

**INVESTIGATING THE HIGH INCIDENCE OF BONE DISORDERS IN A
BROILER FARM**

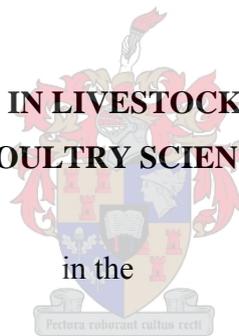
A case study

by

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Assignment presented in partial fulfillment
of the requirements for the Degree

**MASTER OF PHILOSOPHY IN LIVESTOCK INDUSTRY MANAGEMENT
(POULTRY SCIENCE)**



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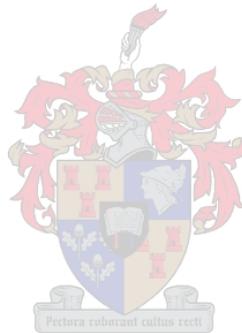
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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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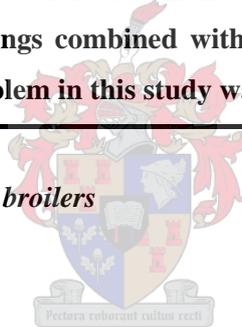
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Abstract

Rickets is described as a disease that affects young growing poultry. Poorly mineralized bones with thickened and irregular growth plates characterize it. The onset of rickets is characterized by a failure of mineralization of cartilage and bone. Other symptoms of rickets include reluctance to movement in affected birds. These birds will sit on their hocks and if startled they use their wings for balance. On necropsy, bones are soft and fragile and they have thickened growth plates. In this study 30% of the chicks aged between 7 and 8 days from a broiler flock, started showing splay leg problems. Affected chicks were unable to support their weight on their legs, some showing paralysis. The bones were soft and rubbery. To try and identify the possible cause, bones from the affected chicks were analyzed for calcium (Ca) and phosphorus (P) to determine the Ca:P ratio. Blood serum was also analyzed for the mineral content. Ca and P were the main focus of the tests as the problem was suspected to be rickets. The feed was analyzed for protein, Ca and P. The bone analysis showed a Ca:P ratio of less than 2:1, while results of the blood serum showed an inverse Ca: P ratio. The analysis results of the feed as well as the bones showed an imbalance in the Ca:P ratio which according to literature and research done is a possible cause for rickets. These findings combined with the symptoms displayed by the affected birds, lead to the suggestion that the problem in this study was rickets.

Key Words: Rickets, calcium, phosphorus, broilers



Opsomming

Ragitis word bekryf as 'n siekte wat jong groeiende pluimvee aantast. Dit word gekenmerk deur swak gemineraliseerde bene met verdikte en ongereelde groeiplate. Die inisiële gevolg van ragitis word gekenmerk deur 'n gebrek aan mineralisering van kraakbeen en been.

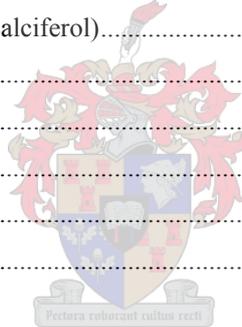
Ander simptome van ragitis behels die weerstand teen beweging van aangetaste voëls. Sulke voëls sit gewoonlik op hul hakke en wanneer hulle skrik gemaak word gebruik hulle hul vlerke om te balanseer. By die nadoedse ondersoek is die bene sag en breekbaar en vertoon verdikte groeiplate. In hierdie studie toon 30% van kuikens, tussen 7 en 8 dae, uitstaanpoot probleme. Aangetaste kuikens was nie in staat om hulle gewig op hul pote te dra nie. Sommige het verlamming getoon. Die bene was sag en rubberig. Met die doel om die oorsaak van die siekte te identifiseer, is die bene van die aangetaste kuikens verwyder en geanaliseer vir kalsium en fosfor om te bepaal wat die Ca:P verhouding is. Bloedserum is ook geanaliseer vir die mineraal inhoud. Kalsium en fosfor was die hoofokus, aangesien die probleem vermoedelik ragitis was. Die voer is geanaliseer vir proteïen, kalsium en fosfor. Die been analisering het 'n Ca:P verhouding getoon van minder as 2:1, terwyl die bloedserum 'n omgekeerde Ca:P verhouding getoon het. Die analisering van die voer, sowel as die bene het 'n wanbalans in die Ca:P verhouding getoon. Volgens die literatuur en navorsing wat gedoen is, is dit 'n moontlike oorsaak van ragitis. Die bevindings, tesame met die simptome wat vertoon word deur die aangetaste kuikens, lei tot die bewering dat die probleem in die studie ragitis was.

Sleutel Worde: Ragitis, kalsium, fosfor, braaikuiken

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SUMMARY OF ABBREVIATIONS

Ca = Calcium

P = Phosphorus

Ca: P ratio = Calcium: Phosphorus ratio

MCP = Mono-Calcium Phosphate

CaBP = Calcium-binding protein

Mg = Magnesium

Mn = Manganese

TD = Tibial Dyschondroplasia

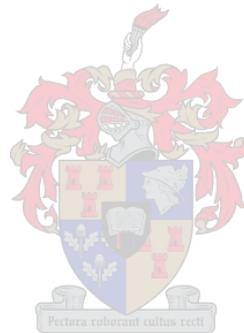
PTH = Parathyroid Hormone

IBV = Infectious Bronchitis Virus

IBD = Infectious Bursitis Disease

NCD = New Castle Disease

BV = Biological Availability



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I would like to thank B. Clyde and B. Coleman for all the help with my case study and Dr. Last from VetDiagnostix for the microscopic evaluations. My sincere appreciation goes to my youngest sister Nosipho who couldn't live to see the results of her encouragement and support; may her soul rest in peace. My deepest gratitude goes to my family for the understanding, love and support throughout my studies. I dedicate this work to my children Khwezi, Bahlebonke and Ndabenhle who received very little attention during the course of my studies but understood my love for them.



CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Nutritional imbalances result in many problems in poultry production, most of them being skeletal deformities, it is therefore critical that where possible the effects are reduced or prevented. Rickets is one of the pathologies caused by nutritional imbalances in young chicks. It is known to be the result of a calcium: phosphorus (Ca: P) imbalance, where sometimes a deficiency in vitamin D could aggravate the situation. Unfortunately there are no straight answers as to what causes the imbalance, situations vary. It is of utmost importance that each situation is analyzed carefully to try and identify the cause of the problem so that all parties involved in the production cycle can focus on preventing the recurrence of events. In that way the feed manufacturer and the farmer will all be careful in their actions and be vigilant for any signs that could result in this type of problem. The broiler producer strives for fast growth and heavier weights in his flock but has little knowledge of what fast growth to heavier weights does to the anatomy and development of the bird itself. This case study was therefore undertaken in order to identify the origin of the problem and prevent a recurrence in the future.

1.2 THE NORMAL DEVELOPMENT OF BONE AND MUSCLE IN CHICKENS

1.2.1 The structure and maintenance of the skeleton

Mainly there are three different types of bone tissue that hold together the skeleton of a domestic fowl, these are cortical bone which is found in the diaphysis of the long bones, the cancellous bone in the vertebrae and the epiphysis of the long bones (Newman & Leeson, 1997). At the end of the long bone is a metaphysis, the growth plate and epiphysis which is covered in articular cartilage. The growth plate is populated by cells called chondrocytes which are in parallel arrangement to the long axis of the bone. It contains several zones which are arranged in succession from the proximal to the distal border, these include: the resting also called reserve zone containing stem cells, the proliferative zone with stacks of flattened cells; the hypertrophic zone containing hypertrophic chondrocytes and the degenerative zone with a partially calcified matrix and invading capillaries (Pines *et al.*, 2005). The bone-forming cells proliferate in the growth plate, and then enlarge. The next step is the mineralization of the cartilage which later gets replaced by bone (Ross Tech, 2001). The normal growth and development of the bone results in variation in leg structure as well as the shape. When this variation goes to the extreme birds are described as having leg problems. It is

well known that abnormal development of the bone occurs due to the failure of the mechanisms involved in controlling growth; this failure can be aggravated by environmental or nutritional stress (e.g. high stocking density or rickets). Leg abnormalities have major economic impacts on commercial poultry production. These abnormalities which include tibial dyschondroplasia (TD) and rickets are caused by nutritional imbalances like a narrow Ca:P or low dietary levels of vitamin D₃. It therefore makes sense to discuss the effects of minerals in detail below.

