Effect of feeding zinc, copper and manganese complexed to two molecules of 2-hydroxy-4-(methylthio) butanoic acid on broiler performance, carcass and intestinal characteristics

By

Gareth Graeme Salmond

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Department of Animal and Wildlife Sciences
Faculty of Natural and Agricultural Sciences
University of Pretoria, Pretoria 0002
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Declaration

I, Gareth Salmond declare that the following dissertation, which I hereby submit for the degree MSc Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me at this or any other tertiary institution.

Signature:

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List of abbreviations

%: Percentage
$(\mathrm{HMTBA})_2$: 2-hydroxy- 4-(methylthio) butanoic acid
°C: Degrees Celsius
BW: Body Weight
BWG: Body Weight Gain
cm: Centimeter
Cu: Copper
CV: Coefficient of Variation
DM: Dry Matter
FCR: Feed Conversion Ratio
FI: Feed Intake
FPD: Foot pad dermatitis
g: Grams
GLM: General Linear Model
$HClO_4$: Perchloric Acid
HNO_3: Nitric Acid
IgA: Immunoglobulin A
m^2: Square meter
Mg/kg: Milligrams per kilogram
mL: Millilitre
Mn: Manganese
N: Newton
NRC: National Research Council
P: Probability
$P < 0.05$: Probability less than 5 percent
$P > 0.05$: Probability greater than 5 percent
RH: Relative Humidity
SE: Standard error
SO_4: Sulphate
uL: Micro litres
Zn: Zinc

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Abstract

Over supplementation of inorganic trace minerals is common practice in the poultry industry which often results in poor bioavailability and low body retention. This can lead to more than 90% of the dietary trace minerals being excreted. The trace minerals Cu, Mn and Zn have been shown to contribute to a healthy skin through their roles in the structural proteins of collagen, keratin and elastin. In this study the effects of replacing industry levels of inorganic Cu, Mn and Zn with lower levels of 2-hydroxy- 4-(methylthio) butanoic acid $(HMTBA)_2$metal chelates of Cu, Mn and Zn was investigated on broiler performance, carcass characteristics, skin and intestinal integrity and the prevalence of foot pad dermatitis.

Two 35-day grow out trials were conducted in an environmentally controlled broiler facility. The purpose of the first trial was to find a litter moisture level to be applied in a subsequent trial in order to simulate most commercial conditions where the prevalence of foot pad dermatitis is relatively high. In Trial 2 a total of 1920 Ross first grade day-old male broiler chicks were randomly distributed into 32 identical concrete floor pens. The dietary treatments in Trial 2 consisted of:

- Inorganic treatment (standard commercial trace mineral levels) containing 10, 100 and 100 mg/kg Cu, Mn and Zn, respectively (from inorganic sulphates)
- Negative control containing reduced inclusion levels of inorganic Cu, Mn and Zn at 8, 32 and 32 mg/kg, respectively (from inorganic sulphates)
- $(HMTBA)_2$ chelated Cu, Mn and Zn at inclusion levels of 8, 32 and 32 mg/kg (from only Mintrex® Zn, Mintrex® Cu and Mintrex® Mn, respectively)
- $(HMTBA)_2$ chelated Cu,Mn and Zn at inclusion levels of 30, 32 and 32 mg/kg (from only Mintrex® Zn, Mintrex® Cu and Mintrex® Mn, respectively).

Broiler performance results of body weight (BW), body weight gain (BWG), feed intake, mortality, and feed conversion ratio (FCR) adjusted for mortality were not significantly affected when broilers were fed lower levels of Cu, Mn and Zn (organic and inorganic) compared with broilers fed the commercial inorganic levels of these trace minerals over the entire trial period. Feeding Cu, Mn and Zn $(HMTBA)_2$ chelates at levels of 8, 32 and 32 mg/kg, respectively, resulted in the largest final mean body weight and body weight gain at 35 days of age, while maintaining a similar FCR. Carcass traits were mostly not affected by the different trace mineral treatments; whilst feeding 30, 32, and 32 mg/kg Cu, Mn and Zn $(HMTBA)_2$ chelates resulted in a significant increase in percentage of carcass yield in comparison to all other treatment diets. At 21 days of age the negative control treatment resulted in a significantly higher presence of foot pad dermatitis in comparison to all other treatments. At 35 days of age the feeding of Cu, Mn and Zn- $(HMTBA)_2$ resulted in a significant reduction of foot pad dermatitis and thus more saleable paws in comparison to the inorganic sulphate treatments. A lower average carcass scratch length and higher ileum strength were both observed from the

supplementation of $(HMTBA)_2$ chelates. The analysed Cu and Zn liver concentrations were significantly similar between the positive control, and the $(HMTBA)_2$ treatments ($P > 0.05$). The Mn liver concentrations were significantly higher for the positive control treatment in comparison to the $(HMTBA)_2$ treatments($P < 0.05$).

The dermis length and dermis area were significantly lower for the negative control treatment in comparison to all other treatments when comparing all grade 1 samples at 21 days of age. The epidermis length of foot pad skin samples were largest for the $(HMTBA)_2$ 8:32:32 treatment when comparing all foot pad dermatitis grade 1 samples taken at 35 days of age . The stratus corneum area and epidermis area increased from a foot pad dermatitis grade of 1 to grade 3 for samples taken at 21 and 35 days of age. At 35 days of age the grade 5 samples had the largest measurement for the stratus corneum area and epidermis area in comparison to all other grades.

From this study it may be concluded that organic trace minerals of $(HMTBA)_2$ metal chelates may be supplied in broiler rations at much lower levels than the currently utilised commercial levels of inorganic trace minerals without having any adverse effects on the performance and carcass characteristics of birds. The results suggest that organic trace minerals may provide a viable tool for improving broiler production and overall profitability through improved flock uniformity, improved foot pad health and increased intestinal strength.

Chapter 1

Introduction

Research and technology in the poultry industry has rapidly advanced in production and management of high performing birds. However, the trace mineral advancements have seemed to lag behind. The trace mineral requirements of broilers provided by the National Research Council (NRC) are outdated and rarely seen as accurate (Dibner *et al.*, 2007). Inorganic oxide and sulphate sources have predominantly been used in the past for trace mineral supplementation in broiler diets. The use of wide safety margins when formulating for trace minerals has been the most practical strategy due to the low cost of inorganic sources and the lack of accurate requirement data (Zhao *et al.*, 2008; Bao and Choct, 2009).

Recently much attention has been devoted to the use of organically complexed trace mineral sources because of the increased pressure from animal welfare organizations, environmentalists and rising production costs. The higher bioavailability of organic trace minerals has been observed to improve animal health and performance at lower trace mineral supplement levels, while also decreasing mineral excretion to the environment (Nollet *et al.*, 2007; Jahanian *et al.*, 2008; Zhao *et al.*, 2008; Richards *et al.*, 2010; Saenmahayak *et al.*, 2010).

The broiler industry is continually under pressure to maintain profits despite rising costs of feed, fuel and labour and also increasing export demands and import competition. This has resulted in the commercial broiler industry becoming an intensely run operation relying on improving tight margins. Downgrades associated with skin problems (cuts and tears) can result in considerable economic losses. Reducing these downgrades and condemnations at the processing plant is of vital importance in the improvement of profits. Skin lesions (such as scabs, sores and scratches) and resultant infections are mostly developed during grow-out and can all contribute to skin tearing during processing. Rossi *et al.* (2007) estimated that skin tears caused about 5 to 7 percent of the downgrades at the processing plant. Nutritional aspects can influence the formation and maintenance of skin integrity to a certain degree and therefore limit the economic losses due to skin tearing (Bilgili *et al.*, 1993; Rossi *et al.*, 2007).

The monitoring of foot pad dermatitis (FPD) relates directly to bird welfare, though it has an important secondary financial implication (Shepherd and Fairchild, 2010; Manangi *et al.*, 2012). A reduction in foot pad dermatitis is important for the welfare of birds, their production performance, prevention of secondary bacterial infections and the market demand for saleable paws (Bilgili *et al.*, 2009). The widespread problem of FPD seems to be caused and prevented mainly by management practices affecting the litter moisture, although the feed composition can also contribute towards FPD (Nagaraj *et al.*, 2007a).

The trace minerals Cu, Mn and Zn have been shown to contribute to a healthy skin through their roles in the structural proteins of collagen, keratin and elastin. These trace minerals also play a role in the immune status and intestinal health, oxidative stress management, bone development and maintenance and production performance in terms of growth and feed conversion (Saenmahayak *et al.*, 2010; Zhao *et al.*, 2010; Bun *et al.*, 2011; Manangi *et al.*, 2012). The 2-hydroxy- 4-(methylthio) butanoic acid $(HMTBA)_2$metalchelates of Cu, Mn and Zn improve micro-mineral retention and the availability of these trace minerals in the bird, thereby allowing improved skin integrity and strength compared to inorganic sources (Richards *et al.*, 2010; Saenmahayak *et al.*, 2010; Zhao *et al.*, 2010). Research indicates that the use of organically complexed trace minerals provides a viable tool in improving the economics, bird welfare and productivity of a financially pressurized broiler industry (Owens *et al.*, 2009; Saenmahayak *et al.*, 2010; Manang i*et al.*, 2012).

Organic trace minerals are a relatively new area of research. The bioavailability of the organically complexed trace minerals must be established and trace mineral inclusion rates must be redefined accordingly (Dibner *et al.*, 2007). The study objective was to evaluate the effect of$(HMTBA)_2$ metal chelates of Cu, Mn and Zn on broiler performance traits, skin and intestinal integrity and the prevalence of foot pad dermatitis. The $(HMTBA)_2$ chelated trace minerals were added to the diets at lower levels than the standard supplementation levels for inorganic minerals. The hypotheses were that:

- H0: Broilers that receive lower levels of Cu, Mn and Zn will have a lower production performance, skin and intestine integrity and a higher foot pad dermatitis incidence than the higher inorganic Cu, Mn and Zn levels.
- H1: The lower levels of Cu, Mn and Zn will be equally/more effective than the higher inorganic Cu, Mn and Zn levels in improving broiler performance, skin and intestine integrity and foot pad dermatitis.

Chapter 2

Literature review

Effects of organically complexed copper, manganese and zinc on broiler performance, skin and intestine quality and the prevalence of foot pad dermatitis

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Abstract

Skin disorders in broilers have increased rapidly in the last 30 years. Cu, Mn and Zn are critical in the synthesis and maintenance of skin and connective tissue structure and therefore influence the incidence of foot pad dermatitis and skin tears at processing. The use of organically complexed trace minerals is suggested to be a viable option in the effort to prevent substantial economic losses from foot pad dermatitis and other skin defects during processing. The higher bio available chelated minerals better supply the biochemical systems of the animal, leading to a wide variety of benefits. Studies have shown many benefits in replacing inorganic trace mineral sources with organic sources including improved immune development and response, oxidative stress management, tissue and bone development and strength and a superior tissue retention of minerals leading to reduced excretion rates. Foot pad dermatitis (FPD) is a type of contact dermatitis which is an ulcerative skin condition affecting the plantar surface of broiler feet. This condition is the major cause of downgrades and condemnations of saleable chicken paws. In addition, foot pad dermatitis is relevant to the broiler industry as it is an animal welfare concern, while severely affected birds show slow weight gains from pain induced loss in appetite, susceptibility to secondary infections and higher prevalence to hock and breast burns. Research suggests the incidence and severity of FPD is dominantly controlled by litter moisture, however, other nutritional, genetic and management factors also play contributing roles. Severe foot pad dermatitis can be kept below 2 percent through improving litter management, ventilation and nutrition.

Keywords: economic losses, bio available, foot pad dermatitis, downgrades, welfare, litter moisture.

2.1 Importance of copper, zinc and manganese for broilers

Dietary intake of trace minerals is essential for the wellbeing of broilers and is vital to allow the modern broiler to obtain its’ genetic potential for performance. Copper (Cu), manganese (Mn) and zinc (Zn) are involved in a variety of bodily functions, primarily as catalysts in many enzyme systems within cells or as a part of enzymes. These are also involved in healthy tissue development, immune defence and hormone secretion pathways (Bao and Choct, 2009; Aksu *et al.*, 2010). In the study of Bun *et al.* (2011), improved trace mineral nutrition in broiler diets enhanced the immune response to coccidiosis vaccination, increased intestinal integrity, lowered lesion scores, and improved performance of flocks.

2.1.1 Functions of zinc

Zn is certainly one of the most important trace minerals and has three essential biological functions, categorized as catalytic, structural and regulatory. Zn plays a catalytic role in the association of more than 300 enzymes. Zn participates as a component or cofactor of enzymes, which are important for growth, reproduction and carbohydrate and protein metabolism (Rossi *et al.*, 2007).Zn’s role in gene regulation is based on the incorporation of Zn in more than 1000 transcription proteins, which suggests alterations in mineral status will be translated into changes in gene expression (Bao and Choct, 2009). Zn is important for all aspects of immunity and is critical for the integrity of the cells involved in the immune responses (Bun *et al.*, 2011). Zn plays a vital role in epithelial cell production which is the first barrier of protection to infectious microorganisms. Zn also plays a role in T-cell function, thymocyte development and thymic integrity (Rossi *et al.*, 2007). Zn is suggested to act as an antioxidant; through increased synthesis and expression of metallothionein, a cysteine-rich protein which scavenges free radicals and through the action of superoxide dismutase (Huang *et al.*, 2009). Zn can also exert a direct antioxidant action through competition with iron and Cu to bind to the cell membrane and decrease the production of free radicals (Kidd, 2004). Decreasing the production of free radicals is not only important for a healthy immune system but also facilitates the maintenance of meat colour (Saenmahayak *et al.*, 2010).

