights were improved when they were supplemented with P during the dry season (Pfeffer *et al*. 2005). Many health problems in cattle were also related to P deficiency as described in previous chapters of this thesis.

The supply of P from the soil to the plants and from the plant to the animal is affected by many factors which professionals need to have knowledge about to ensure they enhance P supply to the animal utilizing the specific forage (Karn 2001). Factors like precipitation effects, environmental conditions, stage of plant maturity, partitioning of P in the different areas of a plant, the interaction between different nutrients in the plant and soil, the management system on the farm, are only a few which will have an effect on the concentration of P in the plant and the availability of the plant P to the animal utilizing these plants. The concentration of P in plants together with the dietary P level was highly correlated with live weight gains in steers in a study by Coates *et al*. (1990) in northern Australia. The P levels in legumes and grass-legume roughages increased after P fertilization according to many studies, although it did not always change the distribution of P in the different plant tissues (Karn 2001). This was not consistent as seen in the study by Jones (1990) where P fertilizer did not improve the P status of the forage over 0.10% P, although cattle in this study showed a preference towards fertilized grass-legume pastures in comparison to the same unfertilized pasture. This preference was confirmed by another study on steers and continued for 11 months after fertilizing grass-legume pastures with P (Jones & Betteridge 1994). The results from this study also showed that the higher intakes in these cattle were not due to changes in pasture nitrogen levels.

Research has not only indicated a DMI response to P supplementation but results also indicated a change in selection of specific pasture species (Coates & Le Feuvre 1998; Hendricksen & Punter 1990). These publications show that cattle with a P deficiency selected less Stylosantes (legume specie) in comparison to cattle which have sufficient P in their diet, and therefore had a sparing effect on the grass pastures. In another study, Coates (1996) has shown that season also affects the botanical selection by cattle in Australia with a peak legume intake (80%) in the late wet season or early dry season in comparison to a peak grass intake during the early wet season and late dry season.

**P response in growing animals**

Researchers around the world published different results regarding growth and reproduction when cattle were supplemented with P due to factors like the cattle's genetic potential, environment, cattle's P status, P availability in their ration, level of P in the trial diets, gastro-intestinal recycling of P and resorption of bone P mechanisms, feeding system (pen fed or pasture fed), nutrient levels of the total diet, age of the cattle, and their production stage (De Brouwer *et al.* 2000; De Waal &

Koekemoer 1997; Karn 2001; Spangenberg 1997). These inconsistencies to P supplementation in growing cattle is an indication that P may be either a primary or a secondary limiting nutrient to other nutrients like protein, energy, and many more in the diet of cattle and therefore may increase or decrease the performance efficiencies due to the total supply of nutrients and the cattle's requirements (Karn 2001).

**P response in mature cattle**

P supplementation became practice in some low P containing grazing areas in South Africa and had a marked effect on the reproduction and growth of cattle in these areas (De Brouwer *et al*. 2000). Cows without supplementation in these areas tended to calve only every two years (Groenewald 1986). Other research groups reported weight loss in cattle when they were supplemented with P lower than their requirement, and also when their protein and energy requirements were not balanced (Ferreira 1976; Van Niekerk & Jacobs 1985). De Brouwer *et al.* (2000) supplemented cattle with 8 g, 4 g and 0 g P per cow per day during the summer in a two-season trial on the western Highveld region in South Africa. The authors gave 10 g P to all cows during the winter and found no differences in reproduction efficiencies between the three treatment groups. The only difference was the higher mean live mass, condition score and bone P of the cows that were supplemented with P during the summer months. It is therefore not advisable to use the same P supplementation strategy in different regions which contain different roughage P levels, because it might be essential in one region but unnecessary in the next region with different animal performances as a result (De Brouwer *et al*. 2000; De Waal *et al.* 1993; De Waal *et al.* 1996; De Waal & Koekemoer 1993 1997; Engels 1981; Read 1984; Read *et al*. 1986; Spangenberg 1997).

Many studies were conducted in the P deficient areas in South Africa to evaluate the effect of P supplementation in mature cattle with the following results:

| | |
|---|---|
| Theiler *et al*. 1924 | Feeding bone meal improved cow fertility. |
| Du Toit & Bisschop 1929 | Feeding bone meal improved cow fertility. |
| Read *et al*. 1986 | P supplementation increased cow weights, reproduction performance, calf birth and weaning weights. |
| | Feed intake was severely decreased in P-deficient cows. |
| De Waal *et al.* 1996 | P supplementation improved cow weights, calf birth weights and calving percentage. Weaning weights were not affected by P supplementation. |

**Inorganic P sources in South Africa**

The factory production process does influence the proportion of the P in a product that can be absorbed or utilized by animals to meet their nutritional requirements for phosphorous (Viljoen 2001). This is due to the chemical reaction and the factors influencing the specific reaction in the production process which dictate all the end products and mixtures of phosphate bonds and compounds. Some manufacturing processes create temperatures that are too high (uncontrolled reaction) which increase the evaporation of the crystallization water to form an anhydrate product, with a detrimental effect on P digestibility (Viljoen 2001). This loss in moisture increases the concentration of P in the DCP products and is therefore a good indicator of the process and type of product. Hydrated DCP products contain more or less 18% phosphorous, while anhydrous DCP products analyse up to 22% P. Phosphorous products are also evaluated with the X-ray defraction method to evaluate the different chemical properties of a commercial product, although the most accurate method is still *in vivo* testing.

Sources of MCP will always contain some DCP, which influence the total P digestibility in the specific commercial product depending on the ratio of DCP to MCP. Products which contain more than 80% as MCP, are classified as a MCP product. On the other hand, MDCP products contain only 50 to 80% as MCP. The rest of the P is then in the DCP form, which influences P bio-availability, depending on the DCP process and the amount of the DCP within a product as seen in Chapter 5.

The MCP to DCP ratio in a product is determined in practice through water solubility tests as well as with a Ca to P ratio evaluation (Viljoen 2001). The P in pure MSP is fully water soluble (100%) and the P in DCP is insoluble (0%), and this can help to calculate the ratio of MCP to DCP within a product. The DCP also contains more Ca, 24%, in comparison to MCP's content of 16%. A product with a Ca to P ratio of 0.8 is an indication that the product contains more than 70% P from MCP. If this ratio is higher than 0.9, it is an indication that a product contains more DCP and less MCP. The manufacturing process of the DCP will then have a much higher effect on the bioavailability of the P within a product which contains high levels of DCP.

Many studies over the past few decades showed distinct differences between different generic products (DCP, MCP, MDCP, DFP, MSP) regarding bioavailability, but this has not been well documented for ruminant animals. Many results are published without detailed descriptions about the manufacturing process as well as the P bioavailability of the specific sources. There are also not many ruminant studies published to evaluate the effect of different P products on the performance of animals fed different sources of P. Cattle on the Armoedsvlakte experimental farm outside Vryburg,

located in the North West region of South Africa, routinely receive supplemental phosphorous throughout the year due to low phosphorous in the soil and plants. De Waal *et al.* (1996) recommended a P supplementation rate of 16 g P per cow per day for a 6-month period during late pregnancy and lactation, and a 9 g daily P supplementation per cow during the other 6 months of the year.

The results from the Kaalplaats research project (Botha *et al.* unpublished; Chapter 4 of this thesis) indicated that phosphorous intake, phosphorous bioavailability, and the phosphorous source in the supplement may be important factors which may have an influence on the prevalence of osteochondrosis in cattle. The mineral differences between the healthy, the osteochondrosis affected animals and the affected animals that were supplemented for three months created more questions regarding the supplementation and source of P, and therefore it was decided to start with a phosphorous source trial over a longer period, as described below.

## Materials and methods

A trial was conducted at the Departmental Livestock Centre at Armoedsvlakte, near Vryburg in the North West province in South Africa, to test the hypothesis that phosphorous sources affect the phosphorous level within cattle and therefore might play a role in the prevalence of osteochondrosis in cattle. This Livestock Centre is known for many phosphorous research trials conducted during the previous century. The facility has a well-managed infrastructure with adequate animal and human resources to conduct research trials.

### Pasture status

The grazing pastures at Armoedsvlakte contain low levels of phosphorous due to the low phosphorous level in the soil in this region (De Waal *et al*. 1996; De Waal & Koekemoer 1997; Prozesky *et al*. 2016; Read *et al*. 1986; Spangenberg *et al*. 1997; Theiler 1912). Since 1882, many cattle experiments were conducted at Armoedsvlakte with results that related health issues with a mineral imbalance, with a strong reference to phosphorous deficiency. Based on the published research, it was decided not to feed any supplement based on no-phosphorous (negative control).

The standard stocking rate on Armoedsvlakte is 8 ha per Large Stock Unit. The Large Stock Unit is a standard unit used in South Africa and the equivalent of an 450 kg ox (live weight), which gains 500 g per day on grazing pastures (with a mean digestible energy of 55%). Eighteen camps with

typical P-depleted extensive grass veld (Ghaap Plateau) of 10 ha each were used in a rotational system system to limit any camp effects.

**Treatments and animals**

Four different commercially available South African feed phosphorous sources, namely three di-calcium phosphate (P18) sources and one mono-calcium phosphate (21% P) were tested as the specific phosphorous source in four treatments.

Thirty-two yearling osteochondrosis unaffected Bonsmara steers (average starting weight 213.9 kg) derived from the cow herd at the Armoedsvlakte experimental farm were selected and randomly allocated to one of the 4 treatments, 8 steers per treatment. The steers had been raised by cows receiving the Yara Animal Nutrition DCP (P18) and had been wintered receiving the same lick after having been weaned during June 2005. For the trial:

- All animals were inspected before the commencement of the trial and were given a condition score.
- All animals were visually assessed with the OPOL system for the presence or absence of osteochondrosis, prior and at the starting date of the trial. Only animals not displaying clinical signs of osteochondrosis were used to evaluate whether phosphorous source induces osteochondrosis.
- The animals were vaccinated with Supavax (Intervet, Schering-Plough Animal Health, Austria) used as an immunization against anthrax, botulism and blackleg, with a dosage of 2ml per animal subcutaneously.
- The standard DCP (P18) plus salt Armoedsvlakte lick supplementation was given to the animals prior to the start of the trial.
- Trial licks were provided *ad lib.* in a mixture of 50% stock salt and 50% of the phosphorous source.
    - The same source of stock salt was used in all treatments.
- Trial groups were rotated through camps to minimize camp effects.
- The trial commenced on 10 November 2005 with an adaptation period on the standard lick and then fed the different treatments from the 7$^{th}$ of December 2005 and the final weight was recorded on the 13$^{th}$ of February 2007. All trial animals were slaughtered on the 13$^{th}$ of February 2007. The duration of the trial was 433 days.

At the start of the trial, all animals were ranked according to live weight and then randomly allocated into the different treatment groups namely,

- **Group "DCP1":** Eight steers were provided the supplement which contained a dicalcium phosphate (18% P) as phosphorous source from a commercial phosphorous supplier A. This DCP was produced in a continuous process operation.
- **Group "DCP2":** Eight steers were provided the supplement which contained the dicalcium phosphate (18% P) as phosphorous source from a commercial phosphorous supplier B. This DCP was produced in a "batch by batch" process operation.
- **Group "DCP3":** Eight steers were provided the supplement which contained a dicalcium phosphate (18% P) as phosphorous source from a commercial phosphorous supplier C. This DCP was produced in a "batch by batch" process operation.
- **Group "MCP":** Eight steers were provided the supplement which contained a mono calcium phosphate (21% P) as phosphorous source from a commercial phosphorous supplier A. This MCP was produced in a continuous process operation.

Table 6.2 is a summary of the wet chemistry mineral results that were done on the four phosphorous sources as well as the salt that were used during the trial. Table 6.3 is a summary of the four treatment mixes and their nutrient content based on the nutrient analysis of the source batches that were received and used for the trial.

Lick was provided *ad lib.* in a mixture of 50% stock salt and 50% phosphorous source. Although the measurement of individual intake would have been preferable, it was more important at the time of this trial that animals be managed under normal grazing conditions, and therefore it was decided to measure all intakes on a per group basis. Ample trough space was provided for the supplements and the supplement intake was monitored to ensure that troughs always contained the specific supplement per group and then calculated every 28 days (during weighing) to ensure all animals received the correct quantities according to their predicted intakes.

**Table 6.2** Mineral analysis on an as is basis of the specific batches of phosphorous sources and the salt used in the osteochondrosis trial conducted at Armoedsvlakte (North West province, South Africa)

| Source † | P % | Ca % | Ca: P | Mg % | Na % | K % | S % | Mn mg/kg | Cu mg/kg | Fe mg/kg | Zn mg/kg | Pb mg/kg | Cr mg/kg | Hg mg/kg | Ni mg/kg | Al mg/kg | Cd mg/kg | Si mg/kg | As mg/kg | Se mg/kg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DCP1 | 20.2 | 24.4 | 1.21 | 1.41 | 0.27 | 0.14 | 0.76 | 326 | 81.0 | 4277 | 58.8 | 1.19 | 18.3 | 0.07 | 15.9 | 1918 | 0.07 | 19.0 | 24.0 | 1.95 |
| DCP2 | 19.8 | 23.0 | 1.16 | 1.34 | 0.27 | 1.06 | 0.71 | 3085 | 75.6 | 4367 | 28.7 | 0.75 | 13.9 | 0.11 | 16.5 | 2237 | 0.06 | 13.1 | 23.8 | 1.76 |
| DCP3 | 21.8 | 20.9 | 0.96 | 1.25 | 0.27 | 0.15 | 0.80 | 2834 | 63.0 | 4403 | 15.9 | 0.66 | 13.2 | 0.06 | 12.4 | 1754 | 0.05 | 12.7 | 22.5 | 1.98 |
| MCP | 21.4 | 17.0 | 0.80 | 1.20 | 0.20 | 0.13 | 0.74 | 268 | 69.7 | 4107 | 14.4 | 0.83 | 15.2 | 0.05 | 13.8 | 1557 | 0.06 | 17.2 | 22.2 | 1.98 |
| Salt | | 0.23 | | 0.17 | 39.1 | 0.19 | 0.10 | 7.86 | 0.46 | 114 | 2.60 | 0.58 | 0.77 | 0.05 | 0.43 | 65.4 | 0.04 | 4.1 | 0.87 | 0.43 |

† The nutrient content of the sources was chemically analyzed on the specific batches that were used during the trial. DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source. DCP1 and the MCP source were from a supplier which produces products within a continuous production process. The same batch of salt was used during the entire trial.

**Table 6.3** Summary of the four treatment mixes and its nutrient values as calculated from the source analysis in Table 6.5 and fed for 433 days to young Bonsmara steers

| | | Treatment mixes and analysis † | | | |
|---|---|---|---|---|---|
| | | **DCP1** | **DCP2** | **DCP3** | **MCP** |
| | P source inclusion | 50% | 50% | 50% | 50% |
| | Salt inclusion | 50% | 50% | 50% | 50% |
| Mixture nutrient content, "as is" | P, % | 10.1 | 9.89 | 10.9 | 10.7 |
| | Ca, % | 12.3 | 11.6 | 10.6 | 8.64 |
| | Na, % | 19.7 | 19.7 | 19.7 | 19.7 |
| | K, % | 0.17 | 0.63 | 0.17 | 0.16 |
| | Mg, % | 0.79 | 0.76 | 0.71 | 0.69 |
| | S, % | 0.43 | 0.41 | 0.45 | 0.42 |
| | Mn, mg/kg | 167 | 1546 | 1421 | 138 |
| | Cu, mg/kg | 40.7 | 38.0 | 31.7 | 35.1 |
| | Fe, mg/kg | 2195 | 2240 | 2258 | 2110 |
| | Zn, mg/kg | 30.7 | 15.6 | 9.23 | 8.52 |
| | Pb, mg/kg | 0.89 | 0.67 | 0.62 | 0.71 |
| | Cr, mg/kg | 9.52 | 7.33 | 6.97 | 8.00 |
| | Hg, mg/kg | 0.06 | 0.08 | 0.06 | 0.05 |
| | Ni, mg/kg | 8.16 | 8.47 | 6.41 | 7.13 |
| | Al, mg/kg | 991 | 1151 | 910 | 811 |
| | Cd, mg/kg | 0.06 | 0.05 | 0.05 | 0.05 |
| | Si, mg/kg | 11.6 | 8.60 | 8.39 | 10.6 |
| | As, mg/kg | 12.5 | 12.4 | 11.7 | 11.5 |
| | Se, mg/kg | 1.19 | 1.10 | 1.21 | 1.21 |

† The nutrient content of the mixtures is based on the nutrient analysis of the batches that were used during the trial. DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source. DCP1 and the MCP source were from a supplier which produces products within a continuous production process

**Measurement summary**

- The season was characterized by abnormal high rainfall from January to May 2006. Despite this most of the observations and data collection actions were completed.
- The supplement per treatment was bagged in colour-coded bags according to the treatment groups which were also colour-coded to ensure all groups received the correct supplement. DCP1 – Yellow; DCP2 – Green; DCP3 – Blue; MCP – Orange.
- Lick intakes was daily monitored and calculated on a “per group” basis to ensure all animals always have lick available in their feed troughs. Supplements were added where necessary to keep supplements available *ad libitum*.
- All the lick ingredients were analysed on arrival for the four treatment groups.

- The four groups of 8 steers were weighed on 16-hour fasted weight every 28 days with the final weight at slaughtering. The dry carcass weight as a percentage of live weight was also measured at slaughter.
- All steers were screened during the trial period for osteochondrosis with the OPOLS system and any steer with musculo-skeletal lesions were photographically recorded every 28 days during the weighing events.
- Tail bone samples (33 steers) and liver biopsies (21 randomly selected steers) were collected from this trial group at the start of the trial to evaluate the mineral status of the population.
- Liver section samples and rib bone samples were collected from all the trial animals during February 2007 (at slaughter).

### 6.1.1 Raw materials in the supplements

Minerals were analyzed in the Nutrilab of the University of Pretoria on all raw material batches that were received and used for the different treatment groups. This was done to calculate the mineral intake of the cattle within a treatment group. The four phosphorous sources and the salt were analyzed through wet chemistry methodology for the following minerals: P, Ca, Mg, Na, K, S, Mn, Cu, Fe, Zn, Pb, Cr, Hg, Ni, Al, Cd, Si, As, and Se (Official Methods of Analysis, 17th edition. 2000. Association of Official Analytical Chemists).

### 6.1.2. Bone and liver samples for micro and macro mineral analysis

Tail bone and liver biopsy samples were collected at the start of the trial to evaluate the mineral status of the steers. Rib bone and liver samples were collected from all the animals at slaughter (13$^{th}$ of February 2007) and sent for mineral analysis at the ARC - Soil, Climate and Water Institute's laboratory in Belvedere Street, Pretoria, South Africa (for details regarding the methodology on tissue and bone analysis see **Appendix 1).** These analyses were used to compare the four treatment groups that were supplemented with the different phosphorous sources for 433 days.

- The 15 cm rib bone samples were collected from the last rib on the left hand side of all the animals.
- The liver samples were collected from all the steers and analyzed for the following minerals: P, Mg, Na, K, Ca, S, Fe, Mn, Zn, Cu, Al, C, and N.

### 6.1.3. Intake of the supplement

All supplements in the treatments were given *ad lib.* during the trial. Phosphorous intakes were monitored daily. Lick supplements were mixed, and the bags were colour-coded to ensure that treatment animals received the correct feed. Intake of the grazed vegetation was not measured due to practical reasons, but groups were rotated in order to eliminate camp effects as far as possible.

### 6.1.4. Pathological examination of the trial animals

All steers were screened for osteochondrosis with the OPOLS system and any steer with musculo-skeletal lesions were photographically recorded every 28 days during the weighing period on all animals within the four treatment groups (n=32). This was done under the supervision of the pathologist, Prof. L Prozesky, and all musculo-skeletal abnormalities were reported. All animals were also clinically screened for osteochondrosis at slaughtering.

Only one steer developed osteochondrosis over the 433 days and this was in the DCP2 treatment. One steer died during the screening process due to an injury.

## Statistical design and evaluation

### 6.2.1. Data analyses

The animals were compared in a randomly designed study and the results were evaluated with the ANOVA regression analysis on GenStat release 17.1 which caters for trials with a "normal" distribution. The treatments were tested at a 5% significance level. The linear mixed model repeated measurement analysis was on "adjusted body weight" to evaluate the differences when starting weight was used as covariate. The BONFERRONI correction was also used as an adjustment to the *P values* when several dependent or independent statistical tests were simultaneously performed on a single data set. The supplement intakes was evaluated on a "per group" basis which created a data basis without any variance within a group, and therefore no statistical evaluation was possible.

### 6.2.2. Hypothesis

($H_0$): There is no difference in the liver and rib bone mineral status, average daily gain, or the prevalence of osteochondrosis in cattle fed different South African phosphorous sources.

($H_1$) The mineral status, the daily growth rates and the prevalence of osteochondrosis differ in cattle fed these different phosphorous sources.

## Results and discussion

### Mineral levels in the different P ingredients and treatment mixes

The average mineral concentration of each phosphorous source as well as the salt source is shown in Table 6.2. and Table 6.3 is a summary of the supplement mixes as well as the nutrient values as calculated from the ingredient analysis. DCP1 and MCP products were produced in the same company that uses a continuous manufacturing process and the other two sources, DCP2 and DCP3, were from different suppliers who use a "batch by batch" manufacturing process. The production process does have an influence on the proportion of P in the final product which can be absorbed or utilized by the animal (Viljoen 2001). Many studies over the past few decades have shown distinct differences between different generic products regarding bioavailability, even between products within the same generic or descriptive P groups, for example dicalcium phosphate, mono calcium phosphate or mono dicalcium phosphate groups (Kemme *et al*. 2001; Khattak *et al*. 2016; Van der Klis & Versteegh 1996; Waibel *et al*. 1984). The continuous production process has been developed to improve the control on heat, generated by the chemical process during manufacturing, with the aim to improve the P bioavailability of a product (Viljoen 2001). The "batch by batch" production process generates much more heat during the process and has more variability from one batch to another, which not only decreases the P bioavailibilty of the product, but it also creates variability between different batches of the final product.

Unfortunately, results from many other studies are published without detailed descriptions on which generic sources were used, and in relation to digestibility, and for example if DCP was used as the source, if it was in the hydrated or anhydrated form. Waibel *et al*. (1984) described the variation in P bioavailability in generic P product groups in the diets for turkeys as 32, 31 and 18-percentage units for mono dicalcium phosphate (MDCP), dicalcium phosphate (DCP) and defluorinated phosphate (DFP) respectively, the analyses of which are shown in Table 5.6. The average bioavailability in the mentioned study were 95.8, 90.3 and 78.6% respectively for MDCP, DCP and DFP, with a large variability between different generic P groups.

DCP1, DCP2 and DCP3 tested higher in P (%) than the expected 18% for a dicalcium phosphate at 20.2, 19.8 and 21.8% P respectively. Products that contain a ratio of 80% MCP and 20%

DCP as ingredients, normally analyze P at 20.4%. Another indication that all three DCP products contained more DCP than expected, was the Ca content which was respectively 24.4, 23.0, and 20.9% for the DCP1, DCP2 and DCP3 products. The Ca and P levels are an indication that all three products contained more DCP, but which cannot be used as the only parameter for P bioavailability in a product. The bioavailability of the DCP sources is also influenced by the heat control during the manufacturing process and this is more difficult in a "batch by batch" production plant in comparison to a continuous manufacturing process (Viljoen 2001). The DCP1 and MCP were both manufactured in a heat-controlled facility to optimize their P bioavailability and less water crystallization loss due to lower heat during the production process. The DCP2 and DCP3 on the other hand were produced in facilities which generate more heat in the vessel which cannot be stopped until the product is out loaded, which extends the heat process and therefore the P bioavailability.

The analyses on all the batches used in this trial showed very similar nutrient values, except for Mn which tested 3085 and 2834 mg / kg respectively for DCP2 and DCP3, in comparison to the 326 mg/kg in the DCP1 and 268 mg/kg in the MCP products, almost ten-fold. Manganese is important in physiological functions for example in the enzymes pyruvate carboxylase, arginase, superoxide dismutase as well as the activator for enzymes like hydrolases, kinases, decarboxylases and transferases (NRC 2016). Manganese also plays an important role in skeletal development (cartilage formation and maintenance) and if fed at low levels, one may expect a higher prevalence in skeletal abnormalities (for example stiffness, twisted legs, enlarged joints, decreased bone strength) in young cattle.

The Mn levels in DCP2 and DCP3 were tenfold higher but still lower than the maximum tolerable concentration of 1000 mg/kg DM in the total diet (NRC 2016). Cunningham *et al*. (1966) reported data which indicated that growth and feed intake only decreased when the Mn levels in the total diet were more than 2000 mg/kg DM, much higher than the level fed in this trial. High dietary intake of phosphorous, potassium, calcium, and iron results in an increase in Mn excretion in the feces presumably due to reduced manganese absorption (Hansen *et al.* 2010).

These batch analyses results were then used to calculate the nutrient values of the final mixes and the results for P were 10.1, 9.89, 10.9 and 10.7% (as is basis) respectively for the DCP1, DCP2, DCP3 and MCP treatments. For Ca, it was 12.3, 11.6, 10.6 and 8.64% (as is) in the final mixes. The sodium level for all the DCP treatments were 19.7 as well as for the MCP treatment. These levels do not mean much without considering the feed intakes to evaluate the actual intake of supplemented minerals per animal per day. This is discussed by the information provided in Table 6.10.

**Mineral levels in the tail bone and liver biopsy samples at the start of the trial**

The average mineral concentrations from the Bonsmara steers in these trials are shown in Table 6.4 for the tail bone samples (32 randomly selected animals) that were taken at the start of the trial and Table 6.5 for liver biopsy samples (21 animals) of steers which were selected for the trial.

**Table 6.4** Mineral analyses of the tail bone samples of the Bonsmara steers at the start of the trial (wet bone basis)

| | Al mg/kg | Ca % | Cd mg/kg | Cr mg/kg | Cu mg/kg | Hg mg/kg | Mg mg/kg | Mn mg/kg | P % | Zn mg/kg |
|---|---|---|---|---|---|---|---|---|---|---|
| Min | 0.07 | 0.81 | 0.01 | 0.09 | 0.18 | 0.01 | 140 | 0.08 | 0.42 | 4.29 |
| Max | 65.6 | 5.56 | 0.32 | 1.21 | 0.69 | 0.08 | 688 | 0.25 | 2.64 | 16.5 |
| Ave | 9.35 | 3.22 | 0.09 | 0.35 | 0.27 | 0.02 | 412 | 0.14 | 1.53 | 8.65 |
| SD | 12.0 | 1.11 | 0.16 | 0.26 | 0.11 | 0.02 | 126 | 0.04 | 0.51 | 2.62 |
| n † | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 | 32 |

† 32 of the 33 steers that were tested were used in the trial group. One animal died due to injury

The mineral results from the tail bone of these steers (without osteochondrosis) tend to be highly variable and cannot be compared to the data set from the osteochondrosis affected and healthy animals in the Kaalplaats trial (Botha *et al.* unpublished, Chapter 4). The ratio of P to Ca was in line with other publications, although it is not accurate to evaluate Ca, P and Mg on a total wet bone basis (McDowell 1997; Pfeffer & Hristov 2005; Underwood & Suttle 1999;). The more accurate method for these comparisons are to evaluate bone minerals on a dry fat-free bone ash basis for Ca, P and Mg, and on a dry fat-free basis for micro minerals, but the high variability indicated that it would have been of no value to evaluate the tail bone minerals in these animals without any data on their feed or feed intakes.

**Table 6.5** Mineral analyses of the liver biopsy samples of the population of steers when the trial started

| | Al mg/kg | Cd mg/kg | Cr mg/kg | Cu mg/kg | Mn mg/kg | Ni mg/kg | Fe mg/kg | Pb mg/kg | Zn mg/kg |
|---|---|---|---|---|---|---|---|---|---|
| Min | 28.5 | 0.18 | 0.73 | 31.5 | 2.52 | 1.42 | 74.3 | 3.59 | 25.4 |
| Max | 5199 | 4.24 | 9.81 | 138 | 25.5 | 21.8 | 1971 | 25.8 | 134 |
| Average | 520 | 0.57 | 2.73 | 54.2 | 6.57 | 5.81 | 313 | 9.33 | 45.8 |
| SD | 1130 | 0.87 | 2.43 | 25.0 | 5.12 | 6.47 | 407 | 6.47 | 23.8 |
| n | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 |

Liver mineral level of randomly selected steers before the start of the trial (DM basis)

The average dry liver Zn levels were 45.8 mg/kg tested in comparison to the published adequate level of 94 – 375 mg Zn/kg (dry matter) as published by Puls (1994). Only one of the 21

steers tested higher than 94 mg/kg for liver Zn and therefore it can be concluded that most animals were low in liver Zn. The average dry liver analysis for the cattle in the Kaalplaats trial (Botha *et al.* unpublished) were much higher at 145, 114 and 80 mg/kg for the osteochondrosis affected, the heathy and the osteochondrosis affected cattle that were supplemented for 90 days, respectively.

According to Puls (1994) the adequate level of Zn to Cu in the liver will give a ratio of almost 1 to 1. The liver mineral results from the 21 steers in this study have shown a Zn to Cu ratio of 0.85 in comparison to the ratio in the Kaalplaats healthy cattle of 0.91, both higher than the ratio of 0.51 for the osteochondrosis affected cattle. Pfeffer and Hristov (2005) considered dry liver Cu to be marginal at a level of 63 to 191 mg/kg, while Puls (1995) recommended levels of 75 to 300 mg/kg as adequate. Although the ratio of Zn to Cu in this trial was acceptable, 18 of the 21 steers tested lower than the marginal 63 mg/kg dry liver Cu, and therefore can be accepted as being low in Cu.

Dry liver Mn levels (as per Table 4.6 in a previous chapter) are marginal between the 5 to 7.5 mg/kg DM band. The average for the 21 steers was 6.57 mg/kg, with 11 of the 21 animals testing under the level of 5 mg/kg DM. All the liver samples from these steers tested low in Pb, with the variability in Al, Cd, Cr and Ni very high and therefore too difficult to interpret.

**Mineral levels in the rib bone and liver samples at the end of the trial**

The average mineral concentrations for the slaughtered Bonsmara steers at the end of this trial (13 February 2007) are shown in Tables 6.6a, 6.6b and Appendix 6 for the rib bone samples (32 animals).

No significant differences were found between the DCP1, DCP2, DCP3, or MCP treatments for percentage fat-free rib bone Ca, P, Mg, moisture, ash, dry fat-free Ca, dry fat-free P, nor dry fat-free rib bone Mg. The dry fat-free rib bone ash Ca values were, respectively, 38.3, 38.9, 38.4 and 38.4 % for the DCP1, DCP2, DCP3 and MCP treatments, and very similar to the published “normal” ranges of 36 to 39.6% for dry fat-free rib bone ash Ca (McDowell 1996 1997; Pfeffer & Hristov 2005; Underwood & Suttle 1999). The dry fat-free rib bone ash Ca in four treatments in this trial was also similar to the values of the healthy animals (37.9%) in the Kaalplaats study. The Kaalplaats osteochondrosis affected animals and the osteochondrosis affected animals supplemented for 3 months had mean dry fat-free bone ash Ca levels of 36.5 and 34.5% respectively and both statistically different and lower than the healthy animals.

Only one animal in this trial, from the DCP2 group, has shown clinical signs of osteochondrosis. The low prevalence of osteochondrosis in this trial did not give answers regarding the effect of different P sources on the prevalence of osteochondrosis, but some answers were found regarding supplementation and growth in cattle. This affected animal cannot be discussed as statistically different, but some of the rib bone data will be mentioned. This animal had a low dry fat-free bone ash % of 54.4%, a low dry fat-free Ca of 21.3% and a low dry fat-free P level of 9.46%. These values were all lower than the mean values of the DCP2 group. Although higher than the mean values in dry fat-free bone ash Ca and P, this animal had a lower dry fat-free ash content which resulted in the possible higher values for dry fat-free bone ash Ca and P.

The mean dry fat-free bone ash Mg % for the DCP1, DCP2, DCP3 and MCP treatments were not different at 0.599, 0.594, 0.599, 0.625, respectively, but lower than the “normal” published level of 1 to 1.2% (McDowell 1997; Underwood & Suttle 1999). The dry fat-free Ca to Mg ratio of DCP1, DCP2, DCP3 and MCP were 64.3, 66.2, 64.8, and 61.6, which according to Suttle (2010) is an indication that all groups were deficient in Mg if the ratio is higher than 50:1. There is no indication from the data in this trial nor the Kaalplaats trial that dry fat-free Mg deficiency is associated with osteochondrosis affected animals.