1.3 THE EFFECT OF MINERALS ON SKELETAL DEVELOPMENT OF BROILER CHICKENS

Calcium (Ca) is the most abundant mineral in the body of an animal, 99 % of this is found in the skeleton (Waldroup, 1995). Phosphorus (P) is the second most in abundance in the body of an animal. Ca and P have a responsibility of building good bones and maintaining them. Minerals like magnesium and boron have certain effects on the absorption of calcium and phosphorus, these effect depend on the amount of these minerals in the diet. These mineral effects are discussed in detail below.

1.3.1 Calcium

The major role of Ca in the body is in bone formation. Ca has other important functions like its requirement for muscle contraction, blood clotting, and together with potassium and sodium; Ca is necessary for normal heart function. The Ca requirements for young birds are said to be around 0.8% to 1.0%, this is assuming that the P level is around 0.45% - 0.5%. Lesson & Summers (2001) stress that a ratio of 2:1 for broilers in the diet is very important, however if higher levels of P are fed then Ca levels must be increased accordingly. As far as Ca toxicity is concerned, these authors suggest that since there is a negative correlation between diet Ca concentration and percentage absorption, birds are able to tolerate high dietary levels of Ca. According to theses authors, toxicity of Ca is said to be explainable on the basis of changes to digesta pH where high levels could affect the solubility and absorption of other minerals.

Edwards (1992) reported that broilers fed low Ca-high P diets developed tibial dyschondroplasia. In studies where birds were fed diets of different Ca and P content, the birds fed diets high in P showed a thickening of the growth plate by 2 weeks of age , which is typical of hypocalcaemic rickets. This condition could easily develop into TD due to decreased hypertrophy of chondrocytes when the bird tries to adapt to the low Ca diet. According to Edwards (1992) this condition can be corrected by increasing dietary Ca. Due to the relationship between Ca and P, the effects of Ca should not be considered by themselves but in relationship with the P content of the diet.

1.3.2 Phosphorus

The major role of P is as a component of bone; however it is also an essential component of organic compounds involved in almost every aspect of metabolism. Every 100 ml of blood contains approximately 35 – 45 mg of P, 10 % of which is in the form of inorganic phosphate. According to Leeson & Summers (2001), there is an inverse relationship between serum diffusible Ca and serum inorganic phosphate. A deficiency of P or a wide imbalance in the Ca: P ratio in the diet can cause rickets. Edwards (1992) reported on a study where cases of rickets were recorded in a group of chicks which received the lowest level of dietary P. However, he states that in later studies growth rate and bone ash were used as a measurement for adequacy of P in a diet; as diets with low growth rate and bone ash were often confirmed as having low P levels. Sanders *et al.* (1991) conducted trials to determine the Ca and total P requirements of the modern broad-breasted white tom turkey for performance and bone ash. Although they estimated Ca and P requirements for optimum growth rate at 12.5 and 10.0g/kg respectively, the maximum bone ash could not be achieved until much higher levels of Ca and P were applied. These authors observed a quadratic response in bone ash due to Ca and P, this response was reportedly very pronounced due to dietary P.

Hengmin & Lingping (1997) conducted experiments on ducklings to evaluate the effects of P deficiency. In the two treatments all the nutrients were adequate except P, the control feed had Ca at 0.80% and P of 0.65% while the P deficient feed (test feed) had Ca at 0.80% and P at 0.366%. On the first week ducklings on the test feed started to show lameness and difficulty in standing, while the ducklings on the control feed did not show any signs. Among other things, the affected birds showed leg weakness, depressed appetite and stunted growth and also abnormal gait. When the experiment ended at 3 weeks the tibial ash, ash Ca and ash P were 61.7%, 60% and 56.6% respectively, below the same parameters in the control feed.

Microscopic examination revealed the hypertrophied zone of the growth plate in the epiphysis of long bones being mildly elongated. The findings of Hengmin & Lingping (1997) in this experiment show the effect of adequate P levels in the normal development of bone and consequently bone components like tibial ash, ash Ca and ash P.

Table 1.1 Tibial ash, ash Ca and P content in ducklings (Hengmin & Lingping, 1997)

Groups	Tibial ash (%)	Ash Ca (%)	Ash P (%)
P deficient	22.67	7.81	4.70
Control	36.74	13.0	8.31

Baker *et al.* (1998) conducted a study to determine the vitamin D₃ requirement of young chicks receiving diets varying in Ca and available P. In this study, broiler chicks on their 2nd and 3rd week of life were fed diets with maize-soyabean meal that were designed to be severely deficient in available P, marginally deficient in Ca or adequate in both available P and Ca. The results showed that when birds were fed diets first limiting in available P, there was a linear increase in weight gain in response to increasing doses of vitamin D₃. The growth response of 37% observed at levels of 0 and 12.5µg vitamin D₃/kg was greater than the response of 11% obtained at levels between 12.5 and 1250µg vitamin D₃/kg in diets which were deficient in P. Also tibia ash concentration and total ash weight per tibia responded linearly between 0 and 1250µg vitamin D₃/kg. When P was adequate in the diet, 5µg vitamin D₃/kg appeared to be sufficient for maximal weight gain which suggests that at adequate levels of Ca and P the requirement for the young chicks is only 5µg vitamin D₃/kg.

1.3.3 Calcium and Phosphorus Availability

The importance of Ca and P are discussed in detail in the sections above. Among other functions, Ca is involved in bone formation and maintenance, in improving efficiency of gain and feed utilization, and is necessary in egg shell formation. On the other hand the functions of P include involvement in bone formation and maintenance, building muscle tissue and egg formation, improving efficiency of gain and feed utilization and others (Waldroup, 1995).

Of utmost relevance are the utilization and metabolism of these two minerals. There are factors that will influence their utilization and metabolism, such as the ratio of the two elements in the diet, the amount of vitamin D in the same diet as well as the biological value of the supplements used to provide the elements, the age and the physiological state of the animal.

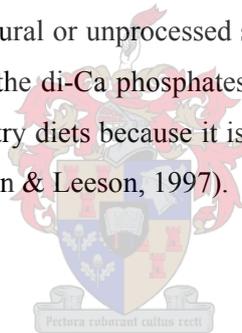
1.3.3.1 Ca and P absorption

Ca is absorbed from the small intestines through an active transport mechanism that is influenced by vitamin D. Absorption of Ca also occurs to a lesser extent by passive ionic diffusion, this may be sufficient for animals which have little Ca demands (Waldroup, 1995). P on the other hand is absorbed mainly in the duodenal area of the small intestine. According to this author, P that is absorbed from the intestines is circulated throughout the body and is readily withdrawn from the blood for bone development. Under normal conditions, 70% of Ca absorption in young chickens is dependant on vitamin D. Hurwitz (1992) reported that

vitamin D also increases permeability to phosphate in the chick's intestine independently of its effect on Ca absorption. In addition to the action of vitamin D₃ on mineral absorption, there is evidence that 1,25(OH)₂ D₃ affects cell differentiation. In vitamin D-deficient animals, the intestinal villus is considerably shorter than normal. Hurwitz, (1992) however states that through promoting proliferation by activating polyamine synthesis, 1,25(OH)₂ D₃ normal length of the villus can be restored.