Zn is required for the synthesis of two important structural proteins, collagen and keratin. Collagen is an important protein of the extracellular matrix and the internal connective tissues of cartilage and bone. Keratin is an important protein ensuring structural soundness of feathers, beaks, claws and skin. Zn is also involved in the cross-linking process of collagen, which provides the tensile strength of skin and plays a vital role in the healing process of wounds (Saenmahayak *et al.*, 2010). A deficiency of dietary Zn can lead to decreased collagen and keratin synthesis rates resulting in a variety of bone abnormalities, reduced tissue strength, poor feathering and dermatitis (Richards *et al.*, 2010). Deficiency may also lead to a reduced appetite, growth, immune response, gene regulation, and defence against antioxidant stress and damage (Richards *et al.*, 2010).

2.1.2 Functions of copper

Similar to Zn, Cu is important for a wide variety of health and performance-related functions in all animal species (Richards *et al.*, 2010). Cu is involved in cellular respiration, immune function, cardiac function, connective tissue development, bone formation, keratinisation and pigmentation of tissue; may indirectly affect haemoglobin biosynthesis as well as myelination of the spinal cord. Cu is mainly combined with the protein ceruloplasmin and it is also found in a large number of metallo-enzymes such as superoxide dismutase, cytochrome oxidase, lysyl oxidase, dopamine hydroxylase and tyrosinase (Bao and Choct, 2009). Functions performed by Zn are often enhanced by Cu-dependent enzymes. The Cu dependent enzyme lysyl oxidase, crosslinks collagen and keratin into mature protein forms, and also crosslinks the structural protein elastin found in connective tissue. Therefore Cu plays an important role in promoting skin, bone, tendon and intestinal strength (Richards *et al.*, 2010).

2.1.3 Functions of manganese

Manganese is important for correct bone growth both in the embryo and after hatch in broilers, for carbohydrate and lipid metabolism, immune system and nervous system function and reproduction. Mn functions as an enzyme activator and as a constituent of metallo-enzymes involved in glycolysation of proteins (Bao and Choct, 2009). Proper bone development is dependent on the proteoglycan matrix containing collagen and elastin. This matrix requires Mn for glycosylation of its protein core molecule. A Mn deficiency may lead to improper endochondral ossification, causing chondrodystrophy and perosis. Mn supplementation allows these defects to be prevented and corrected (Richards *et al.*, 2010).

2.1.4 Copper, manganese and zinc in immune functions

A deficiency in Zn can result in poor efficacy of vaccinations through limited antibody production, resulting in disease susceptibility, poor immunity, increased mortality and economic losses(Richards *et al.*, 2010).Cu, Mn and Zn are vital to the birds immune system defence through their participation in the antioxidant defence systems. The mechanism by which trace minerals exert their antioxidant action is not well defined, however, these minerals are all involved in the activity of superoxide dismutase and the synthesis of metallothionein, a free radical scavenger (Sahin *et al.*, 2009). Two forms of superoxide dismutase include a cytoplasmic Cu and Zn dependent form and a mitochondrial Mn dependent form. The Cu is involved in catalysis and the Zn is important for stability of superoxide dismutase. The superoxide dismutase enzymes form a first-line of defence converting oxygen radicals to hydrogen peroxide (which is a less toxic molecule), followed by conversion of hydrogen peroxide to water. Antioxidant compounds such as superoxide dismutase reduce the severity of infections by ameliorating the degree of intestinal lipid peroxidation (Bun *et al.*, 2011).

Deficiency in trace minerals can therefore result in increased lipid, protein and nucleic acid damage due to the depressed superoxide dismutase activity, leading to induced cell death (Richards *et al.*, 2010). In the study of Bun *et al.* (2011), activities of Cu–Zn superoxide dismutase and glutathione peroxidase were increased with increased Zn levels in both the challenged to coccidiosis and non-challenged groups of birds. Coccidiosis infection increased Zn requirements of broilers and an organic

Zn source might have exerted some anticoccidial effects by maintaining the oxidative balance (Bun *et al.*, 2011). Cell-mediated immune responses are thought to be an important factor for protection against coccidiosis. Sunder *et al.* (2008) reported that cell-mediated immune response was significantly higher in broilers supplemented with greater than 80 mg/kg of Zn than in those supplemented with less than 80 mg/kg of Zn. Secretory IgA of birds receiving dietary Zn supplementation was significantly higher than un-supplemented birds. Birds fed Zn supplementation excreted fewer oocysts in the excreta than those receiving no Zn supplement. The results of this study showed that improved Zn supplementation reduced oxidative stress and improved many immune responses independent of whether birds were healthy or challenged with E. Tenella (Bun *et al.*, 2011).

2.1.5 Trace minerals and bird bone growth

Cu, Mn and Zn are required for the development, growth and maintenance of healthy bones. The bone is a complexed tissue and its development and growth will influence the overall body growth of birds (Bao and Choct, 2009). Availability of these trace minerals are vital for the early development of bone due to the involvement of metallo-enzymes in the development of structural connective tissue (Dibner *et al.*, 2007).As described above, Cu and Zn play important roles in bone development via their actions on collagen. Cu is important for the cross linking of collagen and elastin, providing the tensile strength and elasticity of bone. Even though Zn is required for collagen synthesis, if Cu is limiting, the cross linking of fibrils will be poor and may result in a weak or non-existent structure (Dibner *et al.*, 2007). Lysyl oxidase, a Cu dependent enzyme, is responsible for bone strength and therefore Cu intake is a major determinant of bone strength (Bao and Choct, 2009). In addition to Zn's role in collagen synthesis, it is important in the regulation of hydroxyapatite crystallization, the changes in gene transcription that accompany ossification and the cellular invasion of the cartilage matrix by the osteoblasts (Dibner *et al.*, 2007). Mn-dependent enzymes play an important role in enhancing the formation of the proteoglycan matrix in the cartilage model for developing bone (Richards *et al.*, 2010). Mn deficiency may result in thickened long bones, perosis and tibial dyschondoplasia. Bones lacking Mn are therefore weak and can result in bone breaks (Bao and Choct, 2009). Trace minerals are therefore essential for bone flexibility, tensile strength, compressive strength and provision of a lightweight structural material.

2.2. Trace mineral requirements and supplementation

Historically trace mineral nutrition has lagged behind other areas of nutrition. Inorganic salts are typically supplemented at much higher levels than the recommended NRC guidelines for poultry diets to avoid deficiencies, firstly because of a lack of precise nutrient requirement data; and secondly because of their low cost compared to other nutrients (Bao and Choct, 2009). Trace mineral requirements for poultry were developed in the past based on research with inorganic trace minerals.

These requirement guidelines, especially from the NRC (1994) are outdated and only provide minimum levels for productivity with many areas where discrepancies prevail (Dibner *et al.*, 2007). Many studies have shown a much higher bioavailability of minerals in the sulphate form, the major mineral constituents in commercial broiler diets, in comparison to oxide sources. However, the sulphates may promote free radical formation from reactive metal ions due to the high water solubility of sulphates. This leads to degradation of vitamins, fats and oils, reducing the nutritive value of feeds. The oxides are much less reactive but are less bio available (Jahanian *et al.*, 2008). Many antagonisms cause variable and low bioavailability on minerals when using inorganic salts. The antagonism may be due to the influence of feed constituents and binding factors, macro minerals and even other trace minerals. For example as dietary calcium increases less Cu, Mn and Zn are absorbed. Phytate forms stable and highly insoluble chelates with minerals. There is also competition between absorption sites between Cu, Mn, Zn and iron (Dibner *et al.*, 2007). In the study of Mohanna and Nys (1999), 94% of the Zn ingested from a $ZnSO_4$ source by broiler chickens was excreted under normal commercial dietary conditions.

Even though the importance of trace minerals is well acknowledged, their requirements in practical broiler diets are not well defined and only tentative values are given because of the aforementioned complexities (Bao and Choct, 2009). The use of organic trace minerals appears to provide an answer to reducing trace mineral excretion and meeting broiler requirements in a more efficient manner. However, this is still a relatively new area of research, and requires trace mineral inclusion levels to be redefined for the modern fast growing broiler and higher availability of organic trace minerals (Dibner*et al.*, 2007).The organic Zn inclusion level for broiler chickens has been recommended at 60 mg/kg until 14 days old and 70 mg/kg thereafter. The requirements of other organic trace minerals such as Cu, Mn and Fe are more difficult to determine as it is impossible to produce growth deficiency symptoms under sufficient Zn diets. Filling these gaps of knowledge of inorganic and organic trace minerals is required for optimal mineral nutrition in broiler diets (Dibner *et al.*, 2007).

2.3 Importance of skin and intestine strength in broilers

Skin tears and carcass contamination can result in significant economic losses in broiler production. Rossi *et al.* (2007) estimated that skin tears caused about 5 to 7 percent of the carcass downgrades at the processing plant. In addition, skin defects may result in depressed productivity, decreased product wholesomeness and therefore loss of market value (Bilgili, 1990). The skin lesions, sores, scabs, scratches and/or underlying infections that most commonly develop during the grow-out phase, all contribute to skin tearing during processing. The above skin defects can be caused by poor management such as overcrowding, inadequate feed and drinker space and excessive bird activity or by the improper handling and catching of birds before slaughter.

Another important aspect determining the processing costs of broiler production is the removal of carcass contamination as a requirement of the Hazard Analysis and Critical Control Points plan for each abattoir (Buhr*et al.*, 1998).Carcass contamination is caused by the escape of digesta or faeces from a torn or cut in the digestive tract onto surrounding tissue. The resultant carcass reprocessing or carcass condemnation slows down the operations of the slaughter plant and increases production costs. This has led to considerable research been focused towards the development of new and improved methods which will reduce carcass contamination at the processing plant (Northcutt *et al.*, 1997).The commonly used method of feed withdrawal to empty out the digestive tract, decreases the likelihood of contamination during processing, however results indicate that feed withdrawal decreases tensile strength of the intestines (Zuidhof *et al.*, 2004). Tensile strength is a measure of intestinal integrity and the possibility for intestinal breakage during processing. Increasing the tensile strength of the intestinal tract provides a higher resistance to intestinal breakage at the processing plant, thereby reducing the need for reprocessing or condemnation. Bilgili and Hess (1996) showed that the tensile strength of the intestine was reduced by 19 to 22 percent as the feed withdrawal period was increased from 6 hours to 18 hours (Buhr *et al.*, 1998). Broiler processing plants often attribute carcass contamination to the tearing of the intestines during evisceration and increased fragility of the intestinal tract, instead of the amount of content within the digestive tract (Bilgili and Hess, 1997).The economic loss as a result of skin tearing and carcass contamination emphasizes the importance to optimise all nutritional aspects that may influence the formation and maintenance of tissue integrity (Rossi *et al.*, 2007).

2.4 Prevalence of foot pad dermatitis

2.4.1 Definition and prevalence in flocks

Foot pad dermatitis (FPD)is also known as foot pad lesions, ammonia burns and pododermatitis which are a type of contact dermatitis (Nagaraj *et al.*, 2007c). Contact dermatitis is an ulcerative skin condition which affects the plantar surface of feet, the breast and/or the hocks (Haslam *et al.*, 2007). FPD begins as a discolouration of the foot pad skin, which develops into hyperkeratosis and necrosis of the epidermis. Severe FPD can result in ulcers with inflammatory reactions of subcutaneous tissue (Kjaer *et al.*, 2006).These lesions commonly affect metatarsal pads but can also develop further to cover digital pads of feet. Foot pad dermatitis can form within one week, which further develops into ulcers. These ulcers range from superficial to deep depending on severity (Shepherd and Fairchild, 2010). The condition causes the skin of foot pads to become hard, inflamed, necrotic and scaly often with exudates, litter and faecal material covering the affected areas (Mayne, 2005). The prevalence of FPD varies widely between studies as a result of the multiple factors affecting FPD severity, including management, environment and genetics. In the studies of Ekstrand *et al.* (1997), Haslam *et al.* (2007) and Kapell *et al.*(2012) the mean prevalence of FPD in commercial broiler flocks was found to differ between 11 and 49 percent in European countries. Studies in Denmark and Netherlands suggest prevalence of FPD between 19 to 93 percent (Kjaer *et al.*, 2006; Ask, 2010). FPD plays an integral part in the economic and welfare situation of production systems (Shepherd and Fairchild, 2010). The disorder continues to significantly increase in magnitude, resulting in a great challenge in intensive broiler production. By improving or maintaining proper management and environmental conditions in broiler production, in particular litter management and ventilation, severe dermatitis can be maintained below 5 percent of flocks (Bessei, 2006).

2.4.2 Implications of foot pad dermatitis for the poultry industry

2.4.2.1 Economic implication

A large export market in Asia has resulted in tremendous price increases for the sale of unblemished paws. This has resulted in paws being the third most important component of chicken production, behind the breast and wings (Shepherd and Fairchild, 2010). In addition to the higher selling price, paw prices have remained relatively strong over the past few years while other products have declined in price. The value of chicken paws is entirely based on quality (grade A and B) and size(small, medium or jumbo). Many factors may cause paws to be downgraded at processing plants (such as bruising, cuticle remnants and processing mutilations); however FPD is by far the single highest causative factor (roughly 99 percent of downgrades) (Shepherd and Fairchild, 2010).FPD not only reduce income as there is no real market for grade B paws, but also affect performance and meat production of broilers. Therefore, the financial incentive to produce high quality paws at processing emphasises the importance of FPD prevention (Bilgili *et al.*, 2006).