**Table 6.6a** Mineral analyses of the rib bone samples of the slaughtered Bonsmara steers that were fed *ad lib.* for 433 days on four different phosphorous sources while grazing natural pastures

| Variate, bone samples | Animal groups | Mean | SEM | n | S. E. | cv % | F pr test |
|---|---|---|---|---|---|---|---|
| Fat-free Ca, % | DCP1 | 17.8 | 1.10 | 8 | 1.14 | 6.60 | F pr = 0.28 |
| | DCP2 | 16.7 | 1.15 | 8 | | | |
| | DCP3 | 17.1 | 1.42 | 8 | | | |
| | MCP | 17.1 | 0.82 | 8 | | | |
| Fat-free P, % | DCP1 | 8.10 | 0.51 | 8 | 0.61 | 7.80 | F pr = 0.43 |
| | DCP2 | 7.60 | 0.66 | 8 | | | |
| | DCP3 | 7.73 | 0.71 | 8 | | | |
| | MCP | 7.90 | 0.55 | 8 | | | |
| Fat-free Mg, % | DCP1 | 0.28 | 0.03 | 8 | 0.03 | 12.3 | F pr = 0.41 |
| | DCP2 | 0.26 | 0.04 | 8 | | | |
| | DCP3 | 0.26 | 0.04 | 8 | | | |
| | MCP | 0.28 | 0.03 | 8 | | | |
| Moisture, % | DCP1 | 23.6 | 4.17 | 7 † | 3.25 | 13.3 | F pr = 0.64 |
| | DCP2 | 25.2 | 3.20 | 8 | | | |
| | DCP3 | 25.2 | 3.40 | 8 | | | |
| | MCP | 23.8 | 2.02 | 8 | | | |
| Dry Fat-free Ash, % | DCP1 | 60.2 | 2.60 | 8 | 3.44 | 5.80 | F pr = 0.64 |
| | DCP2 | 58.1 | 3.05 | 8 | | | |
| | DCP3 | 59.4 | 3.80 | 8 | | | |
| | MCP | 58.7 | 4.09 | 8 | | | |
| Dry fat-free Ca, % | DCP1 | 23.1 | 1.42 | 8 | 1.33 | 5.80 | F pr = 0.81 |
| | DCP2 | 22.6 | 1.55 | 8 | | | |
| | DCP3 | 22.8 | 1.20 | 8 | | | |
| | MCP | 22.4 | 1.08 | 8 | | | |
| Dry fat-free bone ash Ca, % | DCP1 | 38.3 | 2.43 | 8 | 2.03 | 5.30 | F pr = 0.93 |
| | DCP2 | 38.9 | 1.32 | 8 | | | |
| | DCP3 | 38.4 | 1.78 | 8 | | | |
| | MCP | 38.3 | 2.39 | 8 | | | |
| Dry fat-free P, % | DCP1 | 10.5 | 0.66 | 8 | 0.82 | 7.80 | F pr = 0.89 |
| | DCP2 | 10.3 | 0.89 | 8 | | | |
| | DCP3 | 10.6 | 0.97 | 8 | | | |
| | MCP | 10.4 | 0.72 | 8 | | | |
| Dry fat-free bone ash P, % | DCP1 | 17.4 | 1.07 | 8 | 1.15 | 6.50 | F pr = 0.88 |
| | DCP2 | 17.7 | 0.96 | 8 | | | |
| | DCP3 | 17.8 | 0.78 | 8 | | | |
| | MCP | 17.8 | 1.60 | 8 | | | |
| Dry fat-free Mg, % | DCP1 | 0.360 | 0.03 | 8 | 0.05 | 12.5 | F pr = 0.80 |
| | DCP2 | 0.346 | 0.05 | 8 | | | |
| | DCP3 | 0.357 | 0.06 | 8 | | | |
| | MCP | 0.366 | 0.03 | 8 | | | |
| Dry fat-free bone ash Mg, % | DCP1 | 0.599 | 0.03 | 8 | 0.06 | 10.5 | F pr = 0.73 |
| | DCP2 | 0.594 | 0.05 | 8 | | | |
| | DCP3 | 0.599 | 0.06 | 8 | | | |
| | MCP | 0.625 | 0.03 | 8 | | | |

SEM = standard error of the mean; DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source. DCP1 and the MCP source were from a supplier which produces products within a continuous production process; F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used); Feeding = healthy animals fed for 433 days with the different supplements, n = number of steers used; † One value was excluded to normalize the data and stabilize variances

**Table 6.6b** Mineral analyses of the rib bone samples of the slaughtered Bonsmara steers that were fed *ad lib.* for 433 days on four different phosphorous sources while grazing natural pastures

| Variate, dry bone samples | Animal groups | Mean | SEM | n | S. E. | cv, % | F pr test |
|---|---|---|---|---|---|---|---|
| Fat-free Na, mg/kg | DCP1 | 4455 | 604 | 8 | 677 | 15.0 | F pr = 0.81 |
| | DCP2 | 4420 | 698 | 8 | | | |
| | DCP3 | 4510 | 609 | 8 | | | |
| | MCP | 4724 | 782 | 8 | | | |
| Fat-free K, mg/kg | DCP1 | 815 | 131 | 8 | 169 | 19.8 | F pr = 0.65 |
| | DCP2 | 863 | 169 | 8 | | | |
| | DCP3 | 917 | 202 | 8 | | | |
| | MCP | 831 | 169 | 8 | | | |
| Fat-free Fe, mg/kg | DCP1 | 29.3 | 9.01 | 8 | 10.9 | 34.0 | F pr = 0.57 |
| | DCP2 | 36.5 | 8.56 | 8 | | | |
| | DCP3 | 32.8 | 11.2 | 8 | | | |
| | MCP | 30.3 | 14.1 | 8 | | | |
| Fat-free Mn, mg/kg | DCP1 | 0.68 | 0.09 | 8 | 0.24 | 31.7 | F pr = 0.66 |
| | DCP2 | 0.79 | 0.26 | 8 | | | |
| | DCP3 | 0.82 | 0.24 | 8 | | | |
| | MCP | 0.79 | 0.33 | 8 | | | |
| Fat-free Zn, mg/kg | DCP1 | 43.1 | 4.75 | 8 | 4.59 | 10.7 | F pr = 0.89 |
| | DCP2 | 43.6 | 6.01 | 8 | | | |
| | DCP3 | 41.9 | 2.99 | 8 | | | |
| | MCP | 43.2 | 4.08 | 8 | | | |
| Fat-free Cu, mg/kg | DCP1 | 0.51 | 0.04 | 8 | 0.05 | 10.5 | F pr = 0.59 |
| | DCP2 | 0.49 | 0.04 | 8 | | | |
| | DCP3 | 0.48 | 0.06 | 8 | | | |
| | MCP | 0.48 | 0.07 | 8 | | | |
| Fat-free Al, mg/kg | DCP1 | 12.0 | 3.27 | 8 | 2.64 | 21.5 | F pr = 0.87 |
| | DCP2 | 12.7 | 2.55 | 8 | | | |
| | DCP3 | 12.6 | 2.40 | 8 | | | |
| | MCP | 11.8 | 2.19 | 8 | | | |
| Fat-free S, mg/kg | DCP1 | 626 | 40.0 | 8 | 44.2 | 6.90 | F pr = 0.60 |
| | DCP2 | 647 | 50.6 | 8 | | | |
| | DCP3 | 646 | 34.9 | 8 | | | |
| | MCP | 655 | 49.5 | 8 | | | |
| Fat-free F, mg/kg | DCP1 | 252 | 57.1 | 8 | 64.7 | 24.0 | F pr = 0.55 |
| | DCP2 | 278 | 58.5 | 8 | | | |
| | DCP3 | 293 | 85.2 | 8 | | | |
| | MCP | 256 | 52.9 | 8 | | | |

SEM = standard error of the mean; DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source. DCP1 and the MCP source were from a supplier which produces products within a continuous production process; F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used); Feeding = healthy animals fed for 433 days with the different supplements, n = number of steers used

No differences were found between the fat-free rib bone analyses between DCP1, DCP2, DCP3 or the MCP treatments for Na, K, S, Fe, Mn, Zn, Cu, Al, and F.

All treatments showed a low mean fat-free Zn level of 43.1, 43.6, 41.9, and 43.2 mg/kg respectively for DCP1, DCP2, DCP3 and MCP, in comparison to the published marginal value of 50 to 71 mg/kg (Pfeffer & Hristov 2005; Underwood & Suttle 1999). Underwood and Suttle (1999) reported fat-free rib bone Mn to be marginal between 1.0 to 1.4 mg/kg, and in this trial all treatment mean values analyzed low (DCP1=0.677; DCP2=0.787; DCP3=0.823; MCP=0.789 mg/kg).

The low prevalence of osteochondrosis in this trial indicated no problems regarding bone formation and therefore it was decided not to do any bone density tests to evaluate the mineral concentrations per unit of bone as many other researchers have already published data regarding this for the Armoedsvlakte farm where this trial was conducted (Little 1972; Little & Minson 1977; Read *et al*. 1986; Spangenberg 1997).

**Mineral levels in the liver samples at the end of the trial**

The results of the mineral analyses of the trial animals are provided in Tables 6.7a and 6.7b. The mean dry liver analysis for DCP1, DCP2, DCP3 and MCP fed animals differed statistically for P, Mg, Na, K, S, and Zn after the 433-day period. No differences between the treatments were found for dry liver Ca (%), C (%), N (%), Fe (mg/kg), Mn (mg/kg), Cu (mg/kg), or Al(mg/kg).

The mean liver phosphorous values (DM) for the MCP fed steers were statistically lower than the DCP1 and DCP3 fed animals (0.336 vs 0.363 and 0.378%, $p = 0.001$), whereas DCP1 did not differ from DCP2 or DCP3 (0.368 vs 0.344 and 0.378%, $p = 0.001$). DCP3 were also different and higher than DCP2 (0.378 vs 0.344%, $p = 0.001$). Potassium followed the same statistical trends as P with mean liver values of 0.333, 0.313, 0.339, and 0.310 for the DCP1, DCP2, DCP3 and MCP treatments, respectively ($p = 0.029$).

Animals store the surplus P and Ca in their bones when the dietary supply of these minerals are higher than their Ca and P requirement (De Waal & Koekemoer 1997; Read *et al*. 1985; Spangenberg 1997). This did not happen in this trial as found with the rib bone values which showed no statistical differences in either Ca or P. They tested within normal published ranges, notwithstanding the liver data which indicated that some groups had higher liver P percentages than others.

The mean dry liver Mg values were the lowest in the MCP (166mg/kg) and DCP2 (177.8 mg/kg) treatments and not statistically different, but both statistically different and lower than the DCP3 (195mg/kg, $p$=0.001) fed steers.

**Table 6.7a** Mineral, carbon and nitrogen analyses of the liver samples of the slaughtered Bonsmara steers that were fed *ad lib.* for 433 days on four different phosphorous sources while grazing natural pastures

| Variate, dry liver samples | Animal groups | Mean | SEM | n | S. E. | cv, % | F pr test |
|---|---|---|---|---|---|---|---|
| P, % | DCP1 | 0.363$^{bc}$ | 0.022 | 8 | 0.02 | 5.70 | F pr = 0.001 |
| | DCP2 | 0.344$^{ab}$ | 0.023 | 8 | | | |
| | DCP3 | 0.378$^{c}$ | 0.019 | 8 | | | |
| | MCP | 0.336$^{a}$ | 0.017 | 7$^{\dagger}$ | | | |
| Ca, mg/kg | DCP1 | 253 | 28.4 | 8 | 34.6 | 14.5 | F pr = 0.24 |
| | DCP2 | 241 | 32.2 | 7$^{\dagger}$ | | | |
| | DCP3 | 243 | 13.0 | 8 | | | |
| | MCP | 218 | 48.4 | 8 | | | |
| Mg, mg/kg | DCP1 | 182$^{bc}$ | 14.7 | 8 | 12.9 | 7.2 | F pr = 0.001 |
| | DCP2 | 178$^{ab}$ | 8.58 | 8 | | | |
| | DCP3 | 195$^{c}$ | 16.3 | 8 | | | |
| | MCP | 166$^{a}$ | 10.6 | 8 | | | |
| Na, mg/kg | DCP1 | 616$^{ab}$ | 50.0 | 8 | 61.3 | 10.0 | F pr = 0.04 |
| | DCP2 | 630$^{b}$ | 56.4 | 8 | | | |
| | DCP3 | 650$^{b}$ | 73.1 | 8 | | | |
| | MCP | 560$^{a}$ | 63.5 | 8 | | | |
| K, % | DCP1 | 0.333$^{bc}$ | 0.020 | 8 | 0.02 | 6.80 | F pr = 0.03 |
| | DCP2 | 0.313$^{ab}$ | 0.020 | 8 | | | |
| | DCP3 | 0.339$^{c}$ | 0.024 | 8 | | | |
| | MCP | 0.310$^{a}$ | 0.023 | 7$^{\dagger}$ | | | |
| S, % | DCP1 | 0.238$^{c}$ | 0.031 | 8 | 0.03 | 11.8 | F pr = 0.03 |
| | DCP2 | 0.210$^{ab}$ | 0.022 | 8 | | | |
| | DCP3 | 0.236$^{bc}$ | 0.026 | 8 | | | |
| | MCP | 0.205$^{a}$ | 0.026 | 7$^{\dagger}$ | | | |
| C, % | DCP1 | 17.1 | 0.790 | 8 | 0.84 | 4.90 | F pr = 0.71 |
| | DCP2 | 16.7 | 0.923 | 8 | | | |
| | DCP3 | 16.9 | 0.792 | 8 | | | |
| | MCP | 17.1 | 0.828 | 8 | | | |
| N, % | DCP1 | 3.55 | 0.200 | 8 | 0.20 | 5.60 | F pr = 0.38 |
| | DCP2 | 3.38 | 0.238 | 8 | | | |
| | DCP3 | 3.50 | 0.154 | 8 | | | |
| | MCP | 3.46 | 0.176 | 8 | | | |

SEM = standard error of the mean; DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source. DCP1 and the MCP source were from a supplier which produces products within a continuous production process; $^{abc}$ Means in the same variate with different superscripts differ ($p < 0.05$); F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used); Feeding = healthy animals fed for 433 days with the different supplements, n = number of steers used; $^{\dagger}$One value was excluded to normalize the data and stabilize variances

All DCP treatments were not different from each other for liver Na with mean values 616, 630 and 650 mg/kg DM, for DCP1, DCP2 and DCP3 respectively (Table 6.7a). The liver Na of DCP1

was not different from the mean liver Na level of MCP (616 vs 560 mg/kg DM*)*, but DCP2 and DCP3 have shown higher and different mean values in comparison with the MCP fed steers (630 and 650 vs 560 mg/kg DM for MCP, *p=0.039).* It was estimated by the ARC (1980) that cattle absorbed 91% of the Na that they consumed. The kidneys play an important role in the absorption and excretion of Na which then influences the concentration of Na in the blood and other tissues. According to Renkema *et al*. (1962), cattle excrete very little Na through their faeces and can therefore survive on relative low Na diets for a long period.

The DCP1 and DCP3 fed steers had the highest mean dry liver S content, 0.238 and 0.236% respectively. DCP2 was lower but did not differ from DCP3 (0.210 vs. 0.236). The MCP fed animals had the lowest mean dry liver S, which differ from DCP1 and DCP3 (*p=0.032*) but not from the DCP2 group (0.205 vs. 0.210%).

The mean dry liver Zn level did not show differences between the three dicalcium fed groups (DCP1 = 30.3, DCP2 = 31.7, DCP3 = 32.6mg/kg), but all three tested higher and different compared to the mono calcium phosphate fed group (26.9 mg/kg, *p=0.001*) (Table 6.7b). The adequate dry liver Zn level for cattle was recommended by Puls (1994) to be 94 – 375 mg/kg. The livers in this trial analyzed lower than the adequate level, but all steers did not show Zn related deficiencies. The mean dry liver Zn levels of all these groups in this trial were also lower than the osteochondrosis affected, the healthy, and the osteochondrosis affected animals that were supplemented in the Kaalplaats trial, which had mean liver Zn values of 145, 114 and 80 mg/kg DM.

The mean dry liver values in all the treatments for Fe, Mn and Cu were not statistically different, but they were all lower than the published adequate range of 100 – 150 mg Fe/kg (Underwood & Suttle 1999), 5 – 18 mg Mn/ kg (McDowell 1997; Puls 1994; Underwood & Suttle 1999), and 75 to 300 mg Cu/kg (Pfeffer & Hristov 2005; Puls 1994; Underwood & Suttle 1999). The low level of Cu in this trial was similar to a study by Wadsworth *et al.* (1988) who also has shown lower liver Cu levels (<50 mg/kg) in steers when P was supplemented through mono-ammonium phosphate in comparison to the liver levels of the un-supplemented P group (> 150mg/kg). This effect of P on liver Cu levels was also reported by Karn and Hofmann (1990) but the mechanism for this animal response was not explained, nor an explanation why these cattle did not show any Cu deficiency.

The low mineral levels in this trial was mainly due to the supplements which did not contain any added vitamin-mineral premixes, but only the inherent nutrients or minerals of the sources as provided in Tables 6.2 and 6.3.

**Table 6.7b** Mineral analyses of the liver samples of the slaughtered steers that were fed *ad lib.* for 433 days on four different phosphorous sources while grazing natural pastures

| Variate, dry liver samples | Animal groups | Mean | SEM | n | S. E. | cv, % | F pr test |
|---|---|---|---|---|---|---|---|
| Fe, mg/kg | DCP1 | 49.80 | 13.5 | 8 | 11.5 | 21.6 | F pr = 0.33 |
| | DCP2 | 57.40 | 7.05 | 8 | | | |
| | DCP3 | 56.70 | 14.5 | 8 | | | |
| | MCP | 49.00 | 9.30 | 8 | | | |
| Mn, mg/kg | DCP1 | 3.23 | 0.50 | 8 | 0.50 | 15.4 | F pr = 0.98 |
| | DCP2 | 3.26 | 0.63 | 8 | | | |
| | DCP3 | 3.23 | 0.49 | 8 | | | |
| | MCP | 3.16 | 0.31 | 8 | | | |
| Zn, mg/kg | DCP1 | 30.3[b] | 2.95 | 8 | 2.72 | 8.90 | F pr = 0.001 |
| | DCP2 | 31.7[b] | 3.21 | 8 | | | |
| | DCP3 | 32.6[b] | 2.51 | 8 | | | |
| | MCP | 26.9[a] | 2.47 | 8 | | | |
| Cu, mg/kg | DCP1 | 31.6 | 4.84 | 8 | 9.82 | 28.1 | F pr = 0.32 |
| | DCP2 | 40.2 | 14.4 | 8 | | | |
| | DCP3 | 35.2 | 8.86 | 8 | | | |
| | MCP | 32.7 | 8.78 | 8 | | | |
| Al, mg/kg | DCP1 | 5.38 | 2.41 | 8 | 2.23 | 40.7 | F pr = 0.27 |
| | DCP2 | 4.64 | 0.71 | 8 | | | |
| | DCP3 | 6.80 | 3.27 | 8 | | | |
| | MCP | 5.13 | 1.60 | 7 † | | | |

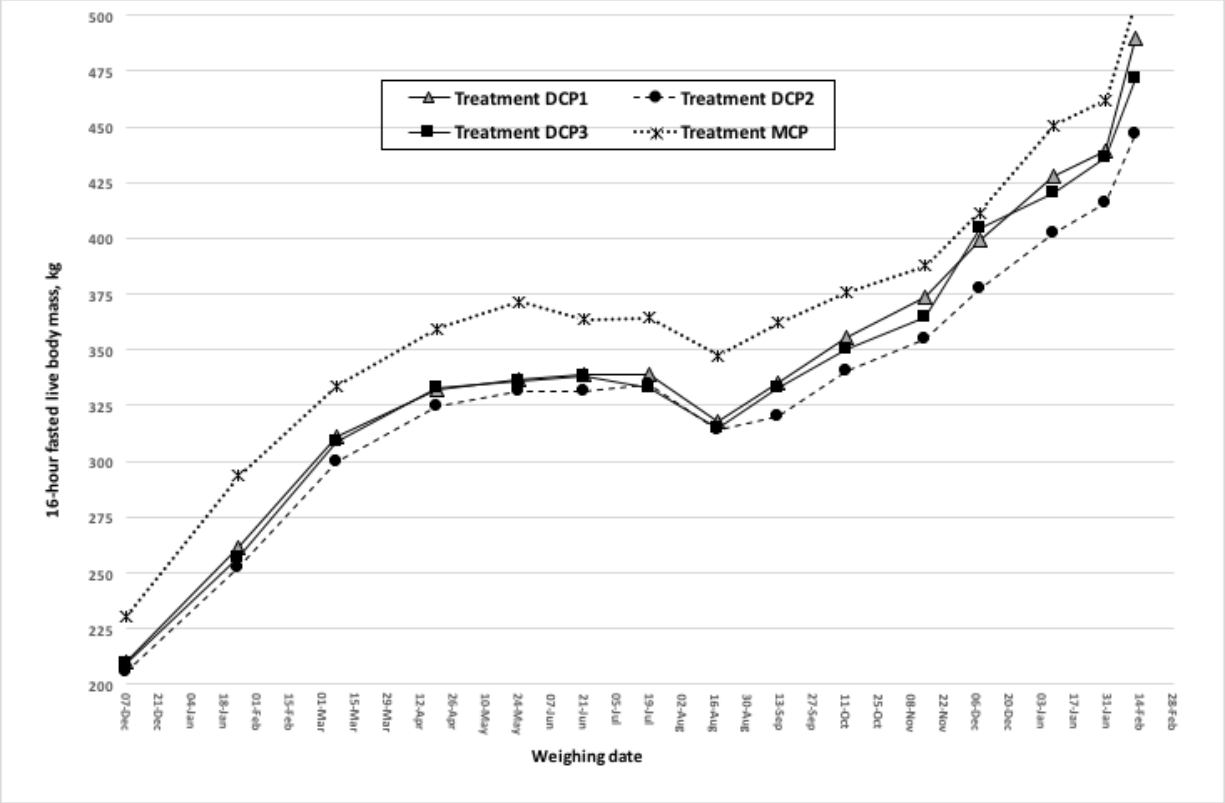
SEM = standard error of the mean; DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source. DCP1 and the MCP source were from a supplier which produces products within a continuous production process; [abc] Means in the same variate with different superscripts differ ($p < 0.05$); F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used); Feeding = healthy animals fed for 433 days with the different supplements, n = number of steers used; † One value was excluded to normalize the data and stabilize variances

**Growth of animals fed with the different P sources**

The growth rate of the Bonsmara steers in the 433-day trial is shown in Tables 6.8 and 6.9, as well as in Appendix 7, 8, 9 and 10. The growth of the animals is also illustrated in Figures 6.1.a. and 6.1.b.

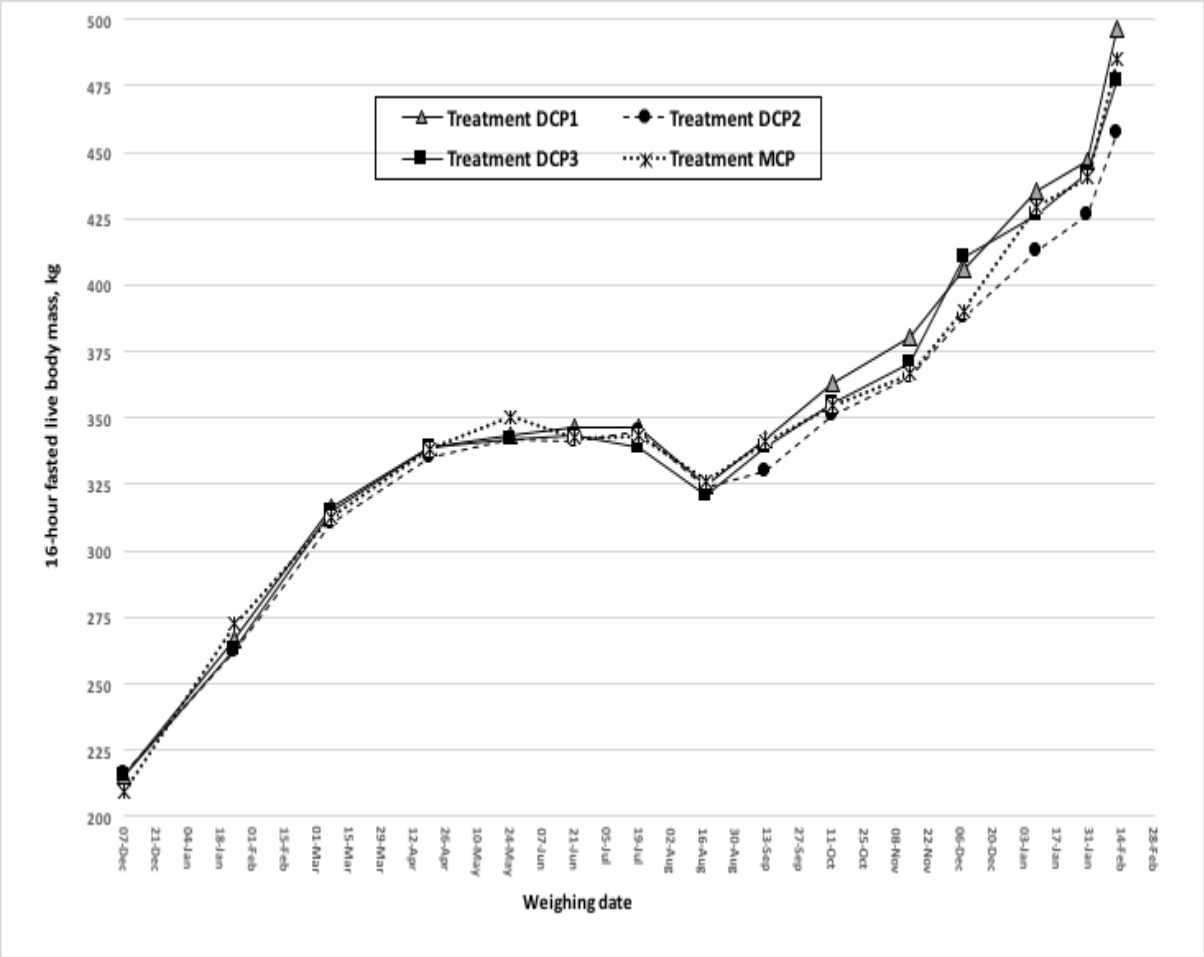
The starting 16-hour fasted live body weights of the Bonsmara steers were 210, 206, 209 and 231 kg for the DCP1, DCP2, DCP3 and MCP treatments, respectively. Figure 6.1a, Appendixes 7 and 8 shows the growth per steer per treatment over the 433-day trial period until slaughtered on the 13th of February 2007.

No correlation was found between body weight over time (*p=0.455)* and therefore the Identity matrix was selected as a model (same as in ANOVA), with fixed effects specified as supplement, day and supplement by day interaction, and random effects as steer and steer by day interaction. The results from this analysis showed that the data must be adjusted for starting weight (*p=0.001)* as a covariate with the results illustrated as per Figure 6.1b and Table 6.8. The starting 16-hour fasted live body weights of the Bonsmara steers were adjusted to 215, 216, 215 and 210 kg for the DCP1, DCP2, DCP3 and MCP treatments, respectively.




DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source; DCP1 and the MCP source were from a supplier which produces products within a continuous production process.

**Figure 6.1a.** The 16-hour fasted live body weights of the 32 Bonsmara steers (8 per treatment) that were fed four different phosphorous supplements over a period of 433 days (8 December 2005 – 13$^{th}$ of February 2007) and **not adjusted** for starting weight as covariate




DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source; DCP1 and the MCP source were from a supplier which produces products within a continuous production process.

**Figure 6.1b** The 16-hour fasted live body weights of the 32 Bonsmara steers (8 per treatment) that was fed four different phosphorous supplements over a period of 433 days (8 December 2005 – 13th of February 2007) and adjusted for starting weight as covariate

The mean body weight (BW) was the lowest for the DCP2 (347kg) group and different from the DCP1, DCP3 and MCP treatments (356, 353, and 354kg, $p<0.001$). Treatments DCP1, DCP3 and MCP did not differ significantly in mean body weight.

As expected, the growth per time period showed statistical differences over the 433-day trial ($p<0.001$). The steers were supposed to be weighed every 28 days, but abnormal high rainfall made it impossible during the first four months and therefore it was only done 3 times during the first 4 months, where after they were weighed according to the planned intervals. The mean 16h-fasted weights over all weighing dates were different, except for:

- 19 April vs. 17 August vs 12 September 2006 that were not different (338 vs. 324 vs. 338kg)

- 19 April (338kg) vs. 24 May (344kg) vs. 21 June (344kg) vs. 19 July (343kg) vs. 12 Sept 2006 (338kg),
- 24 May (344kg) vs. 21 June (344kg) vs. 19 July (343kg) vs. 12 Sept (338kg) vs. 11 October (356kg)
- 11 October (356kg) vs. 14 November (371kg).
- 8 January (426kg) vs. 30 January 2007 (439kg)

The mean weight decreased from 343 on the 19th of July 2006 to 324kg in August 2006, that is 0.672 kg daily weight loss over the 29 days. The low gains from the 19th of April until the 12th of Sept 2006 (0.2kg in 146 days) were due to the winter, which is known for low quality pastures during this period. The mean animal weight gain for the 32 steers increased from 12 Sept 2006 to 11 October by 0.621kg per day for 29 days, and it was not statistical different between treatments. The mean weights of all the steers in this trial increased by 265kg over the 433-day period, on average 0.613kg per day.

The average daily gain (ADG) of the steers over the last 91 days of the trial was 1.28, 1.01, 1.17 and 1.31kg/steer/day (116, 91.1, 107 and 119kg per steer over the period) for the DCP1, DCP2, DCP3 and MCP treatments respectively.

**Table 6.8** Adjusted16-h fasted start weights of Bonsmara steers that were fed *ad lib.* for 433 days on four different phosphorous sources while grazing natural pastures

| Treatment | Mean 16h-fasted body weight per treatment as per weight date, kg. Adjusted for starting weight as covariate. | | | | | | | | | | | | | | | Mean BW, kg | n | SEM | *F pr test* | Fixed model effects | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 07-Dec | 24-Jan | 07-Mar | 19-Apr | 24-May | 21-Jun | 19-Jul | 17-Aug | 12-Sept | 11-Oct | 14-Nov | 07-Dec | 08-Jan | 30-Jan | 13-Feb | | | | | Date | Starting weight (covariate) | Date / Treatment |
| DCP1 | 215 | 266 | 317 | 339 | 343 | 346 | 346 | 325 | 342 | 363 | 381 | 406 | 435 | 447 | 497 | 358[b] | 8 | 1.58 | F pr < 0.001 | F pr < 0.001, SEM = 3.13 | F pr < 0.001 | F pr = 0.46, SEM = 6.09 |
| DCP2 | 216 | 262 | 310 | 335 | 342 | 341 | 345 | 324 | 330 | 351 | 365 | 388 | 412 | 426 | 457 | 347[a] | 8 | | | | | |
| DCP3 | 215 | 263 | 315 | 339 | 342 | 344 | 339 | 321 | 339 | 356 | 370 | 410 | 426 | 442 | 477 | 353[ab] | 8 | | | | | |
| MCP | 210 | 272 | 313 | 338 | 350 | 343 | 343 | 326 | 341 | 355 | 367 | 390 | 429 | 441 | 485 | 354[ab] | 8 | | | | | |
| Weight day† | 0 | 48 | 90 | 133 | 168 | 196 | 224 | 253 | 279 | 308 | 342 | 365 | 397 | 419 | 433 | | | | | | | |
| **Mean BW per weight date** | **214[a]** | **266[b]** | **314[c]** | **338[de]** | **344[ef]** | **344[ef]** | **343[ef]** | **324[cd]** | **338[def]** | **356[fg]** | **371[g]** | **399[h]** | **426[i]** | **439[i]** | **479[j]** | | | | | | | |

Linear mixed model SEM = standard error of the mean; [abcdefghij]Means in the same variate with different superscripts differ ($p < 0.05$); F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used); n = number of steers used; † Mean the day since the starting day

The daily gains per treatment group are shown in Table 6.12, as adjusted for starting weight as covariate, as well as in Appendix 10 (with starting weight not adjusted).

**Table 6.9** Mean daily gains for the Bonsmara steers that were fed *ad lib.* for 433 days on four different phosphorous sources while grazing natural pastures when the adjusted 16-h fasted starting weights were used

| Treatment | Weight day and 16h-fasted body weight (kg) | | n | Mean Average Daily Gain, kg | SEM | F pr test | Fixed model effects |
|---|---|---|---|---|---|---|---|
| | 07-Dec | 13-Feb | | | | | Starting weight, Covariate |
| DCP1 | 215 | 497 | 8 | 0.655[b] | 0.06, (cv% = 9.20) | F pr = 0.03 | F pr = 0.01 |
| DCP2 | 216 | 457 | 8 | 0.566[a] | | | |
| DCP3 | 215 | 477 | 8 | 0.610[ab] | | | |
| MCP | 210 | 485 | 8 | 0.616[ab] | | | |
| | Day 0 | Day 433 | | | | | |

Linear mixed model SEM = standard error of the mean; DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source. DCP1 and the MCP source were from a supplier which produces products within a continuous production process; [abc] Means in the same variate with different superscripts differ ($p < 0.05$); F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used); Feeding = healthy animals fed for 433 days with the different supplements (7 Dec 2005 - 13 Feb 2007), n = number of steers used

DCP1, DCP3 and MCP fed steers showed the highest ADG as shown in Table 6.9 over the total feeding period (0.655 vs. 0.610 vs.0.616 kg/steer/day, *p=0.033).* DCP2 fed steers had a lower mean daily gain in comparison to DCP1 fed steers, whereas DCP2 fed animals did not differ statistically from DCP3 nor MCP animals.

Researchers around the world found inconsistent responses in growth of cattle that are supplemented with P due to factors like the animal's genetic potential, environment, cattle's P status, P availability in their ration, level of P in the trial diets, gastro-intestinal recycling of P and resorption of bone P mechanisms, feeding system (pen fed or pasture fed), nutrient levels of the total diet, age of the cattle, and their production stage (De Brouwer *et al*. 2000; De Waal & Koekemoer 1997; Karn 2001; Spangenberg 1997). Research, furthermore, has not only indicated a DMI response to P supplementation, but results also indicated a change in selection of specific pasture species (Coates & Le Feuvre 1998, Hendricksen & Punter 1990).

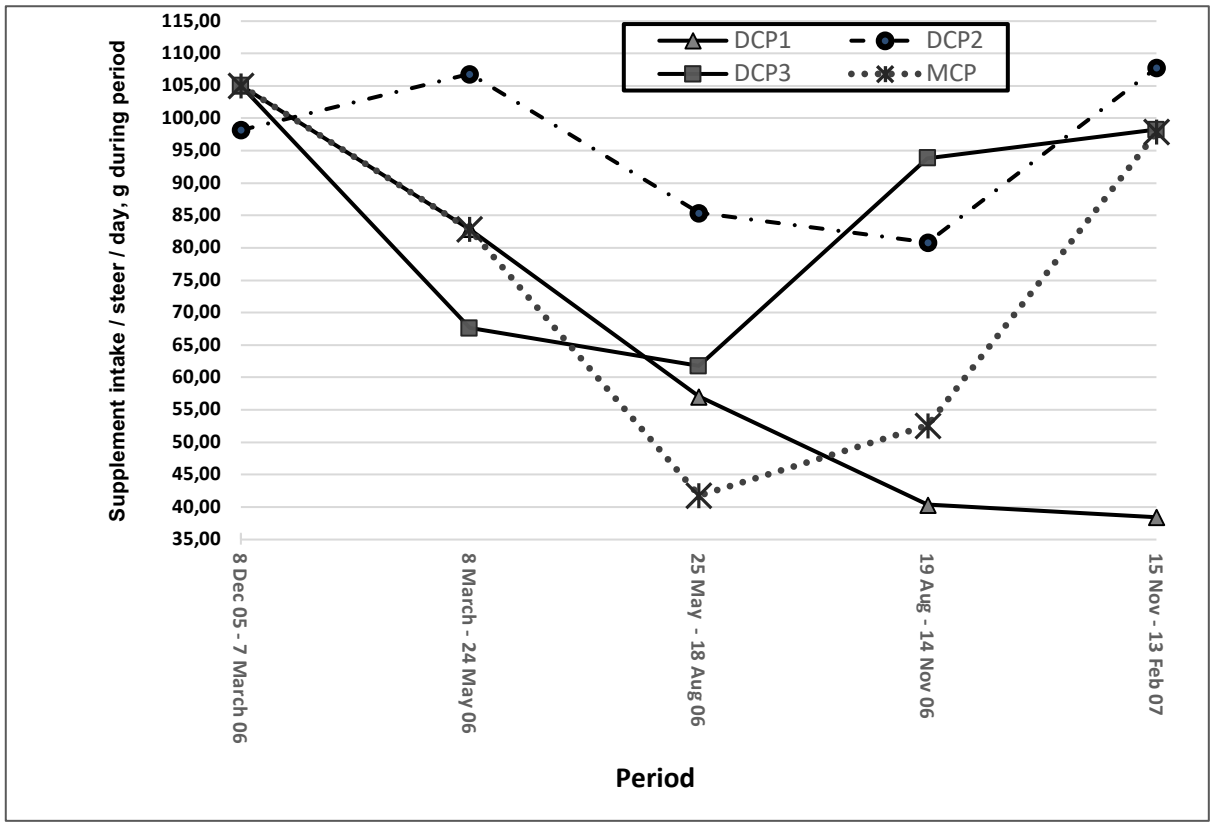
**Supplement intake of animals fed with different P sources**

All supplements were fed on an *ad libitum* basis and all the intakes are reported in Table 6.10, 6.11 and illustrated in Figures 6.2a and 6.2b. It was more important at the time of this trial that animals be managed under normal grazing conditions, and therefore it was decided to measure all intakes on a per group basis. All groups were simultaneously rotated through camps to minimize camp effects. The supplements were colour-coded to ensure the correct supplements were given and intakes were daily monitored and then calculated every 28 days (during weighing) to ensure all animals received the correct quantities according to their group supplement intakes.

Analysis of variance calculations could not be done on the group supplement intake data due to no treatment variance per animal within treatment. For this reason, the data is discussed without any least significant difference analyses and therefore only reported as observations. The growth data in this part of the discussion is explained on measured live weights and not adjusted for starting weight as covariate to evaluate the data more correctly with the real mean 16h-fasted live body weights and real growth rates. The mean weights were also used to calculate the metabolic weights of the animals within treatment to evaluate the supplement intakes against metabolic weight and not only on a total weight basis, and to evaluate the actual nutrient use efficiencies in this trial.

The mean total supplement intake over the 433-day period for the continuous manufacturing process products, DCP1 and MCP were 27.8 and 33.0 kg per animal, respectively. The “batch by batch” manufactured products had a mean total supplement intake of respectively 41.3 and 37.1 kg per animal for the DCP2 and DCP3 treatments. The average daily supplement intakes were 64.3, 95.3, 85.8 and 76.1 g per steer respectively for the DCP1, DCP2, DCP3 and MCP treatments.