1.3.3.2 Ca and P supplementation

A wide range of products are available and used worldwide for supplying Ca and P in animal diets. Sources of concentrated calcium include limestone deposits and marine sources like oyster shell. Limestone and oyster shell are the most used sources because they are both highly soluble and contain approximately 38% Ca. According to Newman & Leeson (1997) a Ca source with large particle size is most beneficial because such particles are retained in the upper digestive tract and dissolve more slowly which provides a more uniform and sustained release of Ca. Ca sources that contain significant levels of Mg should not be used as they may cause diarrhea and are likely to reduce performance (Waldroup, 1995). With P, the variety of sources is even wider. These include natural or unprocessed sources like low-fluorine deposits and guano deposits as well as processed sources like the di-Ca phosphates and defluorinated phosphates. For example di-Ca phosphate is commonly used in poultry diets because it is considered to be 100% available to the bird and it contains 23% Ca and 20% P (Newman & Leeson, 1997).



1.3.3.3 Biological availability

Biological availability (bioavailability, BV) defines the availability of nutrients from the different sources. It provides a measure of the degree to which a P (or Ca) source can support the physiological processes of an animal (Waldroup, 1995). This value can be measured and it gives guidance as to which source is economical in a diet. Newman & Leeson (1997) noted that compounds that are gut acidifying and those substances that form readily soluble metal chelates with Ca enhance its absorption. In general, the factors which aid in dissociating the minerals while holding them in solution are beneficial and those which cause the formation of precipitates or soaps are detrimental to mineral availability.

Phytic acid also known as phytate is an organic complex which is generally regarded as the primary storage form of both P and inositol in plants (Sebastian *et al.*, 1998). The BV of phytate is generally poor depending on the dietary Ca content, inorganic P and vitamin D₃, age and species of the animal but availability is very poor for young growing animals (Waldroup, 1995). Among the negative effects of phytate is its ability to bind dietary cations (e.g. Ca, zinc, copper, manganese, cobalt and iron). Phytate also

binds with proteins, amino acids, carbohydrates and digestive enzymes as well as P and this reduces overall digestibility (Hooge, 2002). Formation of mineral and protein complexes is believed to have a negative effect on phytate hydrolysis by phytase of plant, animal and microbial origin.

The low availability of the P in phytate to poultry is due to the fact that they lack the proper enzyme system to hydrolyze phytate into inorganic P and inositol. This results in manure that has high P concentration from the animals that were fed on diets containing phytate (Sebastian *et al.*, 1998). This over-excretion of P results in accumulation of soil P to levels much more than what is needed by the crops. Another problem is the potential loss of water soluble P from over-fertilized and contaminated soils even when there is minimal soil erosion. These problems have led to the development and use of microbial phytases which limits the P output by monogastric animals. Microbial phytase increases the digestibility of P in the diet by hydrolyzing phosphate from phytate. According to Hooge (2002) reducing non-phytate P and including phytase and 25-OH-cholecalciferol and/ or using high available P maize in poultry feeds, has the following advantages:

- a significant reduction of total and water soluble P excretion by poultry,
- maintaining the fertilizer value of broiler litter and manure,
- subsequently result in lower concentrations of soluble and excretable P in soils than normal litters, and
- reduce the potential for P losses to water by run-off or leaching when manure is managed properly.

Berry (2001) conducted a study to determine whether broiler breeders will respond to microbial phytase in a manner similar to broilers and commercial layers. The results showed improved egg production, improved fertility, improved hatch and improved livability in the phytase-fed birds over the duration of the trial, these are shown in Table 1.2. These results suggests that the improvements attributed to use of phytase in this experiment may be the result of release of energy, protein, amino acids and/ or minerals other than P from the phytate molecule.

Table 1.2 Response of broiler breeders to Phytase feeding (Berry, 2001)

RESPONSE	CONTROL	PHYTASE
Mortality (%)	21	11
Body Weight (kg)	3.69	3.56
Egg Production (% hen/day)	51.06	54.44
Egg Weight (g)	61.19	61.24
Egg Shell Weight (g)	5.46	5.44
Egg Specific Gravity (g)	1.08	1.08
Fertility (%)	94.84	96.05
Hatchability (%)	82.76	85.12
Bone Breaking Strength (kg)	18.17	19.05
Bone Ash (%)	32.8	35.7

Ca bioavailability was explored by comparing different Ca sources (Waldroup, 1995). When Ca carbonate and Ca sulphate were compared to ground oyster shell and two different ground limestone sources, no differences were found in regard to body weight gain or tibia ash. But when bioavailability of Ca was estimated from different P sources, this author reported that there were significant differences in the Ca availability of feed grade phosphates to the chick. He suggested that there is a positive correlation between the availability of Ca and P in feed grade P supplements. The importance of Ca availability to chick performance as explained by Waldroup (1995) is the fact that the chick's P requirement is influenced by particle size of the limestone source.

Boron appears to interact with other nutrients; it also has a regulatory function in the metabolism of minerals like Ca and therefore plays a part in bone metabolism (EVM, 2002). According to Elliot & Edwards (1992), an addition of 1,100 ICU/kg cholecalciferol to a low-calcium, high-phosphorus basal diet containing no cholecalciferol caused a reduction in the incidence of TD and increased percentage tibia bone ash. Boron interactions are discussed below.

1.3.4 Boron

Elliot & Edwards (1992) conducted experiments to determine whether an interaction exists between dietary boron and cholecalciferol and the effects on bone ash and plasma minerals with respect to the development of pathologies such as rickets and TD. The objective of these experiments was to evaluate whether boron supplementation would alleviate reduced performance and increased incidence of rickets and TD in broiler chickens fed diets deficient in cholecalciferol or Ca or both.

The results of these experiments revealed that at a level of 0.90% Ca gave a significant improvement in weight gain when compared to a level of 0.65%. This improvement was more pronounced in diets with a lower level of cholecalciferol. However regardless of boron or Ca level dietary supplementation with 1,100 ICU/kg cholecalciferol resulted in a significant improvement in weight gain compared to 110 ICU/kg. Elliot & Edwards (1992) reported that dietary supplementation with 40 mg/kg boron had no effect on weight gain; furthermore they could not identify any interaction between boron and cholecalciferol or between boron and Ca.

Dietary supplementation with 0.90% Ca and 1,100 ICU/kg did not only improve weight gain, but increased tibia bone ash, increased plasma Ca, decreased plasma magnesium and also reduced the incidence of rickets. However, from these studies there was no indication that supplemental dietary boron could reduce the severity of rickets and TD, which are the results of cholecalciferol deficiency. In fact, supplemental boron failed to delay the onset and severity of a cholecalciferol deficiency. These authors further reported that the effect of a cholecalciferol deficiency and that of a Ca deficiency resulted in increased incidence and severity of TD. The results of these experiments suggest that interactions between boron and Ca and boron and cholecalciferol could not be proven.

Magnesium (Mg) is needed for Ca absorption, without enough Mg Ca cannot be fully utilized (Fuchs, 2002). An increase in Ca levels in the blood stimulates the secretion of calcitonin and suppresses the secretion of the parathyroid hormone (PTH). PTH draws Ca out of the bone while calcitonin increases Ca in the bones. According to Fuchs (2002) sufficient levels of Mg determine this balance. While Mg helps with absorption and retention of Ca, too much Ca prevents the absorption of Mg.

1.3.5 Magnesium (Mg)

According to Edwards (1992) it is excess dietary Mg that has been associated with skeletal abnormalities in chicks and not deficiency. Where diets are marginally deficient in Ca and P, dietary levels of 3.2 g Mg /kg seem to decrease tibia ash values. Leeson & Summers (2001) reported that feeding Mg at 0.9 % to broilers resulted in reduced early growth rate and increased the incidence of leg disorders.