Breast blisters and hock burns are forms of contact dermatitis and are suggested to share the same characteristics and causes to foot pad dermatitis. A positive correlation has been found between hock burns and the incidence of FPD (r =0.76) (Shepherd and Fairchild, 2010). However, lesions on the hocks and breast develop less frequently and at a slower rate. The association of these types of contact dermatitis result in higher proportions of product condemnation and downgrades (Bilgili *et al.*, 2006). FPD is a significant risk factor for the predisposition to the development of lameness; a serious welfare issue facing broiler production. Broilers normally are affected by FPD on both feet making them reluctant to walk, resulting in a pain induced decreased intake of feed and water with a depressed growth rate (Berg, 1998). Lesions are a pathway for bacterial or pathogenic infection, which can hinder broiler performance and result in partial or entire carcass condemnation (Zhao *et al.*, 2008). This gateway for bacteria allows bacteria to infiltrate the blood stream and cause joint inflammation and impairment of product quality in other ways. For example *Staphylococcus aureus* is a common cause of secondary infection in foot pad dermatitis. *Staphylococci* can cause a number of diseases and from the production of enterotoxins can result in food poisoning in humans (Berg, 1998; Bilgili *et al.*, 2006).

FPD is a concern for the poultry industry in terms of food safety, animal welfare and product downgrades. For improved profits broiler production systems need to understand how foot pad dermatitis develops and preventative strategies should be considered (Shepherd and Fairchild, 2010).

2.4.2.2 Animal welfare issues

FPD has recently grown in stature for the characterization of flock health and welfare (Kjaer *et al.*, 2006). In Europe and the United States FPD is used as an objective audit criteria for the assessment of poultry welfare (Bigili *et al.* 2006; Bilgili *et al.* 2009; Shepherd and Fairchild, 2010; Kapell *et al.*2012). Welfare is defined as the ability and state of animals to cope with its' environment which could result in injury or disease; including a pleasurable or unpleasant state of mind. Foot pad dermatitis are suggested to cause pain, depressed health and even lameness in birds, representing a welfare issue (Bessei, 2006).There is evidence that the severity of FPD reflects litter and air quality in broiler houses, indicating additional welfare issues other than pain and lameness (Haslam *et al.*, 2007). The diagnosis of FPD is essential as not only does it identify a potential welfare problem but can alert the producer of management problems such as litter management and feed imbalances that determine overall profitability.

2.5 Possible causes of foot pad dermatitis

The etiology of FPD is extremely complex because of the multiple factors contributing to its' incidence and severity. Potential causes of FPD may be classed into three main categories; management and environment factors (such as litter management, climate and season, stocking density, drinker design and ventilation), factors concerning the animal itself (breed, sex, age and health) and nutritional causes (feed ingredients, mineral deficiencies and imbalances). Many of these factors can cause confounding results in FPD studies through their direct and indirect influences (Shepherd and Fairchild, 2010). In addition to the many contributing factors, FPD is complicated by the difference in severity and time taken for these factors to ascertain a result. The study of Mayne *et al. (*2007b) suggested the external signs of FPD may be observed within 48 hours in poor environmental conditions. The following contributing factors all play a role in the development of FPD.

2.5.1 Management and environmental factors

2.5.1.1 Litter

Litter type, quantity and quality are significant contributors to FPD, health and performance of broilers as they spend almost their entire life in close association with the bedding. The management of litter involves the continual weighing of replacing built up litter against the cost and availability of alternate litter (Bilgili *et al.*, 2009).

2.5.1.2 Litter moisture

Many studies have suggested litter moisture as the single most important cause of FPD (Youssef *et al.*, 2011a). Wet litter predisposes the birds to FPD as it causes the foot pads to be softer and therefore more prone to damage (Shepherd and Fairchild, 2010). An increase in FPD severity is observed with increasing litter moisture, particularly in the presence of sticky faecal droppings. Drying out the litter or replacing the bedding with dry litter results in improved foot pad health (Shepherd and Fairchild, 2010). Adverse effects on broiler health and FPD are commonly observed once litter moisture exceeds 35 percent. Increased moisture levels potentially increase the production of irritant substances such as ammonia (Nagaraj *et al.*, 2007a). Many factors can cause an increase in litter moisture and even more sticky conditions. These include intestinal disorders and diseases (that may induce enteritis, diarrhoea and malabsorption), forced water intake, poor drinker management, high relative humidity and low temperatures, stressful conditions (for example fear, overcrowding or abrupt environment change) and poor litter material. Dietary composition can affect the litter quality through the amount and constituents of the excreta which affect the moisture content and pH of the litter (Nagaraj *et al.*, 2007a; Haslam *et al.*, 2007). Some studies indicated FPD is caused by a combination of high litter moisture, high ammonia levels and other chemical irritants in excreta and litter. Wet litter results in a higher level of volatile ammonia that may contribute to FPD development (Youssef *et al.*, 20011a). Studies have however reported that wet litter alone caused severe foot pad dermatitis, indicating that excreta may not be required (Allain *et al.*, 2009; Youssef *et al.*, 2009).Although majority of research indicates litter moisture is a vital causative agent of FPD, a few studies have found no or insignificant effect of litter moisture on severity of FPD (Eichner *et al.*, 2007; Nagaraj *et al.*, 2007b).

2.5.1.3 Litter material

Variations in bedding availability between and within regions alter the type and quality of bedding utilised in broiler production systems. The most essential characteristics for a superior litter material are the ability to absorb and release moisture (drying time) (Bilgili *et al.*, 2009; Shepherd and Fairchild, 2010).The study of Bilgili *et al. (*2009) suggested that prevalence of FPD increased with higher litter moisture and caking scores. Chipped pine, chopped straw and pine shavings showed the highest severity when compared to mortar sand and ground door filler. The higher moisture binding ability of ground door filler and quick moisture release of mortar sand were the likely reasons for this. Bedding material consisting of sharp edges such as chopped straw and large wood chips may increase incidence of foot pad dermatitis through their abrasive action compared to a soft physical form of lignocellulose (Bilgili *et al.*, 2009).

2.5.1.4 Litter depth

The effect of litter depth has provided inconsistent results. Flocks reared on a thin layer of litter, less than 5cm thick, showed a lower prevalence of FPD compared to flocks on a litter depth greater than 5cm. A thin layer of litter is probably less compact and may result in a greater particle turnover from birds scratching, which improves the ventilation and drying of litter material (Shepherd and Fairchild, 2010).Meluzzi *et al. (*2008b), however, indicated a lower incidence of foot pad dermatitis reared on thicker layers of litter material. This might have been the result of increased material for absorption of the moisture. Inconsistencies between studies may be linked to the differences in the physical and quality properties of the tested materials.

2.5.1.5 Drinker design and management

Drinker design can directly influence litter moisture and therefore the incidence and severity of FPD. Nipple drinkers have become popular in the broiler industry over recent years. Nipple drinkers improve the hygiene of water; reduce water spillage and wastage through evaporation. Shepherd and Fairchild (2010) indicated that nipple drinkers with drip cups are most efficient and result in superior litter conditions when compared to nipple drinkers alone or bell drinkers. The disadvantage of nipple drinkers is the increased time taken for drinking, which may constrain water and therefore feed intake in some individuals. Optimal management of water lines such as correct pressure, height settings, regular flushing and sanitation of water lines can help decrease leakage, thereby keeping litter drier (Mayne *et al.*, 2007b; Shepherd and Fairchild, 2010).

2.5.1.6 Stocking density

The optimum density for broiler production is continually debated among producers, breeders and welfare auditors. Optimum density is determined by profit margins, which reflects a much higher density than recommendations from welfare and individual broiler performance (Bilgili *et al.*, 2009). Published research consistently demonstrates that the welfare and health of birds are adversely affected if stocking density increases above 34-38 kg/m^2 depending on final body weights (Estevez, 2007). Negative consequences of a high stocking density include lower growth performance, feed intake, carcass yield, feed conversion, locomotion; and higher litter moisture, ammonia production, heat stress, skin scratches, foot pad dermatitis and condemnations (Estevez, 2007;Bilgili*et al.*, 2009). The recent study of Estevez (2007) indicates that a higher stocking density beyond 30 kg/m^2 results in a decline in BW (because of a reduction in feed intake), increased incidence of food pad dermatitis and skin scratches. The greatest cause for the increased incidence of FPD with an increasing stocking density is the pronounced deterioration of litter conditions. In the study of Bessei (2006) litter condition rapidly declined and litter moisture significantly increased as stocking density increased. Even though litter quality is difficult to maintain at high stocking densities, many authors have concluded that as long as environmental conditions are precisely maintained stocking density has minimal adverse effects (Shepherd and Fairchild, 2010). In contradiction, the study of Estevez (2007) indicated that above 30 kg/m^2, even with very accurate environment control, a steep rise in frequency of problems is seen. Therefore it is suggested that good health, profit and welfare can be achieved in the broiler industry between a range of densities, most probably between 34-38 kg/m^2. Although a high proportion of research indicates the association of higher stocking densities with increased FPD levels(McIlroy *et al.*, 1987; Ekstrand *et al.*,1997; Haslam *et al.*, 2007; Meluzzi *et al.*, 2008b), other studies have suggested that stocking density plays a minor or even no role in the development of FPD (Sirri*et al.*, 2007; Meluzzi*et al.*, 2008a).

2.5.1.6 Climate

The weather and therefore season of year has been suggested to play a contributing role in development of FPD. FPD is closely correlated to the relative humidity inside and outside of the house. High relative humidity can adversely influence litter conditions. Litter moisture can be increased by condensation that occurs when temperatures are very low and RH is high. Air temperature and humidity are influenced by season and ventilation system (Alchalabi, 2002). Common practice in winter months is the decreased ventilation rates to avoid low temperatures in houses and minimizing heating costs (Shepherd and Fairchild, 2010). This causes an increased RH inside the house. For the above reasons the incidence of FPD is found to be much greater in winter months than summer months (Greene *et al.*, 1985; McIlroy *et al.*, 1987; Haslam *et al.*, 2007; Meluzzi*et al.*, 2008a). The close association between relative humidity and temperatures makes it difficult to determine which of the two are of greater importance. Although a significant number of published studies have observed higher incidence of FPD in winter months or cold conditions, other research has disagreed with this (Shepherd and Fairchild, 2010).

2.5.2 Nutritional factors

The diet of the broilers is a major contributor alongside poor litter conditions in the development of FPD. Different dietary factors influence the incidence and severity of FPD in a variety of ways (Youssef *et al.*, 20011b). Nutrition has a direct influence on faecal droppings and litter moisture which both play important roles in the development of FPD (Shepherd and Fairchild, 2010).

2.5.2.1 Protein level and source

A high protein level and a diet based on all vegetable/ plant based proteins may increase the incidence and severity of foot pad dermatitis. In contrast, broilers fed a low protein diet with the inclusion of animal proteins showed a very low incidence of FPD (Nagaraj *et al.*, 2007b; Shepherd and Fairchild, 2010). Excess crude protein in the diet increases the uric acid excretion, leading to high moisture excreta rich in nitrogen. The consequence is higher ammonia production and wetter litter which predisposes birds to FPD. Higher ammonia released may not only act as an irritant to the skin of broilers but also adversely acts on their respiratory tract. Few studies have suggested controversial results regarding the association between ammonia levels released from the litter and incidence of foot pad dermatitis (Nagaraj *et al.*, 2007a). Inclusion of soybean meal (the most popular commercial protein source) at high levels (>40 percent) in the broiler diet is suggested to cause severe foot pad dermatitis (Youssef *et al.*, 20011c). Soybean meal contains a high carbohydrate level, often exceeding 30 percent of its total dry matter. It is the indigestible carbohydrates known as non-starch polysaccharides (NSPs), in soybeans that are responsible for causing FPD. Oligosaccharides in soya accelerate feed passage while the pectin's and hemicelluloses fractions of NSP are responsible for increased digesta viscosity and lowered nutrient availability (Youssef *et al.*, 20011c). Increased NSP intakes lead to watery/sticky faecal droppings and higher litter moisture. Sticky manure adheres to the feet of birds; causing an enhanced deterioration of their epidermis and keratin layers (Shepherd and Fairchild, 2010). The study of Nagaraj *et al.* (2006; 2007b, 2007c) suggested that the higher prevalence of FPD in broilers fed on high soybean meal may be the result of increased digesta viscosity and an increased nitrogen excretion. Soybean meal may also contribute to FPD through its high content of potassium which increases water intake (Eichner *et al.,* 2007).

2.5.2.2 Grain sources

NSPs are also found in high concentrations in wheat, triticale and barley, amongst others. The anti-nutritional effects of NSPs include the encapsulation of the starch and protein by cell wall NSPs. The more relevant effect to this study is related to the higher digesta viscosity. Increasing inclusion of these grains in diets not only may lower ME values but increases manure adherence to foot pads by increasing digesta viscosity. Shepherd and Fairchild (2010) suggested that viscosity increased simultaneously with the increased inclusion of wheat in the broiler diet. Viscosity was 28.71 percent higher when 20 percent wheat was included while at 40 percent wheat, viscosity increased to 53.07 percent.

2.5.2.3 Mineral elements and imbalances

Many dietary factors can increase water intake and thus litter moisture; however in common ingredient formulations electrolytes play a major role (Eichner *et al.*, 2007). Macro minerals, especially the electrolytes sodium and potassium, are suggested to influence the incidence of FPD through their effects of increasing water intake, causing higher excreta and litter moisture. In addition excretion of chemically irritant minerals may irritate foot pad skin. High levels of sodium, potassium, phosphorus and magnesium (not calcium) increased water intake and litter wetness, predisposing birds to foot pad dermatitis (Eichner *et al.*, 2007; Bilgili, 2009). The studies of Murakami *et al.* (2000 and 2001) observed significant increases in water intake and litter moisture at a sodium level of 2.50 g/kg in the diet; while the study of Youssef (2011b) showed similar outcomes when increasing potassium in the diet to 12g/kg. Formulating for sodium is important as performance traits can be significantly improved through supplementation, however, care is required not to increase water intake, excretion and litter moisture to adverse proportions.