The total P intake per steer over the 433-day period was 2.81, 4.09, 4.06, and 3.53 kg for groups DCP1, DCP2, DCP3 and MCP, respectively. This gave a daily P intake per steer of 6.49, 9.44, 9.37, and 8.14 g over the 433-day period.




DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source; DCP1 and the MCP source were from a supplier which produces products within a continuous production process. DCP1 and MCP were produced in a continuous well controlled production facility. DCP2 and DCP3 were produced in a "batch by batch" production facility with less heat control during the chemical reaction process; Supplement intake was calculated within a group over the period (example 8 Dec to 7 March 06 = mean value for the group)

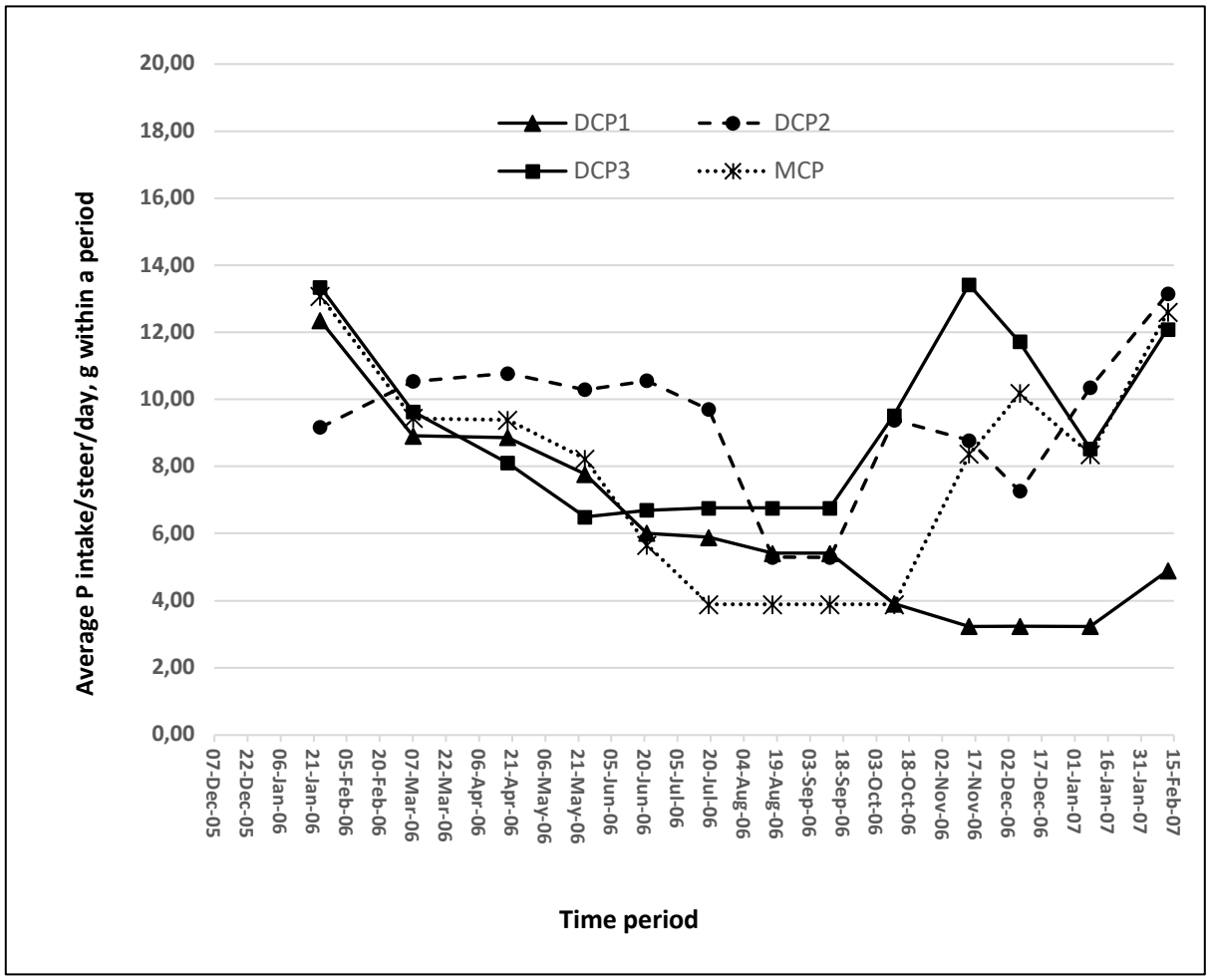
**Figure 6.2a** The average supplement intake per day per Bonsmara steer within a specific time period for the four different phosphorous treatment groups fed over a 433-day period on the Armoedsvlakte farm in the North West province, South Africa

The average daily supplement intake from the 8th of Dec 2005 until the 7th of March were, respectively, 105, 98.2, 105 and 105g per steer for the treatments, DCP1, DCP2, DCP3 and MCP respectively. The daily intake decreased to a winter mean average of 57.0, 85.4, 61.8 and 41.8g respectively for the groups, DCP1, DCP2, DCP3 and MCP. A definite seasonal trend was observed for groups DCP2, DCP3 and the MCP with higher summer intakes and lower winter intakes (Figure 6.2a). Seasonal live body weight gain difference also occurred during the winter period (25 May to 18 August 2006) with weight changes of -18.6, -17.6, -21.1 and -24 kg per steer respectively for treatments DCP1, DCP2, DCP3 and MCP and therefor a possible reason for the lower supplement intake trend during this period. Despite the consistent decrease in daily supplement intake for the

DCP1 treatment, the mean daily gains of the steers were not different from treatments DCP3 or MCP (0.553 vs 0.610, 0.616 kg/steer).

Cattle store the surplus P and Ca in their bones when the dietary supply of these minerals are higher than their Ca and P requirement (De Waal & Koekemoer 1997; Read *et al*. 1986; Spangenberg 1997). The steers in this trial have shown no differences in rib bone fat-free ash Ca and P, irrespective of the different supplement Ca and P intakes and different growth rates. Both DCP1 and MCP (well controlled continuous production process) have shown lower supplement intake levels than the DCP2 and DCP3 sources without recording decreased rib bone Ca, P or Mg mineral levels, which is an indication that bioavailability could have played a role here. Van der Klis and Versteegh (1996) as well as Waibel *et al*. (1984) have shown differences in P bioavailability of 55 – 95.8% in monogastric animals amongst the different DCP, MDCP and MCP sources from different manufacturing processes.

Low P intakes were also observed in the DCP1 and MCP treatment groups without affecting the rib bone mineral level, which was also true for the high P intake groups. This was not due to high P from pasture intake, because Armoedsvlakte is well known for its low P in pasture (De Brouwer *et al*. 2000; De Waal & Koekemoer 1997; Spangenberg 1996). The differences might therefore be associated with P bioavailability, but this will have to be investigated in future trials.




DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source; DCP1 and the MCP source were from a supplier which produces products within a continuous production process. DCP1 and MCP were produced in a continuous well controlled production facility. DCP2 and DCP3 were produced in a "batch by batch" production facility with less heat control during the chemical reaction process; P intake was calculated over the previous period (example 7 Dec to 24 Jan 2005 = average data on position 24 Jan)

**Figure 6.2b** The average P intakes per day per Bonsmara steer within a specific time period for the four different phosphorous treatment groups fed over a 433-day period on the Armoedsvlakte farm in the North West province, South Africa

**Table 6.10** The average supplement intake as well as the weight change per Bonsmara steer within a specific time period when fed four different phosphorous sources over a period of 433-days

| Variate | Period | Treatments | | | |
|---|---|---|---|---|---|
| | | DCP1 | DCP2 | DCP3 | MCP |
| Ave lick intake/steer/day, g during period | 8 Dec 05 - 7 March 06 | 105 | 98.2 | 105 | 105 |
| | 8 March - 24 May 06 | 82.9 | 106.8 | 67.6 | 82.9 |
| | 25 May - 18 Aug 06 | 57.0 | 85.4 | 61.8 | 41.8 |
| | 19 Aug - 14 Nov 06 | 40.4 | 80.9 | 93.4 | 52.6 |
| | 15 Nov - 13 Feb 07 | 38.4 | 108 | 98.3 | 97.9 |
| Ave P intake/steer/day, g | 8 Dec 05 - 7 March 06 | 10.6 | 9.71 | 11.5 | 11.2 |
| | 8 March - 24 May 06 | 8.38 | 10.6 | 7.38 | 8.87 |
| | 25 May - 18 Aug 06 | 5.76 | 8.44 | 6.75 | 4.47 |
| | 19 Aug - 14 Nov 06 | 4.08 | 7.99 | 10.3 | 5.63 |
| | 15 Nov - 13 Feb 07 | 3.88 | 10.7 | 10.7 | 10.5 |
| Tot lick intake/steer/period, g | 8 Dec 05 - 7 March 06 | 9453 | 8839 | 9453 | 9453 |
| | 8 March - 24 May 06 | 6465 | 8333 | 5275 | 6465 |
| | 25 May - 18 Aug 06 | 4905 | 7345 | 5313 | 3593 |
| | 19 Aug - 14 Nov 06 | 3552 | 7116 | 8259 | 4627 |
| | 15 Nov - 13 Feb 07 | 3457 | 9706 | 8844 | 8813 |
| Total lick intake/steer (total trial period), g | | 27832 | 41339 | 37144 | 32951 |
| Total P intake/steer/day, g during period | 8 Dec 05 - 7 March 06 | 955 | 874 | 1032 | 1011 |
| | 8 March - 24 May 06 | 653 | 824 | 576 | 692 |
| | 25 May - 18 Aug 06 | 496 | 726 | 580 | 384 |
| | 19 Aug - 14 Nov 06 | 359 | 703 | 902 | 495 |
| | 15 Nov - 13 Feb 07 | 349 | 959 | 966 | 943 |
| Total P intake/steer (total trial period), g | | 2812 | 4086 | 4056 | 3526 |
| Total live body weight gain per period, kg † | 8 Dec 05 - 7 March 06 | 102 | 94 | 99.6 | 103 |
| | 8 March - 24 May 06 | 23.3 | 31.6 | 26.9 | 37.4 |
| | 25 May - 18 Aug 06 | -18.6 | -17.6 | -21.1 | -24 |
| | 19 Aug - 14 Nov 06 | 55.7 | 41.1 | 49.8 | 40.4 |
| | 15 Nov - 13 Feb 07 | 118 | 91.9 | 107 | 119 |
| Starting live body weight (not corrected), kg | | 210 | 206 | 209 | 231 |
| End live body weight (not corrected), kg | | 488 | 447 | 471 | 507 |
| Total live body weight gain/steer (total trial period), kg ‡ | | 280 | 241 | 262 | 276 |
| Total P intake/kg live weight change per period, g§ | 8 Dec 05 - 7 March 06 | 9.41 | 9.30 | 10.4 | 9.78 |
| | 8 March - 24 May 06 | 28.0 | 26.1 | 21.4 | 18.5 |
| | 25 May - 18 Aug 06 | -26.7 | -41.3 | -27.5 | -16.0 |
| | 19 Aug - 14 Nov 06 | 6.44 | 17.1 | 18.1 | 12.3 |
| | 15 Nov - 13 Feb 07 | 2.97 | 10.4 | 9.04 | 7.94 |
| Total P intake / kg total live weight gain, g ¶ | | 10.1 | 17.0 | 15.5 | 12.8 |
| g P intake / kg metabolic weight gain ++ | | 41.1 | 66.8 | 62.3 | 52.1 |

DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source; DCP1 and the MCP source were from a supplier which produces products within a continuous production process. DCP1 and MCP were produced in a continuous well controlled production facility. DCP2 and DCP3 were produced in a "batch by batch" production facility with less heat control during the chemical reaction process; Statistical analysis was not done on the feed intake data due to the lack of variance because all steers were fed in groups and not individually. † Mean 16h-fasted live weight gain over die period in kg (negative value means cattle lost weight during the period); ‡ The mean 16h-fasted live weight gain from the 8th of Dec 2005 to the 13th of Feb 2007). § The mean total phosphorous intake per metabolic weight to evaluate the P intake for every kg metabolic weight gain per period. Metabolic weight = (16h-fasted

live weight in kg)$^{0.75}$; ¶ Total P intake per kg 16h-fasted live weight gain evaluated over the total period; $^{++}$ The mean g phosphorous intake per kg metabolic weight gain to evaluate the P intake for every kg metabolic weight gain evaluated over the total period

All the treatment groups decreased in mean 16h-fasted body weight during the winter months (25$^{th}$ of May 2006 until the 18$^{th}$ of August 2006), with 18.6, 17.6, 21.1 and 24 kg per steer for DCP1, DCP2, DCP3 and MCP respectively. The steers gained in weight from the 19$^{th}$ of August until the 13$^{th}$ of February 2007 by 172, 133, 157 and 159 kg, with a mean supplement intake of 27.8, 41.3, 37.1, and 33 kg per steer, and a mean P intake of 2.81, 4.09, 4.06 and 3.53 kg per steer respectively for the DCP1, DCP2, DCP3 and MCP treatments (based on the real weights and not corrected for starting weights).

The P intake per kg live weight gain for the total 433-period was, respectively, 10.1, 17.0, 15.5 and 12.8 g per kg live weight gain for the DCP1, DCP2, DCP3 and MCP treatments. The P intake per kg metabolic weight gain was also calculated to evaluate the utilization on a more justifiable nutrient requirement basis. The P intake per kg metabolic weight gain was 41.1, 66.8, 62.3, and 52.1 g for the DCP1, DCP2, DCP3 and MCP sources respectively. No statistical evaluations were possible on the group fed data set, but the values showed a trend in terms of the efficiency (lower value mean lower P/kg metabolic weight gain, therefore lower values = higher efficiencies) of the different sources (DCP1> MCP > DCP3 > DCP2).

The increased mean 16h-fasted total weight gain (corrected for starting weight as covariate) over the 433 days was 282, 262 and 276 kg for groups DCP1, DCP3, and MCP respectively, the difference being not significant. However, the mean total weight gains of these three sources were significantly higher than the DCP2 group (241 kg per steer).

The linear regression analysis between live weight gains or metabolic live weight gains and P intake per animal per day was done to evaluate the possibilities to use prediction equations to model supplemental P and growth which are shown in Table 6.11.

The results from this linear regression analysis showed no correlation between growth rate and phosphorous intake with sources DCP1, DCP2 and DCP3 and indicated that the equations cannot be used. In contrast, MCP showed the most usable equation (*p=0.001)* with a $R^2$ of 0.588 for weight gain and daily P intake or 60.4 when metabolic weight gain is estimated with P intake or *vice versa*. This is still low and an indication that growth and P supplementation are affected by many other factors as seen in Chapter 5.

**Table 6.11** Linear regression analysis between live weight gain (Y1), or metabolic weight gain (Y2), and P intake (X in g/day) in Bonsmara steers fed four different sources of phosphorous over a period of 433-days on natural pasture

| Treatment | Linear Regression Equation | F pr | $R^2$ | SEM |
|---|---|---|---|---|
| DCP1 | Weight gain per day ($Y_1$), kg = 0,547+0.0103(X) | *p*=0.89 | | 0.67 |
| DCP2 | Weight gain per day ($Y_1$), kg = -0,757+0.137(X) | *p*=0.052 | 23,8 | 0.48 |
| DCP3 | Weight gain per day ($Y_1$), kg = -0,975+0,1688(X) | *p*=0.013 | 39,4 | 0.52 |
| MCP | Weight gain per day ($Y_1$), kg = -0.609+0.1542(X) | *p*=0.001 | 58,8 | 0.41 |
| DCP1 | Metabolic weight gain per day($Y_2$), kg = 0,0801+0.0044(X) | *p*=0.726 | | 0.12 |
| DCP2 | Metabolic weight gain per day($Y_2$), kg = -0.128+0.0235(X) | *p*=0.063 | 21,5 | 0.09 |
| DCP3 | Metabolic weight gain per day ($Y_2$), kg = -0,1682+0.02917(X) | *p*=0.013 | 39,0 | 0.09 |
| MCP | Metabolic weight gain per day($Y_2$), kg = -0,1077+0.02676(X) | *p*=0.001 | 60,4 | 0.07 |

DCP1, DCP2 and DCP3 are different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source; DCP1 and the MCP source were from a supplier which produces products within a continuous production process. DCP1 and MCP were produced in a continuous well controlled production facility. DCP2 and DCP3 were produced in a "batch by batch" production facility with less heat control during the chemical reaction process; Mean 16h-fasted live weight gain were used; F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used)

## Carcass information

The animals were slaughtered after 433 days and all the carcass information is provided in Table 6.12 and Appendix 11. No differences were found between the treatments DCP1, DCP2, DCP3 or MCP in carcass slaughter percentage or in Rand income value per carcass (13 February 2007 prices).

**Table 6.12** Carcass information of 32 Bonsmara steers fed on four different P sources for 433 days on natural pasture and then slaughtered

| Treatment | n | Mean cold carcass slaughter % | S.D. | SEM | F pr test | Rand income value per carcass, R† | S.D. | SEM | *F* pr test |
|---|---|---|---|---|---|---|---|---|---|
| DCP1 | 8 | 52.8 | 0.85 | | | 3610 | 300 | | |
| DCP2 | 8 | 53.5 | 0.71 | 0.980, (cv% = 1.90) | F pr = 0.24 | 3351 | 342 | 287 (cv% = 8.10) | F pr = 0.18 |
| DCP3 | 8 | 52.6 | 1.53 | | | 3512 | 143 | | |
| MCP | 8 | 52.8 | 0.51 | | | 3657 | 324 | | |

Linear mixed model SEM = standard error of the mean; F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used); Feeding = healthy animals fed for 433 days with the different supplements, n = number of steers used; †Rand value for the meat was based on the prices on 13 Feb 2007

Based on the average meat prices for at Feb 2007, the Rand income value per carcass was R3 610, R3 351, R3 512, and R3 657 for the treatments DCP1, DCP2, DCP3 and MCP respectively. If the prices change to the average meat prices for October 2017 (www.agrimark.co.za), then the Rand income value per carcass changes to R10 531, R9 933, R10 409 and R10 969 for the treatments DCP1, DCP2, DCP3 and MCP respectively. This is a difference of R1 036 per steer, but it is only a tendency with a *p value* of only 0.12 and therefore not significant (SEM = 823 and a cv% = 7.9).

## Conclusion

Feeding three different dicalcium phosphate sources and one mono calcium phosphate source in four different treatments to healthy Bonsmara steers in a 433-day trial on a farm known for its low phosphorous pastures, did not influence the prevalence of osteochondrosis. The animals were rotated on pasture and 8 per treatment group were fed *ab libitum* with one of the four different supplements. The minerals in the rib bones between the different treatments did not show any differences at slaughter, although the dry fat-free liver P, Ca, Mg, K, S, and Zn were different and lower on the MCP fed supplement. Treatments (or supplements) DCP3 and DCP1 had the highest dry fat-free liver P, Mg, K, and S level. All three DCP supplements gave a higher dry fat-free liver Zn level in comparison to animals fed with the MCP supplement.

Only one steer in the DCP2 group developed osteochondrosis and due to similar Ca and P in the dry fat-free bone ash in all the groups, no bone density tests or mineral per $cm^3$ were evaluated, but in future this may be further investigated to evaluate source vs bone density and its contents.

The mean 16h-fasted body weights for the 433-day period were the highest for the MCP, DCP1 and DCP3 treatments when compared to the DCP2 animals. The DCP2 group did not show the highest rib bone or liver minerals, despite the highest supplemental intake with the DCP2 supplement. DCP2 also showed the lowest average daily gain over the period and therefore it can be concluded that this source may be lower bioavailable and must be further investigated.

Supplement DCP1 resulted in a high average weight gain with the lowest supplement intake as well as mineral intake. In addition, the amount of P per kg metabolic weight gain was also very low in this group, followed by the MCP and DCP3 groups. Nevertheless, there was no apparent negative effect on the health of the steers, such as an increased prevalence of osteochondrosis. The steers in this group also had similar rib bone minerals. As other contributing factors were similar in the trial. This probably implies higher bioavailability of the specific DCP source, which in this case was from a

supplier that controls the chemical heat production during the process better than suppliers that use a "batch to batch" process where it is very difficult to control heat damage on the minerals.

Bioavailability can be estimated by a water solubility test (CEFIC 1999, European Chemical Industry Council) to evaluate the ratio of mono calcium to di-calcium phosphate in the source. The water content of a batch phosphorous can also indicate the level of hydrate or anhydrate DCP in a DCP sample. Unfortunately, this was not tested on the P batches that were used in this trial. The data from the current trial suggest that cattle may be sensitive to the bioavailability of a P source and therefore it is important to evaluate solubility as well as the percentage hydrate vs anhydrate for every batch of phosphorous that is used to ensure that only high bioavailable P sources are used in supplements of cattle.

The effect of bioavailability is not only important for daily gains, but due to the large difference in intakes it would also influence the amount of supplements that cattle producers use. Phosphorous is one of the most expensive nutrients in the diet and if a lower quality P source increases the amount of P used over long periods, then it could affect the profitability of the cattle farming operation. The information from this trial is therefore to advise cattle farmers to also consider the estimated bioavailability in P sources and not only to buy products on the basis of total P. The tendency in this trial to produce a carcass with a lower income value from a supplement with a higher intake will result in financial loss.

Research on P bioavailability in cattle is difficult and very expensive, but the current data indicate that more research needs to be done to fully understand the difference between products or bioavailable P in animal performance when small quantities of a supplement are given. This may be expanded to test new hypotheses regarding the effect of mineral imbalances, possible changes in blood profiles, possible mineral antagonists, nutritional supplement strategies, nutritional management systems, and genetic or breed differences to better understand the high prevalence of osteochondrosis on some farms. Future research must also include cows and lactating cows to evaluate the intakes of these groups over the different seasons and to evaluate the effect of bioavailability of a P source on the performance of other groups as well.

Many farmers use no specific calving season which is intended to match requirements with supply. Also, the requirement of lactating cows is higher compared to dry or growing animals, yet most farms feed the same supplement to all groups of animals. This management strategy may contribute to the prevalence of osteochondrosis, because all animals get the same supplement without considering the production stage of the group, or individual animals within a group which may have specific nutritional requirements, or shy animals which consume inadequate amounts of supplement.

Many of these animals underperform or they are exposed to deficiencies as a result of the supplements they are fed which do not meet their specific requirements.

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# Chapter 7

# EVALUATING THE PREVALENCE OF OSTEOCHONDROSIS IN CATTLE ON COMMERCIAL FARMS WHEN FED WITH SPECIALIZED MINERAL SUPPLEMENTS

## Abstract

F.K. Botha[1*], H.H. Meissner[1], L. Prozesky[1], C.A. Shepstone[1] and C.de Brouwer[2]

Many farmers in the North West Province in South Africa were desperate to decrease the high incidence rate of osteochondrosis in all commercial and non-commercial cattle on their farms in order to minimize the losses due to the high culling rates and poor production efficiency of osteochondrosis affected animals. A total of 24 294 cattle from different pure and cross breeds, and age groups were evaluated with the OPOLS on-farm system on six different farms (KB, KO, FM, JC, TC and CB) for the prevalence of osteochondrosis from August 2008 until August 2011 to study the success of feeding a specialized mineral supplement to decrease the prevalence of osteochondrosis. A total of 11 939 females, 2 913 bulls and 699 oxen were evaluated per hind leg and the other 8 743 animals were only evaluated as healthy or osteochondrosis affected. The trials were conducted on farms, well known for a high prevalence of osteochondrosis, with well-managed infrastructure and adequate animal and human resources to conduct research within a commercial environment. The evaluated animals received a specialized mineral pack, known as Arthrocure, which was formulated by the Onderstepoort Arthrosis research team, and added to the different summer, winter, maintenance or production supplements fed according to the production stage of the animals and the time of year. The Arthrocure product contained highly bio-available magnesium, manganese, iron, zinc, copper, cobalt, iodine, and selenium, and were fed with additional highly bio-available phosphorous. Although the animals were not in a randomly designed study, the numeric values on all six farms show a downward trend of 50.6%, 69.1%, 65.2%, 88.4%, 60.2%, and 44.3% respectively for the prevalence of osteochondrosis in cattle on farms KB, KO, FM, JC, TC and CB after the implementation of the Arthrocure minerals (including the additional higher bio-available P). The correlations ($R^2$) with the downward trends were respectively, 0.93, 0.86, 0.79, 0.98, 0.91, and 0.70, and therefore an indication of a strong relationship between the decreased lesions on the animals over time since the Arthrocure minerals were introduced. Results from this study indicated a strong downward trend in the prevalence of osteochondrosis in cattle when the Arthrocure mineral supplementation plus a highly bio-available P source were fed on commercial farms.

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**Keywords:** Osteochondrosis, cattle, phosphorous, minerals, Arthrocure.

# EVALUATING THE PREVALENCE OF OSTEOCHONDROSIS IN CATTLE ON COMMERCIAL FARMS WHEN FED A SPECIALIZED MINERAL SUPPLEMENT

## Introduction

Many farmers were desperate to decrease the high incidence of osteochondrosis on their farms in order to minimize financial and genetic losses due to high culling rates and poor production efficiency of osteochondrosis affected animals (Prozezky 2010). As many researchers described osteochondrosis as multifactorial with no single factor accounting for the disease (DeGaris & Lean 2008; Prozesky 2010; Van der Veen *et al*. 2017), this could prove a daunting task.

From earlier observations and results of previous projects, researchers (Onderstepoort Arthrosis research team) hypothesized that the possible cause of the osteochondrosis could be related to the bio-availability of the P-source and/or a shortage of bio-available trace minerals fed to cattle when:

- the on-farm evaluation method (OPOLS) was developed and validated
- the evaluation of the liver and bone mineral profile of healthy vs. osteochondrosis affected cattle was compared
- data from cattle fed with different phosphorous sources over a 433-day period, as well as from data when osteochondrosis affected cattle were fed with a mineral supplement for 90 days, and then compared, was collected.
- four different P sources were fed to evaluate the effect on the prevalence of osteochondrosis.

In addition, the mineral levels in water sources vary between farms as well as within different sources on the same farm, which may then affect the bio-availability of the minerals supplied by the feed due to the antagonistic effects between the specific minerals (NRC 2016).

This hypothesis was tested on commercial beef cattle farms in the Vryburg and surrounding areas in the North West province in South Africa, through the supply of minerals by using different supplementation formulations to meet their mineral requirements.

# Materials and methods

## Experimental design and animals

The trials were conducted on six different cattle farms, well known for a high prevalence of osteochondrosis, with well-managed infrastructure and adequate animal and human resources to conduct research within a commercial environment. Cattle on all six farms were managed in three ecological groups, female cattle (pregnant cows/heifers and cows with calves), bulls, and oxen. The animals were managed within the existing habitat and management regime with the aim to optimize animal performance within the limitations of a specific farm.

A total of 24 294 cattle from different pure and cross breeds, and age groups were evaluated for the prevalence of osteochondrosis with the OPOLS on-farm system (Table 3.1) from August 2008 until August 2011. OPOLS data on some farms were done since August 2007 as reference points to the level of osteochondrosis on that specific farm. A total of 11 939 females, 2 913 bulls and 699 oxen were evaluated with the OPOLS system per hind leg and the other 8 743 animals were only evaluated as healthy or osteochondrosis affected animals.

The farms were all commercial units and described as:

- **Farm "KB":** Evaluated from August 2007 until August 2011. A total of 9 032 animals were evaluated on this farm.
- **Farm "KO":** Evaluated from August 2008 until August 2011. A total of 7 718 animals were evaluated on this farm.
- **Farm "FM":** Evaluated from October 2008 until June 2010. A total of 887 animals were evaluated on this farm.
- **Farm "JC":** Evaluated from August 2008 until May 2010. A total of 1 746 animals were evaluated on this farm.
- **Farm "TC":** Evaluated from August 2008 until August 2011. A total of 3 182 animals were evaluated on this farm.
- **Farm "CB":** Evaluated from August 2008 until May 2010. A total of 1 729 animals were evaluated on this farm.

The evaluated animals received a specialized mineral pack, known as Arthrocure, which was formulated by the Onderstepoort Arthrosis research team, and added to the different summer, winter, maintenance or production supplements. The supplements were then fed according to the production stage of the animals and the time of year. The trials were conducted with different "Arthrocure" prototype products, A, B and C, in different phases to evaluate the relative efficacy of each product

under commercial conditions. The Arthrocure specialized mineral packs contained the micro and macro minerals (from highly bio-available sources) needed by the cattle at any particular time of the year, presumably ensuring an animal with a healthy liver and bone structure and reducing the likelihood of the osteochondrosis condition observed. Arthrocure packs were then included into the rest of the supplement and supplied to the different groups on the different farms. The NRC (2001) for beef cattle guidelines were used to calculate the micro and macro mineral requirements for the different groups. Results from work published since the 1880's indicated that animals that are fed without phosphorous and microminerals may have a higher risk to develop health issues and therefor none of the commercial farms used a negative control (no Arthrocure and no P).

During the first phase (from November 2008) of the project, the aim was to determine if the mineral supplement known as "Arthrocure A", would decrease the prevalence of osteochondrosis in commercial cattle. Arthrocure A was formulated to include organic trace minerals with a higher bio-availability as part of the mineral formula (26 to 63% of the total minerals were supplied in this form and the rest as inorganic minerals), mainly to improve the bio-availability of the minerals to the animal. The results from this phase were then used to further develop the mineral pack, called Arthrocure B and then later Arthrocure C, in order to improve the cost effectiveness of the mineral packs within the supplementations. The farms did not switch over between Arthrocure A to B and then to C containing supplements on the same date, due to differences in intakes, habitat and management, although change-overs happened within reasonable time frames of a week or two. The cattle started receiving the different Arthrocure containing mineral packs from the following dates:

Arthrocure A: from November 2008

Arthrocure B: from March 2009

Arthrocure C: from November 2010

Tables 7.1 and 7.2 contain the recommended intakes of the specialized mineral packs as well as the actual mineral composition (as indices) that were formulated and recommended as the so-called Arthrocure mineral strategy for the different seasons as well as for the different animal production groups (supplied by the manufacturer ANH, 7 Murati drive, Centurion, Gauteng, South Africa; admin@animalnh.co.za).

**Table 7.1** The recommended intake levels for the Arthrocure A, B or C products (g per animal per day) when used within different supplements for different animal groups and seasons

| | Winter Maintenance supplements | | Winter Production supplements | | Summer Phosphorous supplements | | Summer Production supplements | | Phase D on the veld supplements | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| Arthrocure A or B premix, g/animal/day | 10 | 15 | 10 | 15 | 7.5 | 15 | 10 | 15 | 15 | 25 |
| Arthrocure C premix, g/animal/day | 5 | 10 | 5 | 10 | 5 | 10 | 5 | 10 | 7.5 | 15 |
| P recommendation, g P intake/animal/day † | 7.5 | | 11 | | 7.5 | | 11 | | 12 | |

† P intake is also based on the usage of a highly bio-available P source from Mono-Calcium Phosphorous. No Bone meal, Tri-calcium phosphate or any other Rock phosphate can be used within this recommendation or supplements. The inclusion of Arthrocure A, B or C is based on the usage of ONLY one of the packs per supplement. Min = minimum, Max = maximum. The inclusion rate of the products per 1000 kg supplement depends on the recommended intake of the total supplement. Salt levels needed to be formulated to achieve the recommended intakes. It was also recommended that farmers inject the animals biannually with vitamin A and E

**Table 7.2** The Arthrocure A, B and C mineral pack formulations expressed as indices of Arthrocure A

| Nutrients | Arthrocure A | Arthrocure B | Arthrocure C |
|---|---|---|---|
| Mg | 100 | 100 | 168 |
| Mn | 100 | 100 | 173 |
| Iron | 100 | 100 | 104 |
| Zinc | 100 | 100 | 167 |
| Copper | 100 | 100 | 156 |
| Cobalt | 100 | 100 | 147 |
| Iodine | 100 | 100 | 168 |
| Selenium | 100 | 100 | 131 |

All minerals in Arthrocure A were supplied from inorganic sources, except for the Se, Fe, Co, Cu, Zn and Mn, of which 26 to 63% of the total mineral was from organic minerals to improve the bio-availability of the specific mineral. Athrocure B and C only contained inorganic minerals. The actual mineral levels cannot be published due to the registered intellectual property of the products. All values are expressed as indices of Arthrocure A. The recommended intakes of Arthrocure A and B were the same, but Arthrocure C was fed at 50% of Arthrocure A or B levels

For the trial:

- All cattle were visually assessed with the OPOLS system for the presence or absence of osteochondrosis lesions, prior and at the starting date of the trial. All animals, displaying clinical signs of osteochondrosis and healthy, were used to evaluate whether the newly formulated mineral "Arthrocure" pack, together with the usage of a highly bio-available phosphorous source (MCP) will decrease the prevalence of osteochondrosis. Cattle were evaluated on farm KB since Aug 2007, farm FM since Oct 2008 and farms KO, JC, TC, and CB since Aug 2008. The reason was to gather information regarding osteochondrosis per gender and per leg as the reference point (as per Table 3.1).

- It was also recommended that farmers inject cattle biannually with vitamin A and E. This recommendation was due to the fact that supplements did not contain any added vitamins, other than the vitamins supplied by the raw materials within the different supplements.
- Animals within groups changed over time and they also changed camps in accordance with the specific farm strategy and management. The age of the different animal groups were also not monitored due to the changes within a specific camp group.
    - Some of the animals were culled or slaughtered based on each farm's commercial strategy.
    - As the season and grass availability changed, decisions were made as to when the respective licks had to be altered, but the focus was always to keep the Arthrocure and P intake as per recommendation.
- The supplements containing the Arthrocure packs were produced by one feed mill to minimize the variation in formulations, raw materials as well as the nutrient values of the ingredients used during manufacturing.
- Ample feed trough space was provided for cattle groups and monitored to ensure that troughs always contained the specific supplement per group, and to ensure all animals received the correct quantities according to their predicted intakes.
    - Although measurement of individual intake would have been preferable, it was more important at the time of the trial that animals be managed under normal grazing conditions, and therefore it was decided to feed the animals *ad libitum* per production group and season on all farms.
    - The predicted intake was calculated, but the actual intakes were not used due to cost constraints to hire professional personnel to accurately monitor intakes. The cost implication was too high due to the high animal numbers, the travel distance between the different groups and the size of farms.
- The trial supplements were produced and feeding commenced in November 2008 with the standard adaptation when licks were introduced or changed and then fed until August 2011 on some farms, although some farms stopped earlier as indicated in Table 7.3 and Appendix 12.1.
- The different groups (females, bulls and oxen) received their measured intake of Arthrocure as per Table 7.1 per supplement.
- All animals were managed to received ample grazing, but different according to the habitat differences between the camps and farms.
- Water was available *ad lib.* to all cattle, although it would have been better to analyse the water sources for mineral levels quarterly, in order to have more data evaluable regarding actual mineral supply from the water sources.

**Measurement summary:**

Animals were evaluated with the OPOLS system per animal and per leg by specialists from the Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria. Evaluations on the cattle were done over two time-frames on most farms;

- The first period was used to measure osteochondrosis with the OPOLS system per gender, per leg, and per time period, in order to analyse data and trends per gender type and per leg.
- The second period where cattle were OPOLS evaluated only as a positive or a healthy animal to follow trends for the total evaluated population on the specific farm. This was done to evaluate cattle quicker and to lower the stress for the animals that were put through the gate crash area.

Supplement intakes were monitored to ensure animals are fed on an *ad lib.* basis, but the data was not used in the trial results. This was done purely to ensure animals were fed the formulated quantity, but the data was not collected by professional technicians.

**Statistical analysis**

**7.1. Data analyses**

The animal data, the supplement type and intakes were not compared in a randomly designed study and therefore no statistical significance could be tested. The OPOLS data were evaluated on a "per farm", "per group" and a "per leg" basis which created a data basis without any variance within a group or farm. Trend line analysis were used to evaluate all results on a farm per evaluated population and gender per ecological group, and per leg over the different trial time period.