Gardiner *et al.* (1961) performed experiments to study the interrelationships between Mg and fluoride and also to ascertain the effect of P level in the diet on the relationship. The results revealed that the inclusion of 0.08% of supplemental fluoride from sodium fluoride reduced growth rate but did not have any effect on bone ash, bone Ca, bone P, plasma Mg or plasma inorganic P. When supplemental Mg was added at 0.25% from Mg carbonate, the result was increased plasma Mg but did not have any effect on the other variables. However when 0.08% fluoride and 0.25% Mg were added in the same diet, the result was a growth depression greater than when fluoride alone was added. This combination of Mg and fluoride in the diet resulted in a characteristic leg weakness, reduced bone ash and a reduction in both Ca and P content of the bone.

In a different study, Lee & Britton (1980) conducted trials to test the effect of Mg toxicity, and the effect on P utilization by broiler chicks. In their experiments, dietary Mg was added to a corn-soya basal diet at levels of 0.3%, 0.5% and 0.9%. The results showed that these levels of Mg were cathartic and they resulted in a significant decrease in body weight to 28 days of age. The addition of Mg also resulted in increased mortality and induced leg abnormalities. Increasing dietary levels of Mg also resulted in reduced body weight and bone ash percentage. However, the authors noticed that increasing dietary P significantly reduced the effect of the Mg toxicity except where Mg was at 0.9%. In bone composition, increasing Mg in the diet resulted in decreased Ca and P. The results of the trials conducted by Lee & Britton (1980) also suggested that excessive dietary Mg increases the need for dietary P and that failure to monitor the Mg content of diets may result in the excretion of P to the environment. The dietary schemes presented in this experiment represent low phosphate diets (0.1% and 0.12% inorganic P) and diets that are similar to those found in the industry (0.24% and 0.48% inorganic P). These authors indicated that practical rations are usually formulated without the level of Mg being considered since it is usually not a problem. However they do agree that when certain types of limestone, natural phosphate sources or mineral supplements are used in feed formulation that might result in high levels of Mg in the diet and as a consequence, Mg toxicity.

1.4 FACTORS THAT AFFECT THE NORMAL DEVELOPMENT OF A CHICKEN

1.4.1 The effect of dietary nutrients

The nutrient requirements of poultry are influenced by factors like the size of the bird, the energy content of diet, the physical form of diet, the environmental temperature and nutritional adequacy of diet. Voluntary intake of feed is directly influenced by the size of the bird, while the energy content of the diet and feed intake are inversely related.

According to Waldroup (1995), using a medium to fine particle size Ca supplement is beneficial in chick diets. Where comparison was done for the different grind sizes of limestone, finer grounds were found to improve availability of Ca to the chicks and result in increased gains and improved feed utilization at low dietary Ca levels. While coarser grinds increased gains and improved feed efficiency at higher Ca levels.

Very high levels of vitamin A in the diet might interfere with absorption or the metabolism of vitamin D thereby inducing rickets. In the same way, an excess of one of the minerals may result in deficiency of another mineral or the interference with the absorption and utilization of the other mineral. Experiments conducted by Aburto & Britton (1998) clearly showed that high levels of vitamin A and E depress bone ash and increase rickets if the vitamin D₃ level of the diet is marginal. Sometimes P and Ca availability may be lower than expected because of the presence of certain fats and high phytate levels (Pillai & Kailasam, 1998). The fact that rickets is more common in meat birds or broilers suggests that due to the requirements for fast growth in this type of birds, the development of rickets occurs more rapidly (Riddell, 1992). In young animals during bone formation minerals are deposited on the bone matrix, giving rise to trabecular bone and as a result the bones elongate. One of the functions of vitamin D₃ is to bring about matrix mineralization. As a result when vitamin D₃ is deficient, the organic matrix fails to mineralize resulting in rickets in young birds and osteomalacia in adult birds (Leeson & Summers, 2001). In Ca and vitamin D deficiencies, the zone of proliferation is thickened and a small zone of hypertrophy is poorly invaded by metaphyseal vessels (Riddell, 1992). While in P deficiency, the hypertrophic zone is thickened and although not mineralized it is invaded by metaphyseal vessels.

It is noteworthy that in some situations, outbreaks of rickets are caused by a nutritional deficiency due to a feed mixing error. In broiler chickens where symptoms are uniformly thickened growth plates, the cause is usually a marginal level of Ca and excess P in the diet (Riddell, 1992). However, in other outbreaks of rickets, nutrients are adequate but factors like excess vitamin A and high fat levels in the feed are

interfering with the utilization and absorption of vitamin D. Riddell (1992) further reported that excess Mg in the diet would produce lesions similar to rickets in chickens.

1.4.2 The effect of different levels of vitamin A, D₃ and E in broiler chicks

Aburto & Britton (2001) conducted a study to evaluate the effect of varying dietary levels of vitamin A, D₃ and E in broiler chicks. One day-old male chicks were fed a corn-soya basal diet in a 16-day feeding trial. In this experiment, three levels of vitamin A (1,500; 15,000 and 45,000 IU/ kg), three levels of vitamin D₃ (500, 1,500 and 2,500 ICU/ kg) and three levels of vitamin E (10, 5,000 and 10,000 IU/ kg) were used. The recommended levels according to the NRC (1984), are 1,500 IU/kg vitamin A; 200 ICU/kg vitamin D and 10 IU/kg vitamin E for broilers of 0-6 weeks of age. In this trial, an increase in the levels of vitamin E resulted in a decrease in body weight and feed consumption at marginal levels of vitamin D₃.

This effect was counteracted when the levels of vitamin D₃ were increased. High levels of vitamin A and E decreased bone ash, but again increasing the levels of vitamin D₃ could prevent this response. More importantly, the authors noticed that the incidence of the vitamin D rickets showed a significant increase as vitamin A and vitamin E levels were increased. This condition was reversed completely by increasing the levels of vitamin D₃. It was further noted in this experiment that high levels of vitamin E significantly reduced plasma Ca, but again this was corrected by adding vitamin D₃. The results of this experiment suggest that high levels of vitamin A and E have adverse effects on the nutritional balance of broilers and can induce the incidence of nutritional rickets.

1.4.3 The effects of vitamin D₃ (cholecalciferol)

According to Whitehead (1992), vitamin D is fat-soluble and for many years it has been regarded as a nutritional factor, which prevents or cures rickets. However, after the discovery of the metabolic conversion of the vitamin to 1,25- dihydroxyvitamin D₃ [1,25(OH)₂ D₃], and the feedback relationships between Ca metabolism and the synthesis of the metabolite, the 1,25(OH)₂ D₃ was classified as a steroid.

Vitamin D₃ is well known for its effect on mineral absorption and therefore the supply of mineral to bone. In addition, vitamin D affects bone development directly by controlling differentiation of its cellular elements. Generally, the function of vitamin D (Leeson & Summers, 2001) is to elevate Ca and P levels in the plasma necessary to support normal body functions. There are two hormones, thyrocalcitonin (calcitonin) and parathyroid hormone (PTH), that influence release of 1,25(OH)₂ D₃ needed to control levels of blood Ca and P. Calcitonin is involved in controlling high serum Ca levels by depressing gut absorption, stopping demineralization and depressing Ca absorption in the kidney. Vitamin D₃ however, brings about an elevation

of plasma Ca and P by stimulating specific pump mechanisms in the intestine, kidney and bone, thereby maintaining blood levels of Ca and P from these body reserves. Vitamin D also plays an important role in improving the immune system through differentiation and proliferation of cells, skin and cancer cells also benefit from the function of vitamin D₃.

1.5 PATHOLOGIES

According to observations of Leach & Gay (1986), it is the inappropriate posture for long periods of walking and standing combined with enhanced muscle development that causes undue stress on cartilage, bones and tendons of the weight-bearing limbs. This is one of the causes for skeletal malformation. The epiphyseal growth plate plays a key role in skeletal development, and therefore factors that influence the metabolism of this tissue can lead to abnormal skeletal development. Cartilage development is mainly influenced by nutrition and genetics, and these are discussed below.