2.5.2.4 Nutritional deficiencies

Amino acids such as methionine, lysine and cysteine, vitamins such as biotin and riboflavin and also trace minerals such as Cu, Mn and Zn are involved in the formation and maintenance of the skin. Deficiencies of these nutrients can therefore predispose birds to the incidence of skin lesions and tears (Youssef *et al.*, 20011d). The study of Shepherd and Fairchild (2010) suggested that the supplementation of Zn and biotin significantly decreased FPD when birds were kept on dry litter, but this result was not found when birds were reared on wet litter. It was suggested that deficiencies in Zn and biotin are therefore not solely responsible for FPD and rectifying a deficiency is not as effective for birds reared in conditions that directly increase the development of foot pad dermatitis.

2.5.2.5 Feed manufacturers

An effect of feed supplier was identified in the study of Ekstrand *et al.* (1998). The feed manufacturer may influence FPD through feed quality and ingredients as discussed above. This will affect the faecal consistency and constituents, which influences litter moisture and pH. The skin integrity can directly be influenced through many micronutrient dietary levels (Haslam *et al.*, 2007).

2.5.3 *Animal factors*

2.5.3.1 Genetics

Clear differences were demonstrated in the prevalence of FPD when different commercial breeds at the same age were compared (Ekstrand *et al.*, 1998; Berg, 2003), while a few studies failed to show significant differences (Ekstrand *et al.*, 1997). Evidence of genetics playing a role in FPD was demonstrated in studies that reported reduced incidence of FPD and hock burns through genetic selection (Kjaer *et al.*, 2006; Allain *et al.*, 2009; Ask, 2010). Some studies on turkeys suggested that in very fast growing strains, the skin of the birds may have insufficient time to mature and adequately strengthen to carry the large body weights of the birds (Breuer *et al.*, 2006).

2.5.3.2 Age

Welfare conditions such as reduced litter conditions and increased kg per m^2deteriorate towards the end of the rearing cycle. This is also when significant increases in severity and incidence of FPD are seen (Shepherd and Fairchild, 2010). Mcilroy *et al.*(1987) found that when litter deteriorated drastically in a house the severity of hock lesions and breast blisters increased by 33 percent and more than trebled respectively.

2.5.3.3 Sex and body size

Some researchers found that females have a higher tendency to develop FPD compared to males (Kjaer *et al.*, 2006; Shepherd and Fairchild, 2010). Other studies however, have demonstrated a higher prevalence in male broilers (Bilgili *et al.*, 2006; Nagaraj *et al.*, 2007b; Shepherd and Fairchild, 2010). A higher incidence of foot pad dermatitis may be caused by a higher growth rate and higher body weight. Males are heavier than females and therefore a greater physical pressure is placed on their feet. The inconsistent effect of body weight and sex on FPD may suggest that these factors are not significant causes of FPD. However Ask (2010) suggested that if body weight continues to increase without including FPD into the breeding program, incidence of FPD will greatly increase in the future.

2.5.3.4 Gastrointestinal infections

The health of the intestinal tract can indirectly influence FPD through increased litter moisture. Diarrhoea can result from bacterial infections, viral infections and intestinal parasites. *E. coli* and *Clostridium perfringens* among others are commonly related to enteritis in chickens. Common viruses associated with occurrence of wet litter include Rotavirus, Adenovirus and Reovirus. Parasites such as coccidia, *Histomonasmeleagridis*, Ascaridia and also mycotoxins may all cause intestinal disease (Custodis and Hafez, 2007).

2.6 Prevention of FP lesions

2.6.1 Control of litter moisture

Litter moisture is seen to be the dominant factor in the cause of incidence and severity of foot pad dermatitis. Many studies have suggested litter moisture be maintained below 30 percent in order to minimise FPD (Youssef *et al.*, 2011a). Good litter control is a key area for minimizing and preventing FPD and can even result in rapid healing of already formed lesions. In broilers transferred from wet to dry litter material, lesions healed within two weeks (Mayne *et al.*, 2007b). Such a situation is extremely rare in commercial flocks, except when flocks are sometimes thinned as birds are sold (Youssef *et al.*, 2010).Litter moisture should be controlled by proper litter management strategies including appropriate litter depth, regular assessment of litter material, regular turning, regular top dressing and/or regular replacement of wet litter with fresh litter. Running fans may help to dry out the litter faster. Litter material used should be clean, have a high water-holding capacity and be free of dust and hard sharp edges (Berk, 2007). Wood shavings have proven to be a superior bedding material because of its’ high water absorption capability (Shanawany, 1992). Management should look to optimise the environment, nutrition and enteric health in order to maintain sufficient litter conditions. Litter moisture can be improved through correct ventilation and heating to maintain house temperature and relative humidity (Haslam *et al.*, 2007). Good ventilation does not only ensure fresh air flow through the house but also prevents humidity and moisture build up inside the house. Particular attention should be given to ventilation and relative humidity during winter months. The study of Weaver and Meuerhof (1991) showed birds reared at a RH of 45 percent resulted in three times less FPD in comparison to birds reared at 75 percent RH. Proper drinker management, such as the use of nipple drinkers, height adjustments and water line sanitation, is also necessary to control litter moisture (Berg, 1998).

2.6.2 Application of litter amendments

Litter amendments offer an extra tool to improve and control litter conditions while also reducing ammonia volatilization (Parsons, 2006). Examples of litter amendments include applications of sodium bisulphate, aluminium sulphate, ferric sulphate and propionic acid. The acidifiers are most commonly used which act by decreasing litter pH as well as inhibiting growth of ammonia producing bacteria thereby reducing ammonia volatilization. Nagaraj *et al.* (2007a) found that the addition of $NaHS0_4$ to litter lowered the incidence and severity of FPD.

2.6.3 Nutritional aspects

Sound knowledge of the condition of FPD is essential for feed formulations and manufacturers. A good relationship between the broiler producer and feed manufacturer is vital in order to discuss the problems and subsequent solutions relating to the health and performance of birds. High nutrient dense feeds and feeding ingredients at high inclusion levels that contain high levels of NSPs without the inclusion of complementary exogenous enzymes should be avoided. Protein levels should be cautiously balanced to precisely meet requirements as excess protein may increase PFD incidence (Nagaraj *et al.*, 2007b). The inclusion of maize gluten meal and/or animal proteins has been found to reduce the severity and incidence of FPD compared to an all-vegetable diet (Saenmahayak *et al.*, 2010). Minerals such as sodium, potassium and chloride in the diet should be balanced and not excessively supplied. In conditions where bird density is high or ventilation is insufficient, reducing sodium levels to resist increased litter moisture can be advantageous. Therefore dietary sodium should be decreased to effectively control litter moisture and thereby FPD incidence.

2.6.4 Dietary prophylaxis

Dietary supplementation of certain nutrients in the diet may reduce and prevent FPD. The most significant additives include biotin, Zn, enzymes and prebiotics (Youssef *et al.*, 2011d).

2.6.4.1 Biotin

Biotin is a cofactor for many enzymes in protein synthesis, fatty acid synthesis and carbohydrate metabolism and is therefore important for the formation, maintenance and repair of skin. It is well documented that signs of a biotin deficiency usually include scaly dermatitis lesions. Some studies demonstrated that biotin supplementation lowered FPD incidence. A high biotin level in diets of young birds may possibly prevent FPD development, whereas once lesions have become more severe remedy via biotin supplementation is difficult (Youseff *et al.*, 2011d). According to Mayne (2005), biotin may be more effective in preventing FPD in birds reared on dry litter than on wet litter.

2.6.4.2 Zinc

Zn plays an integral role in skin health, bird health and bird growth and development. A Zn deficiency (less than 30 mg/kg) has been suggested to induce a higher incidence of foot pad dermatitis(Youssef *et al.*, 2011d). Zn supplementation can significantly alleviate FPD under poor environment conditions. The study of Bilgili (2009) reported that organic dietary Zn reduced the incidence and severity of foot pad dermatitis under conditions of high stocking density.

2.6.4.3 Enzymes

The use of exogenous enzymes have resulted in many benefits including enhanced bird performance, feed conversion and also decreased environmental problems (Saenmahayak *et al.*, 2010).The suggested decrease in FPD is a consequence of the reduced nitrogen excretion, ammonia emissions, decreased water excretion and reduction in digesta viscosity. Exogenous glycanase added to broiler diets, cleave NSPs into smaller polymers, removing their ability to form viscous digesta and improving diet digestibilities. Recently the use of exogenous enzymes for use in maize-soybean based diets have risen tremendously (Nagaraj *et al.*, 2007c). The use of these exogenous enzymes in soybean meal based diets has improved digestibility of the complex carbohydrates in soybean meal which are poorly digested by endogenous enzymes. Research suggests that the sticky and wet excreta conditions are significantly reduced, therefore removing the predisposition to FPD. Examples of enzymes on the commercial market include protease, carbohydrase, α-amylase; and multi-enzyme preparations containing xylanase, arabinofuranosidase, β-glucanase, glucosidase, galactosidase and cellulose (Nagaraj *et al.*, 2007c).

2.6.5 Enteric health

Maintaining gut health is essential in the prevention of FPD as it is important for the maintenance of dry and good quality litter. Good management and environmental conditions is the best method of maintaining a healthy gut. These include adequate sanitation and biohazard programs, regular assessment of flock performance and proper vaccination and coccidial programs. In addition to these the use of feed additives may enhance gut health such as the use of exogenous enzymes and prebiotics such as mannan-oligosaccharide (Hooge, 2003).

2.6.6 Genetics

It has been suggested that through genetic selection the incidence of FPD can be reduced (Kestin *et al.*, 1999; Kjaer *et al.*, 2006; Allain *et al.*, 2009; Ask, 2010). The study of Ekstrand *et al.* (1998) reported different prevalence in FPD among different strains. Kjaer *et al.* (2006) found a moderate heritability of FPD and a low genetic correlation to body weight indicating genetic selection for FPD can be significantly worthwhile. Therefore, it appears as if strains that are less susceptible to FPD can be selected and used.

2.7 Effects of chelated trace minerals

2.7.1 Definition and description

In recent years much research has focussed on the effects of organic trace minerals in broiler diets replacing the inorganic mineral forms. An organic mineral is a combination of a metal ion with an organic ligand such as amino acids, proteins, polysaccharides, yeast, or organic acids. Different compounds are commercially available including metal proteinates, metal-amino acid complexes, metal amino chelates, metal yeast complexes, metal-polysaccharide complexes and metal-organic acid complexes (Patton, 1990). The metal ion binds to the organic ligand through ionic or covalent bonding, with the metal ion in a central position of the formed structure. The organic minerals can be formed naturally during normal digestion and metabolism in birds while synthetic mineral complexes are supplemented to diets to enhance mineral absorption across the intestinal mucosa. Efficacy of organic mineral complexes rely upon the stability constant, which is required to be high enough for intact absorption of the complex and low enough for removal at the metabolic utilization point (Hess *et al.*, 2001). Interactions among these factors are the likely cause of the many variable and contradictory research trial results on organic mineral bioavailability (Owens *et al.*, 2009). However, a well-defined effective increase in mineral bioavailability and tissue retention is weighted in favour of organic mineral use for poultry (Hess *et al.,* 2001; Bao and Choct, 2009; Aksu *et al.*, 2010). The organic forms of Cu, Mn and Zn are widely used in poultry production.

2.7.2 Comparison and effects of replacing inorganic trace minerals with organic sources

The major advantage of organic trace minerals is there stability in the upper gastro intestinal tract. Organic trace minerals resist dissociation in the crop, proventriculus and gizzard allowing the supplemented trace mineral to be delivered to the absorptive epithelium of the small intestine. This is in comparison to the dissociation of inorganic salts in the upper tract which can lead to it binding other minerals, nutrients and non-nutritive components of digesta such as fibre and phytase. These resulting insoluble forms are excreted. During chelation the positive charge of minerals is shielded. Therefore the mineral avoids binding to the negative charged mucin layer and leads to less competition between minerals of a similar charge in their uptake from the gut and transfer to the enterocyte (Nollet *et al.*, 2007). Increased availability of organic Cu, Mn and Zn compared to inorganic forms has been widely demonstrated leading to improved dietary formulations and broiler performance (Dibner *et al.*, 2007).Many studies have indicated that organically complexed trace minerals are better absorbed and utilised than their inorganic salts or oxides, providing a pathway to benefit the environment without compromising bird performance (Bao and Choct, 2009). The study of Bao and Choct (2009) showed that organic complexes of elements were at least 30 percent higher in bioavailability and excretion was significantly reduced compared to their inorganic forms when fed to broilers. Bioavailability refers to the absorption, transportation to the site required and conversion to a physiologically active form (Owens *et al.*, 2009). Organic complexes utilising lysine and methionine as a ligand have been preferred because of their potential higher bioavailability. Zn-methionine fed to chicks on a predominantly maize-soybean diet had a 206 percent bioavailability, while Zn oxide showed a 61 percent bioavailability relative to Zn sulphate at 100 percent. Jahanian *et al.* (2008) suggested Mn-methionine chelates to be 30-40 percent more bio available than the oxide source. Combinations of metal-amino acid sources and sulphates of trace minerals may result in improved retention, such as an increase in tibia Zn when Zn-Amino Acid and Zn sulphate were supplied (Burrell *et al.*, 2004). More absorption sites or transporters in the intestine might be involved when both organic and inorganic Zn sources are supplied which could explain the higher bioavailability (Burrell *et al.*, 2004).