**7.2. Hypothesis**

($H_0$): There is no difference in the prevalence of osteochondrosis in cattle on commercial farms in the North West Province that receive the specialized "Arthrocure" mineral pack compared to prevalence of osteochondrosis before they received the formulated mineral blend.

($H_1$): The prevalence of osteochondrosis decreased when commercial cattle receive the Arthrocure mineral pack.

## Results and discussion

The 24 294 cattle were evaluated on the six farms described as farms **KB, KO, FM, JC, TC** and **CB** with the results summarized in Tables 7.3.

No analysis of variance calculations could be done on the groups on the farms, between the farms for supplement intakes, as well as for the prevalence of osteochondrosis after the supplement containing the Arthocure mineral pack as well as the highly bio-available P were fed. This was due to no treatment variance within the trial design. For this reason, the data is discussed without any least significant difference analyses and therefore only reported as observations. The results are also discussed as averages in specific periods and treated as weighted averages due to the variation in group sizes between farms and evaluation periods. The discussions on cattle legs in this chapter are all based on hind leg evaluation as per OPOLS system. Other important points why the data are discussed as observations only are:

- Some animals went out of specific groups due to culling, mortalities and health issues, as well as their production cycle. Animals within the groups did therefore change from time to time, although the aim was to keep them consistent as far as possible.
- All groups were not simultaneously rotated through the camps and were managed according to the specific farm's grazing conditions.
- All groups on the farms were not simultaneously changed to a specific supplement, but they were changed according to the grazing conditions. All farms received a supplement containing the Arthrocure mineral pack as well as a high bio-available P source according to the levels as per Table 7.1.
- Supplement intakes were group fed and therefore no individual intakes were available. All supplements were fed on an *ad libitum* basis and within the limitations of supplemental feeding regimes as discussed in Chapter 5.

**Table 7.3a** A summary of the data from the six commercial cattle farms in the Vryburg / North West Province which supplemented cattle with the Arthrocure products and a highly bio-available P source (all animals and females)

| Farm | Date evaluated | All Animals evaluated | | | | | Female animals evaluated | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total animals evaluated | % of evaluated population with lesions | % of evaluated population with lesions on the left leg | % of evaluated population with lesions on the right leg | % of evaluated population with lesions on both left and right legs | Number of females evaluated | % of evaluated females with lesions | % of evaluated females with lesions on the left leg | % of evaluated females with lesions on the right leg | % of evaluated females with lesions on both left and right legs |
| KB | Aug-07 | 1088 | 32.0% | | | | | | | | |
| | May-08 | 1373 | 21.6% | 16.97% | 12.89% | 8.23% | 679 | 22.4% | 16.9% | 15.5% | 10.0% |
| | Aug-08 | 874 | 22.3% | 14.9% | 15.8% | 8.35% | 713 | 25.5% | 17,0% | 18,1% | 9,54% |
| | Nov-08 | 1581 | 17.4% | 11.8% | 13.0% | 7.34% | 830 | 18.0% | 12.4% | 13.4% | 7.83% |
| | Feb-09 | 1064 | 11.4% | 7.05% | 7.71% | 3.38% | 867 | 12.5% | 7.84% | 8.42% | 3.81% |
| | May-09 | 997 | 12.8% | 8.53% | 9.43% | 5.12% | 796 | 13.7% | 9.17% | 10.2% | 5.65% |
| | Jul-10 | 1507 | 8.16% | 5.24% | 4.71% | 1.79% | 873 | 8.71% | 5.50% | 5.04% | 1.83% |
| | Aug-11 | 548 | 11.3% | 8.21% | 5.66% | 2.55% | 548 | 11.3% | 8.21% | 5.66% | 2.55% |
| KO | Aug-08 | 843 | 16.5% | 12.5% | 8.90% | 4.86% | 645 | 18.4% | 14.0% | 10.2% | 5.74% |
| | Dec-08 | 888 | 7.88% | 4.95% | 4.95% | 2.03% | 685 | 9.34% | 5.99% | 5.84% | 2.48% |
| | Jan-09 | 931 | 4.62% | 3.76% | 1.93% | 1.07% | 686 | 5.98% | 4.81% | 2.62% | 1.46% |
| | Nov-09 | 1631 | 7.48% | | | | | | | | |
| | Jun-10 | 1581 | 4.05% | | | | | | | | |
| | Sep-10 | 1017 | 3.24% | | | | | | | | |
| | Aug-11 | 827 | 2.06% | | | | | | | | |
| FM | Oct-08 | 229 | 18.8% | 12.7% | 12.7% | 6.55% | 227 | 18.9% | 12.8% | 12.8% | 6.61% |
| | Jan-09 | 168 | 7.74% | 5.36% | 3.57% | 1.19% | 168 | 7.74% | 5.36% | 3.57% | 1.19% |
| | Jun-09 | 168 | 8.33% | 3.57% | 5.95% | 1.19% | 168 | 8.33% | 3.57% | 5.95% | 1.19% |
| | Nov-09 | 172 | 3.49% | | | | | | | | |
| | Jun-10 | 150 | 6.67% | | | | | | | | |

| Farm | Date evaluated | All Animals evaluated | | | | | Female animals evaluated | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total animals evaluated | % of evaluated population with lesions | % of evaluated population with lesions on the left leg | % of evaluated population with lesions on the right leg | % of evaluated population with lesions on both left and right legs | Number of females evaluated | % of evaluated females with lesions | % of evaluated females with lesions on the left leg | % of evaluated females with lesions on the right leg | % of evaluated females with lesions on both left and right legs |
| JC | Aug-08 | 400 | 20.5% | 12.8% | 13.0% | 5.25% | 371 | 20.2% | 11.9% | 13.7% | 5.39% |
| | Feb-09 | 478 | 4.60% | 3.56% | 1.88% | 0.84% | 424 | 3.77% | 2.83% | 1.65% | 0.71% |
| | Sep-09 | 400 | 1.75% | 1.00% | 0.75% | 0.00% | 371 | 1.89% | 1.08% | 0.81% | 0.00% |
| | May-10 | 468 | 0.64% | | | | | | | | |
| TC | Aug-08 | 487 | 20.7% | 15.4% | 11.5% | 6.16% | 476 | 20.8% | 15.3% | 11.8% | 6.30% |
| | Mar-09 | 660 | 12.7% | 9.70% | 8.03% | 5.00% | 660 | 12.7% | 9.70% | 8.03% | 5.00% |
| | Jun-09 | 660 | 7.27% | 4.09% | 4.70% | 1.52% | 660 | 7.27% | 4.09% | 4.70% | 1.52% |
| | Nov-09 | 429 | 7.69% | 5.59% | 3.96% | 1.86% | 429 | 7.69% | 5.59% | 3.96% | 1.86% |
| | May-10 | 375 | 6.67% | | | | | | | | |
| | Sep-10 | 361 | 7.20% | | | | | | | | |
| | Aug-11 | 210 | 2.86% | | | | | | | | |
| CB | Aug-08 | 283 | 14.8% | 11.7% | 6.01% | 2.83% | 205 | 15.6% | 12.2% | 6.83% | 3.41% |
| | Nov-08 | 385 | 12.5% | 8.57% | 9.61% | 5.71% | 284 | 14.1% | 9.15% | 11.3% | 6.34% |
| | Feb-09 | 198 | 13.6% | 8.08% | 9.60% | 4.04% | 174 | 14.4% | 8.62% | 10.3% | 4.60% |
| | Sep-09 | 360 | 6.39% | | | | | | | | |
| | May-10 | 503 | 6.76% | | | | | | | | |

OPOLS data evaluated on 6 different commercial farms from 2008 to 2011. The trial was done commercially and not in a randomly design. Animals from three different ecological groups, cows, bulls and oxen. Animals on the farms received different pastures in different camps and managed within the specific limitations of the farm. Arthrocure A mineral pack was provided from November 2008, Arthrocure B from March 2009 and Arthrocure C from November 2010. All farms did not change the Arthrocure products at the same date, but within reasonable time. These Arthrocure products were fed with a highly bio-available P source (MCP) within a supplement formulated for the Winter, Summer, Maintenance and Production supplements. Animals were from different breeds, age and production groups. Animals were not kept in the same groups over the 3-year period and did change on some farms. Animals were also culled from the groups according to the normal practices on these farms. Animals were evaluated on some farms from a specific date onwards only as osteochondrosis affected or healthy and not as per gender or per leg

**Table 7.3b** A summary of the data from the six commercial cattle farms in the Vryburg / North West Province which supplemented cattle with the Arthrocure products and a highly bio-available P source (bulls and oxen)

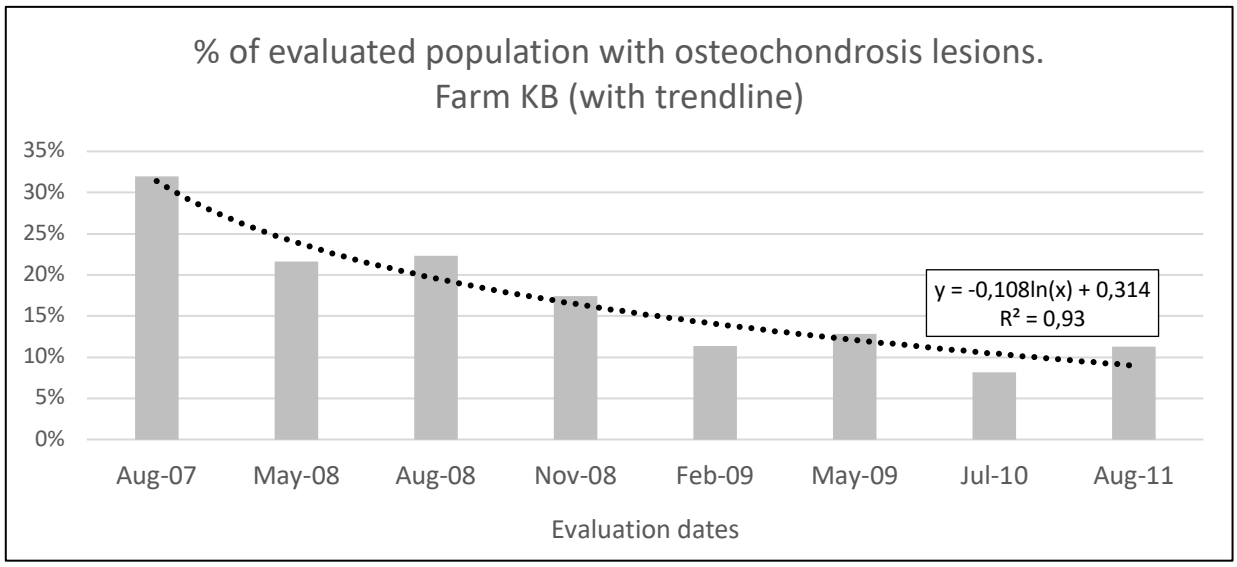
| Farm | Date evaluated | Bull animals evaluated | | | | | Oxen animals evaluated | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of Bulls evaluated | % of evaluated bulls with lesions | % of evaluated bulls with lesions on the left leg | % of evaluated bulls with lesions on the right leg | % of evaluated bulls with lesions on both left and right legs | Number of oxen evaluated | % of evaluated oxen with lesions | % of evaluated oxen with lesions on the left leg | % of evaluated oxen with lesions on the right leg | % of evaluated oxen with lesions on both left and right legs |
| KB | Aug-07 | | | | | | | | | | |
| | May-08 | 344 | 19.5% | 18.0% | 7.56% | 6.10% | 350 | 22.3% | 16.0% | 13.1% | 6.86% |
| | Aug-08 | 118 | 8.47% | 6.78% | 5.93% | 4.24% | 43 | 6.98% | 2.33% | 4.65% | 0.00% |
| | Nov-08 | 682 | 15.0% | 9.53% | 10.70% | 5.28% | 69 | 34.8% | 26.1% | 30.4% | 21.7% |
| | Feb-09 | 170 | 1.18% | 0.59% | 0.59% | 0.00% | 27 | 40.7% | 22.2% | 29.6% | 11.1% |
| | May-09 | 171 | 2.34% | 1.75% | 1.17% | 0.58% | 30 | 50.0% | 30.0% | 36.7% | 16.7% |
| | Jul-10 | 609 | 7.22% | 4.93% | 3.94% | 1.64% | 25 | 12.0% | 4.00% | 12.0% | 4.00% |
| | Aug-11 | | | | | | | | | | |
| KO | Aug-08 | 173 | 11.6% | 8.67% | 5.20% | 2.31% | 25 | 0.00% | 0.00% | 0.00% | 0.00% |
| | Dec-08 | 188 | 3.19% | 1.60% | 2.13% | 0.53% | 15 | 0.00% | 0.00% | 0.00% | 0.00% |
| | Jan-09 | 229 | 0.87% | 0.87% | 0.00% | 0.00% | 16 | 0.00% | 0.00% | 0.00% | 0.00% |
| | Nov-09 | | | | | | | | | | |
| | Jun-10 | | | | | | | | | | |
| | Sep-10 | | | | | | | | | | |
| | Aug-11 | | | | | | | | | | |
| FM | Oct-08 | 2 | 0.00% | 0.00% | 0.00% | 0.00% | | | | | |
| | Jan-09 | | | | | | | | | | |
| | Jun-09 | | | | | | | | | | |
| | Nov-09 | | | | | | | | | | |
| | Jun-10 | | | | | | | | | | |

| Farm | Date evaluated | Bull animals evaluated | | | | | Oxen animals evaluated | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of Bulls evaluated | % of evaluated bulls with lesions | % of evaluated bulls with lesions on the left leg | % of evaluated bulls with lesions on the right leg | % of evaluated bulls with lesions on both left and right legs | Number of oxen evaluated | % of evaluated oxen with lesions | % of evaluated oxen with lesions on the left leg | % of evaluated oxen with lesions on the right leg | % of evaluated oxen with lesions on both left and right legs |
| JC | Aug-08 | 5 | 20.0% | 20.0% | 0.00% | 0.00% | 24 | 25.0% | 25.0% | 4.17% | 4.17% |
| | Feb-09 | 3 | 0.00% | 0.00% | 0.00% | 0.00% | 51 | 11.8% | 9.80% | 3.92% | 1.96% |
| | Sep-09 | 5 | 0.00% | 0.00% | 0.00% | 0.00% | 24 | 0.00% | 0.00% | 0.00% | 0.00% |
| | May-10 | | | | | | | | | | |
| TC | Aug-08 | 11 | 18.2% | 18.2% | 0.00% | 0.00% | | | | | |
| | Mar-09 | | | | | | | | | | |
| | Jun-09 | | | | | | | | | | |
| | Nov-09 | | | | | | | | | | |
| | May-10 | | | | | | | | | | |
| | Sep-10 | | | | | | | | | | |
| | Aug-11 | | | | | | | | | | |
| CB | Aug-08 | 78 | 12.8% | 10.3% | 3.85% | 1.28% | | | | | |
| | Nov-08 | 101 | 7.92% | 6.93% | 4.95% | 3.96% | | | | | |
| | Feb-09 | 24 | 8.33% | 4.17% | 4.17% | 0.00% | | | | | |
| | Sep-09 | | | | | | | | | | |
| | May-10 | | | | | | | | | | |

OPOLS data evaluated on 6 different commercial farms from 2008 to 2011. The trial was done commercially and not in a randomly design. Animals from three different ecological groups, cows, bulls and oxen. Animals on the farms received different pastures in different camps and managed within the specific limitations of the farm. Arthrocure A mineral pack was provided from November 2008, Arthrocure B from March 2009 and Arthocure C from November 2010. All farms did not change the Arthrocure products at the same date, but within reasonable time. These Arthrocure products were fed with a highly bio-available P source (MCP) within a supplement formulated for the Winter, Summer, Maintenance and Production supplements. Animals were from different breeds, age and production groups. Animals were not kept in the same groups over the 3-year period and did change on some farms. Animals were also culled from the groups according to the normal practices on these farms. Animals were evaluated on some farms from a specific date onwards only as osteochondrosis affected or healthy and not as per gender or per leg

## Individual farms

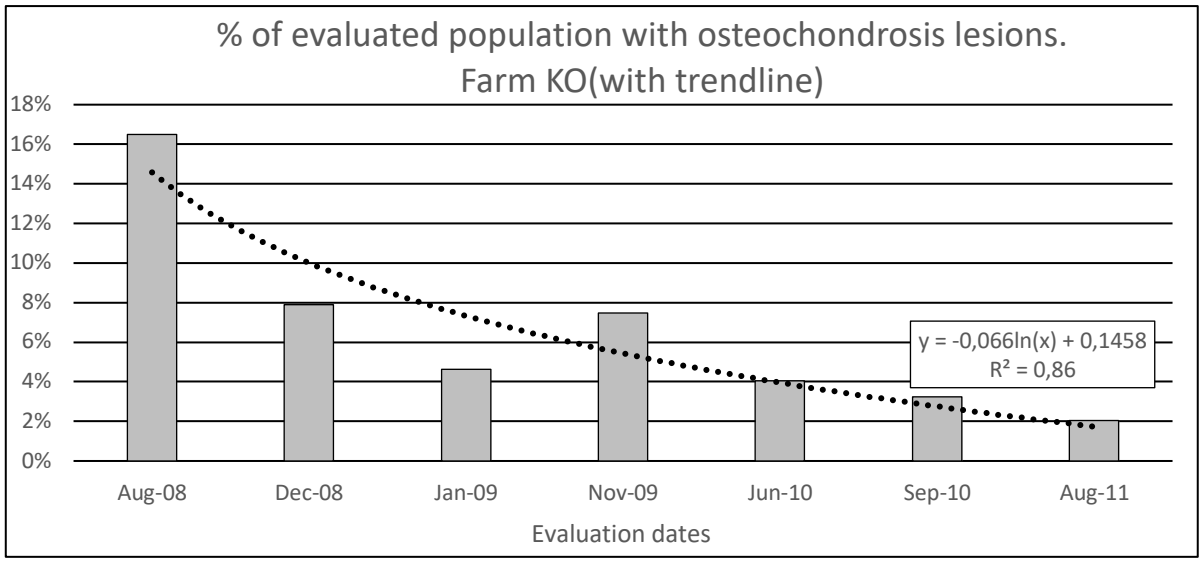
The prevalence of osteochondrosis in cattle on farm **KB** was 32% in August 2007 with an average positive identified prevalence of 25.2% during the pre-treatment period of August 2007 to August 2008 (n=3335). Cattle were fed with the specialized minerals from 1 November 2008 and the prevalence of osteochondrosis changed to 12.4% (n=5697) from the end of November 2008 to August 2011, when the Arthrocure minerals were fed. Although, this could not be evaluated within a statistical model, the numbers over the large quantity of animals seems to have substantially lowered as shown in Figure 7.1. The logarithmic equation over the three-year period indicated a trend of decreased osteochondrosis within the 9 032 evaluated population on this farm ($R^2$=0.93).




Cattle were evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from 1 November 2008. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. A high bio-available P source was also included in the supplement. Logarithmic trend line. Animals changed camps and some animals did leave the herd due to standard culling practices

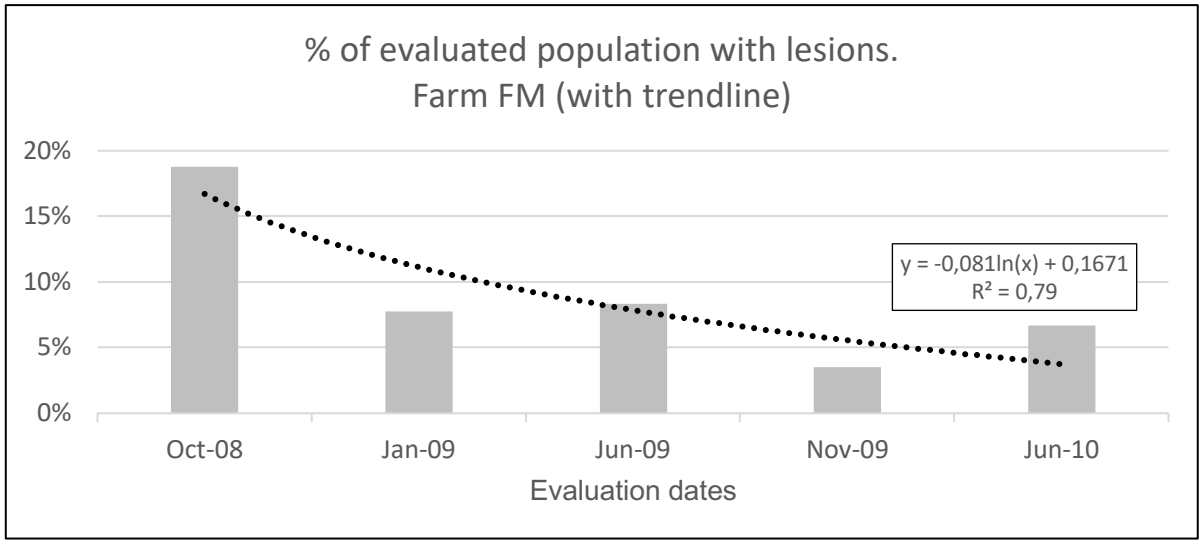
**Figure 7.1** The prevalence of osteochondrosis in cattle on a commercial farm **(KB)** in the North West Province over a four-year period and supplemented with the Arthrocure minerals for three years

Figures 7.2, 7.3, 7.4, 7.5 and 7.6 show the same trends as Figure 7.1 for farms **KO, FM, JC, TC** and **CB**, but with different correlations as indicated.



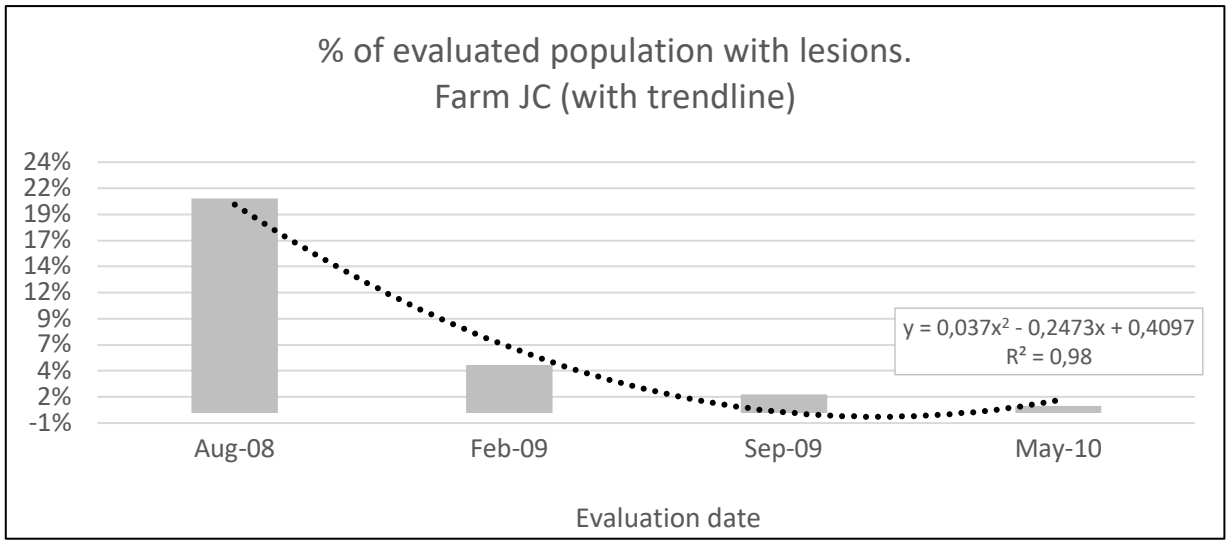

Cattle were evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Logarithmic trend line. Animals changed camps and some animals did leave the herd due to standard culling practices

**Figure 7.2** The prevalence of osteochondrosis in cattle supplemented with the Arthrocure minerals for three years on a commercial farm **(KO)** in the North West Province



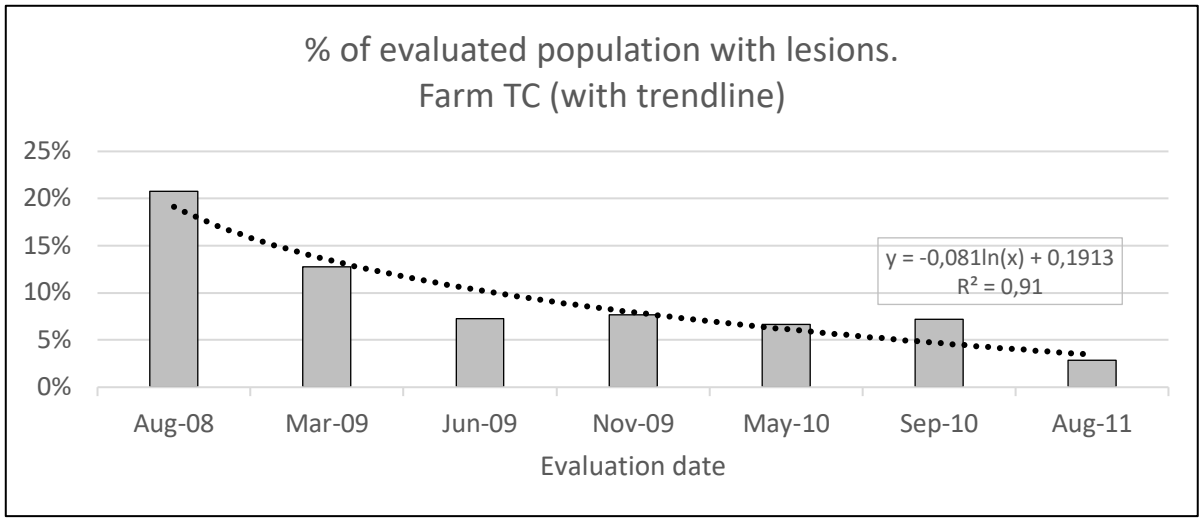

Animals were evaluated with the OPOLS system. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Logarithmic trendline. Animals changed camps and some animals did leave the herd due to standard culling practices

**Figure 7.3** The prevalence of osteochondrosis in cattle supplemented with the Arthrocure minerals for 20 months on a commercial farm **(FM)** in the North West Province



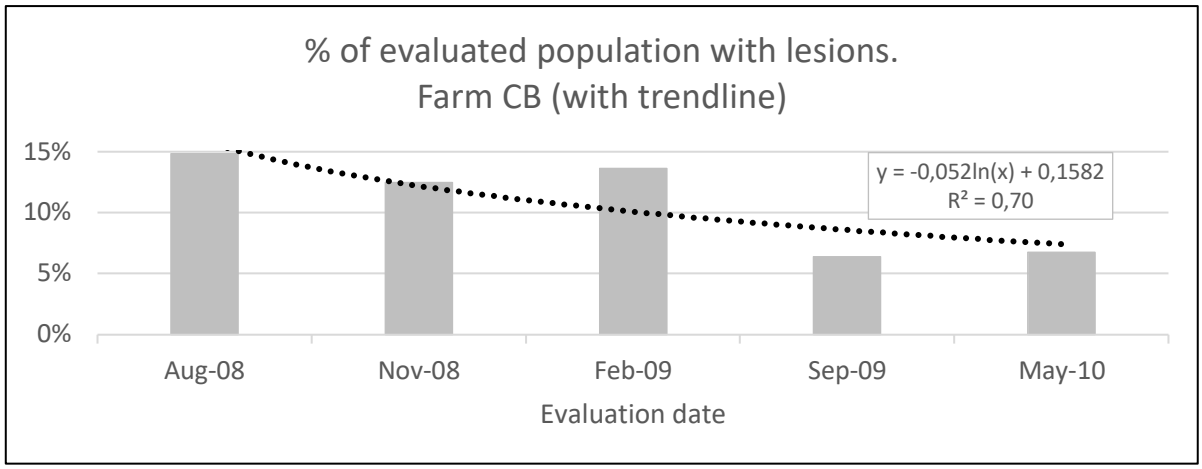

Cattle were evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Polynomial trend line. Animals changed camps and some animals did leave the herd due to standard culling practices

**Figure 7.4** The prevalence of osteochondrosis in cattle supplemented for 21 months with the Arthrocure minerals on a commercial farm **(JC)** in the North West Province




Cattle were evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Logarithmic trend line. Animals changed camps and some animals did leave the herd due to standard culling practices

**Figure 7.5** The prevalence of osteochondrosis in cattle supplemented with the Arthrocure minerals for three years on a commercial farm **(TC)** in the North West Province




Cattle were evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Logarithmic trend line. Animals changed camps and some animals did leave the herd due to standard culling practices

**Figure 7.6** The prevalence of osteochondrosis in cattle supplemented with the Arthrocure minerals for 21 months on a commercial farm **(CB)** in the North West Province

Although the animals were not in a randomly designed study, the numeric values on all six farms show a downward trend of 50.6%, 69.1%, 65.2%, 88.4%, 60.2%, and 44.3% respectively for the prevalence of osteochondrosis in cattle on farms **KB, KO, FM, JC, TC** and **CB** after the implementation of the Arthrocure minerals (including the higher bio-available P, n=24 294). The correlations ($R^2$) with the downward trends were respectively, 0.93, 0.86, 0.79, 0.98, 0.91, and 0.70, and therefore an indication of a strong relationship between the decreased lesions on the animals over time since the Arthrocure minerals were introduced. A correlation($R^2$) of 0.50 indicates an intermediate relationship between any two variables and 1.00 a direct and strong relationship. The above-mentioned values of 0.70 to 0.98 indicates a strong relationship with the equations used and therefore a downward trend over time in the osteochondrosis condition on all six farms.

Tables 7.4, 7.5, Appendixes 12.1, 12.2, 12.3, 12.4, 12.5, 12.6, 12.7, 12.8, 12.9, 12.10, 12.11, 12.12, 12.13, 12.14, 12.15, 12.16, 13, and 14 contain more OPOLS leg related data for the females, bulls and oxen evaluated on farms **KB**, **KO**, **FM**, **JC**, **TC** and **CB.** Tables 7.4 and 7.5 are the summaries of the logarithmic trend line correlations for the prevalence of osteochondroses in females, bulls and oxen on six commercial cattle farms in the Vryburg area (North West Province, South Africa) when the Arthrocure products and a highly bio-available P source were added to the supplements of the cattle.

**Table 7.4** A summary of the logarithmic trend line correlations for the prevalence of osteochondroses in females, bulls and oxen on six commercial cattle farms in the Vryburg area (North West Province), when supplemented with the Arthrocure products and a highly bio-available P source

| | | % of evaluated females with lesions | % of evaluated bulls with lesions | % of evaluated oxen with lesions |
|---|---|---|---|---|
| Farm **KB** | equation | y = -4,412ln(x) + 46,906 | y = -4,974ln(x) + 52,782 | y = -1,422ln(x) + 15,341 |
| | correlation | $R^2$ = 0,58 | $R^2$ = 0,24 | $R^2$ = 0,004 |
| Farm **KO** | equation | y = -31,62ln(x) + 334,96 | y = -27,61ln(x) + 292,49 | † |
| | correlation | $R^2$ = 1.00 | $R^2$ = 1.00 | |
| Farm **FM** | equation | y = -15,54ln(x) + 164,7 | ‡ | § |
| | correlation | $R^2$ = 0,58 | | |
| Farm **JC** | equation | y = -18,09ln(x) + 191,68 | y = -19,64ln(x) + 208,14 | y = -25,09ln(x) + 265,91 |
| | correlation | $R^2$ = 0,80 | $R^2$ = 0,72 | $R^2$ = 1.00 |
| Farm **TC** | equation | y = -12,28ln(x) + 130,24 | ‡ | § |
| | correlation | $R^2$ = 0,87 | | |
| Farm **CB** | equation | y = -2,685ln(x) + 28,579 | y = -9,699ln(x) + 102,81 | § |
| | correlation | $R^2$ = 0,59 | $R^2$ = 0,68 | |

† 56 oxen were evaluated with the OPOLS system, but no positives found within the period. ‡ Bulls were evaluated with the OPOLS system, but no positives were found over the period. § No oxen were evaluated on this farm

**Table 7.5** A summary of the logarithmic trend line correlations for the prevalence of osteochondrosis per leg on six commercial cattle farms in the Vryburg area (North West Province) when supplemented with the Arthrocure products and a highly bio-available P source

| Farm evaluated | % of evaluated population with lesions on the left leg | % of evaluated population with lesions on the right leg | % of evaluated population with lesions on both left and right legs | % of evaluated females with lesions on the left leg | % of evaluated females with lesions on the right leg | % of evaluated females with lesions on both left and right legs | % of evaluated bulls with lesions on the left leg | % of evaluated bulls with lesions on the right leg | % of evaluated bulls with lesions on both left and right legs | % of evaluated oxen with lesions on the left leg | % of evaluated oxen with lesions on the right leg | % of evaluated oxen with lesions on both left and right legs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Farm **KB** | $R^2 = 0,48$ | $R^2 = 0,69$ | $R^2 = 0,69$ | $R^2 = 0,53$ | $R^2 = 0,71$ | $R^2 = 0,71$ | $R^2 = 0,29$ | $R^2 = 0,18$ | $R^2 = 0,38$ | $R^2 = 0,04$ | $R^2 = 0,004$ | $R^2 = 0,01$ |
| Farm **KO** | $R^2 = 1.00$ | $R^2 = 0,94$ | $R^2 = 1.00$ | $R^2 = 0,99$ | $R^2 = 0,94$ | $R^2 = 1.00$ | $R^2 = 0,99$ | $R^2 = 0,95$ | $R^2 = 1.00$ | † | † | † |
| Farm **FM** | $R^2 = 0,79$ | $R^2 = 0,37$ | $R^2 = 0,62$ | $R^2 = 0,79$ | $R^2 = 0,37$ | $R^2 = 0,62$ | ‡ | ‡ | ‡ | `§ | § | § |
| Farm **JC** | $R^2 = 0,88$ | $R^2 = 0,79$ | $R^2 = 0,84$ | $R^2 = 0,84$ | $R^2 = 0,77$ | $R^2 = 0,82$ | $R^2 = 0,72$ | ‡ | ‡ | $R^2 = 0,97$ | $R^2 = 0,83$ | $R^2 = 1.00$ |
| Farm **TC** | $R^2 = 0,81$ | $R^2 = 0,94$ | $R^2 = 0,78$ | $R^2 = 0,81$ | $R^2 = 0,94$ | $R^2 = 0,79$ | ‡ | ‡ | ‡ | § | § | § |
| Farm **CB** | $R^2 = 0,85$ | $R^2 = 0,75$ | $R^2 = 0,18$ | $R^2 = 0,86$ | $R^2 = 0,56$ | $R^2 = 0,16$ | $R^2 = 1.00$ | $R^2 = 0,08$ | $R^2 = 0,10$ | § | § | § |

† 56 oxen were evaluated with the OPOLS system, but no positives found within the period. ‡ Bulls were evaluated with the OPOLS system, but no positives were found over the period. § No oxen were evaluated on this farm

## Comparison and trends on all farms

The results from the combined 24 294 OPOLS evaluated cattle indicated that 11.3% of the evaluated cattle had osteochondrosis lesions, of which 69.6% was on the left leg, 63.2% on the right leg, and 32.8% on both the left leg and right leg. The female cattle had 13.3% lesions, 68.8% on the left leg, 64.4% on the right leg, and 33.2% on both the left and right leg. The bulls had 9.61% lesions of which 74.3% were on the left leg, 55.4% on the right leg, and 29.3% on both hind legs. The oxen had numerically a higher incidence rate of osteochondrosis lesions at 20.9%, of which 69.9% were on the left leg, 64.4% on the right leg, and 34.4% on both hind legs. The percentage lesions per leg was therefore very similar in females, bulls and oxen as can be seen in Appendix 12, although, some variation was evident between the gender groups between farms and similar to the results seen during trials when the OPOLS lesions scoring system were validated (Chapter 3).