1.5.1 Dwarfism (Chondrodystrophy)

1.5.1.1 Manganese (Mn) deficiency

Chondrodystrophy is an abnormality that is characterized by a disproportionate shortening of the long bones. Research shows that nutrient deficiencies are mostly responsible for this problem. For example, Mn deficiency has been identified as one of the causes of the abnormality. Leach & Gay (1986) noticed that where Mn deficiency is experienced in early embryonic development or in newly hatched chickens the result is severe retardation of long bone development. The affected bones become shortened and thickened and the histological examination shows a narrow growth plate. The reason for this is that Mn is required for the glycosyltransferases that function in the synthesis of the glycosaminoglycan side chains of the proteoglycan. The deficiency of Mn therefore impairs the bone's ability to synthesize proteoglycans and result in the abnormality.

1.5.1.2 Genetic factors

A specific genetic mutation does occur and it results in severe retardation of limb development in the chick embryo. The condition is called nanomelia and is due to the reduction in proteoglycan synthesis (Leach & Gay, 1986). These authors further explain that there is another mutation which has similar characteristics as the nanomelia, this is called micromelia, and it causes severe reduction in the size of the limbs, parrot beak and cartilage. This is also due to decreased proteoglycan content. Dwarfism can also be caused by a dwarfing gene which influences longitudinal bone growth in growing chickens.

1.5.1.3 Deficiency symptoms of vitamin D₃

When chicks are fed diets deficient in vitamin D₃, the first signs are retarded growth and severe leg weakness. Also, beaks and claws will become soft and pliable. Affected chicks usually have difficulty walking, they take a few steps then they squat on their hocks. While resting, they often sway from side to side suggesting a loss of balance or equilibrium. Feathering is usually poor and abnormal banding of feathers has been reported with colored breeds. The spinal column may bend downward and the sternum can also deviate to one side (Leeson & Summers, 2001). These structural changes reduce the size of the thorax with subsequent crowding of the internal organs. However, a study reported by Leeson & Summers (2001) suggests that adding the synthetic 1.25(OH)₂ D₃ to the diet reduces the incidence of these problems. Affected chicks also show beading of the ribs at the junction of the spinal column along with a downward and posterior bending. According to Hurwitz (1992) the decrease in feed intake, which accompanies vitamin D deficiency in chicks, could result from a decreased peristalsis of the intestine due to sustained hypocalcaemia.

Vitamin D deficiency symptoms include hypertrophied cartilage zone of variable length, epiphyseal vessels elongated, irregular junction pre-hypertrophied and hypertrophied cartilage, disorganization of proliferating chondrocytes, metaphyseal secondary spongiosa lengthened. The important fact (Whitehead, 1992) is that Ca: P ratios are central to the development of rickets and that high dietary P has a similar effect to low dietary Ca concentration. Marginal concentrations of dietary Ca do not cause rickets in chickens but they can increase the incidence and severity of dyschondroplasia. However, if the dietary concentration of vitamin D₃ is also low then some proximal tibiotarsus will exhibit mild rachitic change.

Bar *et al.* (1987) conducted a study to determine the cause of leg disorders in turkeys. Out of 32 cases, 22 showed low percentage of bone ash and this was considered as field rickets. Most of these also had reduced plasma Ca and inorganic P (Table 1.3). In an effort to examine the cause of the problem in the 22 cases, the vitamin D activity in diets, plasma 25-hydroxyvitamin D₃ (25-OH D₃), and intestinal Ca-binding protein (CaBP) were determined.

Table 1.3 Bone ash, plasma calcium, inorganic P (Pi), 25-hydroxyvitamin D₃ (25-OH D₃) and duodenal calcium-binding protein (CaBP) concentrations in turkeys diagnosed as having field rickets and normal and vitamin D₃-deficient controls (Bar *et al.*, 1987).

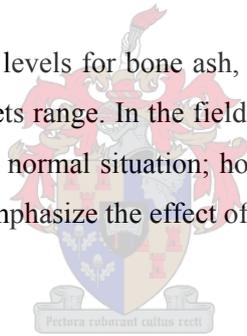
Variable	Field Rickets	Normal	Vitamin D ₃ -deficient
Bone ash, % of DM	¹ 33.1 ± ² 0.6 ³ (22)	40.8 ± 2.7(10)	29.6 ± 3.0(6)
Plasma Ca, mg/dl	9.1 ± 0.9(22)	11.0 ± 0.4(10)	9.3 ± 1.2(6)
Plasma Pi, mg/dl	4.7 ± 1.3(22)	7.5 ± 0.7(9)	4.3 ± 0.8(6)
Plasma 25-OH D ₃ , ng/ml	8.1 ± 2.9(9)	19.4 ± 5.1(4)	1.6 ± 1.2(3)
Duodenal CaBP, µg/g	0.5 ± 0.2(3)	2.6 ± 0.5(4)	0.1 ± 0.1(3)

¹ mean,

² STDEV,

³ number of observations.

The results in Table 1.3 show low levels for bone ash, plasma Ca, inorganic P, 25-hydroxyvitamin D₃ and Ca-binding protein in the field rickets range. In the field rickets analysis results the values of Ca, Pi, vitamin D₃ and CaBP are lower than in the normal situation; however analysis results are even lower in the vitamin D₃-deficient range. These results emphasize the effect of the levels of the Ca, P and vitamin D₃ in the case of rickets.



1.5.1.4 Excessive vitamin D₃

When vitamin D₃ is taken in excessive amounts, symptoms include a syndrome characterized by resorption of bone salts and abnormal deposition of Ca in the viscera and soft tissues. Smooth muscle is very susceptible to abnormal Ca deposition. According to Leeson & Summers (2001), the most notable pathological sequences are inflammation, cellular degeneration, and finally calcification. Ca deposits are often found in the vascular system, and urinary and respiratory tract. Very high levels of cholecalciferol in chicks can lead to renal damage, due to calcification of the kidney tubules while the aorta and other arteries may also become calcified. According to these authors, the toxicity of vitamin D₃ and metabolites is always accentuated when diets are also high in Ca and P.

1.5.2 Tibial dyschondroplasia

Tibial dyschondroplasia (TD) is a skeletal abnormality that occurs in the proximal ends of the *tibia-tarsus* and *tarsus-metatarsus* of rapidly growing birds. Sanders & Edwards (1990) reported in their study that one of the nutritional factors that induce TD is a narrow Ca to P ratio as well as the low levels of dietary vitamin D₃. The lesion of TD is a non-mineralized-non-vascularized plug of cartilage that extends from the growth plate to the metaphysis (Leach and Gay, 1986). Research has shown that TD develops as a result of a series of metabolic and structural changes. The first notable changes are in the prehypertrophic cells of the affected growth plate. This is accompanied by low levels of Ca and P than normal in the mitochondria. As the prehypertrophic zone thickens, this may cause separation of cells from the nutrient supply resulting in energy depletion in the cell. This and other changes that follow in the cell including the occurrence of the flocculent material in the mitochondria are definite signs of irreversible cell death.

Pillai and Kailasam (1998) describe TD as a developmental condition of the growing tibia in which cells derived from the growth plate fail to develop properly and remain as cartilage instead of being a bone tissue. The cause of this in broilers is marginal dietary deficiency of Ca. This causes sub-clinical rickets in birds of 2 weeks of age, which is subsequently seen as TD at 4-6 weeks of age.

Xu & Soares (1993) did a series of experiments to test the hypothesis that vitamin D₃ resistance may be involved in the etiology of TD. The basal diet contained 1.0% Ca and 0.45% available P and no supplemental vitamin D₃. They then took chicks from low and high TD lines and fed them diets supplemented with various levels of vitamin D metabolites. These chicks were then examined for rickets and TD. When chicks were fed a diet containing 1.25µg vitamin D₃/kg, high TD chicks had a higher incidence of severe rickets than lower TD chicks. Lower TD chicks did not exhibit TD when fed diets with 20µg vitamin D₃/kg. These authors further noticed that low TD chicks that were fed a diet containing 5µg vitamin D₃/kg showed a TD incidence of 22%. When high TD chicks were fed diets supplemented with vitamin D₃ and 1.25 (OH)₂ D₃ (20 and 5µg vitamin D₃/kg respectively), the incidence of TD was reduced in all treatments compared to the control (5µg vitamin D₃/kg). These results led to the conclusion that vitamin D₃ is involved in the development of TD.