2.7.3 Benefits on performance and environment

Over supplementation of inorganic trace minerals is a common practice in the broiler industry as a result of the large safety margins used to prevent deficiencies and enhance performance (Zhao *et al.*, 2008). This practice of over supplementation exacerbates the poor trace mineral bioavailability and low body retention, leading to over 90 percent of the supplemented trace minerals being excreted into the environment. The excretion and accumulation in litter of such trace mineral levels can result in toxicity to plants and subsequently the animals feeding on these plants or the chicken litter (Zhao *et al.*, 2008). High mineral supplementation leads to wasteful practice and environmental contamination. Concerns about mineral pollution continue to increase, with research concentrating on the alleviation of this problem. The supplementation of higher bioavailable organically chelated minerals at lower concentrations seems to provide the answer on how to reduce mineral excretion without negatively affecting the performance of animals (Aksu *et al.*, 2010). The study of Zhao *et al.* *(*2008) compared high inorganic concentrations of Cu and Zn to lower concentrations of amino acid chelates. Faecal Cu and Zn excretion was significantly reduced using amino acid chelates while production performance of birds was not adversely affected. The use of chelated trace minerals in trials with their higher bioavailability has translated into many beneficial outcomes. These include enhanced immune and intestinal health, growth and body weights, feed conversion; improved tissue and bone development and integrity, decreased foot pad dermatitis, improved oxidative stress management, processing yield and meat quality characteristics (Zhao *et al.*, 2008). In addition, these improved performance traits were achieved with lower levels of trace minerals compared to inorganic sources, lowering their excretion levels (Richards *et al.*, 2010). In the studies of Dibner *et al.* (2007) and Richards *et al.* (2010), the supplementation of diets with chelated trace minerals Cu, Mn and Zn, resulted in reduced tibial dyschondoplasia, increased bone breaking strength and reduced lameness. The study of Bun *et al.* (2011) indicated organic Zn sources (Zn methionine and $HMTB_2$ chelated Zn) enhanced cellular or antibody responses to vaccination. In addition, chelated minerals resulted in lower levels of lipid hydroperoxides in the blood indicating lower oxidative stress in birds. This is of significance as poor oxidative stress and vaccination response can lead to enormous economic losses through reduced performance and meat quality, compromised immune response and increased morbidity (Richards *et al.*, 2010).

2.7.4 Role of chelated Cu, Mn and Zn on skin integrity, intestinal integrity and foot pad dermatitis

Cu, Mn and Zn are critical in the synthesis and maintenance of epithelial and connective tissue structure and therefore influencing incidence of lesions and tears at processing (Manangi *et al.*, 2010). Zn is required for the synthesis of collagen, keratin, nucleic acid of the skin, the cross-linking process of collagen, a role in epithelial cell layers and is important for wound healing (Richards *et al.*, 2010). Cu is critical for the cross-linking of elastin and collagen which gives tissue its strength and elasticity (Zhao *et al.*, 2008).Collagen is the major fibrous constituent of skin and intestine, providing it's' flexibility and resilience allowing protection from tearing during handling and processing of broilers (Rossi *et al.*, 2007; Saenmahayak *et al.*, 2010). Broilers fed higher organic Zn resulted in increased collagen content, collagen turnover rates and number of epithelial cell layers causing increased epithelial tissue strength. Skin and intestine strength plays a major role in animal health and post-mortem processing. The increased collagen content in birds fed increased levels of organic trace minerals helps to tightly hold cells together in the tissues (Rossi *et al.*, 2007). An increased number of epithelial cell numbers at the body surface of birds provides increased protection against tearing and invasion by pathogens (Rossi *et al.*, 2007).

Due to the role of Zn in immune response, it has been reported that increasing dietary organic Zn reduces inflammation of skin (Rossi *et al.*, 2007). The above discussion suggested that the use of organic chelated trace minerals is preferred for high skin and intestine strength and integrity, while enhancing foot pad health (Zhao *et al.*, 2008). Many studies have resulted in improved skin quality and foot pad lesion incidence and severity with the feeding of organic chelated Cu, Mn and Zn (Manangi *et al.*, 2010; Richards *et al.*, 2010; Saenmahayak *et al.*, 2010). Resistance to skin tearing was significantly improved as a result of higher collagen content, increased epithelial cell layers and decreased inflammation of skin providing a much improved carcass quality (Rossi *et al.*, 2007). In the study of Manangi *et al.* (2010) comprising 120 000 broilers, supplementation of 8 mg/kg Cu, 32 mg/kg Mn and 32mg/kg Zn at reduced levels as $(HMTBA)_2$ chelates resulted in significantly enhanced foot pad health in comparison to commercially fed sulphates at much higher levels of 125 mg/kg Cu, 100 mg/kg Zn and 90 mg/kg Mn.

2.8 Conclusion

Organic Cu, Mn and Zn supplementation improve broiler performance, immune development and response, tissue and bone development and integrity, cellular growth and division and protection against oxidative stress (Richards *et al.*, 2010). The improved bioavailability of organic trace mineral sources compared to inorganic sources allows the performance of birds to be maintained or even increased at a reduced inclusion level. Research indicates that the use of organic complexed trace minerals may provide a viable tool in avoiding the many problems faced in the broiler industry. These include efforts to minimize condemnations and downgrades during processing by improving skin quality and foot pad health, reductions of mineral concentrations in the litter, improved bird welfare and improved feed formulations for higher bird requirements.

The prevention of FPD is essential for saleable paws as approximately 99 percent of paw downgrades are caused by FPD (Shepherd and Fairchild, 2010). The prevalence of FPD is also used in assessing animal welfare conditions, especially indicating overall litter quality. The widespread problem of FPD seems to be caused and prevented mainly by management practices affecting the litter moisture, together with feed composition (Nagaraj *et al.*, 2007c). The dietary nutrients and ingredients indirectly influence FPD through their effects on water excretion and excreta adherence. Trials on supplemented minerals, vitamins, feed ingredients, bird age, weight and also management practices such as stocking density and house temperatures and relative humidity to reduce FPD have resulted in many contradictions (Berg, 1998; Haslam *et al.*, 2007). However, published results on the effects of litter moisture are more strongly in agreement. Minimizing the prevalence of FPD can be achieved through ensuring optimal nutrition, environment and enteric health, which will help reduce the incidence of wet litter so that litter quality may be maintained throughout the life span of the broilers.

Chapter 3

The control of litter moisture for the induction of foot pad dermatitis

3.1 Introduction

Results from published literature indicate that litter moisture percentage plays a significant contribution to the incidence of foot pad dermatitis. The aim of the trial was to determine the quantity of moisture to be added to the litter to simulate most commercial conditions where dry litter is not a priority, and the prevalence of foot pad dermatitis is significantly high. A high incidence of foot pad dermatitis was necessary to enable the evaluation of the organic minerals' efficacy to ameliorate the problem in the subsequent trial. The hypothesis was that:

- H0: Water application to the litter will have no effect on the incidence of FPD.
- H1: Water application to the bedding will increase the incidence of FPD compared litter where no water was added.

3.2 Materials and methods

3.2.1 Animals and management

The trial was conducted in an environmentally-controlled experimental broiler house at the University of Pretoria, South Africa. This experiment was approved by the University of Pretoria Animal Ethics Committee (AEC 21-13). A total of 1800 Ross 308, as hatched, first grade day-old broiler chicks were randomly distributed into 30 identical concrete floor pens of 3.5m^2 each, 60 birds / pen (17.1 birds/m^2).The pens were covered with clean pine shavings of 8cm depth. The trial continued for 35 days. Five treatments were applied consisting of six replicates per treatment.

Each pen was fitted with 2 bell drinkers and 2 tube feeders. On arrival of chicks an extra 2 feed trays and fountain drinkers were placed in each pen, which were removed on day 7. The house was fitted with electrical heaters for heating. Before the arrival of chicks, the house was pre-heated to 32 °C; held at this temperature for 3days and thereafter gradually decreased to 18 °C by 35 days. Birds had *ad libitum* access to feed and water throughout the trial. A lighting schedule of 23L: 1D was imposed for the first week of the experimental period. Thereafter the length of daylight was reduced to 14 hours per day for the following two weeks and again increased to 16 hours of daylight during the last two weeks. The broiler breeder guide for precise management of Ross broilers and their environment was followed (Aviagen, 2009).

3.2.2 General husbandry

The environmentally controlled broiler house was cleaned using Vet One Plus and thereafter disinfected with Vet GL 20 (Immunovet services, Johannesburg). A foot dip (Vet Fluid-O, Immunovet services, Johannesburg) was strictly used at the entrance to the broiler house for the entire trial period. The chicks were vaccinated at the hatchery against New Castle disease and Infectious Bronchitis Virus. Broilers were once again vaccinated against Infectious Bursa disease at 10 and 16 days of age and Newcastle disease at 21 days of age. Three times daily, animals and housing facilities were inspected for the general health, constant feed and water supply as well as temperature and ventilation. All bird mortalities were recorded on the date of death.

3.2.3 Diets

Birds were fed a commercial maize-soya based (from Epol Feeds, Pretoria West, Gauteng) broiler starter diet (crumble) from day 1 of age until 10 days of age, a broiler grower diet (pellets) from 11 days until 28 days of age and a finisher diet (pellets) from 29 days to 35 days of age (Table 3.1). Changeover of diets was at 08h00 in the morning. When changing to a different feeding phase, previous feed was cleaned from pens and leftover feed weights recorded.

Table 3.1. Chemical composition of the trial diet (Epol Feeds)

Nutrients	Unit	Broiler starter (1 -10 days)	Broiler grower (11-28 days)	Broiler finisher (29-35 days)
Protein	g/kg	220 (min)	180 (min)	170 (min)
Total Lysine	g/kg	12 (min)	9 (min)	8 (min)
Total methionine	g/kg	4.56 (min)	3.42 (min)	-
Moisture	g/kg	120 (max)	120 (max)	120 (max)
Fat	g/kg	25 (min)	25 (min)	25 (min)
Fibre	g/kg	50 (max)	70 (max)	70 (max)
Calcium	g/kg	8 (min)	8 (min)	7 (min)
Calcium	g/kg	12 (max)	12 (max)	12 (max)
Total phosphorus	g/kg	6 (min)	5 (min)	5 (min)

*Zn Bacitracin was included in the vitamin-mineral premix of the starter and grower diets.

*Cycostat (33 mg of robenidine per kg of feed) as an aid in prevention of coccidiosis was included in the vitamin-mineral premix of the starter and grower diets.

3.2.4 Treatments

The following five treatments (six replications per treatment) were applied to test the effect of various litter moisture level on the incidence of FPD. All house, management and dietary conditions remained the same throughout the trial except for the changes brought on by the treatments shown in table 3.2.

Table 3.2. Water application rate for each treatment

Treatment #	**Bedding at start**	**Water treatment**	**Days on which water was sprayed**
1	Standard new, clean pine shavings, at a depth of 8cm	Dry, only droppings	Not applicable
2	Standard new, clean pine shavings, at a depth of 8cm	9 L water evenly sprayed	Days 7, 14, 21, 28 and 35
3	Standard new, clean pine shavings, at a depth of 8cm	6 L water evenly sprayed	Four consecutive days per week between days 7 to 35 (i.e. 8-11, 16-19, 24-27 and 32-35).
4	Standard new, clean pine shavings, at a depth of 8cm	3 L water evenly sprayed	On a daily basis between day 4 to 13 and again between day 20 and 29.
5	Standard new, clean pine shavings, at a depth of 8cm	9 L (4.5 L two times a day) water evenly sprayed	On a daily basis between day 4 to 13 and again between day 20 and 29.

3.2.5 Measurements

3.2.5.1 Performance

Body weights were determined per pen at 0, 7, 14, 21, 28 and 35 days of age. Weekly and cumulative feed intake, weight gain and FCR (feed intake/weight gain) were recorded and calculated. Mortality and culled birds were recorded during daily inspection. Dead birds were weighed and feed intake determined in order to correct FCR for mortality.

3.2.5.2 Foot pad dermatitis and breast blisters

All birds were inspected for the incidence of FPD and breast blisters at day 22 and 35 (slaughter age). FPD was scored according to incidence and severity using a visual scoring system. On day 22, birds were only inspected for the presence or absence of FPD. The following scale (Table 3.3) regarding FPD was used at day of slaughter according to Manangi *et al.* (2012).

Table 3.3.Foot pad dermatitis scoring system

Grade	Description
1	no lesions
2	lesion the size of 2 mm (width) or less
3	lesion the size of 2 mm (width) to 7 mm
4	lesion the size larger than 7 mm (width) without lesions on toes
5	lesion the size larger than 7 mm (width) and includes lesion on toes

*The percentage of each category was calculated for each treatment.

All broilers were inspected for the presence or absence of breast lesions on both days 22 and 35. Mortalities and culls occurring before day 22 of age were also inspected for FPD and breast lesions and these were included in the results at day 22. Mortalities and culls occurring after day 22 of age were recorded for FPD and breast lesions and these were included in the results at day 35.

3.2.6 Statistical analysis

All data was analysed as a randomized block design. Significance between treatments was determined by an analysis of variance with the GLM model of SAS Institute (2009). In all cases the level of statistical significance was $P < 0.05$. Means and standard deviations were calculated. Treatment means were separated using Fishers least significance difference test at the 5% level of significance.

3.3 Results

3.3.1 Broiler performance

There was no significant difference between treatments for chick mean body weights at the start of this trial. The increasing water application to the litter did not significantly affect broiler body weight, body weight gains and mortality throughout the trial period ($P > 0.05$; Table 3.4 and 3.5). Cumulative feed intake from day 1 to 21 days of age was significantly lower for the highest water application treatment (Treatment 5) compared to the other treatments; however between 22 and 35 days of age the cumulative feed intake was significantly higher for this treatment in comparison to the other treatments ($P < 0.05$). No significant differences between treatments were observed for cumulative feed intake over the entire trial period ($P > 0.05$). Feed conversion ratio over the entire trial period was significantly better for the treatment with no water application (1.65; Treatment 1) and poorest for the treatment with the highest water application level (1.71; $P < 0.05$). The results from the trial indicated a poorer broiler performance in respect to feed conversion with an increasing water application level from Treatment 1 to Treatment 5.