The percentage decrease in lesions on individual farms on the left leg after the implementation of the Arthrocure minerals was 49.1 ($R^2$=0.48), 65.3 ($R^2$=1.00), 64.8 ($R^2$=0.79), 81.3 ($R^2$=0.88), 57.3 ($R^2$=0.81), and 28% ($R^2$=0.85) respectively, for the farms **KB, KO, FM, JC, TC** and **CB.** The corresponding decrease in lesions on the right leg was 39.4 ($R^2$=0.69), 61.8 ($R^2$=1.00), 62.4 ($R^2$=0.37), 89.5 ($R^2$=0.79) and 49.7 ($R^2$=0.81), respectively. The results of farm **CB** on the other hand, showed the opposite with an increase of 60% ($R^2$=0.75) in lesions on the right leg of the evaluated animals.

The perception of farmers that osteochondrosis was more prone on a specific side of cattle, was proven not true during the trials when the OPOLS lesions scoring system was validated (Chapter 3). The present large commercial trial with 24 294 Opols evaluated cattle also confirmed no trend for this perception.

For the animals which had lesions on both the left and right leg, the decrease in lesions was 48.3 ($R^2$=0.69), 68.5 ($R^2$=1.00), 81.8 ($R^2$=0.62), 91.2 ($R^2$=0.84), and 52.8 ($R^2$=0.78), respectively on farms **KB, KO, FM, JC and TC** and a numerical increase of 81.6% ($R^2$=0.18) on farm **CB**. The correlation in the latter was however weak and therefore probably not an accurate reflection of the trend. A more pronounced downward trend was also noticed on farm **CB** only after Feb 2009 with 6.6% positive animals during Feb 2009 to May 2010 (n=863) period compared to 12.9% during Nov 2008 to Feb 2009, and 14.8% pre-treatment. The greater decrease in lesions on this farm (**CB**) was observed from 10 months after the implementation of the Arthrocure strategy, when a decrease of 55.5% was seen in comparison to a decrease of only 13.1% within the first 3 months. This is important to mention because most of the cattle were evaluated per gender group and per leg during

the first 3-month period, which might be the reason for the different values compared to the other herds, where the decrease in lesions occurred numerically quicker. Similar trends were also observed for the female cattle and the bulls, except for the decreased trend on the percentage of bulls with lesions on both left and right legs, but with a low correlation ($R^2$=0.10).

Farms **KB, KO, FM, JC, TC** and **CB** showed a trend of decreased lesions on all female cattle with correlations ($R^2$) of 0.58, 1.00, 0.58, 0.80, 0.87 and 0.59 as summarized in Table 7.4. Farms **KB, KO, JC,** and **CB** showed a trend of decreased lesions on all bulls, with correlations ($R^2$) of 0.24, 1.00, 0.72 and 0.68. The trend for farm **KB** is unconvincing (low correlation) due to low numbers. Farms **KB** and **JC** showed a decreased trend in lesions in the oxen, but with big differences in correlations ($R^2$) of 0.004 and 1.00 respectively, and therefore one cannot pay much attention to these trends. Fifty-six oxen were also evaluated on farm **KO**, but all without any lesions pre- and post-treatment. The total number of oxen evaluated for lesions was only 2.9% (Table 7.1) of the total evaluated population and therefore their results are inconclusive. Results from the original OPOLS validation trials indicated that the number of cattle is very important for accuracy and with the low animal numbers in this trial for the bull and oxen groups on some farms, maybe this is the reason for the variation between the results on some of the farms (Chapter 3).

## Arthrocure products

Supplements were fed on an *ad libitum* basis and due to the logistical challenge in such a big trial with 24 924 animals spread over a large geographical area and too many different personnel, it was decided not to evaluate the intake on a per animal per day basis. All the farms used Arthrocure A(+P) and B(+P), but Arthrocure C(+P) was only used on farms **KB, KO** and **TC**, where animals were evaluated after November 2010 when Arthrocure C was implemented.

The average lesion percentage on Farm **KB** changed from 25.2% (pre-treatment, n=2461) to 12.53% (period with Arthrocure A or B, n=6023), and then to 11.31% (the period with Arthrocure C, n=548). The lesion percentages for the same comparable periods were respectively, 16.5 (n=843) vs. 4.95 (n=6048) vs. 2.06% (n=827) for farm **KO** and 20.7 (n=487) vs. 8.69 (n=2485) vs 2.86% (n=210) for farm **TC**. The number of lesions were numerically lower with the same downward trend over time, but no statistical evaluations for comparison between farms were conducted. The osteochondrosis lesion results did not show any negative trends when Arthrocure C was implemented on the three farms, bearing in mind that the total number of animals fed with Arthrocure C were low (6.4% of the total evaluated cattle).

Arthrocure A, B and C were formulated to give a mineral pack as per Table 7.2 but if intakes are brought into consideration the indices and prices (April 2018) are as per Table 7.6.

**Table 7.6** The Arthrocure A, B and C mineral pack formulations and prices expressed as indices with intake included as per recommendation

| Nutrients | Arthrocure A premix | Arthrocure B premix | Arthrocure C based on 50% intake |
|---|---|---|---|
| Mg | 100 | 100 | 84 |
| Mn | 100 | 100 | 87 |
| Iron | 100 | 100 | 52 |
| Zinc | 100 | 100 | 83 |
| Copper | 100 | 100 | 78 |
| Cobalt | 100 | 100 | 74 |
| Iodine | 100 | 100 | 84 |
| Selenium | 100 | 100 | 65 |
| Price as index of Arthrocure A | 100 | 19 | 14 |

All minerals in Arthrocure A were supplied from inorganic sources, except for the Se, Fe, Co, Cu, Zn and Mn, of which 26 to 63% of the total minerals was from organic minerals to improve the bio-availability of the specific mineral. Arthrocure B and C only contained inorganic minerals. The actual mineral levels cannot be published due to the registered intellectual property of the products. All values are expressed as indices of Arthrocure A. The recommended intakes of Arthrocure A an B were the same, but Arthrocure C was supplemented at 50% of Arthrocure A or B levels

The main reason for the changes from Arthrocure A to B, and then to C was the cost of the products per animal per day. Arthrocure A with organic minerals was very expensive and theoretically formulated to improve mineral supplementation on all farms and to decrease any antagonistic effects from the habitat and water sources (NRC 2016). The cost of Arthrocure B was 5.3 times and Arthrocure C 7.1 times cheaper than the cost of Arthrocure A. With this in mind and no visible lesion trend change from this data set, the usage of Arthrocure B and C can be more cost effective in cattle compared to Arthrocure A under normal conditions. The outcome may change drastically in areas where the water contains high levels of antagonistic minerals, which could have a negative impact on the bio-availability of the minerals from the diet.

The results from this trial indicated a downward trend in the prevalence of osteochondrosis, similar to the results as published by Prozesky (2016) when osteochondrosis affected cattle were fed with minerals, but contradictary to the results observed by Van den Veen *et al.* (2017), and therefore an indication that more research is necessary to further investigate this inconsistent response in affected animals. Figures 3 and 4 are some photo's of osteochondrosis affected cattle. Figure 4 are photos of some of the Arthrocure fed cattle showing trends of healing cartilage areas.

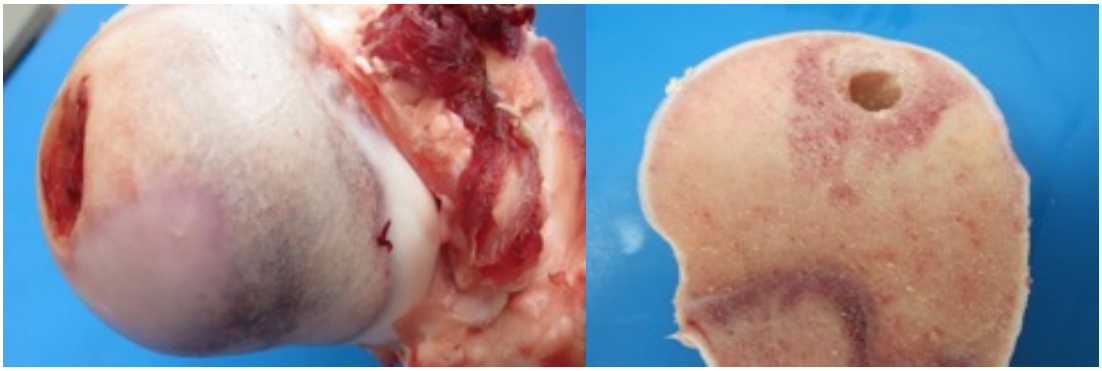


**Figure 3** Cartilage lesions in osteochondrosis affected animals (supplied by Prof. Leon Prozesky)

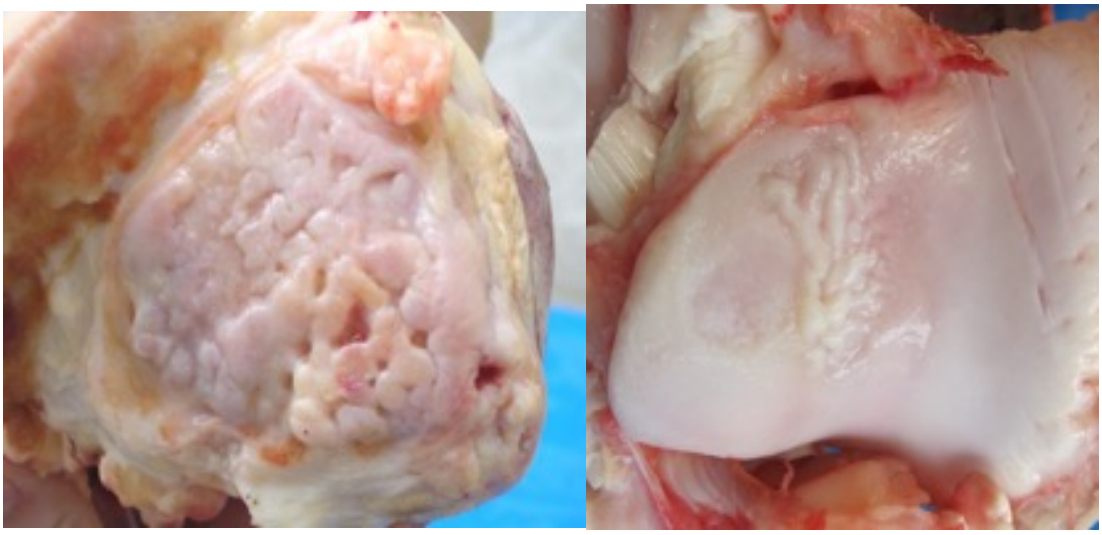


**Figure 4** Healing of the cartilage lesions in osteochondrosis affected animals fed a supplement containing Arthrocure supplement. Note the irregular fibrous articular cartilage covering the femur condyle on the left photo (supplied by Prof. Leon Prozesky)

## Conclusions

Farmers need to feed supplements to their cattle to supply the deficient nutrients from the pastures when cattle are grazing. Supplements must be fed according to the production stage of the animals (nutrient requirements) and balanced keeping factors in mind like soil-plant-mineral interactions, mineral status of the water, dietary cation-anion difference of the diet, bio-availability and palatability of the mineral sources, to only mention a few.

Feeding the specialized Arthrocure mineral supplements to cattle, with the specific aim to decrease the prevalence of osteochondrosis, showed a tendency to decrease the percentage lesions on all six commercial farms. Osteochondrosis was still noticed in some animals, perhaps because all animals do not eat supplements. The percentage lesions on the left and right leg of the animals was very similar on all the farms, and similar to the OPOLS validation data during earlier trials from Onderstepoort. All the numbers in this trial showed similar decreases in the prevalence of osteochondrosis lesions in females and in bulls, although the oxen numbers were low and therefore unreliable.

Research on mineral bio-availabilities in cattle are expensive and very difficult. Nevertheless, more research is required to fully understand all the interactions with the mineral levels from the habitat and water sources of cattle on extensive grazing farms. The minerals in water sources are highly variable (Spears 2003) and therefore another focus area for research, especially with the confounding factor of current and previous mining activities which may contaminate the sources.

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# CHAPTER 8

## RECOMMENDATIONS AND FURTHER RESEARCH

### Industry recommendations

Results from the phosphorous trial (Chapter 6), the bone mineral comparisons between healthy and osteochondrosis affected cattle (Chapter 4), and the commercial trial when cattle were supplemented with specialized minerals (Chapter 7), supported the hypothesis that osteochondrosis are related to the diet of the animals. The "Arthrosis" research team evaluated many different farm management and feeding systems, in the North West Province, since the early 2000's to get answers related to the high prevalence of osteochondrosis within this province. Feed recipes, raw material differences within the fed diets, forage sources and supplemental intakes were evaluated and compared between farms and even between camps within the same farm.

Several hypotheses have already been discussed in the different chapters in this thesis, but there are some unanswered questions which will be discussed in the recommendations for future research. One very important finding, that was brought under the attention of the industry as well as the legislators, is the low minimum trace mineral requirements for all registered cattle supplements as published in the South African legislation through Act 36 of 1947 (Appendix 15). This act regulates the minimum and maximum level of some nutrients and used by the Animal Feed industry to register products for commercial usage, for example cattle supplements.

Some important shortcomings of the minimum trace mineral levels for cattle supplements in the regulations of Act 36 were presented by Mr. Botha and Dr. Meissner at an Animal Feed Manufacturing Association (AFMA) Technical Committee meeting, and included senior management members of Act 36.

The shortcomings were summarized as being the following:

- Act 36 uses the same minimum and maximum mineral value for all the different cattle classes. The differences in requirements for cattle with different ages, gender or productivity, as published by for example the NRC 2016 for beef cattle, are therefore not addressed through this legislation.
- Act 36 specify minimum trace mineral levels only for a Trace Mineral supplement registration. All three other supplement classes in the act, the mineral, protein and energy supplements, can be registered and manufactured without containing any trace minerals. Added trace and macro minerals are expensive and this shortcoming created an opportunity for some supplement industry companies to register and sell

cheaper supplements which do not contain important minerals to meet the requirements of the animals. This may negatively affect the health and productivity of cattle in some mineral poor areas, for example the North West Province which is known for low P levels in the pastures.

- Act 36 do not specify the chemical form that relates to a specific mineral. Companies can therefore use any mineral form based on price, which may affect the bio-availability of the mineral to the animal. Farmers only see the mineral values printed on the bags or bag tag and not the chemical form of the mineral.

Table 8.1 shows the minimum requirements for minerals as published for beef cattle by the NRC 2016 for three cattle groups as examples. These levels are compared with the minimum mineral levels published in Act 36 (Appendix 15) in Tables 8.2.a. and 8.2.b.

**Table 8.1** The minimum mineral requirements for three different cattle groups as published by the NRC 2016 for beef cattle

| | | | | Nutrients, mg ‡ | | | | | | | Nutrients, g ‡ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cattle group** | **DMI, kg / animal / day †** | **Feed intake, kg on 88 % DM basis** | | **Fe** | **Mn** | **Cu** | **Co** | **Zn** | **Se** | **I** | **Ca** | **P** |
| **NRC Beef, 533 kg Cow with calf ¶** | 12 | 13.6 | per kg, as is | 44 | 35.2 | 8.8 | 0.13 | 26.4 | 0.09 | 0.44 | | |
| | | | per animal /day | 600 | 480 | 120 | 1.80 | 360 | 1.2 | 6 | 36.0 | 24.0 |
| **NRC Beef, 533 kg cow late gestation** | 10.7 | 11.8 | per kg, as is | 44 | 35 | 8.8 | 0.13 | 26.4 | 0.09 | 0.44 | | |
| | | | per animal /day | 519 | 415 | 104 | 1.56 | 312 | 1.04 | 5.19 | 29.8 | 18.1 |
| **NRC growing cattle** | 6.9 | 7.8 | per kg, as is | 44 | 17.6 | 8.8 | 0.13 | 26.4 | 0.09 | 0.44 | | |
| | | | per animal /day | 345 | 138 | 69 | 1.04 | 207 | 0.7 | 3.45 | 38.0 | 16.6 |

† DMI = dry matter intake; ‡ NRC Beef Cattle 2016 recommendations in general; § Growing cattle = 300 kg Shrunk body weight, 1.2 kg ADG; ¶ Based on a diet with a 60% TDN (DM) and 60 days after calving; Bio-availability not taken into account

**Table 8.2.a** The minimum mineral requirement for three different cattle groups as published by Act 36 (1947) for a Trace Mineral Supplement and compared with the requirements published in the NRC 2016 for beef cattle

| | | | | Nutrients, mg ‡ | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cattle group** | **DMI, kg / animal / day †** | **Feed intake, kg on 88 % DM basis** | | **Fe‡** | **Fe (Act 36)** | **Fe (Act 36 vs. NRC)** | **Mn‡** | **Mn (Act 36)** | **Mn (Act 36 vs. NRC)** | **Cu‡** | **Cu (Act 36)** | **Cu (Act 36 vs. NRC)** | **Co‡** | **Co (Act 36)** | **Co (Act 36 vs. NRC)** |
| **NRC Beef, 533 kg Cow with calf ¶** | 12 | 13.6 | per animal / day | 600 | 100 | 16.7% | 480 | 40 | 8.3% | 120 | 20 | 16.7% | 1.80 | 0.2 | 11.1 % |
| **NRC Beef, 533 kg cow late gestation** | 10.7 | 11.8 | per animal / day | 519 | 100 | 19.3% | 415 | 40 | 9.6% | 104 | 20 | 19.3% | 1.56 | 0.2 | 12.8 % |
| **NRC growing cattle** | 6.9 | 7.8 | per animal / day | 345 | 100 | 29.0% | 138 | 40 | 29.0% | 69 | 20 | 29.0% | 1.04 | 0.2 | 19.3 % |

† DMI = dry matter intake; ‡ NRC Beef Cattle 2016 recommendations in general; § Growing cattle = 300 kg Shrunk body weight, 1.2 kg ADG; ¶ Based on a diet with a 60% TDN (DM) and 60 days after calving; Bio-availability not taken into account

**Table 8.2.b** The minimum mineral requirements for three different cattle groups as examples, as published by Act 36 (1947) for a Trace Mineral Supplement and compared with the requirements published in the NRC 2016 for beef cattle

| | | | | Nutrients, mg ‡ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cattle group** | **DMI, kg / animal / day †** | **Feed intake, kg on 88 % DM basis** | | **Zn ‡** | **Zn (Act 36)** | **Zn (Act 36 vs. NRC)** | **Se ‡** | **Se (Act 36)** | **Se (Act 36 vs. NRC)** | **I ‡** | **I (Act 36)** | **I (Act 36 vs. NRC)** |
| **NRC Beef, 533 kg Cow with calf ¶** | 12 | 13.6 | per animal / day | 360 | 60 | 16.7% | 1,2 | 0.2 | 16.7% | 6 | 1 | 16.7% |
| **NRC Beef, 533 kg cow late gestation** | 10.7 | 11.8 | per animal / day | 312 | 60 | 19.3% | 1.04 | 0.2 | 19.2% | 5.19 | 1.00 | 19.3% |
| **NRC growing cattle** | 6.9 | 7.8 | per animal / day | 207 | 60 | 29.0% | 0.7 | 0.2 | 28.6% | 3.45 | 1 | 29.0% |

† DMI = dry matter intake; ‡ NRC Beef Cattle 2016 recommendations in general; § Growing cattle = 300 kg Shrunk body weight, 1.2 kg ADG; ¶ Based on a diet with a 60% TDN (DM) and 60 days after calving; Bio-availability not taken into account

Many commercially available supplements in South Africa are formulated based on the minimum requirements specified in Act 36, although some companies formulate their supplements for the specific needs of animals based on published scientific investigations. Supplying nutrients to cattle based on the minimum nutrient values published in Act 36 (1947) created an industry where many animals receive sub-optimal mineral levels. These low-mineral containing supplements are therefore based on price only, and not on the specific needs of the supplemented animals.

Tables 8.2.a. and 8.2.b. are calculated values according to the NRC 2016 for beef cattle predicted intakes and then compared with the Act 36 minimum trace mineral levels. Feeding a Trace Mineral supplement based on the minimum mineral levels (Act 36) will only supply 8.30 to 29.0% of the total requirements of the mentioned cattle groups.

These calculations and the high incidence of osteochondrosis in cattle on farms in the North West province created an urgent need to change the criteria used by Act 36 for registering supplements as presented by the Onderstepoort research team to the AFMA Technical committee and the personnel of Act 36. The results and in-depth focus into the supply of minerals from this Onderstepoort research team were fundamental in creating this awareness with the legislating authorities.

The AFMA Technical committee decided to immediately start a process to evaluate all nutrient requirements, as published by Act 36 of 1947, with the aim to implement new regulations to meet the requirement of all animals and therefore improving the health of animals. This process was started, not only for beef cattle, but also for all the other species as one of the main projects within AFMA and as an important part of the new Animal Feeds Bill. All the different recommendations by the seven different "species committee's" will be completed in 2018 for approval by the industry. The "species committees" were requested to address the following aspects in the new regulations, as set out by the Onderstepoort Osteochondrosis research team:

- To create different animal groups within a specie.
- To recommend the minimum nutrient requirements for the different animal groups (for example age, gender, and productivity) within a specie.
- To recommend the chemical form of the minerals as the reference point to the minimum requirements to be used within supplements and feeds.
- To standardize the terminology used between the different species (concentrates, meals, supplements, and many more).

Research by Van der Veen *et al*. (2017) indicated that the DCAD of supplements may also play a role in the efficacy of the specific supplement. Water in some areas in South Africa contains

high sulphur levels and with supplement feed troughs close to the water troughs, it is not known how the combined effect of a low DCAD supplement and the high sulphur in water will affect the cattle's plasma pH. Another important point was that supplements are formulated since the 1980's with some sulphur containing raw materials, like mono-ammonium phosphate, ammonium sulphate, lower quality MCP's, to mention only a few. These raw materials contain high levels of sulphur which decreased the DCAD values in some of the supplements on the evaluated farms to as low as -1419 meq/kg. Results from DeGaris and Lean (2008) have shown that a lower DCAD will induce a metabolic acidosis and aciduria, although it is still not well documented in beef cattle with supplements. Research published by different groups have also found a link between the parathyroid hormone (PTH) changes and mineral changes in the plasma and urine due to metabolic acidosis (Horst *et al*. 1997; La Perle & Capen 2006). Van der Veen *et al.* (2017) also found a lower blood pH in some of the cattle groups in his preliminary blood profile work on commercial farms, well known for their higher incidence rate for osteochondrosis. The Onderstepoort osteochondrosis research group therefore also recommended that the industry formulate cattle supplements for growing and lactating animals with low S raw materials to keep the DCAD value positive (meq/kg) to prevent metabolic acidosis, until the hypothesis is well tested. The recommendation also stated that S is formulated to only meet the animal's S requirement and not to overfeed S, one of the major anions affecting the DCAD value of supplements.

**Future research projects**

1. Further research to validate the accuracy of the sub-categories, within the OPOLS system, may also add value to professionals to evaluate the size of lesions from one evaluation period to another, although one may expect more inaccuracy when more sub-categories are added.

2. The mineral levels in the rib bone and liver of osteochondrosis affected and healthy animals, together with the mineral levels in the supplement were used as a starting point for the follow-up trials to better understand the aetiology of osteochondrosis. Research on these minerals as well as the physiological processes involved, needs to be expanded to test new hypotheses regarding the effect of mineral imbalances, possible mineral antagonists, possible changes in blood profiles, nutritional supplements, nutritional management systems, and phosphorous sources in order to better understand the high prevalence of osteochondrosis on some farms. Future research must also include cows and lactating cows to evaluate the intakes of these groups over seasons and to evaluate the effect of bioavailability of a P source on the performance of other groups as well.

3. All cattle do not consume free-choice supplements, and therefore it is very important to optimize the supplement supply to the different groups with different management and supply systems to minimize the number of cattle not eating the supplement. New mineral feeding systems need to be created and researched to improve the intake of minerals in commercial beef cattle.

4. Research is also necessary to evaluate the effect of feeding a low or high positive DCAD supplement (50 and 450 meq/kg) in comparison to a low or high negative DCAD (-100 and -450 meq/kg) supplement on the plasma pH and the mineral balances within the animal's system. It must also be evaluated what the effect might be on PTH as well as mineral metabolism within the body, especially the bone. This might explain the higher incidence of osteochondrosis on some farms which fed a low DCAD supplement to animals which also drink water containing high levels of S. A project has started to evaluate the affect of low plasma pH on blood PTH and the prevalence of osteochondrosis over a five-year period with three different supplements, based on different DCAD levels. This trial started on two different farms, Armoedsvlakte Research Station (Vryburg) and the ARC Research Farm (Potchefstroom). Due to a lack of funds, these trials were stopped during 2017, but the "Arthrosis" research team believe this DCAD hypothesis must be tested and may give important answers to some factors which increase the prevalence of osteochondrosis in cattle.

5. Research on mineral bio-availabilities in cattle is expensive and difficult. Nevertheless, more research is required to fully understand all the interactions with the mineral levels from the habitat and water sources of cattle on extensive grazing farms. The minerals in water sources are highly variable (Spears 2003) and maybe another focus area for research, especially with the confounding factor of current and previous mining activities in specific areas, which may contaminate the sources.

6. Histone deacetylases (Hdacs) activity plays important roles in the development of all organs and tissues, including the mineralized skeleton (Bradley *et al*. 2011). Endochondral ossification is a highly organized and tightly controlled development process and Hdacs play a very important role in its orchestration and this gives rise to the majority of bones in the skeleton. Given the important role of Hdac activity in bone cell function and skeletal development, it is not surprising that aberrant class II Hdac activity is associated with skeletal diseases in humans, including osteoarthritis and osteoporosis. There are 18 genes that encode Hdacs in the human and mouse genomes, although there are greater than 1800 transcription factor genes which are important to regulate gene expression. Hill *et al.* (1998) did a study on extensively managed Brahman cattle, grazing native pastures, and found clinically observed osteochondrosis in several lame bulls necropsied, all related to a common ancestral sire. The role of heritability in the development of osteochondrosis in cattle is therefore still unclear and an area where research is necessary to solve this hypothesis. There are many gene-mapping projects running in South Africa which may help to solve the question related to gene activity and osteochondrosis affected animals.

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# CHAPTER 9

## SHORTCOMINGS OF THE RESEARCH

### EVALUATING THE MINERAL PROFILE OF BEEF CATTLE WITH AND WITHOUT OSTEOCHONDROSIS AND THE RESPONSE OF OSTEOCHONDROSIS AFFECTED ANIMALS TO A SHORT TERM MINERAL SUPPLEMENT

- The trace mineral levels within the *E. curvula* and water supplied during the trial for the osteochondrosis fed animals (group S), were not determined. This could also be a reason why the shift in some liver and bone mineral levels was different to typical changes, as seen in other published research for sick animals, and could not be explained.
- The chemical form of mineral sources within the commercial supplement used in this study was not known, which resulted in some unanswered questions.

### COMPARING FOUR DIFFERENT PHOSPHOROUS SOURCES IN SUPPLEMENTS FED TO BEEF CATTLE ON THE PREVALENCE OF OSTEOCHONDROSIS IN THE NORTH WEST PROVINCE

- The trial was done on a research farm in an area well-known for phosphorous related health problems and therefore it was decided not to feed a supplement without phosphorous, as the control group. The health risk with such a control supplement, over a period of 433 days in growing steers, was too high according to the published research done in the North West province since 1912 (Prozesky 2016; Spangenberg 1997).
- The steers were supplemented in an extensive grazing system on a group basis and therefore all intake data was collected per group and not on an individual basis. Feeding only one group per treatment together with no individual intakes created a data set where analysis of variance calculations were not possible due to no treatment variance per animal within treatment. All the supplement intake data was therefore discussed only as observations.
- It was also not possible to do pasture intakes under the extensive conditions over such a long period. Pasture intakes per group per season could have helped to explain some of the results, but it is too costly and difficult to simulate what cattle eat within these extensive grazing conditions.
- The different P-source batches used in this trial were not tested for water soluble phosphorous as an evaluation of the ratio of mono calcium to di-calcium phosphate within a product. This must be included in future trials to give more insight into animal performance from different phosphorous sources.

**EVALUATING THE PREVALENCE OF OSTEOCHONDROSIS IN CATTLE ON COMMERCIAL FARMS WHEN FED WITH SPECIALIZED MINERAL SUPPLEMENTS**

- The trial was done on commercial farms in the North West province (South Africa), well-known for their osteochondrosis health problems. These farms were also in a P-depleted area and therefore it was decided not to feed a supplement without phosphorous, as the control group. The health and reproduction risk with such a negative control supplement were too high according to the published research done in the North West province since 1912 (De Waal 1993; Spangenberg 1997).
- The water and habitat sources were not evaluated for minerals due to the costs and complexity to do it within the different pasture camps on the different farms. Nevertheless, more research is required to fully understand all the interactions with the mineral levels from the habitat and water sources of cattle on extensive grazing farms.
- It was decided not to monitor supplemental intake on the different farms due to the logistical challenges as well the differences in personnel on the farms.
- Another shortcoming of this trial was to add a treatment group per farm where only phosporous are supplied without the Arthrocure mineral pack. Using the combination of P plus Arthrocure minerals resulted in a confounding effect, which created unanswered questions to pinpoint the main cause of osteochondrosis.

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# LIST OF APPENDIXES

**Appendix 1** Liver and bone sampling and analysis methodology used in trials to do mineral comparisons in cattle

| **Liver** | |
|---|---|
| Methods used | Nitric + Perchloric acid digestion.<br>P, Fe, Ca, Al, Mg, K, Mn, Cu, Na, Zn levels determined by using the ICP OES method.<br>Cr, Hg, Pb, Ni, Cd, Co levels determined by using the ICP MS method<br><br>Nitric acid digestion method<br><br>**As, Se**, Fe, P, Mg, K, Mn, Cu, Zn, Na and scanning can be determined by using ICP MS.<br>If scanning is required, it is recommended to use the Nitric acid digestion method. |
| Method of collection | Cut a transect from left to right of the liver, approximately 3 cm wide, and the depth of the liver; roll sample and place into one plastic container, This will give a representative sample of the liver. |
| Storage | Store in a plastic container (bag or bottle) (1 container per animal).<br>Kept frozen if possible (- 20°C), otherwise in formalin<br>If formalin is used, digestion and analysis needs to be done within one month of collection to get reliable results. |
| Time | Collection: as fresh as possible<br>Storage: as soon as possible, try prevent autolysis |

| **Bone** | **Rib bone sample** |
|---|---|
| Methods used | ***Ashing***<br><br>Ash percentage<br>Ash – from ash Ca, Mg, P. method: aqua regia dissolution method.<br>Ash - F levels can be determined with the ION Selective electrode. (of iron)<br>Nitric + Perchloric acid digestion of Dry Bone.<br>P, Fe, Ca, Al, Mg, K, Mn, Cu, Zn levels determined by using the ICP OES method<br>Cd, Cr, Hg, Pb, Ni levels determined by using the ICP MS method<br><br>If scanning is required it is recommended to use the Nitric acid digestion method. |

| | |
|---|---|
| Method of collection | Rib: fifteen centimetres of the 13th rib on the left hand side of the carcass |
| Storage | Store in a clean plastic container (bag or bottle) (1 container per animal).<br>Kept frozen if possible (- 20°C). |
| Time | Collection: as fresh as possible<br>Storage: as soon as possible, try prevent autolysis |
| **Key things to remember** | |
| | 1. *Species / breed of animal*<br>2. sex of animal<br>3. age of animal<br>4. possible diagnosis or history<br>5. area of origin |

**Appendix 2** Method used to sample water on cattle farms

| **Water quality** | |
|---|---|
| Microbiological | Coliform counts, *Microcystis aeruginosa* etc. (see bacteriology and toxicology sampling) |
| Chemical | A wide variety of the above mentioned minerals could be tested using the ICP quantitative scan method. |
| **Sampling criteria** | |
| Microbiological sample | Use a sterile water holder (plastic container), approximately 100 ml (collect sample "as is"). |
| Chemical sample | Two bottles needed:<br>1. Clean bottle (volume: 500ml), used to determine pH, TDS and other chemical factors.<br>2. 100ml (container) water sample with 5ml Nitric acid (to determine amounts of different metals in solution, Prepared bottles available from the Institute of Soil Climate and Water). |
| **Method** | |
| Surface water (dam, trough) | Nearest point of abstraction |
| Ground water (Borehole) | Purge for 3 minutes (at point of use), allowing EC and pH to stabilize. |
| **Key things to remember** | |
| | 1. Best to sample water at point of use (what the animal is drinking)<br>2. Rinse out the container with the sample water after its been purged (about 3 times). Use sterile glass bottle.<br>3. Fill the pre-treated sample bottle (50ml container with 5ml Nitric acid), from the collected sample.<br>4. Top the sampling container up.<br>5. Clearly mark all containers |
| Time | Handed in for analysis within 1 week of collection. |
| Contact details | Institute of Soil Climate and Water: 012 310 2500 (Chemical Analysis)<br>Veterinary Public health (Mev C Watermeyer): 012 529 8461 (Microbiological) |

**Appendix 3** Methods used to sample and analyse grass, soil and supplement samples

| **Feed Quality** | |
|---|---|
| Chemical | Nutrient elements; Dry matter, Ash, Crude protein, energy, fibre fractions (grass only).<br>Minerals; B, Ca, P, Cu, Mg, Fe, Na, F, Mn, Mo, Zn, Al, Cd, Cr, Se, Co, oxalates and nitrates. |
| **Collection Method** | |
| Grass | Grass: within a 1-hectare camp, randomly select 1m by 1m square patches of grass to be collected. Cut the grass approximately 8cm from the soil surface (imitating what a cow would have eaten). Place the sample in a plastic bag and put it in a freezer asap.<br><br>Dry the freezed grass sample in a drying oven at 60 $^{0}$C for approximately 48 hours, weight before drying and after to determine the dry matter of the sample. The grass will then be milled and a representative sample will be placed in a sample holder in a dark dry room until the analysis is done.<br><br>5 camps per 1000 hectares |
| Soil | Soil samples can be taken in the 1 hectare camps. Numerous samples will be collected within the one-hectare camp using the bicycle soil auger (this augur has a bag attached to the back of it, allowing the 1$^{st}$ 10cm of soil to be augured (collected) and stored as the total hectare gets sampled).<br><br>Soil samples will be dried in a drying oven at 60 degrees Celsius for 48 hours. Weight before drying and after will be measured and Dry weight will be calculated. Soil will be sieved and stored in a sample holder in a dark dry room till analysis is done. |
| Supplement | A representative sample of the supplement will be collected as discribed in the "Methods" per trial from all farms.<br>Both the delivered (bagged) lick and the final mix fed to the animals will be collected.<br>Bags: 7 bags will be randomly selected, opened and mixed thoroughly. Three grab samples per bag will be collected and placed in a plastic bucket. Mix this combined sample thoroughly and take a 500 g sample to be stored in a sample holder in a dark dry room until analysis is done.<br>Final mix: The Salt-Supplement final mix sample is collected at mixing. Random samples of supplement are then collected from mixed supplement and thoroughly mixed in a bucket. A 500 g sample is then taken to be stored in a sample holder in a dark dry room till analysis is done. |
| **Key things to remember** | |
| | Clearly mark all containers/ plastic bags / paper bags |