1.5.3 Rickets

Rickets is a pathology characterized by a widened disorganized growth plate associated with vitamin D₃, Ca or P deficiency and/or Ca: P imbalance in the diet (Whitehead, 1992), which results in low levels of Ca and P in the blood. Nutritional rickets is one of the skeletal problems which are purely induced by nutritional deficiencies or imbalances between minerals and vitamins. In Ca and vitamin D rickets there is a

substantial increase in the width of the epiphyseal growth plate that was due primarily to an increase in the proliferating and prehypertrophic zones of chondrocytes, this results in the loss of orientation of the chondrocytes. Leach and Gay (1986) observed that feeding a high Ca diet restores the normal length as well as the chondrocyte arrangement to the proliferative zone. In addition to the lack of organization in chondrocytes, an elongated degenerative zone of hypertrophic chondrocytes is also noticeable. This is probably due to low P in the diet. Characteristic lesions for P deficiency include an increase in the length of the degenerating hypertrophied zone and the metaphyseal primary spongiosa. These findings lead these authors to the deduction that Ca is necessary for chondrocyte hypertrophy, while P is needed for the resorption of hypertrophic chondrocytes.

1.5.3.1 The effects of vitamin D in the development of rickets

Research has shown that vitamin D has an important role to play in Ca and P metabolism, in skeletal development and in eggshell strength (Waldroup, 1995). Severe deficiency of this vitamin results in skeletal deformities, beak and eggshells in poultry. In young chickens or turkeys, vitamin D deficiency is usually associated with decrease in blood Ca, an increase in blood P, an enlargement of the cartilaginous epiphysis, and a failure to effectively mineralize the developing bone. This, as defined by Vaiano *et al.* (1994) is rickets. Hurwitz (1992) however, describes rickets as the classical manifestation of vitamin D deficiency in birds even under normal Ca or phosphate nutritional levels. Described in chemical terms, rickets is characterized by a low bone ash i.e. Ca and P content. Other symptoms of vitamin D deficiency include atrophy of cartilage cells at the growth plate and invasion of cartilage by blood vessels. According to Vaiano *et al.* (1994) the osseous elements are progressively reduced leading to a widening of the cartilaginous growth plate, this is visible to the naked eye.

1.5.3.2 Vitamin D₃ requirements

Factors that affect the requirements of vitamin D in poultry include the age and physiological status of the bird. On estimating values for vitamin D₃, consideration should be made for the P source in the diet, the ratio of Ca to P and the extent of exposure to sunlight (Leeson & Summers, 2001). According to these authors recommended levels of vitamin D₃ can in the absence of sunlight produce normal growth, calcification, production and reproduction, only if the diets contain adequate levels of Ca and P. However, narrowing or widening of the Ca: P ratio increases the requirement for vitamin D₃. Laying hens need vitamin D₃ for optimum egg production and for eggshell quality, while in breeding hens vitamin D₃ is necessary for good hatchability and to provide a reserve for the hatching chicks. It has been reported that the chick does

not have an optimum supply of the enzyme cholecalciferol-25-hydroxylase until about two weeks of age. Therefore it is possible for the chicks hatching from eggs where breeders have been fed a diet low in vitamin D₃ to show rickets even though the chick's diet contains sufficient levels of vitamin D (Leeson & Summers, 2001).

Cholecalciferol is said to be synthesized in birds from 7-dehydrocholesterol (previtamin D) in skin exposed to ultraviolet irradiation. The previtamin D₃ is then converted to vitamin D₃ by a temperature-dependant isomerisation (Hurwitz, 1992). Leg skin contains approximately 10 times more previtamin than back skin, whereas feathers contain none. The important fact is that vitamin D₃ can be supplied in food from which it is absorbed in the intestine at an efficiency of 60- 70%.

1.6 CONCLUSION

Rickets is a disease of the growth plate in which the proliferative zone of the growth plate is enlarged, the arrangement of the chondrocytes is completely disorganized from the normal columnar arrangement and there are additional chondrocytes in each column (Pines *et al.*, 2005). It is described as a nutritional imbalance that is caused by Ca:P imbalance or a deficiency of one of the minerals that could be combined with a vitamin D deficiency. Factors that promote rickets are unavailability of Ca and P to the bird, and these in turn depend on factors like contaminated Ca sources, high levels of vitamin A or E in the diet which interfere with the absorption of vitamin D. Mg toxicity has been identified as a cause of leg problems with lesions similar to the rickets lesions, Mg toxicity also results in reduced body weight and increased mortalities. Limestone sources that are used should therefore be carefully analyzed for impurities as they interfere with Ca availability in a diet. A lot of work has been done on rickets and the causal agents have been identified. However as this type of pathology is still found in the field and it results in devastating economic losses, it remains very critical to accurately identify the real cause in each field case so as to prevent recurrences.

CHAPTER 2

INVESTIGATING A SUSPECTED CASE OF FIELD RICKETS

2.1 Environment

The poultry farm in this case study had 6 open-sided houses with concrete floors. Each house accommodated 5000 birds housed at a stocking density of 15 birds/ m². Summer temperatures ranged between 24 °C and 33 °C with an average of 26 °C, while in winter temperatures range between 14 °C minimum and 26 °C maximum with an average of 20 °C. The houses were not provided with any environmental control devices, only portable fans that were used in hot weather to help circulate the air.

2.2 Clinical History

In each flock about 30% of the birds between 7 and 8 days of age suddenly showed leg problems. The affected birds were unable to walk and showed severe paralysis. These birds supported their weight with their hocks, and when forced to move they would balance with their wings but were unable to stand up. These chicks refused to eat or drink. Mortalities, which could have been considered as expected from any broiler flock by 9 days (about 2 – 3 chicks/day) increased to 15 – 20 chicks per day in each house at about 11 days, and 15 days of age the percentage of affected birds had increased to about 60%. From the symptoms displayed by the affected birds, there were several viral diseases that could cause the problem. The potential involvement of these viruses was investigated. However, due to the characteristic symptoms observed in the birds it was suspected that this could be a case of rickets. Several samples were taken for post-mortem analysis. The results will be discussed in detail in the relevant section.

During the clinical evaluation of the birds, it became evident that the bones were incredibly soft and rubbery and they could be bent to extremes without breaking. Mortality in affected birds was 100% while morbidity was about 60%.

2.3 Feed and laboratory analyses

Feed samples were analyzed for the relevant nutrients, and the results are shown in the Table 2.2 and Table 2.3. Lameness could be attributed to several infectious and non-infectious diseases or a combination of the two and sometimes a synergistic interaction of both (Ross Tech, 2001). The most common causes of lameness are of nutritional or infectious nature. It is for these reasons that tests were done for viral diseases which could also be responsible for the symptoms displayed by the affected birds.