Table 3.4. Effects of increasing water application onto broiler litter on the growth, feed intake and performance of broilers

	Parameter	Treatment 1^{1}	Treatment 2^{2}	Treatment 3^{3}	Treatment 4^{4}	Treatment 5^{5}	P-value	SE
1-21 Days of age	Cumulative BWG (g)	770.9	747.06	737.89	772.6	746.14	0.14	11.46
	Cumulative FI (g)	1191.34^{BA}	1203.83^{A}	1198.79^{A}	1204.97^{A}	1160.51^{B}	0.04	10.98
	FCR^{6}	1.56	1.61	1.63	1.6	1.61	0.38	0.03
	Mortality (%)	0.19	0.19	0.19	0.14	0.14	0.95	0.06
22-35 Days of age	Cumulative BWG (g)	1096.37	1066.57	1079.37	1069.69	1117	0.21	16.8
	Cumulative FI (g)	1936.77^{BA}	1915.21^{BA}	1881.11^{B}	1905.83^{BA}	1974.54^{A}	0.02	24.42
	FCR^{6}	1.8	1.82	1.81	1.85	1.87	0.39	0.03
	Mortality (%)	0.23	0.33	0.28	0.14	0.09	0.32	0.09
1-35 Days of age	Cumulative BWG (g)	1867.29	1813.61	1817.24	1842.26	1863.1	0.35	23.25
	Cumulative FI (g)	3128.1	3119.03	3079.89	3110.8	3135.1	0.51	23.35
	FCR^{6}	1.65^{B}	1.70^{BA}	1.70^{BA}	1.70^{BA}	1.71^{A}	0.03	0.02
	Mortality (%)	0.42	0.51	0.47	0.28	0.23	0.32	0.11

$^{A-B}$ Means within a row with the same superscript do not differ significantly at $P \leq 0.05$.

[1] No water application was undertaken.

[2] 9L water evenly sprayed on days 7, 14, 21, 28 and 35.

[3] 6L water evenly sprayed on four consecutive days per week between days 7 to 35 (i.e. 8-11, 16-19, 24-27 and 32-35).

[4] 3L of water evenly sprayed on a daily basis between day 4 to 13 and again between day 20 and 29.

[5] 9L water sprayed on a daily basis between day 4 to 13 and again between day 20 and 29.

[6] Feed conversion ratio adjusted for mortality and cull weight.

Table 3.5. Effects of increasing water application onto broiler litter on the body weights of broilers

Parameter	Treatment 1[1]	Treatment 2[2]	Treatment 3[3]	Treatment 4[4]	Treatment 5[5]	P-value	SE
BW day 0 (g)	39.37	39.86	39.79	39.76	39.83	0.72	0.28
BW day 7 (g)	164.2	159.73	159.54	160.07	160.5	0.5	2.08
BW day 21 (g)	810.26	786.93	777.7	812.4	785.99	0.14	11.44
BW day 35 (g)	1906.64	1853.5	1857.07	1882.04	1902.94	0.36	23.26

$^{A-B}$ Means within a row with the same superscript do not differ significantly at $P \leq 0.05$.

[1] No water application was undertaken.

[2] 9L water evenly sprayed on days 7, 14, 21, 28 and 35.

[3] 6L water evenly sprayed on four consecutive days per week between days 7 to 35 (i.e. 8-11, 16-19, 24-27 and 32-35).

[4] 3L of water evenly sprayed on a daily basis between day 4 to 13 and again between day 20 and 29.

[5] 9L water sprayed on a daily basis between day 4 to 13 and again between day 20 and 29.

3.3.2 Foot pad dermatitis and breast lesions

The incidence of FPD at 21 days of age showed significant increases ($P < 0.05$)with an increasing level of water application from Treatment 1 (9.83%), to Treatment 5 (68.20%; Table 3.6). At 22 days of age the incidence of breast lesions was not significantly different between treatments ($P > 0.05$).

Results at day 35 showed a similar scenario as the incidence of FPD increased with increasing water application (Table 3.7). Treatment 1 showed the highest percentage of saleable paws (67.90; sum of grade 1 and 2; $P < 0.05$). Treatment 2 and 3 showed a similar percent of saleable paws, 42.73% and 39.22%, respectively ($P > 0.05$). Treatment 4 and 5 had a significantly lower percentage of saleable paws compared to the other treatments, 11.74% and 7.68%, respectively ($P < 0.05$). The incidence of breast lesions was significantly higher for treatment 4 (4.16%) compared to the other treatments at day 35 (Table 3.7; $P < 0.05$). Treatment 5 was 0.83% lower ($P > 0.05$) than treatment 4 regarding breast lesion incidences, while Treatment 1 and 2 resulted in zero breast lesions throughout the trial ($P < 0.05$).

Table 3.6.Effects of increasing water application onto broiler litter on the incidence of foot pad dermatitis and breast lesions at day 21 of age

Parameter	**Foot pad dermatitis present (%)**	**Breast lesions present (%)**
Treatment 1^{1}	9.83^{D}	0
Treatment 2^{2}	30.32^{C}	0
Treatment 3^{3}	41.50^{CB}	0.27
Treatment 4^{4}	49.42^{B}	0.53
Treatment 5^{5}	68.20^{A}	0.47
P-Value	<.0001	0.30
SE	5.51	0.12

$^{A\text{-}D}$Means within a column with the same superscript do not differ significantly at $P \leq 0.05$.

*Means represent entire populations of broilers per treatment.

^{1}No water application was undertaken.

^{2}9L water evenly sprayed on days 7, 14, 21, 28 and 35.

^{3}6L water evenly sprayed on four consecutive days per week between days 7 to 35 (i.e. 8-11, 16-19, 24-27 and 32-35).

^{4}3L of water evenly sprayed on a daily basis between day 4 to 13 and again between day 20 and 29.

^{5}9L water sprayed on a daily basis between day 4 to 13 and again between day 20 and 29.

Table 3.7. Effects of increasing water application onto broiler litter on the incidence of foot pad dermatitis and breast lesions at day 35 of age

Parameter	**Sum of grades 1+2 (%)**	**Grade 3 (%)**	**Sum of grades 4+5 (%)**	**Breast lesions present (%)**
Treatment 1^{1}	67.90^{A}	10.82^{B}	21.28^{B}	0^{C}
Treatment 2^{2}	42.73^{B}	25.30^{A}	31.97^{B}	0^{C}
Treatment 3^{3}	39.22^{B}	29.37^{A}	31.42^{B}	1.50^{BC}
Treatment 4^{4}	11.74^{C}	37.13^{A}	51.13^{A}	4.16^{A}
Treatment 5^{5}	7.68^{C}	32.87^{A}	59.45^{A}	3.33^{BA}
P-Value	0.0001	0.0056	0.0001	0.0007
SE	2.22	4.74	5.05	0.75

$^{A\text{-}C}$Means within a column with the same superscript do not differ significantly at $P \leq 0.05$.

^{1}No water application was undertaken.

^{2}9L water evenly sprayed on days 7, 14, 21, 28 and 35.

[3] 6L water evenly sprayed on four consecutive days per week between days 7 to 35 (i.e. 8-11, 16-19, 24-27 and 32-35).

[4] 3L of water evenly sprayed on a daily basis between day 4 to 13 and again between day 20 and 29.

[5] 9L water sprayed on a daily basis between day 4 to 13 and again between day 20 and 29.

3.4 Discussion

Adverse effects on broiler health and FPD are commonly observed once litter moisture exceeds 35 percent. The trial results were in agreement to the studies of Shepherd and Fairchild (2010) and Youssef *et al.* (2011a) that indicated increased litter moisture as being a major contributing factor to the prevalence of FPD in broilers. Increased moisture levels potentially increase the production of irritant substances such as ammonia that may contribute to the formation of foot pad dermatitis (Nagaraj *et al.*, 2007a). The reasonably high incidence of FPD for Treatment 1 at day 35may have been caused by the significantly high ammonia presence in the broiler house from day 21 and onwards. The increasing litter moisture in this trial showed a reduction in broiler welfare through the increased incidence of FPD and also reduced broiler performance through poorer feed conversions.

3.5 Conclusion

The treatment results were compared to one another in order to ascertain the treatment which would be most effective in inducing foot pad dermatitis. The least severe treatment (least amount of water added to pens) required to induce a foot pad incidence of 40 percent and above, was preferentially chosen. This was done in order to best preserve the welfare of the broilers while allowing effective testing of an organic mineral source in ameliorating FPD. Treatment 3, application of 6 litres of water for a period of four consecutive days per week between days 7 to 35 (i.e. 8-11, 16-19, 24-27 and 32-35), was chosen as the most effective treatment and recommended to be the standard litter condition for the following trace mineral trial (Chapter 4).

From recommendations and practical experience the standard conditions were slightly adjusted for the subsequent trial (Chapter 4), in that the amount of water application applied during the second half of the trial was reduced to 4 litres per day of application. Thus for days 8 to 11 and 16 to 19, 6L of water per pen was used; while for days 24 to 27 and 31 to 34, 4L of water per pen was used. The amount of water application in the second half of the trial was reduced because during the first half of the trial the bedding remained fairly dry as it dried out quicker. Later in the trial the litter remained wetter due to increased excreta output and previous build-up of litter moisture.

Chapter 4

Effect of feeding copper, manganese and zinc complexed to two molecules of 2-hydroxy-4-(methylthio) butanoic acid on broiler performance, carcass and intestinal characteristics

4.1 Introduction

The objective of this research was to evaluate the effects of $(HMTBA)_2$ metal chelates of Cu, Mn and Zn added at levels lower than typical levels of minerals from inorganic sources, on the performance, intestinal integrity, skin integrity and prevalence of FPD in broilers. Two formulations of $(HMTBA)_2$ metal chelates were tested. Cu, Mn and Zn were either provided at levels of8, 32 and 32mg/kg or at levels of 30, 32 and 32 mg/kg, respectively. The hypothesis was that:

- H0: The lower levels of $(HMTBA)_2$ metal chelates of Cu, Mn and Zn will not improve broiler performance, skin and intestine integrity and FPD when compared to higher levels of Cu, Mn and Zn from inorganic sources.
- H1: The lower levels of $(HMTBA)_2$ metal chelates of Cu, Mn and Zn will improve broiler performance, skin and intestine integrity and FPD when compared to higher levels of Cu, Mn and Zn from inorganic sources.

4.2 Materials and Methods

4.2.1 Animals and management

This experiment was approved by Animal Ethics Committee, University of Pretoria (ECO21-13). The trial was conducted in an environmentally-controlled experimental broiler house at the University of Pretoria, South Africa. A total of 1920 male Ross 308 first grade day-old broiler chicks (Eagles Pride Hatchery) were randomly distributed into 32 identical concrete floor pens of $3.5m^2$ each. 60 birds were placed in each pen at a stocking density of 17.1 birds/m^2. The 32 pens were divided into four dietary treatments using a completely randomized block design, resulting in 8 replicates per treatment. The pens were covered with clean pine shavings of 8cm depth. The trial continued for 35 days. Each pen was fitted with 2 bell drinkers and 2 tube feeders. On arrival of chicks an extra 2 feed trays and fountain drinkers were provided, which were removed on day 7. Electrical heaters were fitted to the broiler house for heating. Before the arrival of chicks, the house was pre-heated to 32 °C; held at this temperature for 3days and thereafter gradually decreased to 18 °C by 35 days. Birds had *ad libitum* access to feed and water throughout the trial. A lighting schedule of 23L: 1D was imposed for the first week of the experimental period. There after the length of daylight was reduced to 14 hours per day for the following two weeks and again increased to 16 hours of daylight in the last two weeks. The breeder company's guide for precise management of Ross 308 broilers was followed (Aviagen, 2009).

4.2.2 Diets and treatments

Birds were fed a broiler starter diet (crumbles) from day 1 until 10 days of age, a broiler grower diet (pellets) from 11 days until 28 days of age and a finisher diet (pellets) from 29 days of age to 35 days of age. Changeovers of diets took place at 08h00 in the morning. When changing to a different feeding phase, leftover feed was cleaned from pens and weights recorded. The maize-soybean basal diet was formulated according to industry standards. The compositions of the basal diets are given in Table 4.1.Proximate analysis of feed was undertaken at the University of Pretoria Nutrilab (AOAC, 2000). Four dietary treatments, listed in Table 4.3, with different mineral sources and levels were added to the basal vitamin-mineral premixes (Table 4.2).Treatment 1 (positive control) consisted of Cu, Mn and Zn at inclusion levels of 120, 100 and 100 mg/kg respectively, from inorganic sulphate mineral sources. Treatment 2 (negative control) consisted of Cu, Mn and Zn at inclusion levels of 8, 32 and 32 mg/kg respectively, from inorganic sulphate mineral sources. Treatment 3 ($(HMTBA)_2$ 8:32:32) consisted of $(HMTBA)_2$ metal chelates of Cu, Mn and Zn at inclusion levels of 8, 32 and 32 mg/kg respectively from only Mintrex® Cu, Mintrex® Mn and Mintrex® Zn (Novus International Inc., St. Charles, MO). Treatment 4 ($(HMTBA)_2$ 30:32:32) consisted of $(HMTBA)_2$ metal chelates of Cu, Mn and Zn at inclusion levels of 30, 32 and 32 mg/kg respectively from only Mintrex® Cu, Mintrex® Mn and Mintrex® Zn (Novus International Inc., St. Charles, MO).All treatment diets were adjusted to maintain the same amount of 2-hydroxy- 4-(methylthio) butanoic acid $(HMTBA)_2$as a methionine source.