**Appendix 4** A complete summary of the osteochondrosis OPOLS on-farm observation results on stifle bones of test cattle compared to the X-ray, post-mortem, and combined X-ray and post-mortem examinations

| Test characteristic | X-ray data : Summary of data on the two hind legs over two observation times. | Post mortem data: Summary of data on the two hind legs. | Post mortem + X-ray combined data: Summary of data on the two hind legs. † |
|---|---|---|---|
| Chi-square | 0.862 | 0.286 | 0.209 |
| Probability | 0.353 | 0.593 | 0.647 |
| Sensitivity | 0.900 | 0.857 | 0.886 |
| 95% confidence interval for Sensitivity | ( 0.833, 0.942 ) | ( 0.743, 0.926 ) | ( 0.831, 0.925) |
| Specificity | 0.292 | 0.571 | 0.395 |
| 95% confidence interval for Specificity | ( 0.149, 0.492 ) | ( 0.326, 0.786 ) | ( 0.256, 0.553 ) |
| True prevalence | 0.833 | 0.800 | 0.822 |
| False negative fraction FNF | 0.100 | 0.143 | 0.114 |
| False positive fraction FPF | 0.708 | 0.429 | 0.605 |
| Positive predictive value PV+ | 0.864 | 0.889 | 0.872 |
| Negative predictive value PV- | 0.368 | 0.500 | 0.429 |
| Relative Risk RR | 1.271 | 2.000 | 1.464 |
| Odds ratio or Prevalence ratio | 3.706 | 8.000 | 5.087 |
| Odds ratio for diagnosis on right leg | 8.300 | 10.500 | 12.072 |
| Odds ratio for diagnosis on left leg | 2.300 | 6.800 | 3.154 |
| likelihood ratio of a positive test result LR+ | 1.271 | 2.000 | 1.464 |
| likelihood ratio of a negative test result LR- | 0.343 | 0.250 | 0.288 |
| Observed agreement | 0.799 | 0.800 | 0.799 |
| Expected agreement (chance) | 0.745 | 0.663 | 0.717 |
| Apparent or post test prevalence AP | 0.868 | 0.771 | 0.836 |
| Prevalence P | 0.833 | 0.800 | 0.822 |
| Kappa coefficient | 0.210 | 0.408 | 0.290 |
| Total observations | 144 | 70 | 214 |

† The data is the combined data of the groups for methods X and P. Animals were examined either by X-ray or post-mortem method, and not by both methods on the same animal; Sensitivity = true positive rate; Specificity = true negative rate; Odds ratio = how much more likely will the test make a correct diagnosis than an incorrect diagnosis in animals with osteochondrosis; NND = the number of animals needed to give 10 positive tests

**Appendix 5** A degree of lameness as described by Sprecher *et al.* (1997) has been developed to determine the incidence and severity of lameness in cattle

- Grade 1 (score 1: normal gait).
- Grade 2 (score 2: mildly lame).
- Grade 3 (score 3: moderately lame).
- Grade 4 (score 4: lame)
- Grade 5 (score 5: severely lame)


(adapted from www.zinpro.com from Zinpro incorporated from the Firststep program)

**Appendix 6** Mineral analyses of rib bone samples of the slaughtered Bonsmara steers that were fed *ad lib.* for 433 days on four phosphorous sources while grazing on natural pastures

| | Animal groups | Mean | SEM | n | S. E. | cv, % | F pr test |
|---|---|---|---|---|---|---|---|
| Fat-free Ca, % | DCP1 | 17.84 | 1.097 | 8 | 1.142 | 6.60 | F pr = 0.275 |
| | **Variate, bone samples** DCP2 | 16.73 | 1.151 | 8 | | | |
| | DCP3 | 17.06 | 1.419 | 8 | | | |
| | MCP | 17.07 | 0.819 | 8 | | | |
| Fat-free P, % | DCP1 | 8.09 | 0.51 | 8 | 0.611 | 7.80 | F pr = 0.430 |
| | DCP2 | 7.60 | 0.66 | 8 | | | |
| | DCP3 | 7.73 | 0.71 | 8 | | | |
| | MCP | 7.90 | 0.55 | 8 | | | |
| Fat-free Mg, % | DCP1 | 0.2789 | 0.026 | 8 | 0.03313 | 12.30 | F pr = 0.409 |
| | DCP2 | 0.2561 | 0.038 | 8 | | | |
| | DCP3 | 0.2611 | 0.041 | 8 | | | |
| | MCP | 0.2782 | 0.025 | 8 | | | |
| Moisture, % | DCP1 | 23.60 | 4.168 | 7* | 3.252 | 13.30 | F pr = 0.643 |
| | DCP2 | 25.20 | 3.204 | 8 | | | |
| | DCP3 | 25.18 | 3.400 | 8 | | | |
| | MCP | 23.81 | 2.017 | 8 | | | |
| Dry Fat-free Ash, % | DCP1 | 60.20 | 2.60 | 8 | 3.438 | 5.80 | F pr = 0.640 |
| | DCP2 | 58.07 | 3.05 | 8 | | | |
| | DCP3 | 59.42 | 3.80 | 8 | | | |
| | MCP | 58.72 | 4.09 | 8 | | | |
| Dry fat-free Ca, % | DCP1 | 23.06 | 1.42 | 8 | 1.327 | 5.80 | F pr = 0.807 |
| | DCP2 | 22.6 | 1.55 | 8 | | | |
| | DCP3 | 22.79 | 1.20 | 8 | | | |
| | MCP | 22.44 | 1.08 | 8 | | | |
| Dry fat-free bone ash Ca, % | DCP1 | 38.34 | 2.43 | 8 | 2.032 | 5.30 | F pr = 0.931 |
| | DCP2 | 38.91 | 1.32 | 8 | | | |
| | DCP3 | 38.41 | 1.78 | 8 | | | |
| | MCP | 38.33 | 2.39 | 8 | | | |
| Dry fat-free P, % | DCP1 | 10.45 | 0.66 | 8 | 0.818 | 7.80 | F pr = 0.888 |
| | DCP2 | 10.27 | 0.89 | 8 | | | |
| | DCP3 | 10.59 | 0.97 | 8 | | | |
| | MCP | 10.39 | 0.72 | 8 | | | |
| Dry fat-free bone ash P, % | DCP1 | 17.37 | 1.07 | 8 | 1.146 | 6.50 | F pr = 0.876 |
| | DCP2 | 17.67 | 0.96 | 8 | | | |
| | DCP3 | 17.80 | 0.78 | 8 | | | |
| | MCP | 17.76 | 1.60 | 8 | | | |
| Dry fat-free Mg, % | DCP1 | 0.36 | 0.03 | 8 | 0.045 | 12.50 | F pr = 0.804 |
| | DCP2 | 0.346 | 0.05 | 8 | | | |
| | DCP3 | 0.357 | 0.06 | 8 | | | |
| | MCP | 0.366 | 0.03 | 8 | | | |
| Dry fat-free bone ash Mg, % | DCP1 | 0.599 | 0.03 | 8 | 0.0636 | 10.50 | F pr = 0.732 |
| | DCP2 | 0.594 | 0.05 | 8 | | | |
| | DCP3 | 0.599 | 0.06 | 8 | | | |
| | MCP | 0.625 | 0.03 | 8 | | | |
| Dry fat-free Ca : Mg | DCP1 | 64.30 | 5.05 | 8 | | | |
| | DCP2 | 66.15 | 7.24 | 8 | | | |
| | DCP3 | 64.77 | 7.63 | 8 | | | |
| | MCP | 61.62 | 4.37 | 8 | | | |

SEM = standard error of the mean; DCP1, DCP2 and DCP3 is different di-calcium phosphorous sources and MCP is a mono-calcium phosphorous source. DCP1 and the MCP source were from a supplier which produces products within a continuous production process; F pr means Fisher's protected least significant different test at 5% level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used); Feeding = healthy animals fed for 433 days with the different supplements, n = number of steers used; † One value was excluded to normalize the data and stabilize variances

**Appendix 7** Average full bodyweight (kg) of Bonsmara steers that were fed *ad lib* for 433 days on four phosphorous sources while grazing on natural pastures (per treatment as per weight date and **not adjusted** for starting weight as covariate)

| Date | Day in trial | Treatment | n | Mean | SEM | Date | Day in trial | Treatment | n | Mean | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 07/12/2005 | 0 | DCP1 | 8 | 209.4 | 22.40 | 12/09/2006 | 279 | DCP1 | 8 | 335.0 | 37.77 |
| | | DCP2 | 8 | 205.9 | 26.43 | | | DCP2 | 8 | 319.9 | 39.08 |
| | | DCP3 | 8 | 209.4 | 17.38 | | | DCP3 | 8 | 333.2 | 21.55 |
| | | MCP | 8 | 230.6 | 24.05 | | | MCP | 8 | 362.3 | 19.46 |
| 24/01/2006 | 48 | DCP1 | 8 | 261.1 | 29.55 | 11/10/2006 | 308 | DCP1 | 8 | 355.7 | 37.07 |
| | | DCP2 | 8 | 252.1 | 39.44 | | | DCP2 | 8 | 340.8 | 39.04 |
| | | DCP3 | 8 | 256.8 | 20.60 | | | DCP3 | 8 | 350.0 | 22.63 |
| | | MCP | 8 | 293.4 | 24.21 | | | MCP | 8 | 375.8 | 25.34 |
| 07/03/2006 | 90 | DCP1 | 8 | 311.4 | 32.97 | 14/11/2006 | 342 | DCP1 | 8 | 373.4 | 38.70 |
| | | DCP2 | 8 | 299.9 | 46.91 | | | DCP2 | 8 | 355.0 | 42.97 |
| | | DCP3 | 8 | 309.0 | 17.00 | | | DCP3 | 8 | 364.6 | 22.80 |
| | | MCP | 8 | 334.0 | 20.66 | | | MCP | 8 | 387.8 | 21.31 |
| 19/04/2006 | 133 | DCP1 | 8 | 332.1 | 39.01 | 07/12/2006 | 365 | DCP1 | 8 | 398.9 | 39.03 |
| | | DCP2 | 8 | 324.9 | 45.65 | | | DCP2 | 8 | 377.6 | 44.11 |
| | | DCP3 | 8 | 333.2 | 14.57 | | | DCP3 | 8 | 404.5 | 33.28 |
| | | MCP | 8 | 359.4 | 27.97 | | | MCP | 8 | 411.5 | 19.59 |
| 24/05/2006 | 168 | DCP1 | 8 | 336.3 | 44.78 | 08/01/2007 | 397 | DCP1 | 8 | 427.7 | 47.01 |
| | | DCP2 | 8 | 331.5 | 43.26 | | | DCP2 | 8 | 402.2 | 45.59 |
| | | DCP3 | 8 | 335.9 | 18.17 | | | DCP3 | 8 | 420.6 | 18.49 |
| | | MCP | 8 | 371.4 | 23.34 | | | MCP | 8 | 450.5 | 29.85 |
| 21/06/2006 | 196 | DCP1 | 8 | 339.3 | 40.05 | 30/01/2007 | 419 | DCP1 | 8 | 439.3 | 49.01 |
| | | DCP2 | 8 | 331.2 | 44.87 | | | DCP2 | 8 | 416.1 | 43.55 |
| | | DCP3 | 8 | 338 | 20.30 | | | DCP3 | 8 | 436.0 | 20.59 |
| | | MCP | 8 | 363.6 | 26.26 | | | MCP | 8 | 461.8 | 33.41 |
| 19/07/2006 | 224 | DCP1 | 8 | 339.0 | 38.78 | 13/02/2007 | 433 | DCP1 | 8 | 489.4 | 55.62 |
| | | DCP2 | 8 | 334.8 | 46.35 | | | DCP2 | 8 | 446.9 | 49.85 |
| | | DCP3 | 8 | 333.2 | 17.69 | | | DCP3 | 8 | 471.4 | 25.70 |
| | | MCP | 8 | 364.4 | 22.70 | | | MCP | 8 | 506.5 | 34.91 |
| 17/08/2006 | 253 | DCP1 | 8 | 317.7 | 41.51 | | | | | | |
| | | DCP2 | 8 | 313.9 | 40.33 | | | | | | |
| | | DCP3 | 8 | 314.80 | 19.26 | | | | | | |
| | | MCP | 8 | 347.4 | 25.07 | | | | | | |

SEM = standard error of the mean; n=number of steers per treatment. Data is not adjusted for starting weigh as a covariate.
Trial period = 7 Dec 2005 - 13 Feb 2007

**Appendix 8** Average full body 16-h (fasted) weights (kg) per treatment as per weight date of Bonsmara steers that were fed *ad lib.* for 433 days on four different phosphorous sources while grazing natural pastures (not adjusted for starting weight as a covariate)

| Treatment | Date of weighing | | | | | | | | | | | | | | | Mean BW, kg | n | SEM | *F pr test* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 07 Dec | 24 Jan | 07 Mar | 19 Apr | 24 May | 21 Jun | 19 Jul | 17 Aug | 12 Sep | 11 Oct | 14 Nov | 07 Dec | 08 Jan | 30 Jan | 13 Feb | | | | |
| DCP1 | 210 | 261 | 311 | 332 | 336 | 339 | 339 | 318 | 335 | 356 | 373 | 399 | 428 | 439 | 489 | 350 [b] | 7* | 8.16 | F pr < 0.001 |
| DCP2 | 206 | 252 | 300 | 325 | 332 | 331 | 335 | 314 | 320 | 341 | 355 | 378 | 402 | 416 | 447 | 337 [a] | 8 | | |
| DCP3 | 209 | 257 | 309 | 333 | 336 | 338 | 333 | 315 | 333 | 350 | 365 | 405 | 421 | 436 | 471 | 347 [b] | 8 | | |
| MCP | 231 | 293 | 334 | 359 | 371 | 364 | 364 | 347 | 362 | 376 | 388 | 412 | 451 | 462 | 507 | 375 [c] | 8 | | |
| Weight day | 0 | 48 | 90 | 133 | 168 | 196 | 224 | 253 | 279 | 308 | 342 | 365 | 397 | 419 | 433 | | | | |
| Mean BW per weight date | 214$^{a}$ | 266$^{b}$ | 314$^{c}$ | 337$^{cde}$ | 343$^{de}$ | 343$^{de}$ | 342$^{de}$ | 323$^{cd}$ | 337$^{cde}$ | 355$^{ef}$ | 370$^{f}$ | 398$^{g}$ | 425$^{h}$ | 438$^{h}$ | 478$^{i}$ | | | | |

SEM = standard error of the mean; n=number of steers per treatment. Data is not adjusted for starting weigh as a covariate. Trial period 7 Dec 2005 - 13 Feb 2007

**Appendix 9** Fixed model effects for the average full body 16-h (fasted) weights (kg) per treatment as per weight date of Bonsmara steers that were fed *ad lib.* for 433 days on four different phosphorous sources while grazing natural pastures (not adjusted for starting weight as a covariate)

| Treatment | **Date of weighing** | | Mean BW, kg | n | SEM | *F pr test* | Fixed model effects | | |
|---|---|---|---|---|---|---|---|---|---|
| | 07-Dec | 13-Feb | | | | | Date | Treatment | Date / Treatment |
| DCP1 | 210 | 489 | 350 [b] | 7* | 8.16 | F pr < 0.001 | F pr < 0.001, SEM = 9.00 | F pr < 0.001, SEM = 8.16 | F pr = 1.000, SEM = 12.3 |
| DCP2 | 206 | 447 | 337 [a] | 8 | | | | | |
| DCP3 | 209 | 471 | 347 [b] | 8 | | | | | |
| MCP | 231 | 507 | 375 [c] | 8 | | | | | |
| Weight day | 0 | 433 | | | | | | | |
| Mean BW per weight date | 214[a] | 478[i] | | | | | | | |

SEM = standard error of the mean; n=number of steers per treatment. Data is not adjusted for starting weigh as a covariate. Trial period 7 Dec 2005 - 13 Feb 2007

**Appendix 10** Mean daily gains for the Bonsmara steers that were fed *ad lib.* for 433 days on four different phosphorous sources while grazing natural pastures when the 16-h fasted starting weights (not adjusted for starting weights as covariate)

| Treatment | Weight day and mean 16h-fasted weight (kg) | | n | Mean Average Daily Gain, kg | SEM | F pr test |
|---|---|---|---|---|---|---|
| | 07-Dec | 13-Feb | | | | |
| DCP1 | 210 | 489 | 8 | 0.65 [b] | 0.06, (cv% = 10.1) | F pr = 0.027 |
| DCP2 | 206 | 447 | 8 | 0.56 [a] | | |
| DCP3 | 209 | 471 | 8 | 0.61 [ab] | | |
| MCP | 231 | 507 | 8 | 0.64 [b] | | |
| | Day 0 | Day 433 | | | | |

Linear mixed model SEM = standard error of the mean; abc Means in the same variate with different superscripts differ ($p < 0.05$); F pr means Fisher's protected least significant different test at 5 % level of significance (if F pr < 0.05 then p-value <0.05 correct, if F pr > 0.05 then "comparison of mean" not used); Feeding = healthy animals fed for 433 days with the different supplements, n = number of steers used; Data not adjusted for starting weight as a covariate

**Appendix 11** Carcass information from the slaughter facility after the steers were fed for 433 days in four different treatments with different phosphorous sources

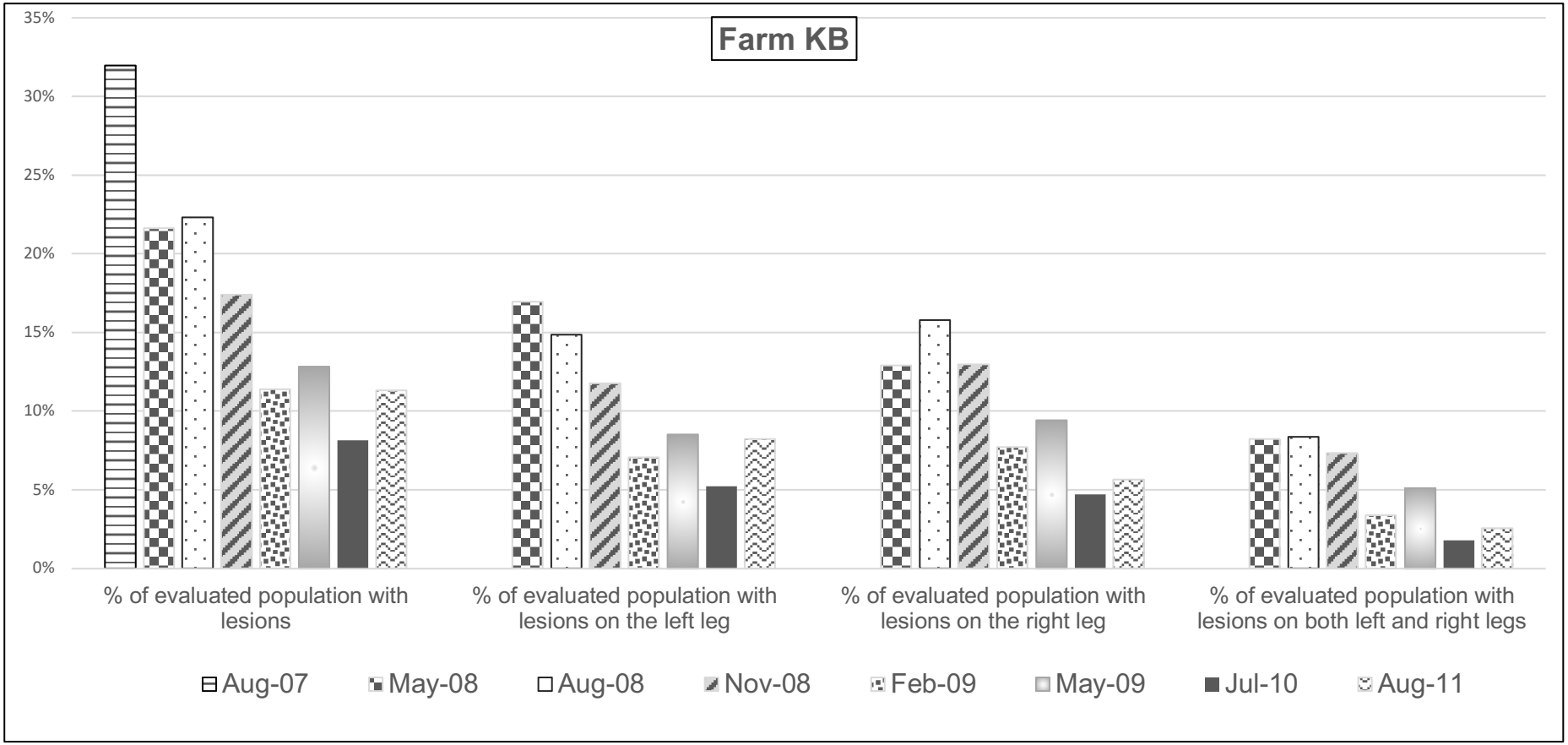
| Paramaters | Minimum | Mean | Maximum | Animal numbers |
|---|---|---|---|---|
| Empty Body weight 24 hours, kg | 346 | 437 | 514 | 32 |
| Carcass cold slaughter weight, kg | 186 | 231 | 268 | 32 |
| Slaughter (dressing) % | 50.8 | 52.9 | 55.1 | 32 |
| Age of steers, days | 795 | 837 | 893 | 32 |

**Appendix 12.1** A summary of the data from the six commercial cattle farms in the Vryburg / North West Province which supplemented cattle with the Arthrocure products and a highly bio-available P source

| Farm | Date evaluated | All Animals evaluated | | | | | Female animals evalutated | | | | | | Bull animals evalutated | | | | | | Oxen animals evalutated | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total animals evaluated | Number of evaluated animals with lesions | Total animals : Lesions on left leg | Total animals : Lesions on right leg | Total animals : with lesions on both left and right legs | Number of females evaluated | Females with lesions | Females : Total Lesions | Females with lesion on left leg | Females with lesion on right leg | Females with lesion on both legs | Number of Bulls evaluated | Bulls : with lesions | Bulls : Total lesions | Bulls with lesion on left leg | Bulls with lesion on right leg | Bulls with lesion on both legs | Number of oxen evaluated | Oxen with lesions | Oxen : Total lesions | Oxen with lesion on left leg | Oxen with lesion on right leg | Oxen with lesion on both legs |
| KB | Aug-07 | 1088 | 348 | | | | | | | | | | | | | | | | | | | | | |
| KB | May-08 | 1373 | 297 | 233 | 177 | 113 | 679 | 152 | 220 | 115 | 105 | 68 | 344 | 67 | 88 | 62 | 26 | 21 | 350 | 78 | 102 | 56 | 46 | 24 |
| KB | Aug-08 | 874 | 195 | 130 | 138 | 73 | 713 | 182 | 250 | 121 | 129 | 68 | 118 | 10 | 15 | 8 | 7 | 5 | 43 | 3 | 3 | 1 | 2 | 0 |
| KB | Nov-08 | 1581 | 275 | 186 | 205 | 116 | 830 | 149 | 214 | 103 | 111 | 65 | 682 | 102 | 138 | 65 | 73 | 36 | 69 | 24 | 39 | 18 | 21 | 15 |
| KB | Feb-09 | 1064 | 121 | 75 | 82 | 36 | 867 | 108 | 141 | 68 | 73 | 33 | 170 | 2 | 2 | 1 | 1 | 0 | 27 | 11 | 14 | 6 | 8 | 3 |
| KB | May-09 | 997 | 128 | 85 | 94 | 51 | 796 | 109 | 154 | 73 | 81 | 45 | 171 | 4 | 5 | 3 | 2 | 1 | 30 | 15 | 20 | 9 | 11 | 5 |
| KB | Jul-10 | 1507 | 123 | 79 | 71 | 27 | 873 | 76 | 92 | 48 | 44 | 16 | 609 | 44 | 54 | 30 | 24 | 10 | 25 | 3 | 4 | 1 | 3 | 1 |
| KB | Aug-11 | 548 | 62 | 45 | 31 | 14 | 548 | 62 | 76 | 45 | 31 | 14 | | | | | | | | | 0 | | | |
| KO | Aug-08 | 843 | 139 | 105 | 75 | 41 | 645 | 119 | 156 | 90 | 66 | 37 | 173 | 20 | 24 | 15 | 9 | 4 | 25 | 0 | 0 | 0 | 0 | 0 |
| KO | Dec-08 | 888 | 70 | 44 | 44 | 18 | 685 | 64 | 81 | 41 | 40 | 17 | 188 | 6 | 7 | 3 | 4 | 1 | 15 | 0 | 0 | 0 | 0 | 0 |
| KO | Jan-09 | 931 | 43 | 35 | 18 | 10 | 686 | 41 | 51 | 33 | 18 | 10 | 229 | 2 | 2 | 2 | 0 | 0 | 16 | 0 | 0 | 0 | 0 | 0 |
| KO | Nov-09 | 1631 | 122 | | | | | | | | | | | | | | | | | | | | | |
| KO | Jun-10 | 1581 | 64 | | | | | | | | | | | | | | | | | | | | | |
| KO | Sep-10 | 1017 | 33 | | | | | | | | | | | | | | | | | | | | | |
| KO | Aug-11 | 827 | 17 | | | | | | | | | | | | | | | | | | | | | |
| FM | Oct-08 | 229 | 43 | 29 | 29 | 15 | 227 | 43 | 58 | 29 | 29 | 15 | 2 | 0 | 0 | 0 | 0 | 0 | | | | | | |
| FM | Jan-09 | 168 | 13 | 9 | 6 | 2 | 168 | 13 | 15 | 9 | 6 | 2 | | | | | | | | | | | | |
| FM | Jun-09 | 168 | 14 | 6 | 10 | 2 | 168 | 14 | 16 | 6 | 10 | 2 | | | | | | | | | | | | |
| FM | Nov-09 | 172 | 6 | | | | | | | | | | | | | | | | | | | | | |
| FM | Jun-10 | 150 | 10 | | | | | | | | | | | | | | | | | | | | | |
| JC | Aug-08 | 400 | 82 | 51 | 52 | 21 | 371 | 75 | 95 | 44 | 51 | 20 | 5 | 1 | 1 | 1 | 0 | 0 | 24 | 6 | 7 | 6 | 1 | 1 |
| JC | Feb-09 | 478 | 22 | 17 | 9 | 4 | 424 | 16 | 19 | 12 | 7 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 51 | 6 | 7 | 5 | 2 | 1 |
| JC | Sep-09 | 400 | 7 | 4 | 3 | 0 | 371 | 7 | 7 | 4 | 3 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 |
| JC | May-10 | 468 | 3 | | | | | | | | | | | | | | | | | | | | | |
| TC | Aug-08 | 487 | 101 | 75 | 56 | 30 | 476 | 99 | 129 | 73 | 56 | 30 | 11 | 2 | 2 | 2 | 0 | 0 | | | | | | |
| TC | Mar-09 | 660 | 84 | 64 | 53 | 33 | 660 | 84 | 117 | 64 | 53 | 33 | | | | | | | | | | | | |
| TC | Jun-09 | 660 | 48 | 27 | 31 | 10 | 660 | 48 | 58 | 27 | 31 | 10 | | | | | | | | | | | | |
| TC | Nov-09 | 429 | 33 | 24 | 17 | 8 | 429 | 33 | 41 | 24 | 17 | 8 | | | | | | | | | | | | |
| TC | May-10 | 375 | 25 | | | | | | | | | | | | | | | | | | | | | |
| TC | Sep-10 | 361 | 26 | | | | | | | | | | | | | | | | | | | | | |
| TC | Aug-11 | 210 | 6 | | | | | | | | | | | | | | | | | | | | | |
| CB | Aug-08 | 283 | 42 | 33 | 17 | 8 | 205 | 32 | 39 | 25 | 14 | 7 | 78 | 10 | 11 | 8 | 3 | 1 | | | | | | |
| CB | Nov-08 | 385 | 48 | 33 | 37 | 22 | 284 | 40 | 58 | 26 | 32 | 18 | 101 | 8 | 12 | 7 | 5 | 4 | | | | | | |
| CB | Feb-09 | 198 | 27 | 16 | 19 | 8 | 174 | 25 | 33 | 15 | 18 | 8 | 24 | 2 | 2 | 1 | 1 | 0 | | | | | | |
| CB | Sep-09 | 360 | 23 | | | | | | | | | | | | | | | | | | | | | |
| CB | May-10 | 503 | 34 | | | | | | | | | | | | | | | | | | | | | |
| | **Total** | **24294** | **2734** | **1405** | **1274** | **662** | **11939** | **1591** | **2120** | **1095** | **1025** | **529** | **2913** | **280** | **363** | **208** | **155** | **83** | **699** | **146** | **196** | **102** | **94** | **50** |

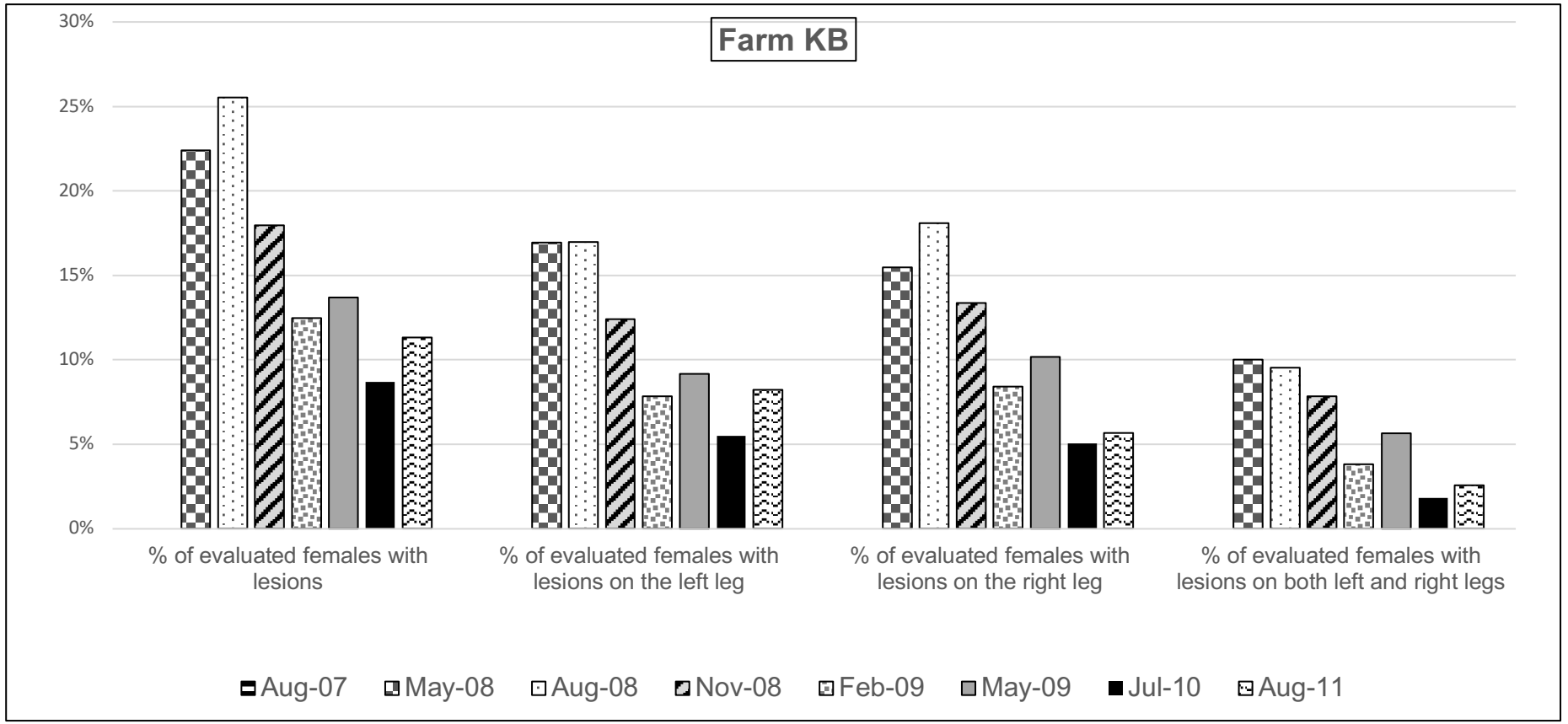
OPOLS data evaluated on 6 different commercial farms from 2008 to 2011. The trial was done commercially and not in a randomly design. Animals from three different ecological groups, cows, bulls and oxen. Animals on the farms received different pastures in different camps and managed within the specific limitations of the farm. Arthrocure A mineral pack was provided from November 2008, Arthrocure B from March 2009 and Arthocure C from November 2010. All farms did not change the Arthrocure products at the same date, but within reasonable time. These Arthrocure products were fed with a highly bio-available P source (MCP) within a supplement formulated for the Winter, Summer, Maintenance and Production supplements. Animals were from different breeds, age and production groups. Animals were not kept in the same groups over the 3-year period and did change on some farms. Animals were also culled from the groups according to the normal practices on these farms. Animals were evaluated on some farms from a specific date onwards only as osteochondrosis affected or healthy and not as per gender or per leg

**Appendix 12.2** The prevalence of osteochondrosis per leg in the cattle fed with the Arthrocure minerals on a commercial farm (KB) in the North West Province



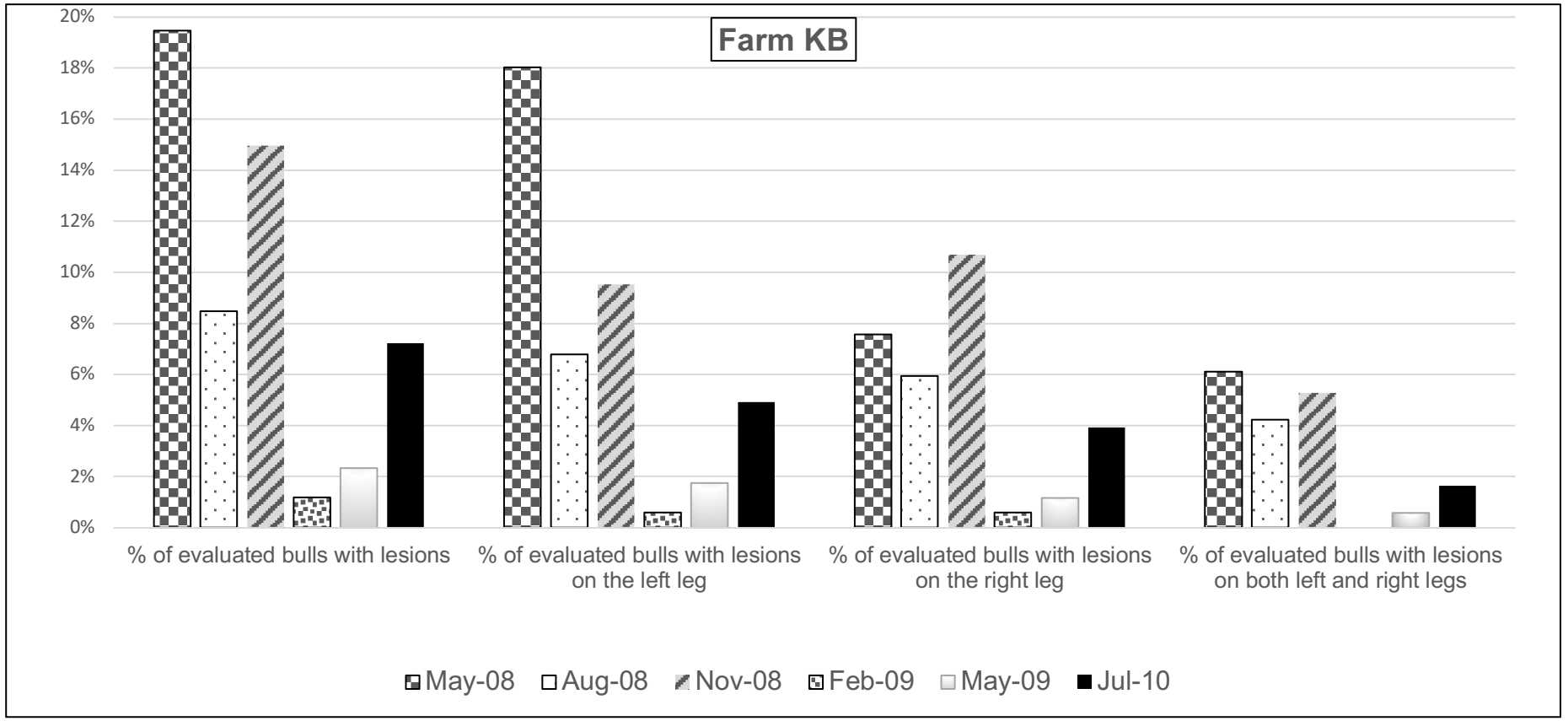

Farm KB was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.3** The prevalence of osteochondrosis per leg in female cattle fed with the Arthrocure minerals on a commercial farm (KB) in the North West Province