The laboratory performed serology tests on the blood of the 10 chicks to check for Newcastle Disease (NCD), Infectious Bronchitis Virus (IBV) or Infectious Bursitis Disease (IBD) which are viral in nature. This was purely to eliminate any possibility of viral causes to the high mortality in young chicks which can also be caused by IBV. Although in NCD cases symptoms are not the same as in this case, if nervous disorders develop paralysis of one or both wings and legs can be observed. Post-mortem analysis was done for Marek's disease since in acute cases of Marek's affected birds also show unilateral paralysis, high morbidity and high mortality. Bone and blood samples for Ca and P content were taken. At this stage twenty chicks which died at 10 days of age were submitted to VetDiagnostix for post-mortem. The number of chicks sent for analysis was limited to reduce costs as the intention was to identify the problem and not to conduct an experiment. Bones analysis for Ca: P ratio was performed by Vet-Diagnostix Pathology Laboratory*. Tibias were cleaned of all flesh. The whole tibia was then incinerated in an oven for ash determination on a dry fat-free basis (Association of Official Agricultural Chemists, 1995). The Ca and P contents of the ash were then determined. Blood serum from the 20 birds was also analyzed for Ca and P. The results of blood serum analysis are presented in Table 2.1 As the problem was suspected to be rickets, microscopic examination was conducted by VetDiagnostix on the *tibia-tarsus* of the dead chicks, to look carefully into the growth plate and the proliferative columns. The nervous tissue and the heart tissue were also examined microscopically for any abnormalities that could lead to the correct diagnosis of the problem. The findings of the microscopic examinations are discussed in the results and discussions.

Feed samples were taken from each of the three sources at the farm, namely: from the tube feeders, a store sample (from the shelf) and from the bulk bin for nutrient analysis to determine nutrient variations if there were any. This set of samples was sent to an independent laboratory by the feed manufacturer to be analyzed for crude protein, Ca and P. The results were compared to the formulation specification. A similar set of samples was sent by the farmer to a different laboratory (Biochemical and Scientific Consultants) for Ca and P analysis.

2.4 Results and Discussion

The analysis results of the blood serum showed inverted Ca:P ratios in all the chicks, these are presented in Table 2.1 below. Vaiano *et al.* (1994) undertook sequential studies using Australian broiler flocks which represented 3 major genetic lines at weekly intervals from 1 to 28 days. The intention was to identify the relationship between serum total Ca, P and 1.25-dihydroxycholecalciferol [1,25(OH)₂D₃]. In the studies, results of the serum analysis revealed that the serum Ca and P remained within normal levels in all flocks between 7 and 21 days, however the serum P was high relative to serum Ca throughout this period. According to Vaiano *et al.* (1994), the high P concentrations relative to Ca observed in the first 3 weeks of

broiler chicks can interfere with vitamin D metabolism by reducing the rate of synthesis of the physiologically active metabolite. This means the high serum P relative to Ca may be capable of suppressing the synthesis of $1,25(\text{OH})_2 \text{D}_3$ by the kidney, which in turn induces a vitamin D-dependant rickets. The findings of Vaiano *et al.* (1994) could explain the blood serum results presented in the Table 2.1.

The results of the feed analysis done by the feed manufacturer are presented in Table 2.2. These results showed a consistently low P in all three samples, (all samples were about 17 % below formulation minimum). In the samples sent in by the farmer to a separate laboratory (presented in Table 2.3), the Ca analyzed at 1.08 % and P analyzed at 0.45 %, the P level here was even lower than in the feed samples.

The samples had low P compared to what was expected. This resulted in a lower than expected Ca: P ratio for growing chicks, which should be between 1.0:1 and 2.2:1 (Leeson & Summers., 2001). A ratio of 2.5:1 appears to be slightly marginal, while a ratio of 3.3:1 was found to produce a high incidence of rickets (Leeson & Summers, 2001). The low Ca levels found in the feed were concerning, and raised a suspicion that an error during the mixing of the P source could have occurred. This was clarified later on, when the mixing process what evaluated. It was found that the mono-Calcium Phosphate (MCP) was added manually, which could result in a mixing error.

Table 2.1: Blood serum analysis for the 20 Chicks.

Identity	Ca (mmol/l)	PO4 (mmol/l)	Identity	Ca (mmol/l)	PO4 (mmol/l)
A7-1	1.43	2.12	A7-11	1.35	3.13
A7-2	1.9	2.38	A7-12	2.03	2.24
A7-3	1.89	1.99	A7-13	1.93	2.71
A7-4	1.68	2.45	A7-14	1.45	2.9
A7-5	0.75	2.27	A7-15	1.67	2.67
A7-6	1.78	2.26	A7-16	2.18	2.46
A7-7	1.69	2.68	A7-17	1.8	2.18
A7-8	1.88	2.18	A7-18	1.92	2.35
A7-9	1.81	2.34	A7-19	1.6	2.88
A7-10	1.92	2.26	A7-20	1.78	2.27

Observations	20 (Ca)	20 (PO4)
Mean	1.72	2.44
Std Dev	0.31	0.30

Table 2.2 Analysis results for broiler Starter Crumbles (Producer's Samples).

	Crude protein (%)		Ca (%)		P (%)	
	Actual	Std Min*	Actual	Std Min*	Actual	Std Min*
Tube Feeder	24.0	22.0	1.13	1.00	0.58	0.70
Store sample	24.2	22.0	0.85	1.00	0.61	0.70
Bulk Bin	24.5	22.0	0.98	1.00	0.58	0.70

*Std Min as per formulation specification.

**Results are on As Is basis.

Table 2.3 Analysis results for broiler Starter Crumbles (Farmer Samples).

Nutrient	Analysis Result (%)
Ca	1.08
P	0.45

*Results are on As Is basis.

The blood results for NCD, IBV or IBD showed antibody titer levels which were not consistent with those expected with field virus challenge. An evaluation of the symptoms presented by the birds suggested that this could be an outbreak of Marek's disease. In acute cases, there is a high morbidity and can result in high mortality rates. Marek's disease is also characterized by paralysis. Symptoms include swelling of the peripheral nerves; particularly the nerves of the leg (Miller Hatcheries, 2005). Affected birds become unable to reach food or water and the end result of this is death. During an outbreak of this disease, the chicks are also affected early in life and also get severe paralysis. A successful identification of this disease can be done based on post-mortem findings, as birds affected by Marek's disease have swollen nerves. The results of the microscopic examination showed a lack of pleocellular lymphocytic infiltrates in the peripheral nerve tissue and viscera. These findings excluded Marek's as a possible cause. The results of the bone analysis revealed a Ca: P ratio of 1.63:1, while the ash was only 31.0%. This is a deviation from the normal Ca: P ratio of 2:1 and it is actually a sign of hypocalcaemic (low Ca:P ratio) rickets. This would effectively result in a hypocalcaemic state which correlates well to the macroscopic evaluation and histopathological findings in these birds.

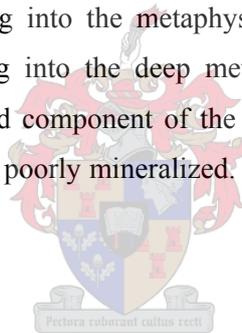
The inversed and low Ca:P ratio revealed by the blood serum analysis agree with the suggestion of Leeson & Summers (2001) that the low Ca:P ratio is the cause for hypocalcaemic rickets. Further on, Leeson & Summers (2001) suggest that the dietary Ca:P ratio greater than 2.2:1 may be necessary for the optimum bone integrity in young chicks. This ratio gets reduced as the birds get older. The symptoms of the chicks before death as well as the laboratory findings on the feed samples and the post-mortem results indicated that this was a case of rickets.

2.5 Microscopic Examination

A microscopic investigation conducted revealed the following:

2.5.1 Bones

The *tibia-tarsus* showed an extension of the proliferative zone of cartilage. There was also a marked hypertrophy of cells in the hyperplastic zone of the growth plate with thickening of the proliferative columns. Persistence of cartilage columns extending into the metaphysis of the *tibia-tarsus* was also noted. The cartilage columns were retained extending into the deep metaphysis with irregular border between the retained cartilage columns and the myeloid component of the marrow. Bone trabeculae were found to be very thin and irregular; they were also very poorly mineralized. All chicks sent for post-mortem examination showed these lesions.



2.5.2 Nervous tissue

Diffuse congestion was observed, with engorgement of meningeal and parenchymal vessels. Intramyelin oedema could be observed in the central white matter neurophil giving the white matter a spongiotic appearance. The peripheral sciatic nerves were found to be within normal histologically limits. There was no evidence of intramyelinic oedema or Schwann cell proliferation noted.