The same basal starter, grower and finisher diets as described above were divided into four parts. The four dietary treatments with different mineral sources and levels were added and mixed to these basal diets at Penville Feeds (Pretoria) using a fountain mixer. The analysed mineral levels in each dietary treatment are shown in Table 4.4. Mineral analysis was conducted at the University of Pretoria Nutrilab, performed by acid heat digestion (20 mL of HNO_3 and 10 mL of $HClO_4$ into 2 grams of feed sample for 30 minutes) and an atomic absorption spectrophotometer (AOAC, 2000).

Table 4.1. Composition of basal diet

Parameter	**Starter** **(0-10 days)**	**Grower** **(11-28 days)**	**Finisher** **(29-35 days)**
Ingredient %			
Yellow maize (fine)	57.8	58.9	62.8
Soya oilcake meal	29.4	21.2	17.4
Gluten 60	2.00	5.71	5.00
Extruded full fat soya	6.80	10.0	10.0
Monocalcium phosphate	1.12	0.73	0.49
Limestone	1.42	1.35	1.19
Salt fine	0.43	0.44	0.43
L-Lysine HCL	0.24	0.24	0.24
L Threonine	0.05	-	-
Methionine hydroxy analog[1]	0.31	0.20	0.21
Soya Oil	-	0.91	1.88
Sodium Bicarbonate	0.07	0.03	-
Antibiotic growth promoter[2]	0.02	0.02	0.02
Coccidiostat[3]	0.05	0.05	0.05
500 Phytase[4]	0.01	0.01	0.01
Vitamin-mineral premix[5]	0.30	0.25	0.25
Calculated nutrient analysis (as is basis)			
DM, %	88.0	88.4	88.0
CP, %	21.1	20.3	18.6
GE, MJ/kg	15.8	16.2	16.4
Ash, %	4.93	4.32	3.93
Ca, %	0.87	0.79	0.68
Total P, %	0.63	0.53	0.45
Na, %	0.16	0.15	0.13
K, %	0.69	0.70	0.73

[1] Alimet (Novus International Inc., St. Charles, MO), feed supplement providing 88% Methionine activity.

[2] Olaquindox 10% included at 200 mg/kg.

[3] Salinomycin 12% included at 500 mg/kg.

[4] Phyzime TPT at 500 FTU.

[5] The basal vitamin-trace mineral premixes are shown in Table 4.2. The Cu, Mn and Zn dietary treatments were added to the vitamin-mineral premix as specified in table 4.3. The total methionine activity included as $(HMTBA)_2$ was balanced to maintain an equimolar quantity of $(HMTBA)_2$ between all treatment diets.

Table 4.2. Vitamin and mineral premix levels (per unit premix) used in the starter, grower and finisher basal diets

Compound	**Starter**	**Grower**	**Finisher**
Vitamin A	11000 I.U	9 000 I.U	7500 I.U
Vitamin D3	5000 I.U	4000 I.U	3300 I.U
Vitamin E	60 mg	50 mg	40 mg
Vitamin K3	2mg	1.5 mg	1.3 mg
Vitamin B1	2 mg	1.7 mg	13 mg
Vitamin B2	5 mg	4 mg	3.3 mg
Niacin (B3)	50 mg	42 mg	33 mg
Cal Panthonate (B5)	12 mg	10 mg	8 mg
Vitamin B6 (Pyridoxine)	3 mg	2.5 mg	2 mg
Folic acid (B9)	2 mg	1.7 mg	1.3 mg
Vitamin B12	0.01 mg	0.008 mg	0.0065 mg
Biotin	0.1 mg	0.08 mg	0.07 mg
Antioxidant	125 mg	125 mg	125 mg
Iron	40 mg	35 mg	25 mg
Cobalt	0.5mg	0.4 mg	0.35 mg
Iodine	2 mg	1.6 mg	1.3 mg
Selenium	0.3 mg	0.25 mg	0.2 mg
Choline	300 mg	250 mg	200 mg

Table 4.3. Dietary trial mineral treatments

Treatment #	**Treatment name**	**Cu, Mn, Zn levels (mg/kg complete feed)**	**Source of minerals**
1	Positive control	Cu = 120; Mn = 100; Zn = 100	Sulphates
2	Negative control	Cu = 8; Mn = 32; Zn = 32;	Sulphates
3	(HMTBA)$_2$[1] 8:32:32	Cu = 8; Mn = 32; Zn = 32	Cu (HMTBA)$_2$,Mn (HMTBA)$_2$and Zn (HMTBA)$_2$
4	(HMTBA)$_2$ 30:32:32	Cu = 30; Mn = 32, Zn = 32;	Cu (HMTBA)$_2$,Mn (HMTBA)$_2$and Zn (HMTBA)$_2$

[1] 2-hydroxy- 4-(methylthio) butanoic acid.

Table 4.4.Analysed mineral analysis of the dietary treatments (as is basis)

Mineral	**Phase**	**Positive control**[1]	**Negative control**[2]	**$(HMTBA)_2$ 8:32:32**[3]	**$(HMTBA)_2$ 30:32:32**[4]
Cu(mg/kg)	Starter	101.45	20.01	15.00	33.98
	Grower	106.52	13.99	16.50	37.99
	Finisher	99.50	12.99	13.00	35.00
Mn (mg/kg)	Starter	125.44	74.29	77.25	72.00
	Grower	108.52	59.47	63.26	73.24
	Finisher	101.25	56.73	56.22	60.49
Zn(mg/kg)	Starter	128.44	65.03	78.50	67.00
	Grower	124.02	63.47	72.01	62.99
	Finisher	129.00	59.98	54.47	69.49

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn- $(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn- $(HMTBA)_2$.

Analysed mineral values may differ slightly between treatments with the same theoretical mineral premix levels in the diet because of the different mineral levels throughout the raw materials, sampling of feed and laboratory discrepancies.

4.2.3 General husbandry

The environmentally controlled broiler house was cleaned using Vet One Plus and thereafter disinfected with Vet GL 20 (Immuno vet services, Johannesburg). A foot dip (Vet Fluid-O, Immuno vet services, Johannesburg) was strictly used at the entrance to the broiler house for the entire trial period. The chicks were vaccinated at the hatchery against New Castle Disease and Infectious Bronchitis virus. Broilers were once again vaccinated against Infectious Bursa disease at 10 and 16 days of age and Newcastle disease at 21 days of age. Three times daily, animals and housing facilities were inspected for the general health, constant feed and water supply as well as temperature and ventilation. All bird mortalities were recorded on the date of death.

4.2.4 Measurements

4.2.4.1 Performance

Body weight was determined on a per pen basis at 0, 7, 14, 21 and 28 days of age. At slaughter (35 days) all broilers were weighed individually to calculate uniformity within treatments. Weekly and cumulative feed intake, weight gain and feed conversion ratio (feed intake/weight gain) were recorded and calculated. Based on the period and cumulative feed intakes, the ingestion rates and cumulative intakes of the Cu, Mn and Zn of the respective sources and inclusion rates were calculated. Mortality and culled birds were recorded during daily inspection. Dead birds were weighed and feed intake determined in order to correct FCR for mortality. The variability of bird size in each pen population was calculated as the coefficient of variation (CV), which was then converted into percent uniformity. CV% = [Standard deviation (g) ÷ average body weight (g)] x 100.

4.2.4.2 Carcass parameters

5 broilers per replicate (40 birds per treatment) were randomly selected at day 35 of age. All selected broilers had a body weight within a 100 grams range of the pen mean body weight. A feed withdrawal period of two hours at slaughter was uniformly maintained for the sampled birds by placing the birds in crates and collecting them at specific time intervals before processing. Birds were slaughtered by electrically stunning (7-9 second contact time) and there after severing the jugular and left to bleed out for 3 minutes. Birds were scalded in hot water for 30 seconds at 60 degrees Celsius and then placed in a rotary de-feathering machine for a further 30 seconds. Carcass mass was measured after manual evisceration by the removal of the head, feet, abdominal fat pad, and viscera. The entire length of the intestinal tract from attachment to the gizzard to the cloaca was measured for each carcass. Whole carcass weight was expressed as percentage of live BW. Thereafter the abdominal fat pad, whole breast, breast muscle, leg portion and wing masses were all measured and expressed as a percentage of carcass mass.

4.2.4.3Foot pad dermatitis and breast blisters

All birds were inspected for the incidence of foot pad dermatitis and breast blisters at day 22 and at slaughter (days 35). FPD was scored according to incidence and severity using a visual scoring system. On day 22, birds were only inspected for the presence or absence of FPD. The following scale (Table 4.5) regarding FPD was used at day of slaughter according to Manangi *et al. (*2012).

Table 4.5.Foot pad dermatitis scoring system

Grade	Description
1	no lesions
2	lesion the size of 2 mm (width) or less
3	lesion the size of 2 mm (width) to 7 mm
4	lesion the size larger than 7mm (width) without lesions on toes
5	lesion the size larger than 7 mm (width) and includes lesion on toes

*The percentage of each category was calculated for each treatment.

All broilers were inspected for the presence or absence of breast lesions on both days 22 and 35. Mortalities and culls occurring before day 22 of age were also inspected for FPD and breast lesions and these were included in the results at day 22. Mortalities and culls occurring after day 22 of age were recorded for FPD and breast lesions and these were included in the results at day 35.

4.2.4.4 Litter scoring

Litter samples were taken at day 21 and day 35, for the testing of litter moisture content. Litter was collected by using an empty 200-mL beaker; a quantity of the full depth of litter was taken from 4 different areas in each pen. Samples were taken from under the drinkers, at the side of the entrance gate and from the middle of each pen. The 4 collected samples were then mixed in a plastic bag. A duplicate (below 5% standard error only accepted) subsample of 30g from each pen was weighed and oven dried at 105 °C for 24 hours. Moisture content was determined from the loss in weight of each sample (AOAC, 2000).

4.2.4.5 Skin scratches and bruising

Skin scratches and carcass bruises were evaluated during processing whereby presence or absence of scratches and bruises was determined (binomial system). The length of the scratches was measured, average scratch length per carcass and average per treatment were obtained. All visual scoring of defects was done by the same person. Any carcass defects caused through malfunction of equipment or human error was neglected and another bird selected for sampling.

4.2.4.6 Skin and intestinal strength

Skin and intestinal samples were taken from the same 5 broilers per pen (40 birds per treatment) that were taken for carcass measurements on the day of slaughter. The breast skin was removed in its entirety with a sharp scalpel blade, carefully separating the subcutis from the underlying muscle. The separated skin was pinned to a wooden board with the feather tracts facing upwards and clearly visible. About 8 pins were evenly spaced around the edges of the skin to prevent distortion on cutting. Skin samples were cut between the feather tracts, using a pair of sharp scissors, along measured outlines that were pressed into the skin using a specially adapted knife to press a uniform shape and size. The uniform shape was formed by mounting safety shaving razor blades to a wooden block into pre-cut slits that cut the skin into a dumbbell shape of 8cm in length, 2cm width at the sample ends and 1cm width at the sample centre, forming the required dimensions. Samples were taken precisely between the two sternal feather tracts, equidistant from the proximal and distal ends of each tract. A dumbbell shape was necessary to ensurethat the skin sample would tear at its narrowest point as opposed to around the grips, provided the sample was cut to the correct dimensions and inserted accurately between the grips of the Shumadzu EZ-LTensile machine (Casey *et al.*, 1992). From each of these birds' two sections of the intestinal tract both 8 cm in length were taken. Jejunum samples were taken by measuring 10 centimetres caudal to the duodenal loop and the ileum samples were taken from 10 centimetres caudal to the Meckel's diverticulum from each bird (Miles *et al.*, 2006). Samples were cleaned from the adhering fat and connective tissue, and gently squeezed to remove the digesta contents.

Any skin or intestine that was torn or defected during processing was recorded and another bird selected for sampling. Skin and intestine samples were placed in 10% buffered neutral formalin for fixation, gently shaken to remove any adhering contents and stored at 4 °C. Tensile strength measurements were made on each skin, jejunum and ileum sample, using a Shumadzu EZ-L Tensile tester**.** Peak force at break point (N) as well as peak stress (N/mm) to break point was recorded. The Tensile tester apparatus was under computer control, enabling precise testing procedure and measurements. The test parameters were as follows: crosshead speed of 75 mm per minute; load cell range 200 Newton and a gauge length of 40mm. Rough sandpaper was fitted to the clamps to prevent the samples from slipping. Prior to insertion between the grips, pH neutral tissue paper was used to dry each sample to further deter against slipping of samples between the grips. The grips were tightened to a similar point by using the same number of turns for tightening. Any sample alleged of slipping, irregular tearing or tearing at the clamps was rejected from the analysis results.

4.2.4.7 Tissue analysis

Whole liver samples were taken from each bird at processing (5 birds per pen) and pooled together for each pen providing 8 replicate liver analyses per dietary treatment. Liver samples were preserved by deep freeze at -4 degrees Celsius until processing. The liver samples were thawed to room temperature, oven dried at 60 degrees celsius for 36 hours and finely milled through a 1mm screen prior to mineral (Cu, Mn and Zn) analysis by atomic absorption spectrophotometry (AOAC, 2000).

4.2.4.8 Foot pad histological analysis

To investigate the histopathology of foot pads and the development of foot pad dermatitis, two broilers per replicate (16 birds per treatment) were randomly selected at days 21 of age and again at 35 days of age. Birds were killed by cervical dislocation by an experienced supervisor at 21 days of age; whereas birds were slaughtered by electrically stunning (7-9 second contact time) and thereafter severing the jugular and left to bleed out for 3 minutes at 35 days of age. The centre of the foot pad skins (5mm x 5mm) were removed using a sharp scalpel, samples were placed in 10% buffered neutral formalin for fixation and stored at 4 °C. The samples were labelled according to their foot pad dermatitis grade and dietary treatment. Foot pad samples were sectioned (2mm thick) and stained with haematoxylin and eosin by the department of pathology at Onderstepoort veterinary institute. All stained samples were photographed using a digital camera linked to a light microscope at the Department of Animal Sciences, University of Pietermaritzburg. The program ImageJ, Version 1.4, was used to analyse the computerized images. The length and area of the stratus corneum, epidermis and dermis was measured as illustrated in Figure 3 using a scale bar of 500 µm. All samples categorised as a foot pad dermatitis grade 1 were compared with one another between treatments, also the results were compared between the different foot pad dermatitis grades.