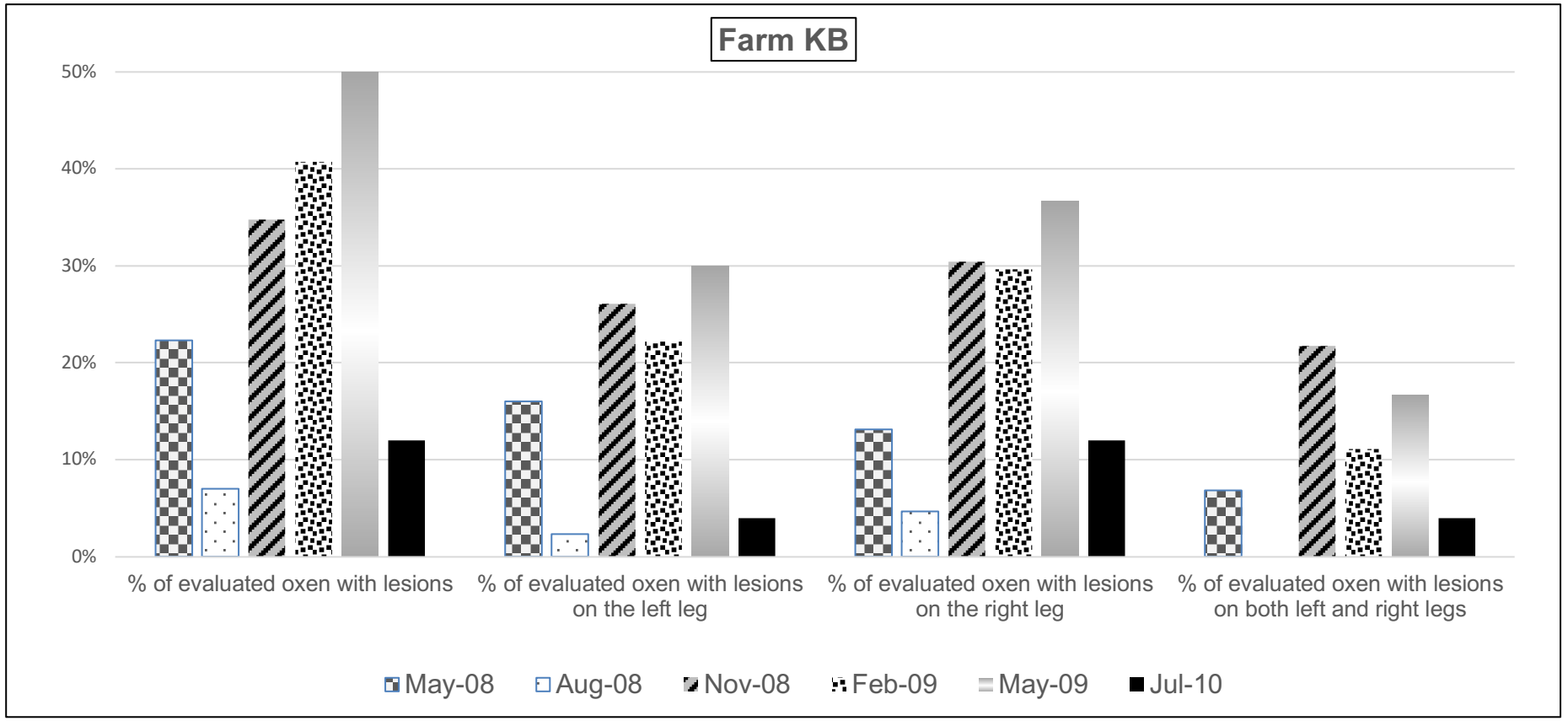

Farm KB was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.4** The prevalence of osteochondrosis per leg in bulls fed with the Arthrocure minerals on a commercial farm (KB) in the North West Province



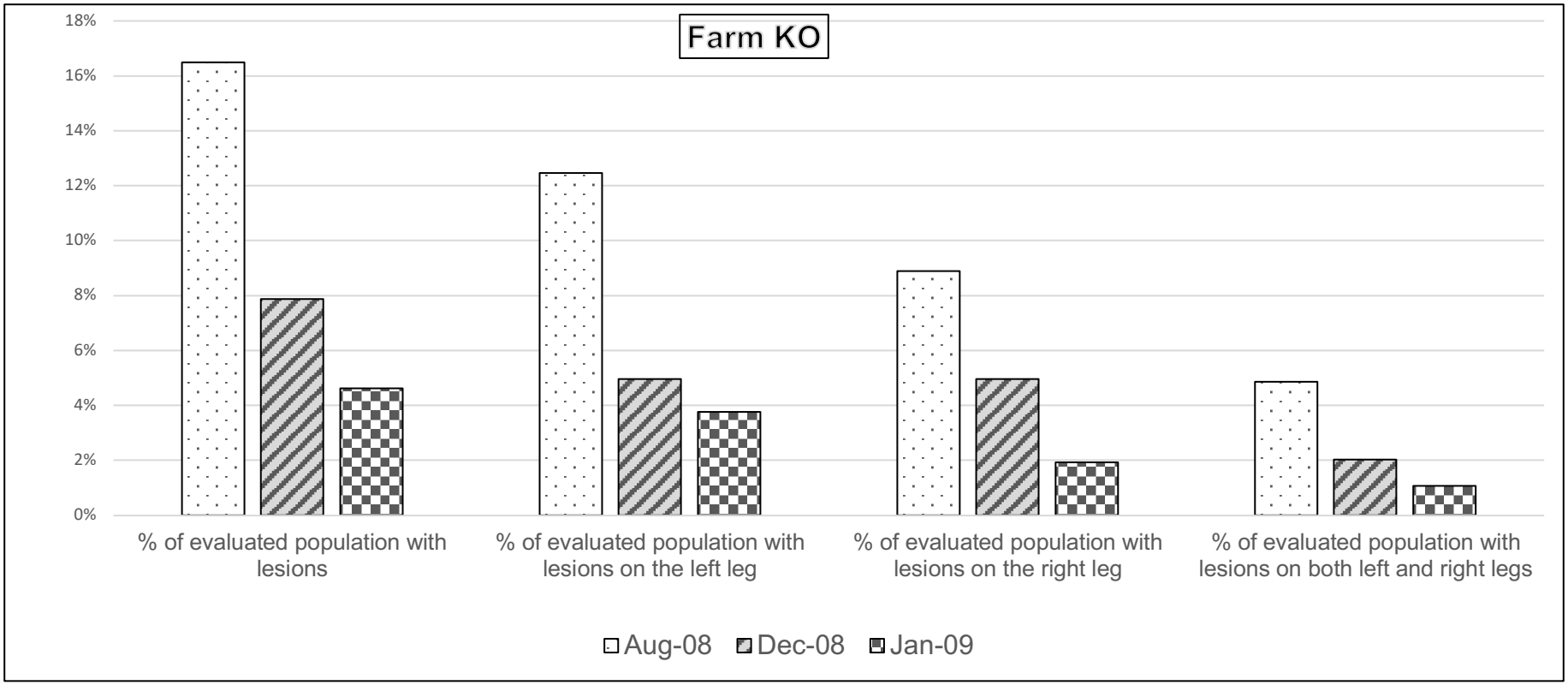

Farm KB was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.5** The prevalence of osteochondrosis per leg in oxen fed with the Arthrocure minerals on a commercial farm (KB) in the North West Province



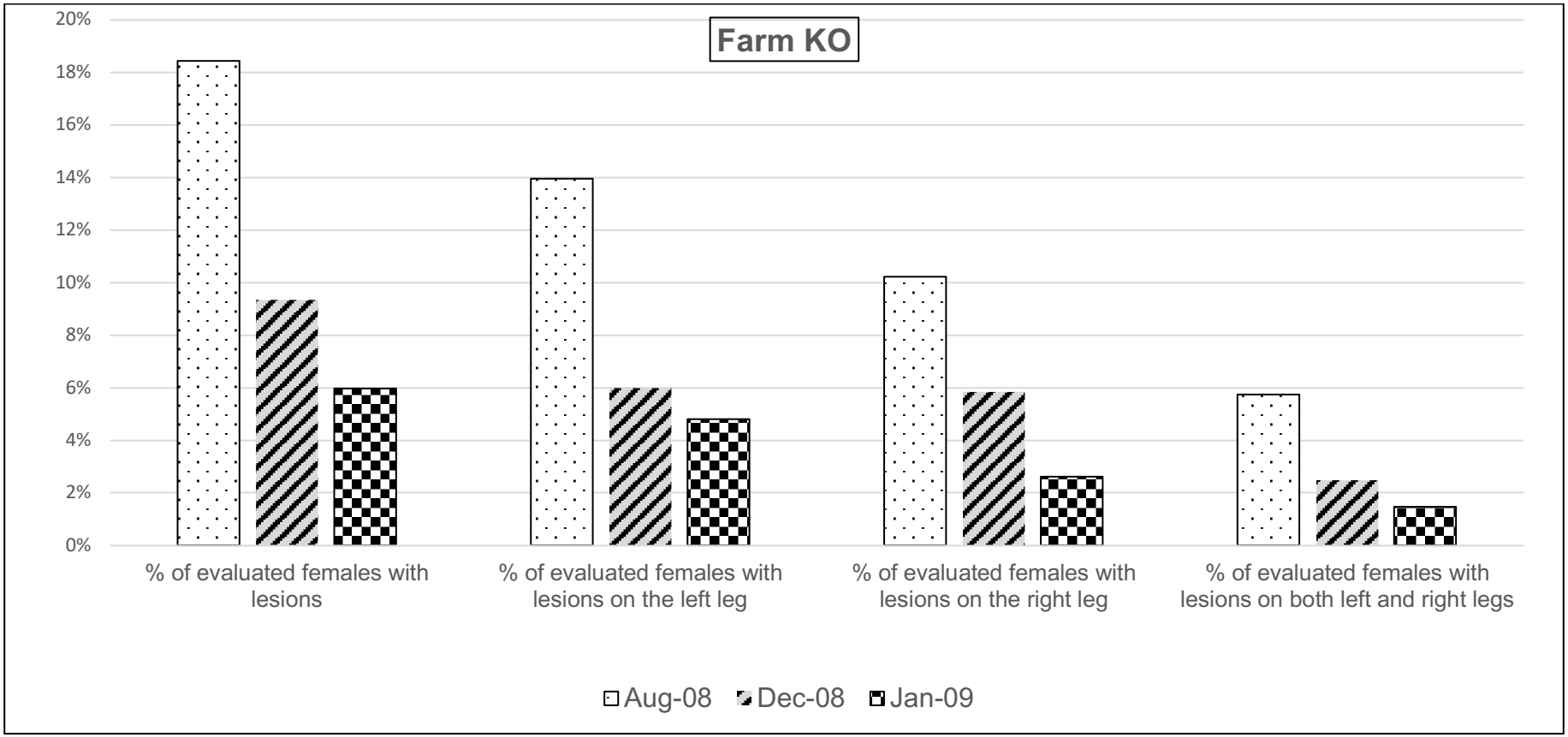

Farm KB was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.6** The prevalence of osteochondrosis per leg in the cattle fed with the Arthrocure minerals on a commercial farm (KO) in the North West Province



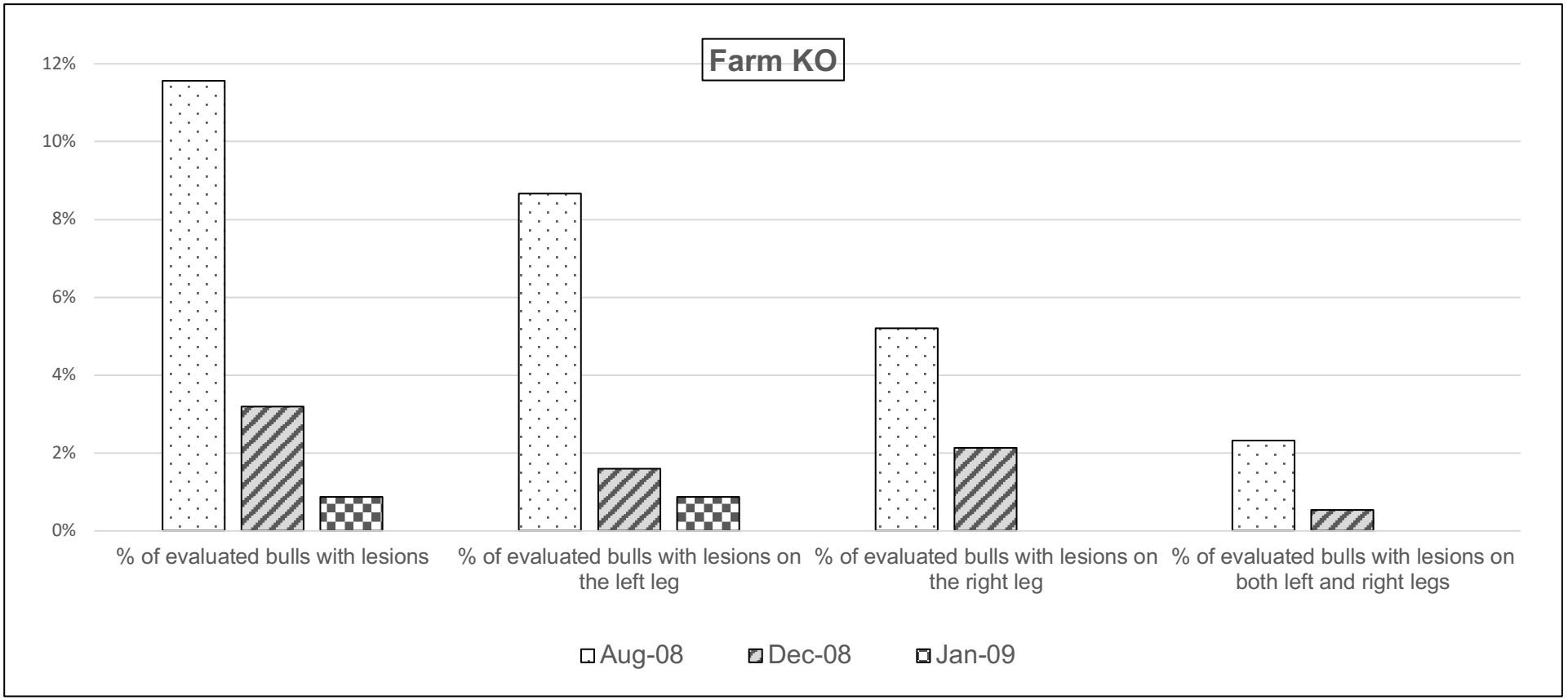

Farm KO was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.7** The prevalence of osteochondrosis per leg in female cattle fed with the Arthrocure minerals on a commercial farm (KO) in the North West Province



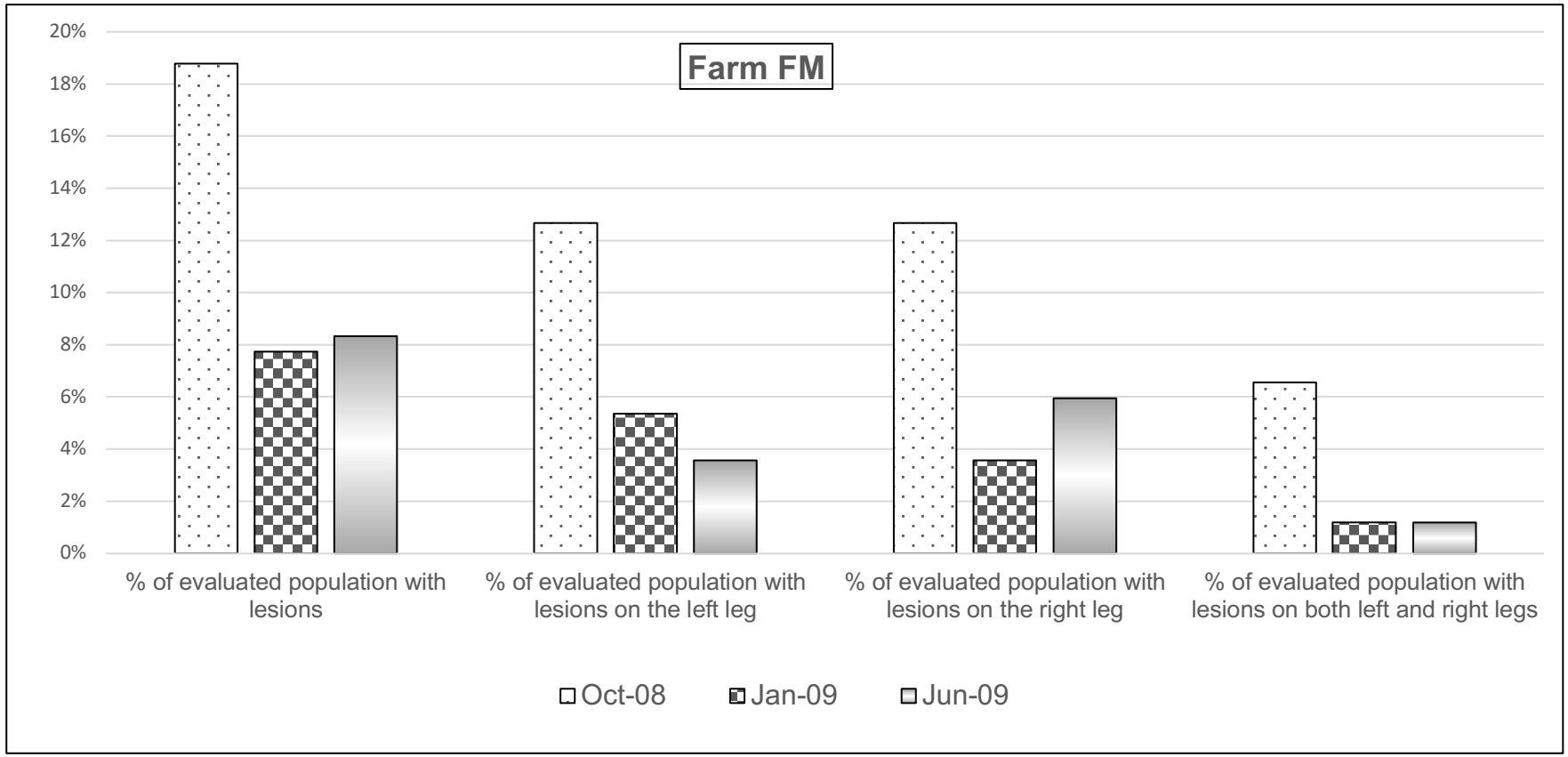

Farm KO was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.8** The prevalence of osteochondrosis per leg in bulls fed with the Arthrocure minerals on a commercial farm (KO) in the North West Province



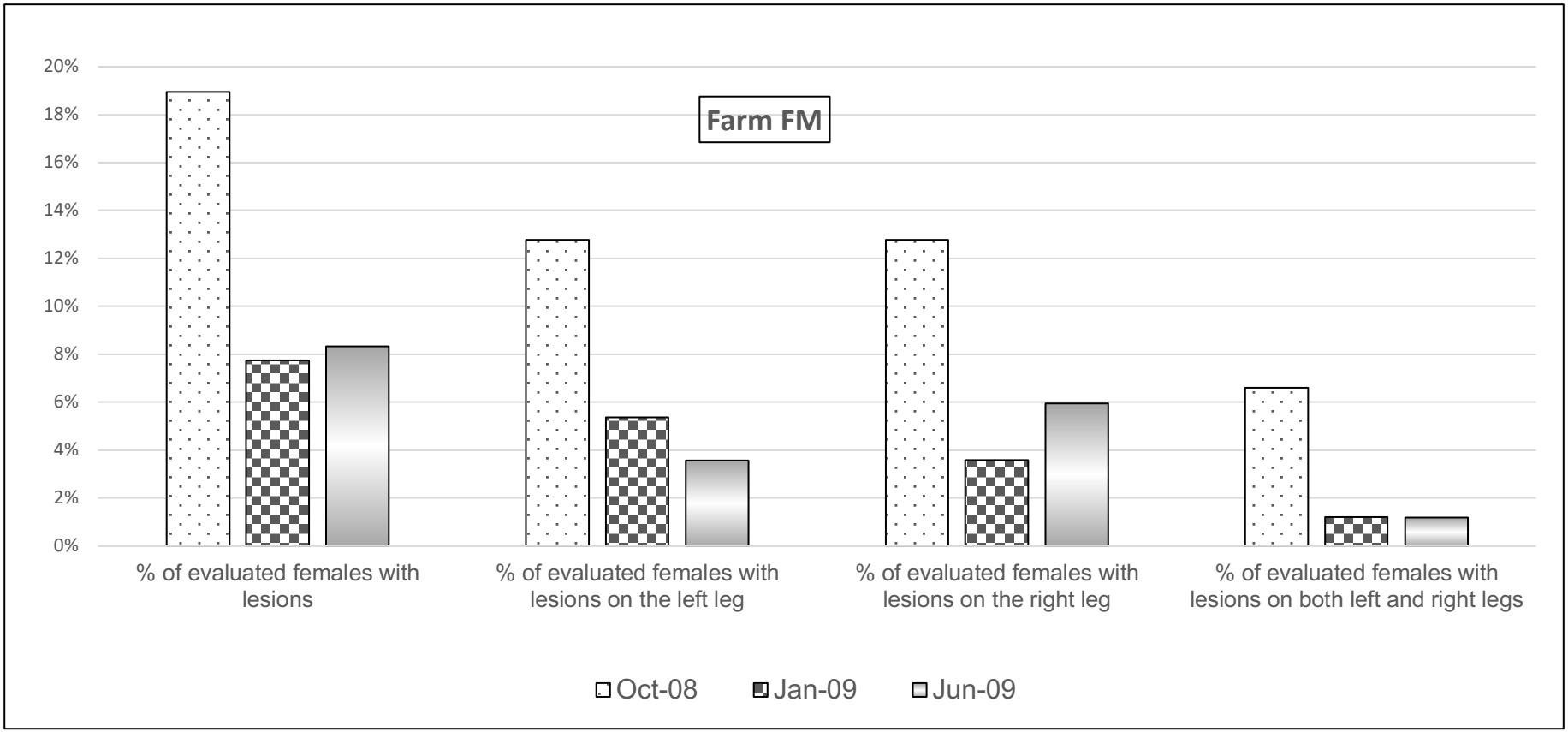

Farm KO was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.9** The prevalence of osteochondrosis per leg in cattle fed with the Arthrocure minerals on a commercial farm (FM) in the North West Province



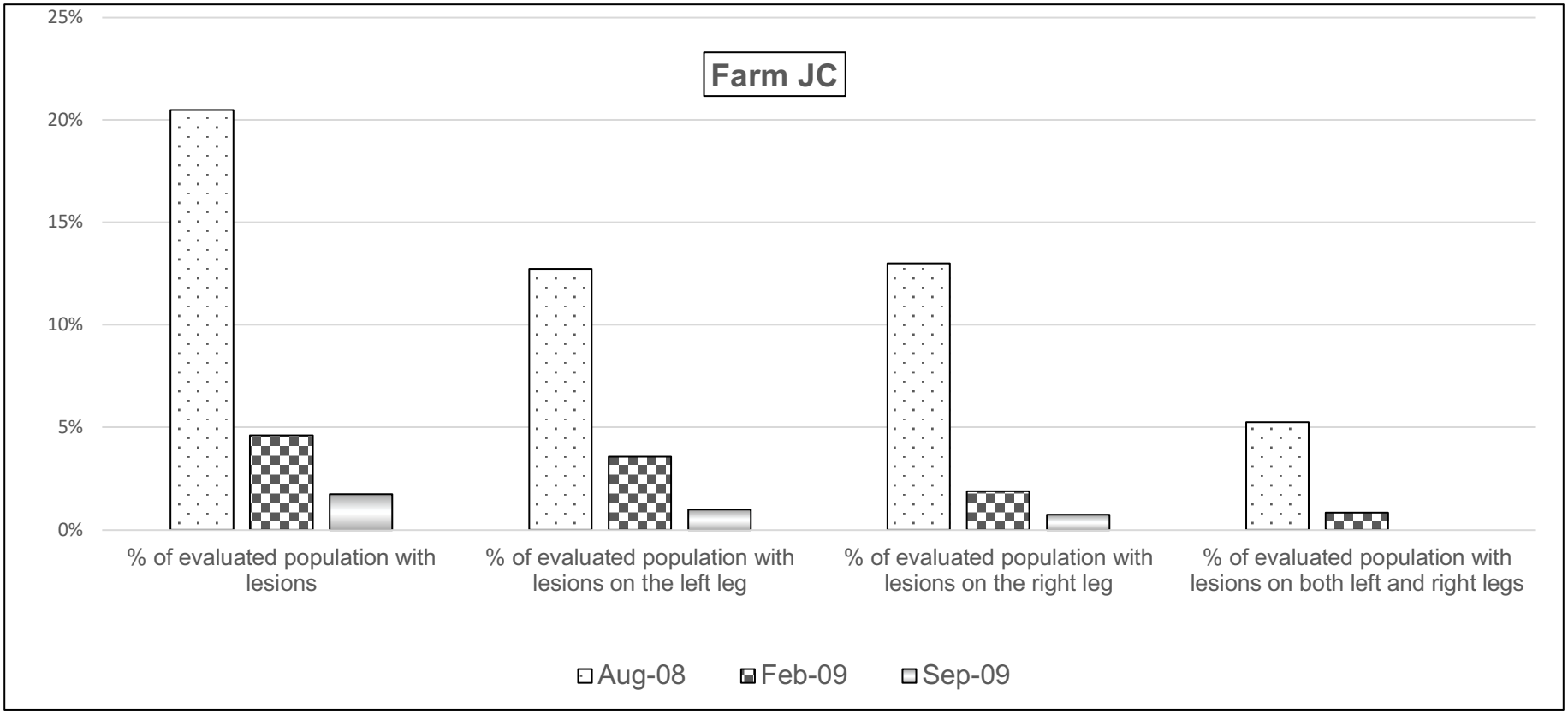

Farm FM was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.10** The prevalence of osteochondrosis per leg in female cattle fed with the Arthrocure minerals on a commercial farm (FM) in the North West Province



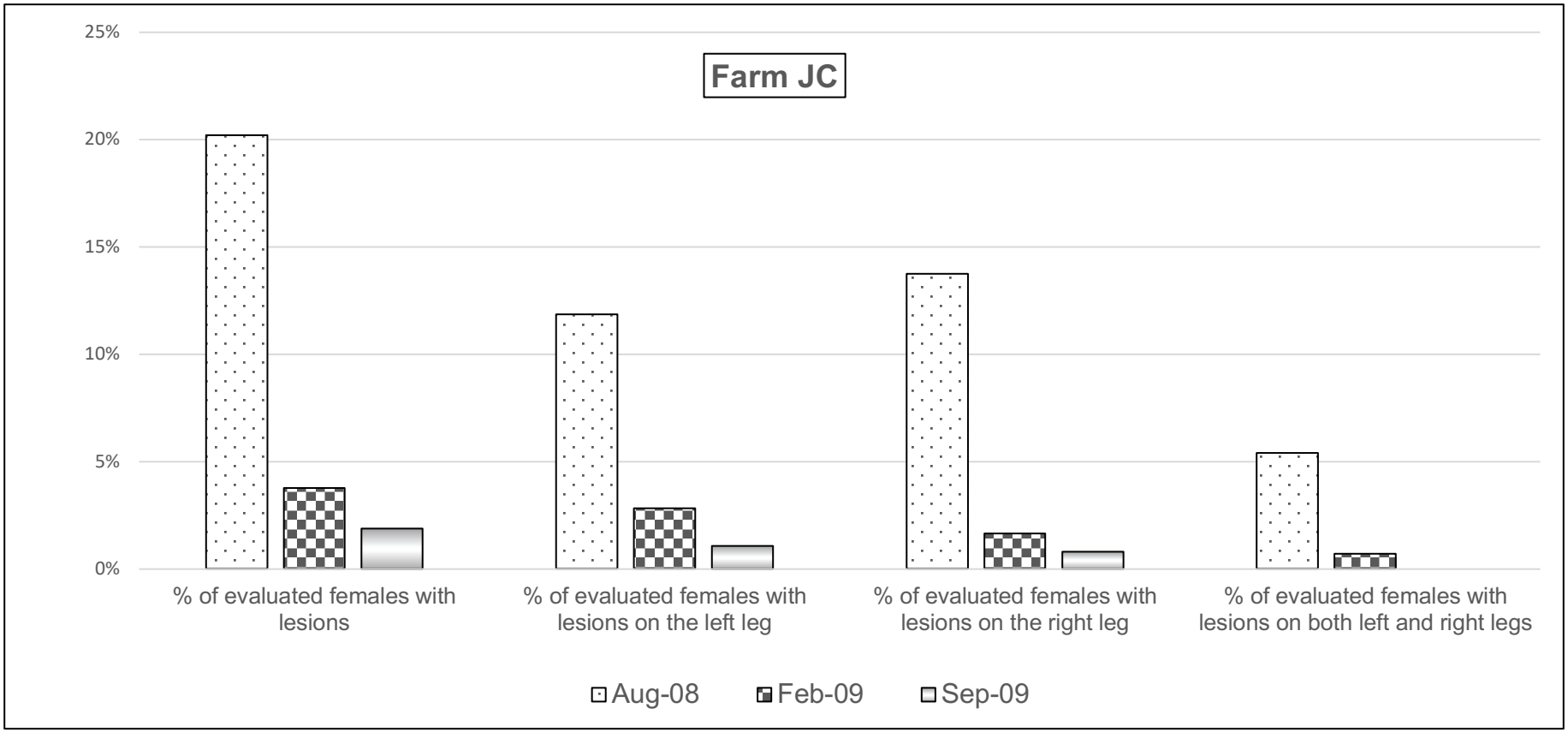

Farm FM was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.11** The prevalence of osteochondrosis per leg in cattle fed with the Arthrocure minerals on a commercial farm (JC) in the North West Province



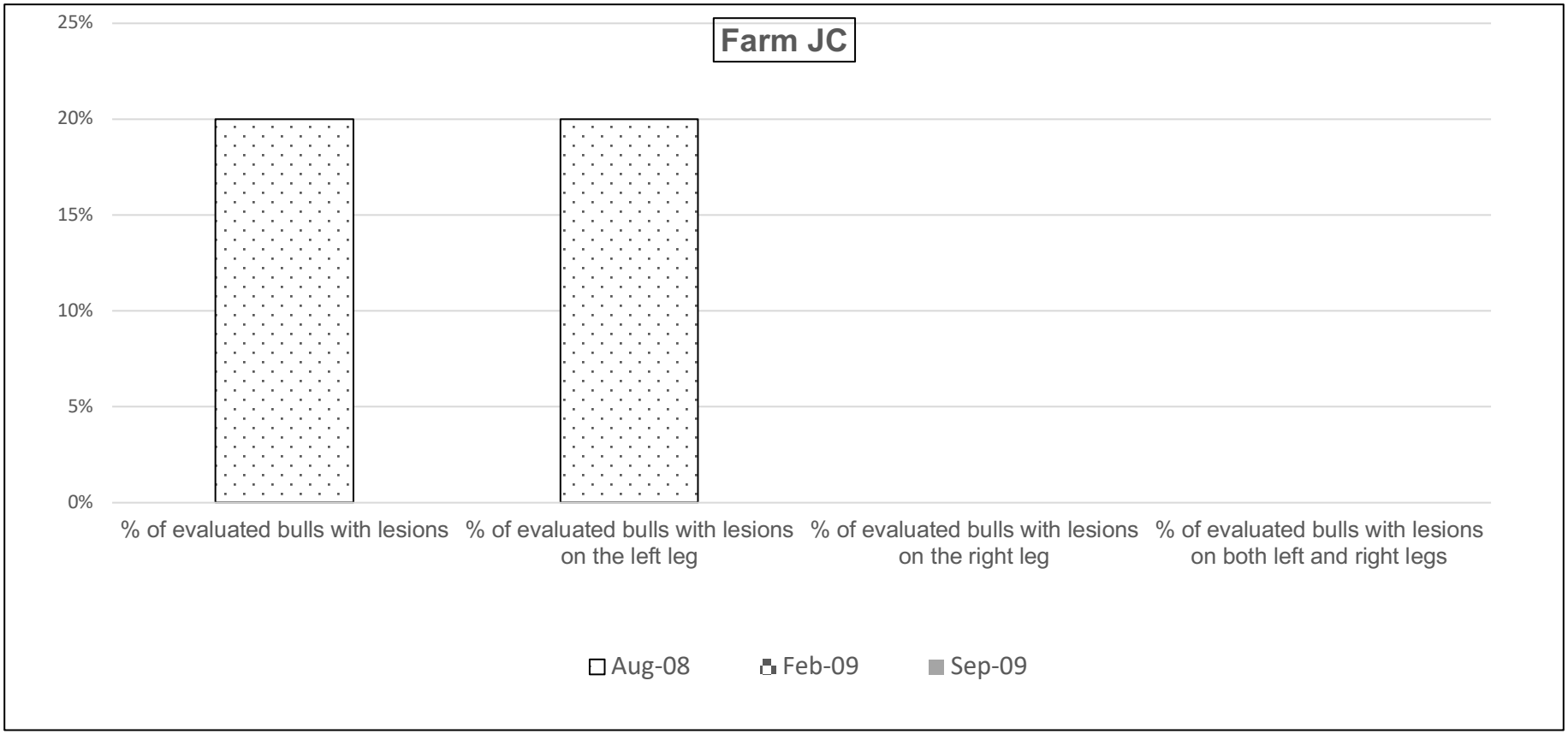

Farm JC was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.12** The prevalence of osteochondrosis per leg in female cattle fed with the Arthrocure minerals on a commercial farm (JC) in the North West Province



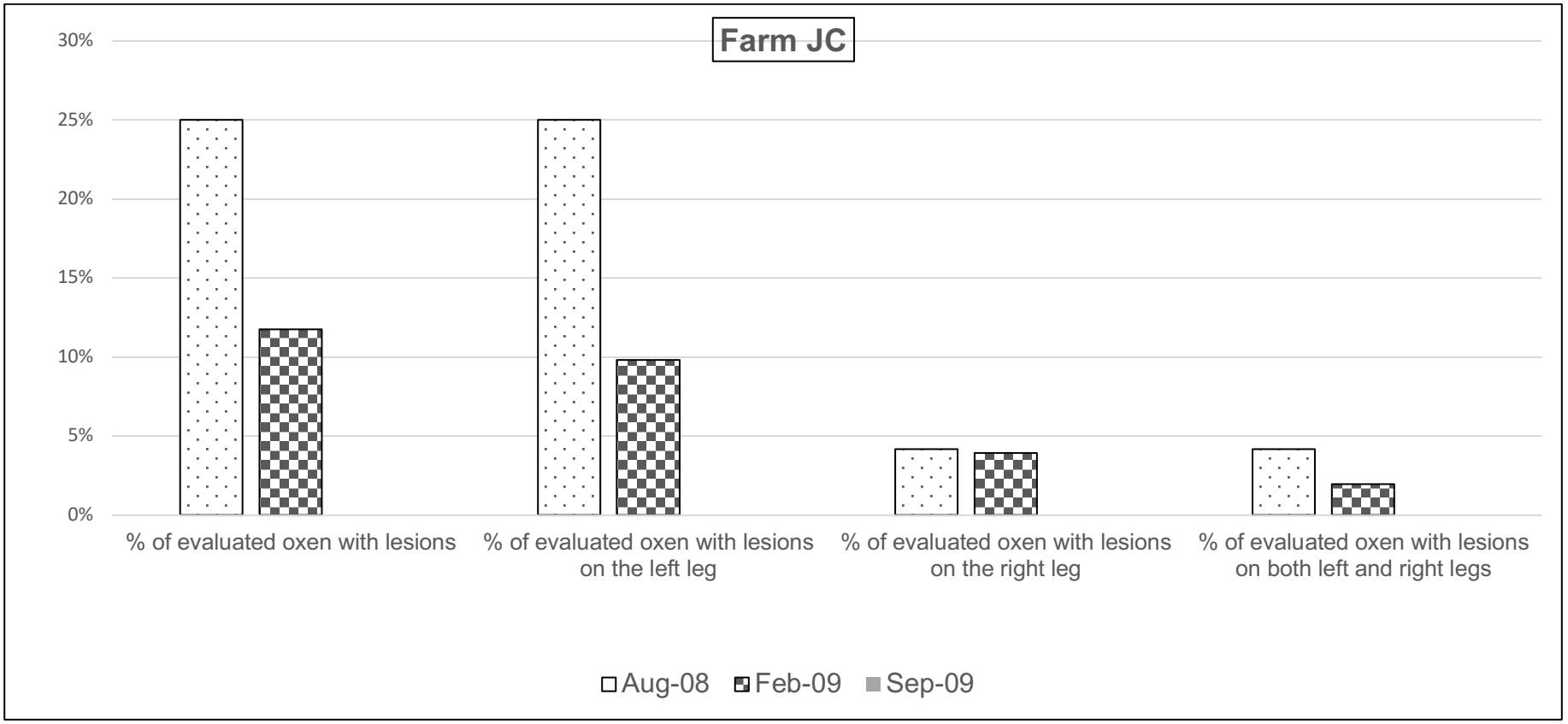

Farm JC was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.13** The prevalence of osteochondrosis per leg in bulls fed with the Arthrocure minerals on a commercial farm (JC) in the North West Province