2.5.3 Heart tissue

The myocardium had diffuse congestion with engorgement of myocardial capillaries. There was interstitial oedema with tearing apart of myofibres. The heart contained a mixed inflammatory infiltrate consisting of heterophils, lymphocytes, plasma cells and macrophages (pericarditis).

The results of the post-mortem i.e. the histological observations confirmed that of the nutritional diagnosis. The imbalances of Ca and P seem most likely with the elongation of cartilage columns being the

predominant histological change in the growth plate of the tibiotarsus (R Last, personal communication). It has been discussed in previous sections that the imbalance between Ca and P and vitamin D₃ adversely affect bone formation as well as mineralization. The changes associated with excess Ca and deficient P include lengthening of cartilage columns, lining of columns by eosinophilic seams of cartilaginous material, disorientation of trabeculae and poor mineralization. In hypocalcaemic rickets, thickening of the epiphyseal plate is due to an accumulation of proliferating chondrocytes accompanied by a variable increase in the length of the perforating epiphyseal vessels (Whitehead, 1992). While in hypophosphataemic rickets, hypertrophic chondrocytes accumulate without any change in the epiphyseal vessels. When a Ca deficiency occurs, changes include persistence of epiphyseal blood-vessels, irregular junction of pre-hypertrophied and hypertrophied cartilage, variation in length, shape and orientation of cartilage columns (Whitehead, 1992).

The potential involvement of vitamin D could not be investigated in this study due to the lack of facilities that could perform this test. However, the findings in the details of the mixing process together with the symptoms of Vitamin D deficiency could lead to the suspicion that this vitamin could be deficient in the diets used in this particular farm.

Conclusion

In this study the feed and blood samples were found to be low in Ca and P giving an indication that it is the imbalance of the two minerals that caused rickets in this particular flock. Mono-Calcium Phosphate (MCP) was added manually at the mill where the feed was produced which could result in errors in terms of over- or under-weighing the P source. However, vitamin and mineral influences on absorption of Ca and P cannot be ruled out from the possible causes of the problem. The possible contamination with Mg of the limestone used for these diets could not be ruled out. As well as the potential deficiency of Vitamin D could not be identified. The findings from this study indicate that the cause of the outbreak in this farm was a case of rickets due to a Ca and P imbalance in the feed.

References

- Aburto, A and Britton, W. M.** (1998). Effects and Interactions of Dietary Levels of Vitamin A and E and Cholecalciferol in Broiler Chickens. *Poultry Science*. **77**: 666-673.
- Aburto, A and Britton, W. M.** (2001). Effects of Different Levels of Vitamins A, D₃ and E in Broiler Chicks. *Poultry Science Suppl.* **74**, S1.
- Association of Official Agricultural Chemists.** (1995). Official Methods of Analysis. 16th ed. Association of Official Agricultural Chemists, Washington, DC.
- Baker, D. H., Biehl, R. R., and Emmert, J. L.** (1998). Vitamin D₃ requirement of young chicks receiving diets varying in Calcium and available Phosphorus. *British Poultry Science*. **39**: 413 – 417.
- Bar, A., Rosenberg, J., Perlman, R. and Hurwitz, S.** 1987. Field Rickets in Turkeys: Relationship to Vitamin D. *Poultry Science*. **66**:68-72.
- Berry, W.** (2001). Use of Phytase in Broiler Breeder Feeds. *The Informed Poultry Professional*. Issue: **51**.
- Edwards, H. M., Jr.** (1992). Nutritional factors and leg disorders. Chapter 10, In: *Bone Biology and Skeletal Disorders in Poultry*. Editor C.C Whitehead. Carfax Publishing Co. Abingdon, Oxfordshire, England.
- Elliot, M. A. and Edwards, H. M.** (1992). Studies to determine whether an interaction exists among Boron, Calcium and Cholecalciferol on the Skeletal Development of Broiler Chickens. *Poultry Science*. **71**: 677-690.
- EVM (Expert Group on Vitamins).** (2002). Review of Boron. EVM/99/23.
- Fuchs, N, K.** (2002) Magnesium: A Key to Calcium Absorption. www.mgwater.com/calmagab.shtml.
- Gardiner, E. E., Rogler, J. C., and Parker, H. E.** (1961). Inter-relationships between magnesium and fluoride in chicks. *Journal of Nutrition*. **75**: 270 – 274.
- Hengmin, C. and Lingping, L.** (1997). Phosphorus deficiency heavily affects ducklings. *World Poultry-Misset* Volume 13, No.4.
- Hooge, D.** (2002). Poultry Nutrition and Immunity. *eDigest* Volume 2, No. 12.
- Hurwitz, S.** (1992). The role of vitamin D in poultry biology. Chapter 6, In: *Bone Biology and Skeletal Disorders in Poultry*. Editor C.C Whitehead. Carfax Publishing Co. Abingdon, Oxfordshire, England.
- Leach, R. M and Gay, C. V.** (1986). Role of Epiphyseal Cartilage in Endochondral Bone Formation. Department of Poultry Science and Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, PA 16802.
- Lee, S. and Britton, W. M.** (1980). Magnesium Toxicity: Effect on Phosphorus Utilization by Broiler Chicks. *Poultry Science*. **59**: 1989 – 1994.

- Leeson, S. and Summers, J. D.** (2001). Nutrition of the Chicken. 4ed. University Books, Guelphs, Ontario, Canada.
- Miller Hatcheries.** (2005). Marek's Disease in Chickens. Millerhatcheries.com.
- National Research Council.** (1984). Nutrient Requirements of Poultry. 8th Ed. National Academy Press, 2101 Constitution Ave. N.W. Washington, DC 20418.
- Newman, S and Leeson, S.** (1997). Skeletal integrity in layers at the completion of egg production. World's Poultry Science Journal. **53**: 265-277.
- Pillai, R. B., and Kailasam, K.** (1998). Flock Health. Poultry Times of India.
- Pines, M., Hasdai, A. and Monsonogo-ornan, E.** 2005. Tibial dyschondroplasia-tools, new insights and future prospects. World Poultry Science Journal. **61**:285-297.
- Riddell, C.** (1992). Non-infectious skeletal disorders of poultry. Chapter 8, In: Bone Biology and Skeletal Disorders in Poultry Carfax Publishing Co. Abingdon, Oxfordshire, England.
- Ross Tech.** 2001. Leg Health in Broilers. No.40. aviageninc@aviagen.com.
- Sanders, A. M. and Edwards, H. M. Jr.** (1990). The effects of 1.25- Dihydroxycholecalciferol on Performance and Bone Development in the Turkey Poult. Poultry Science. **70**:853-866.
- Sanders, A.M., Edwards, H.M. and Rowland, G.N.** (1991). Calcium and phosphorus requirements of the very young turkey as determined by response surface analysis. British Journal of Nutrition. **67**:421-435.
- Sebastian, S., Touchburn, S.P. and Chavez, E.R.** (1998). Implications of phytic acid and supplemental microbial phytase in poultry nutrition: a review. World's Poultry Science Journal. **54**: 27-47.
- Vaiano, S.A., Azuolas, J. K. and Parkinson, G. B.** (1994). Serum Total Calcium, Phosphorus, 1.25-Dihydroxycholecalciferol, and Endochondral Ossification Defects in Commercial Broiler Chickens. Poultry Science. **73**: 1296-1305.
- Waldroup, P. W.** (1995). Calcium and Phosphorus for Poultry Feeds. MITA (P), No 083/21/94. Vol PO 27.
- Whitehead, C. C.** (1992). Bone Biology and Skeletal Disorders in Poultry. Carfax Publishing Co. Abingdon, Oxfordshire, England.
- Xu, T. and Soares, J. H.** (1993). Poultry Science Suppl. **72**. No 109.