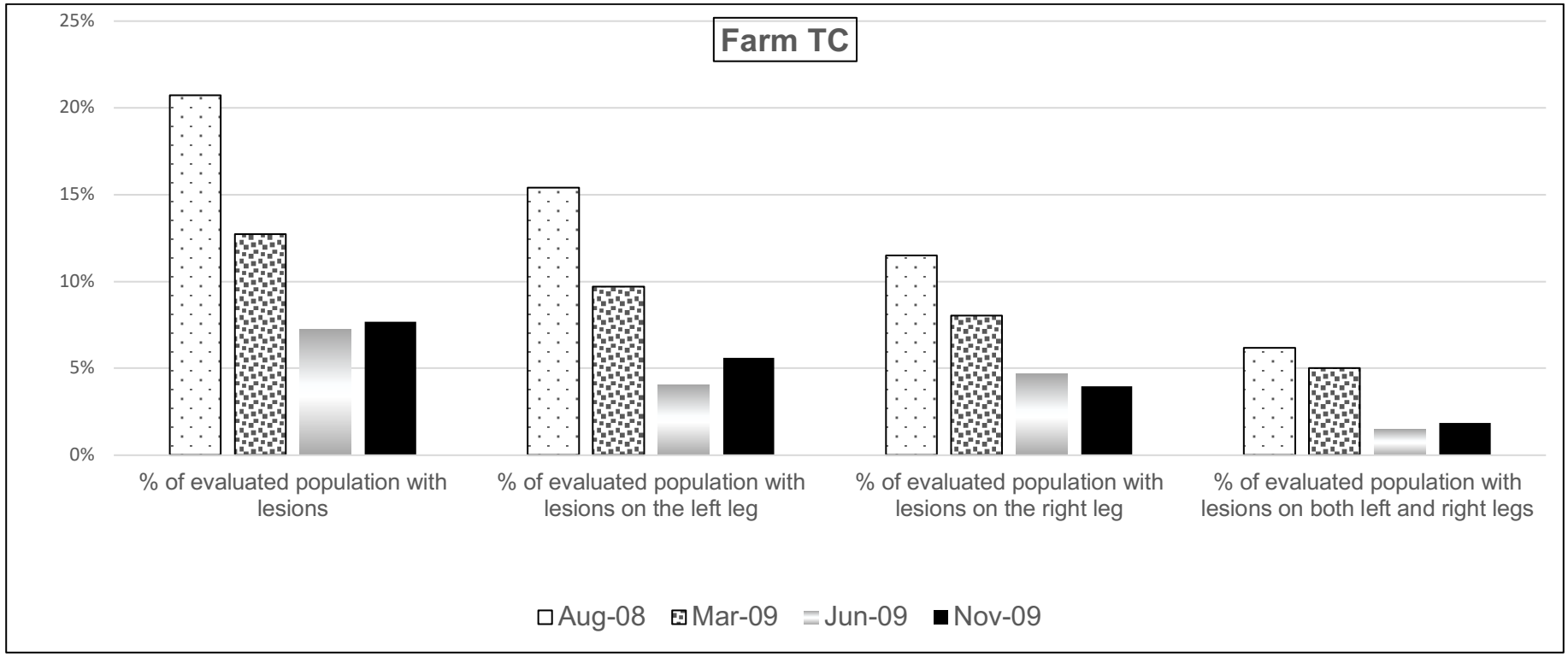

Farm JC was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.14** The prevalence of osteochondrosis per leg in oxen fed with the Arthrocure minerals on a commercial farm (JC) in the North West Province



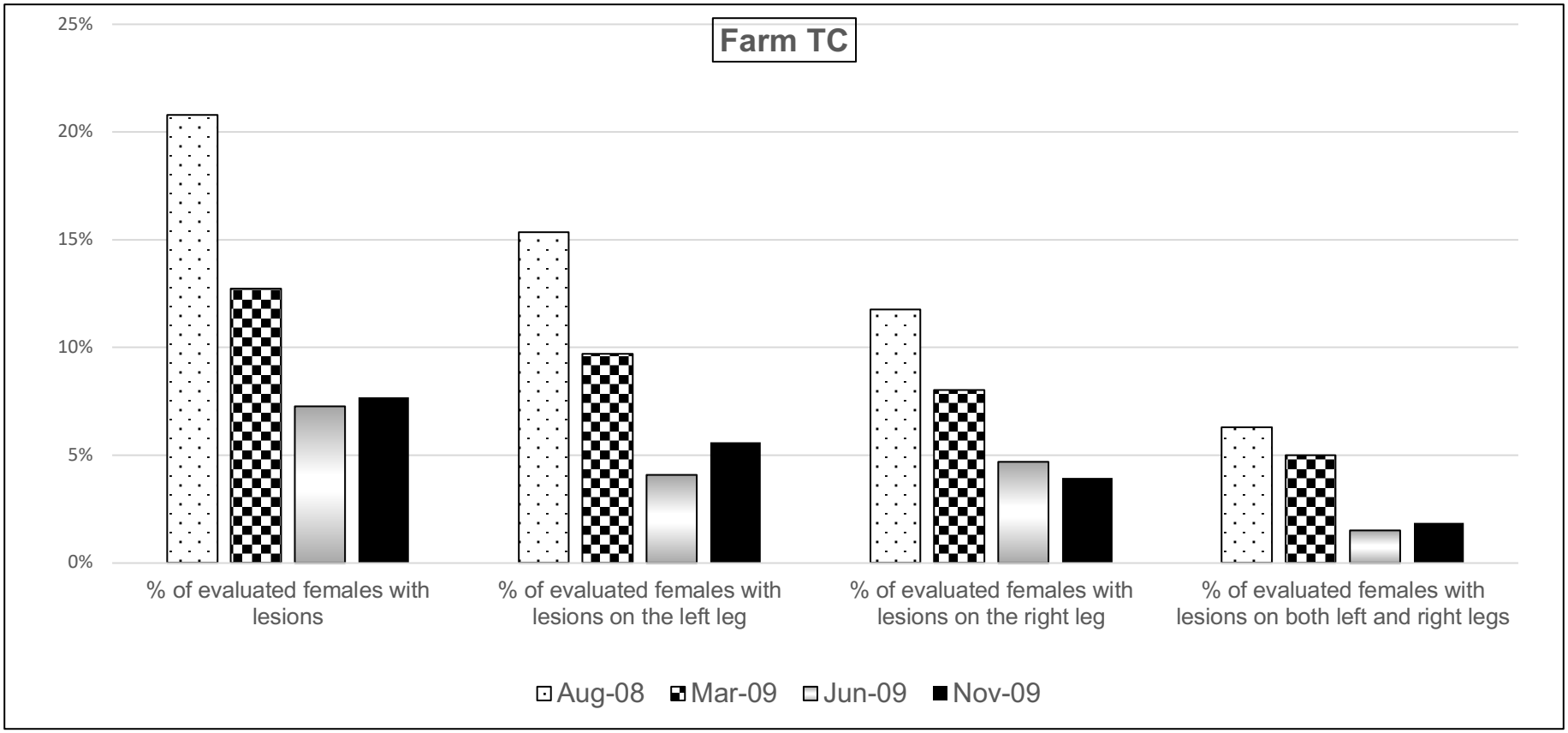

Farm JC was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.15** The prevalence of osteochondrosis per leg in cattle fed with the Arthrocure minerals on a commercial farm (TC) in the North West Province



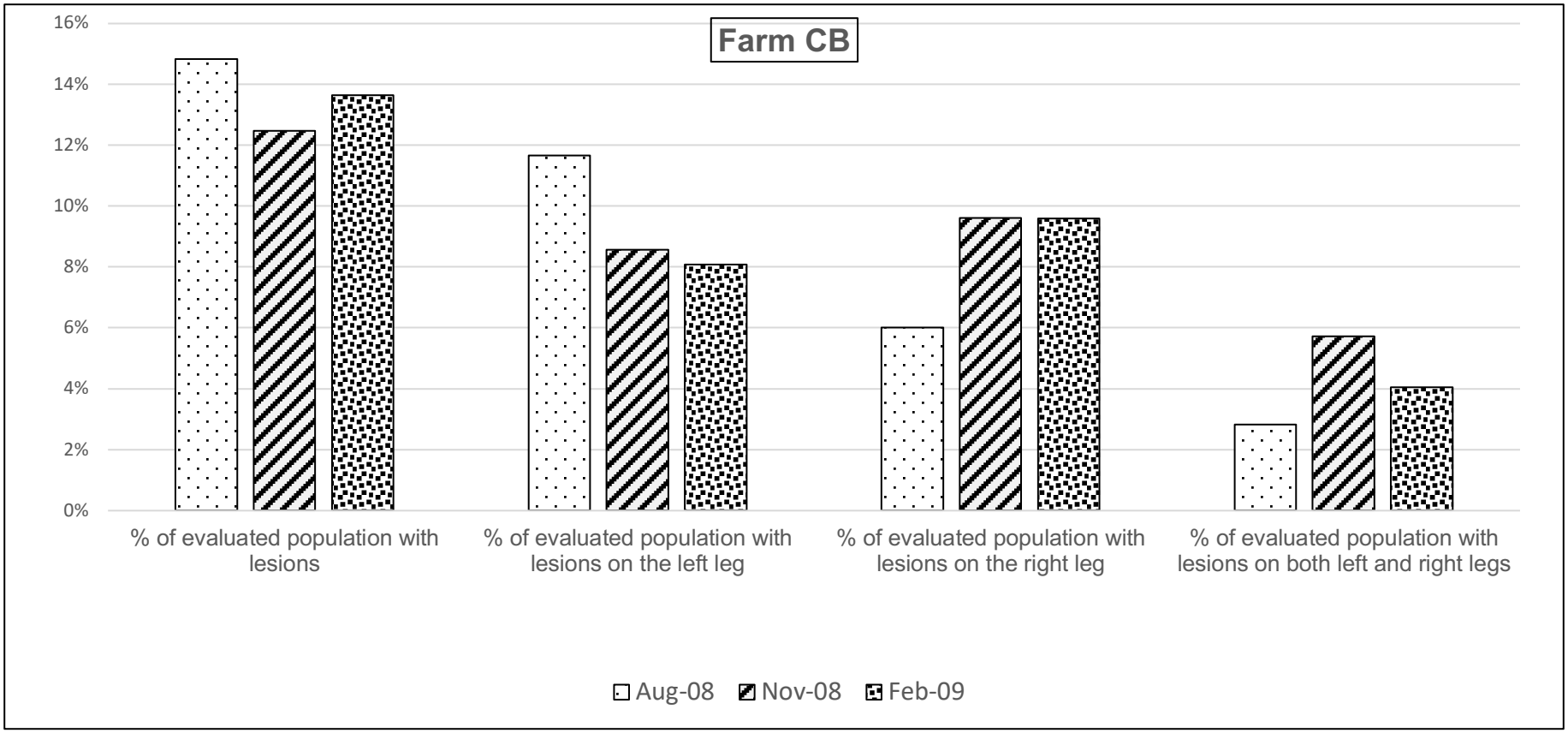

Farm TC was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.16** The prevalence of osteochondrosis per leg in female cattle fed with the Arthrocure minerals on a commercial farm (TC) in the North West Province



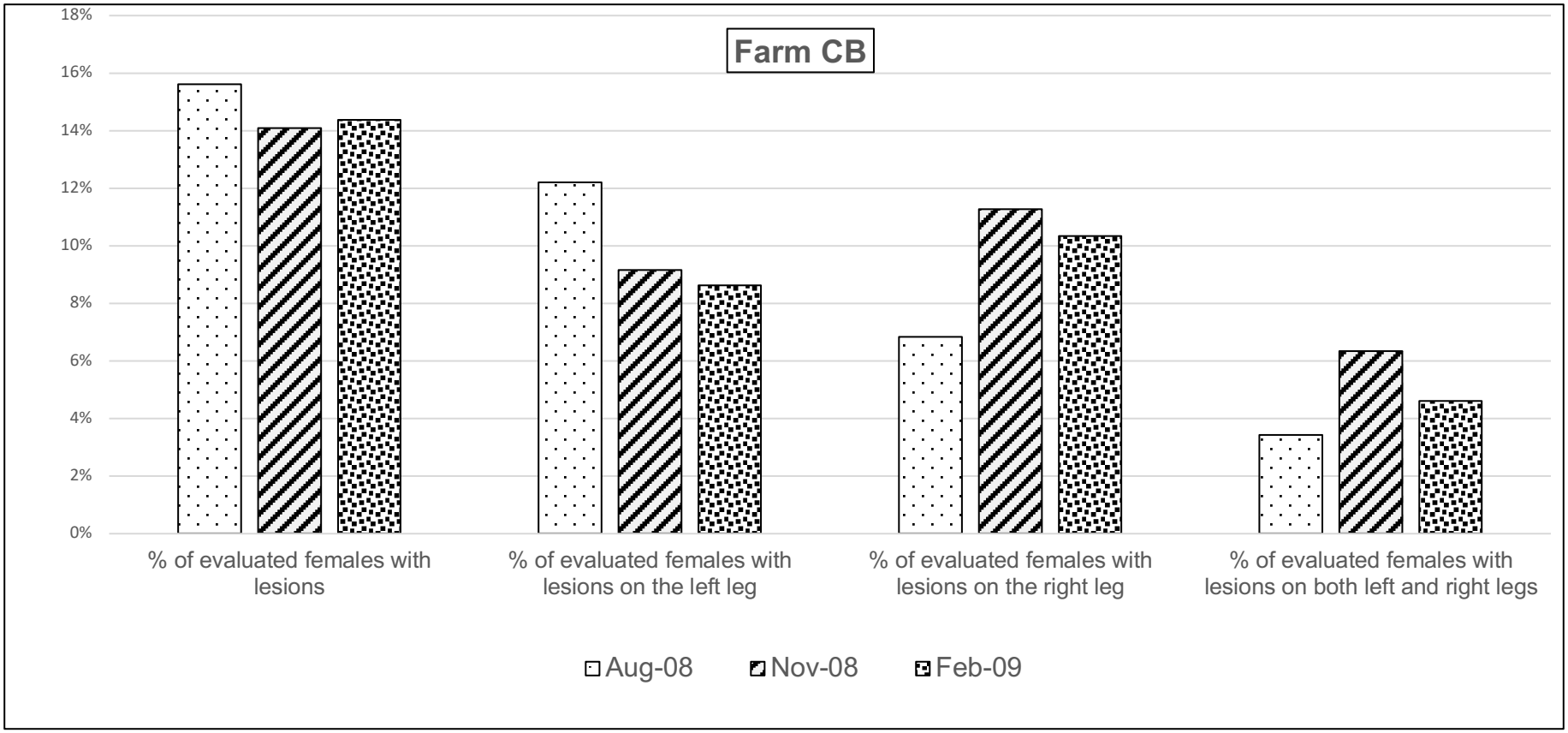

Farm TC was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.17** The prevalence of osteochondrosis per leg in cattle fed with the Arthrocure minerals on a commercial farm (CB) in the North West Province



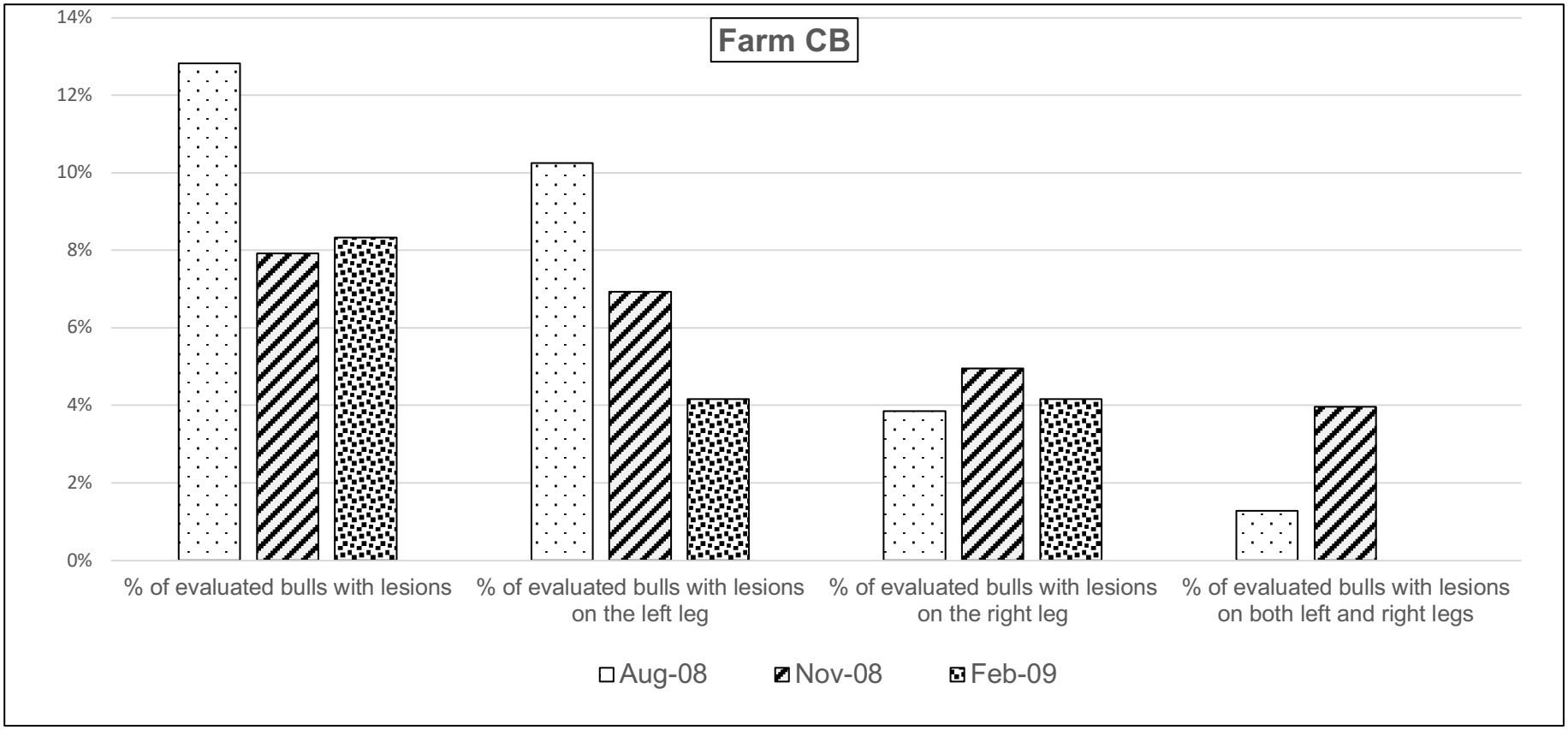

Farm CB was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.18** The prevalence of osteochondrosis per leg in female cattle fed with the Arthrocure minerals on a commercial farm (CB) in the North West Province




Farm CB was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 12.19** The prevalence of osteochondrosis per leg in bulls fed with the Arthrocure minerals on a commercial farm (CB) in the North West Province




Farm CB was well known for a high incidence of osteochondrosis. Animals evaluated with the OPOLS system by trained professionals. Arthrocure products were fed from November 2008. A high bio-available P source was also included in the supplement. Arthrocure A was changed to Arthrocure B since March 2009 and then to Arthrocure C from November 2010. Animals changed camps and some animals did leave the herd due to standard culling practices

**Appendix 13** A summary of the logarithmic and poynomial trend line equations and correlations for the prevalence of osteochondroses per leg on six commercial cattle farms in the Vryburg area (North West Province) when supplemented with the Arthrocure products and a highly bio-available P source (total population and females)

| Farm evaluated | | % of evaluated population with lesions on the left leg | | % of evaluated population with lesions on the right leg | | % of evaluated population with lesions on both left and right legs | | % of evaluated females with lesions on the left leg | | % of evaluated females with lesions on the right leg | | % of evaluated females with lesions on both left and right legs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Logarithmic trend line | Polynomial trend line | Logarithmic trend line | Polynomial trend line | Logarithmic trend line | Polynomial trend line | Logarithmic trend line | Polynomial trend line | Logarithmic trend line | Polynomial trend line | Logarithmic trend line | Polynomial trend line |
| Farm KB | Equation | $y = -2{,}797\ln(x) + 29{,}744$ | $y = 2E\text{-}07x^2 - 0{,}0176x + 355{,}55$ | $y = -3{,}232\ln(x) + 34{,}349$ | $y = 1E\text{-}07x^2 - 0{,}009x + 181{,}9$ | $y = -2{,}156\ln(x) + 22{,}902$ | $y = 9E\text{-}08x^2 - 0{,}007x + 142{,}65$ | $y = -3{,}111\ln(x) + 33{,}077$ | $y = 2E\text{-}07x^2 - 0{,}0171x + 344{,}4$ | $y = -3{,}938\ln(x) + 41{,}833$ | $y = 1E\text{-}07x^2 - 0{,}0117x + 236{,}8$ | $y = -2{,}637\ln(x) + 28{,}005$ | $y = 1E\text{-}07x^2 - 0{,}0089x + 180{,}85$ |
| | Correlation | $R^2 = 0{,}48$ | $R^2 = 0{,}93$ | $R^2 = 0{,}69$ | $R^2 = 0{,}81$ | $R^2 = 0{,}69$ | $R^2 = 0{,}86$ | $R^2 = 0{,}53$ | $R^2 = 0{,}91$ | $R^2 = 0{,}71$ | $R^2 = 0{,}86$ | $R^2 = 0{,}71$ | $R^2 = 0{,}91$ |
| Farm KO | Equation | $y = -23{,}11\ln(x) + 244{,}77$ | $y = 1E\text{-}06x^2 - 0{,}1196x + 2387{,}5$ | $y = -16{,}6\ln(x) + 175{,}84$ | $y = -4E\text{-}06x^2 + 0{,}338x - 6706{,}7$ | $y = -9{,}67\ln(x) + 102{,}44$ | $y = -5E\text{-}07x^2 + 0{,}0387x - 763{,}01$ | $y = -24{,}37\ln(x) + 258{,}15$ | $y = 2E\text{-}06x^2 - 0{,}143x + 2852{,}9$ | $y = -18{,}22\ln(x) + 192{,}98$ | $y = -4E\text{-}06x^2 + 0{,}3513x - 6969$ | $y = -10{,}97\ln(x) + 116{,}17$ | $y = -4E\text{-}07x^2 + 0{,}0327x - 644{,}77$ |
| | Correlation | $R^2 = 1.00$ | $R^2 = 1{,}00$ | $R^2 = 0{,}94$ | $R^2 = 1.00$ | $R^2 = 1.00$ | $R^2 = 1.00$ | $R^2 = 0{,}99$ | $R^2 = 1.00$ | $R^2 = 0{,}94$ | $R^2 = 1.00$ | $R^2 = 1.00$ | $R^2 = 1.00$ |
| Farm FM | Equation | $y = -13{,}91\ln(x) + 147{,}41$ | $y = 3E\text{-}06x^2 - 0{,}222x + 4430{,}8$ | $y = -9{,}31\ln(x) + 98{,}689$ | $y = 5E\text{-}06x^2 - 0{,}3761x + 7497{,}6$ | $y = -7{,}927\ln(x) + 83{,}997$ | $y = 2E\text{-}06x^2 - 0{,}1913x + 3814{,}8$ | $y = -14{,}07\ln(x) + 149{,}16$ | $y = 3E\text{-}06x^2 - 0{,}226x + 4510{,}2$ | $y = -9{,}475\ln(x) + 100{,}44$ | $y = 5E\text{-}06x^2 - 0{,}3801x + 7577$ | $y = -8{,}012\ln(x) + 84{,}902$ | $y = 2E\text{-}06x^2 - 0{,}1933x + 3855{,}8$ |
| | Correlation | $R^2 = 0{,}79$ | $R^2 = 1.00$ | $R^2 = 0{,}37$ | $R^2 = 1.00$ | $R^2 = 0{,}62$ | $R^2 = 1.00$ | $R^2 = 0{,}79$ | $R^2 = 1.00$ | $R^2 = 0{,}37$ | $R^2 = 1.00$ | $R^2 = 0{,}62$ | $R^2 = 1.00$ |
| Farm JC | Equation | $y = -11{,}66\ln(x) + 123{,}54$ | $y = 1E\text{-}06x^2 - 0{,}0766x + 1532{,}6$ | $y = -12{,}08\ln(x) + 128{,}04$ | $y = 1E\text{-}06x^2 - 0{,}1112x + 2222$ | $y = -5{,}194\ln(x) + 55{,}044$ | $y = 5E\text{-}07x^2 - 0{,}0405x + 809{,}19$ | $y = -10{,}67\ln(x) + 113{,}07$ | $y = 1E\text{-}06x^2 - 0{,}0824x + 1648{,}2$ | $y = -12{,}75\ln(x) + 135{,}06$ | $y = 2E\text{-}06x^2 - 0{,}1247x + 2491$ | $y = -5{,}327\ln(x) + 56{,}447$ | $y = 6E\text{-}07x^2 - 0{,}0447x + 892{,}68$ |
| | Correlation | $R^2 = 0{,}88$ | $R^2 = 1.00$ | $R^2 = 0{,}79$ | $R^2 = 1.00$ | $R^2 = 0{,}84$ | $R^2 = 1.00$ | $R^2 = 0{,}84$ | $R^2 = 1.00$ | $R^2 = 0{,}77$ | $R^2 = 1.00$ | $R^2 = 0{,}82$ | $R^2 = 1.00$ |
| Farm TC | Equation | $y = -9{,}491\ln(x) + 100{,}64$ | $y = 6E\text{-}07x^2 - 0{,}0442x + 885{,}85$ | $y = -6{,}978\ln(x) + 73{,}993$ | $y = 2E\text{-}07x^2 - 0{,}0124x + 251{,}25$ | $y = -4{,}24\ln(x) + 44{,}951$ | $y = 6E\text{-}08x^2 - 0{,}005x + 102{,}18$ | $y = -9{,}434\ln(x) + 100{,}04$ | $y = 5E\text{-}07x^2 - 0{,}0437x + 876{,}54$ | $y = -7{,}213\ln(x) + 76{,}49$ | $y = 2E\text{-}07x^2 - 0{,}0143x + 289{,}73$ | $y = -4{,}366\ln(x) + 46{,}289$ | $y = 7E\text{-}08x^2 - 0{,}006x + 122{,}8$ |
| | Correlation | $R^2 = 0{,}81$ | $R^2 = 0{,}90$ | $R^2 = 0{,}94$ | $R^2 = 0{,}95$ | $R^2 = 0{,}78$ | $R^2 = 0{,}79$ | $R^2 = 0{,}81$ | $R^2 = 0{,}90$ | $R^2 = 0{,}94$ | $R^2 = 0{,}96$ | $R^2 = 0{,}79$ | $R^2 = 0{,}80$ |
| Farm CB | Equation | $y = -7{,}737\ln(x) + 82{,}029$ | $y = 2E\text{-}06x^2 - 0{,}1222x + 2433{,}8$ | $y = 7{,}7568\ln(x) - 82{,}063$ | $y = -2E\text{-}06x^2 + 0{,}1701x - 3385$ | $y = 2{,}6257\ln(x) - 27{,}765$ | $y = -3E\text{-}06x^2 + 0{,}2143x - 4260{,}7$ | $y = -7{,}725\ln(x) + 81{,}907$ | $y = 1E\text{-}06x^2 - 0{,}1179x + 2347{,}2$ | $y = 7{,}5998\ln(x) - 80{,}39$ | $y = -3E\text{-}06x^2 + 0{,}252x - 5012{,}3$ | $y = 6E\text{-}05x - 2{,}5082$ | $y = -3E\text{-}06x^2 + 0{,}2191x - 4356{,}3$ |
| | Correlation | $R^2 = 0{,}85$ | $R^2 = 1.00$ | $R^2 = 0{,}75$ | $R^2 = 1.00$ | $R^2 = 0{,}18$ | $R^2 = 1.00$ | $R^2 = 0{,}86$ | $R^2 = 1.00$ | $R^2 = 0{,}56$ | $R^2 = 1.00$ | $R^2 = 0{,}16$ | $R^2 = 1.00$ |

**Appendix 14** A summary of the logarithmic and poynomial trend line equations and correlations for the prevalence of osteochondroses per leg on six commercial cattle farms in the Vryburg area (North West Province) when supplemented with the Arthrocure products and a highly bio-available P source (bulls and oxen)

| Farm evaluated | | % of evaluated bulls with lesions on the left leg | | % of evaluated bulls with lesions on the right leg | | % of evaluated bulls with lesions on both left and right legs | | % of evaluated oxen with lesions on the left leg | | % of evaluated oxen with lesions on the right leg | | % of evaluated oxen with lesions on both left and right legs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Logarithmic trend line | Polynomial trend line | Logarithmic trend line | Polynomial trend line | Logarithmic trend line | Polynomial trend line | Logarithmic trend line | Polynomial trend line | Logarithmic trend line | Polynomial trend line | Logarithmic trend line | Polynomial trend line |
| Farm KB | Equation | $y = -4{,}891\ln(x) + 51{,}881$ | $y = 7E\text{-}07x^2 - 0{,}0545x + 1091{,}4$ | $y = -2{,}346\ln(x) + 24{,}896$ | $y = 3E\text{-}07x^2 - 0{,}0204x + 408{,}36$ | $y = -2{,}262\ln(x) + 23{,}995$ | $y = 2E\text{-}07x^2 - 0{,}0182x + 364{,}24$ | $y = -3{,}424\ln(x) + 36{,}44$ | $y = -1E\text{-}06x^2 + 0{,}0976x - 1948{,}9$ | $y = 1{,}1337\ln(x) - 11{,}799$ | $y = -2E\text{-}06x^2 + 0{,}126x - 2519{,}1$ | $y = -0{,}869\ln(x) + 9{,}301$ | $y = -8E\text{-}07x^2 + 0{,}0622x - 1242{,}2$ |
| | Correlation | $R^2 = 0{,}29$ | $R^2 = 0{,}82$ | $R^2 = 0{,}18$ | $R^2 = 0{,}38$ | $R^2 = 0{,}38$ | $R^2 = 0{,}74$ | $R^2 = 0{,}04$ | $R^2 = 0{,}57$ | $R^2 = 0{,}004$ | $R^2 = 0{,}71$ | $R^2 = 0{,}01$ | $R^2 = 0{,}43$ |
| Farm KO | Equation | $y = -21{,}04\ln(x) + 222{,}85$ | $y = -21{,}04\ln(x) + 222{,}85$ | $y = -12{,}52\ln(x) + 132{,}62$ | $y = -3E\text{-}06x^2 + 0{,}2253x - 4468{,}9$ | $y = -5{,}946\ln(x) + 62{,}98$ | $y = -2E\text{-}07x^2 + 0{,}0132x - 258{,}87$ | # | | | | | |
| | Correlation | $R^2 = 0{,}99$ | $R^2 = 0{,}99$ | $R^2 = 0{,}95$ | $R^2 = 1.00$ | $R^2 = 1.00$ | $R^2 = 1.00$ | | | | | | |
| Farm FM | Equation | ## | | | | | | ### | | | | | |
| | Correlation | | | | | | | | | | | | |
| Farm JC | Equation | $y = -19{,}64\ln(x) + 208{,}14$ | $y = 3E\text{-}06x^2 - 0{,}2193x + 4381$ | ## | | | | $y = -25\ln(x) + 264{,}95$ | $y = 9E\text{-}07x^2 - 0{,}0738x + 1483{,}3$ | $y = -4{,}271\ln(x) + 45{,}274$ | $y = -4E\text{-}07x^2 + 0{,}0345x - 684{,}46$ | $y = -4{,}182\ln(x) + 44{,}318$ | $y = 7E\text{-}08x^2 - 0{,}0056x + 114{,}12$ |
| | Correlation | $R^2 = 0{,}72$ | $R^2 = 1.00$ | | | | | $R^2 = 0{,}97$ | $R^2 = 1.00$ | $R^2 = 0{,}83$ | $R^2 = 1.00$ | $R^2 = 1.00$ | $R^2 = 1.00$ |
| Farm TC | Equation | ## | | | | | | ### | | | | | |
| | Correlation | | | | | | | | | | | | |
| Farm CB | Equation | $y = -13{,}16\ln(x) + 139{,}41$ | $y = 3E\text{-}07x^2 - 0{,}0267x + 537{,}59$ | $y = 0{,}694\ln(x) - 7{,}3069$ | $y = -1E\text{-}06x^2 + 0{,}0887x - 1763{,}3$ | $y = -2{,}764\ln(x) + 29{,}293$ | $y = -4E\text{-}06x^2 + 0{,}3117x - 6194{,}7$ | ### | | | | | |
| | Correlation | $R^2 = 1.00$ | $R^2 = 1.00$ | $R^2 = 0{,}08$ | $R^2 = 1$ | $R^2 = 0{,}10$ | $R^2 = 1{,}00$ | | | | | | |

# 56 oxen were evaluated with the OPOLS system, but no positives found within the period. ## Bulls were evaluated with the OPOLS system, but no positives were found over the period. ## no oxen were evaluated on this farm

**Appendix 15** Regulations as published in Table 9.11 (b) in Act 36 (1947) for the registration of supplement products for catlle. These levels are minimums on an *"as fed"* basis (88 % dry matter basis)

| SUPPLEMENT-VEEVOEDSEL SUPPLEMENT FARM FEED | MINERAAL SUPPLEMENT MINERAL SUPPLEMENT | PROTEIEN SUPPLEMENT PROTEIN SUPPLEMENT | ENERGIE SUPPLEMENT ENERGY SUPPLEMENT | SPOORMINERAAL SUPPLEMENT TRACE MINERAL SUPPLEMENT |
|---|---|---|---|---|
| **MINERALE / MINERAL**<br>Inname / dag g (min)<br>Intake / day g (min)<br>**Ca**<br>**P**<br>**Mg**<br>**K**<br>**S** | <br><br><br>7.5 – 120<br>6 – 40<br>2.5 – 32<br>14 –112<br>3 - 24 | | | |
| **RU -PROTEIEN/CRUDE PROTEIN:**<br>Inname / dag g (min)<br>Intake / day g (min) | | 150 | | |
| **METABOLISEERBARE ENERGIE (BERAAM): METABOLIZABLE ENERGY (ESTIMATED)**<br>Inname / dag MJ (min)<br>Intake / day MJ (min) | | | 8 | |
| **SPOORMINERALE /TRACEMINERALS:**<br>Inname per dag (mg)<br>Intake per day (mg)<br>**Fe**<br>**Co**<br>**Cu**<br>**Mn**<br>**Zn**<br>**I**<br>**Se**<br>**Mo** | | | | <br><br><br>100 – 800<br>0,2 –1,6<br>20– 160<br>40 – 640<br>60 – 480<br>1 – 8<br>0,2 – 1,6<br>0 - 20 |

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