4.2.5 Statistical analysis

All data was analysed as a randomized block design. Significance between treatments was determined by an analysis of variance with the GLM model of SAS Institute (2009). In all cases the level of statistical significance was $P<0.05$.Means and standard deviations were calculated. Treatment means were separated using Fishers' protected least significant difference (LSD) at the 5% level of significance.

4.3 Results

4.3.1 Broiler performance (feed intake, average daily gain, body mass, feed conversion ratio, mortality and uniformity)

FCR (adjusted for mortality) of birds fed $(HMTBA)_2$ 30:32:32 was significantly improved for the period between 28 to 35 days of age compared to all other treatments. FI of birds fed $(HMTBA)_2$ 30:32:32 was significantly improved for the period between 28 to 35 days of age compared to the negative control and (HMTBA)2 8:32:32 treatments ($P < 0.05$; Table 4.6).

All other performance results (BW, BWG, feed intake, mortality, and FCR adjusted for mortality) were not significantly affected when broilers were fed lower levels of Cu, Mn and Zn (organic and inorganic) compared with broilers fed the commercial levels of these trace minerals (Treatment 1) ($P > 0.05$; Table 4.6 and 4.7).

Table 4.6.Effects of feeding Cu, Mn and Zn from (HMTBA)$_2$ sources in comparison with the respective inorganic sulphate trace minerals on the growth performance of broilers

	Parameter	Positive control[1]	Negative control[2]	(HMTBA)$_2$ 8:32:32[3]	(HMTBA)$_2$ 30:32:32[4]	P-value	SE
1-7 Days of age	BWG (g)	131.24	130.22	130.99	128.24	0.57	1.65
	FI (g)	169.19	167.01	167.85	169.75	0.84	2.37
	FCR[5]	1.39	1.38	1.38	1.42	0.6	0.01
8-14 Days of age	BWG (g)	215.46	217.28	221.18	217.06	0.6	3.08
	FI (g)	293.38	295.05	295.91	296.58	0.94	3.83
	FCR[5]	1.46	1.46	1.44	1.47	0.3	0.01
15-21 Days of age	BWG (g)	459.74	470.05	467.76	456.98	0.11	4.27
	FI (g)	612.4	614.78	619.15	630.63	0.27	6.9
	FCR[5]	1.48	1.46	1.48	1.53	0.39	0.01
22-28 Days of age	BWG (g)	584.98	588.23	592	590.51	0.94	8.64
	FI (g)	956.42	958.04	966.72	981.24	0.53	13.05
	FCR[5]	1.62	1.64	1.64	1.66	0.42	0.02
28-35 Days of age	BWG (g)	718.63	745.23	749.63	738.27	0.12	9.54
	FI (g)	1283.49^{BA}	1331.82^{A}	1333.42^{A}	1246.60^{B}	0.02	21.86
	FCR[5]	2.06^{A}	2.05^{A}	2.06^{A}	1.97^{B}	0.01	0.02
1-21 Days of age	BWG (g)	806.44	817.56	819.93	802.27	0.32	7.72
	FI (g)	1074.97	1076.84	1082.9	1096.96	0.42	10.12
	FCR[5]	1.44	1.43	1.43	1.47	0.18	0.01
	Mortality %	3.31	3.55	3.12	2.51	0.79	0.77
22-35 Days of age	BWG (g)	1303.61	1333.46	1341.63	1328.79	0.34	15.19
	FI (g)	2239.91	2289.85	2300.14	2227.84	0.3	31.97
	FCR[5]	1.84	1.84	1.85	1.82	0.28	0.01
	Mortality %	5.41	3.97	5.21	5.46	0.67	0.98
1-35 Days of age	BWG (g)	2110.05	2151.02	2161.55	2131.06	0.19	17.62
	FI (g)	3314.88	3366.69	3383.04	3324.8	0.5	36.42
	FCR[5]	1.60	1.60	1.60	1.61	0.89	0.01
	Mortality	8.72	7.51	8.33	7.97	0.89	1.15

%						
CV%[6]	11.31	10.78	10.30	10.38	0.36	0.46

*Means were calculated from40 chickens per treatment.

$^{A\text{–}C}$Means within a row with the same superscript do not differ significantly at $P \leq 0.05$.

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

[5] Feed conversion ratio adjusted for mortality and cull weight.

[6] The average coefficient of variation per treatment was calculated from the individual body weights of birds from each pen.

A low CV indicates a uniform flock while a high CV indicates an uneven flock. The calculated coefficient of variation for the inorganic sulphate treatments (Treatment 1 and 2) were non-significantly higher in comparison to the $(HMTBA)_2$ treatments (Treatment 3 and 4; P > 0.05). The Positive control treatment resulted in a CV of 11.31%, while the $(HMTBA)_2$ 8:32:32 and $(HMTBA)_2$ 30:32:32 treatments resulted in a CV of 10.30% and 10.38%, respectively.

Table 4.7. Effects of feeding Cu, Mn and Zn from $(HMTBA)_2$ sources in comparison with the respective inorganic sulphate trace minerals on the body weights of broilers

Body weight	**Positive control[1]**	**Negative control[2]**	**$(HMTBA)_2$ 8:32:32[3]**	**$(HMTBA)_2$ 30:32:32[4]**	**P-value**	**SE**
Day 0 (g)	37.05	37.23	37.25	36.99	0.48	0.14
Day7 (g)	168.29	167.45	168.24	165.23	0.54	1.68
Day 14 (g)	383.75	384.73	389.42	382.28	0.69	4.4
Day 21 (g)	843.49	854.79	857.18	839.26	0.31	7.74
Day 28 (g)	1428.47	1443.01	1449.18	1429.78	0.62	13.02
Day 35 (g)	2147.1	2188.25	2198.81	2168.05	0.19	17.67

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

4.3.2Intake of trace minerals

The actual mineral ingestion rates of Cu, Mn and Zn were calculated as grams per bird from the recorded feed intakes and the analysed mineral concentrations in each dietary treatment for each pen, allowing the mean average to be calculated from 8 pen replications per treatment (Table 4.7b). As expected the positive control treatment of 120, 100 and 100 mg/kg Cu, Mn and Zn respectively was significantly higher for all these trace minerals in comparison to all other treatments ($P < 0.05$). The Cu intakes for the $(HMTBA)_2$ treatment of 30 mg/kg Cu (Treatment 3) also agreed with theoretical values, as was significantly higher in comparison to the negative control and $(HMTBA)_2$treatments consisting of 8 mg/kg Cu; while was significantly lower than the positive control treatment consisting of 120 mg/kg Cu ($P < 0.05$).Zn intake over the entire trial (1-35 days) per bird was 0.01 grams less for the negative control treatment when compared to the same theoretical dietary Zn concentration level of both $(HMTBA)_2$ treatments at 32 mg/kg Zn ($P < 0.05$). The Mn intake over the entire trial (1-35 days) per bird was 0.03 and 0.01 grams less for the negative control treatment when compared to the same dietary Mn concentration level of the $(HMTBA)_2$ 30:32:32 ($P < 0.05$) and (HMTBA)2 8:32:32 ($P > 0.05$) treatments respectively at 32 mg/kg Mn. The differences between theoretical and actual mineral ingestion rates were not directly related to differences in feed intakes, carcass traits or lesion scoring.

Table 4.7b. Calculated actual mineral ingestion rates per treatment (g/bird).

	Positive control[1]	**Negative control**[2]	**$(HMTBA)_2$ 8:32:32**[3]	**$(HMTBA)_2$ 30:32:32**[4]	**P-value**	**SE**
Zn intake 1-21 days	0.13^A	0.07^C	0.08^B	0.07^C	<0.0001	0.001
Zn intake22-35 days	0.28^A	0.14^B	0.14^B	0.15^B	<0.0001	0.003
Zn intake 1-35 days	0.42^A	0.21^C	0.22^B	0.22^B	<0.0001	0.003
Cu intake 1-21 days	0.11^A	0.02^C	0.02^C	0.04^B	<0.0001	0.003
Cu intake 22-35 days	0.24^A	0.03^C	0.03^C	0.08^B	<0.0001	0.002
Cu intake 1-35 days	0.34^A	0.05^C	0.05^C	0.12^B	<0.0001	0.004
Mn intake 1-21 days	0.12^A	0.07^C	0.07^C	0.08^B	<0.0001	0.001
Mn intake 22-35 days	0.23^A	0.13^C	0.14^C	0.15^B	<0.0001	0.003
Mn intake 1-35 days	0.35^A	0.20^C	0.21^C	0.23^B	<0.0001	0.003

*All intake values represent accumulated mineral intake values calculated as grams per bird.

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

4.3.2 Carcass Measurements

Feeding the $(HMTBA)_2$ 30:32:32 treatment resulted in a significant increase in percentage of carcass yield in comparison to the inorganic negative control ($P < 0.05$; Table 4.8); while resulting in a non-significant increase in comparison to the positive control and $(HMTBA)_2$ 8:32:32 treatments.

Table 4.8.Effects of feeding Cu, Mn and Zn from $(HMTBA)_2$ sources in comparison with the respective inorganic sulphate trace minerals on carcass characteristics at 35 days of age

Parameter	**Positive control[1]**	**Negative control[2]**	**$(HMTBA)_2$ 8: 32:32[3]**	**$(HMTBA)_2$ 30: 32: 32[4]**	**P-value**	**SE**
Live Body Weight	2230.00	2241.00	2222.75	2265.50	0.33	17.47
Carcass Mass[5]	1584.50^{BA}	1580.50^{B}	1576.50^{B}	1624.00^{A}	0.09	14.72
Carcass yield[6]	71.02^{BA}	70.50^{B}	70.91^{BA}	71.69^{A}	0.03	0.28
Intestine length	190.43	189.34	185.64	190.01	0.38	2.16
Intestine yield	12.06	12.03	11.82	11.73	0.45	0.17
Abdominal fat weight	30.75	31.75	31.50	39.50	0.24	3.46
Abdominal fat yield	1.95	2.01	2.00	2.45	0.34	0.22
Leg weight	453.50	466.25	460.50	469.00	0.21	5.57
Leg yield	28.66	29.48	29.22	28.89	0.12	0.26
Wing weight	169.00^{BA}	170.50^{BA}	165.00^{B}	176.50^{A}	0.02	2.56
Wing yield	10.68	10.79	10.49	10.87	0.30	0.15
Breast+bone weight	510.00	492.00	494.25	507.50	0.17	7.03
Breast+bone yield	32.19^{A}	31.12^{B}	31.33^{B}	31.22^{B}	0.04	0.30
Breast muscle weight	397.00	393.25	397.50	407.25	0.39	5.92

Breast muscle yield	25.04	24.88	25.21	25.07	0.86	0.27

*Values in the table are the means from 40 birds taken per treatment.

*Yields are the parameter percentages calculated relative to carcass mass.

$^{A-B}$Means within a row with the same superscript do not differ significantly at $P \leq 0.05$.

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn- $(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn- $(HMTBA)_2$.

[5] Carcasses with the head, feet, abdominal fat pad, and viscera removed.

[6] Carcass yield is the carcass mass as a percentage of the live body weight.

Although wing weight was significantly higher for the higher Cu-$(HMTBA)_2$ treatment, the wing yield was not significantly affected, this is most probably due to the slightly higher live body mass in the selected broilers for sampling. The Positive control treatment resulted in a significant increase in breast with bone yield in comparison to the other treatments ($P < 0.05$). All other carcass traits were not significantly affected.

4.3.3 Foot pad dermatitis, breast lesions and litter moisture

4.3.3.1 Foot pad dermatitis and breast lesions at 21 days of age

The negative control treatment resulted in a significantly higher presence of foot pad dermatitis (31.63%) in comparison to all other treatments ($P < 0.05$; Table 4.9). The $(HMTBA)_2$ mineral treatment with a 30 mg/kg Cu inclusion (15.87%) resulted in a non-significant reduction in FPD compared to the positive control and $(HMTBA)_2$ 8:32:32 treatments respectively ($P > 0.05$). Presence of breast lesions was not significantly different between treatments; however the Negative control resulted in the highest percentage (0.86%) of breast lesions ($P > 0.05$).

4.3.3.2 Foot pad dermatitis and breast lesions at 35 days of age

Feeding $(HMTBA)_2$ 30:32: 32 resulted in a significant 10.57% and a non-significant 5.74% increase in saleable paws (grade 1 and 2) in comparison to the negative control and positive control treatments respectively ($P < 0.05$; Table 4.10). Increasing the Cu- $(HMTBA)_2$ to 30 mg/kg in the diet resulted in a non-significant increase of 1.11% in saleable paws in comparison to the lower Cu-$(HMTBA)_2$ treatment at 8 mg/kg.

Table4.9.Effects of feeding Cu, Mn and Zn from (HMTBA)$_2$ sources in comparison with the respective inorganic sulphate trace minerals on foot pad dermatitis and breast lesions of broilers at 21 days of age.

Treatment	**Litter Moisture (%)**	**Foot pad dermatitis present (%)**	**Breast lesions present (%)**
Positive control	50.701	18.62^{A}	0.43
Negative control	50.298	31.63^{B}	0.86
(HMTBA)$_2$ 8:32:32[1]	49.270	18.67^{A}	0.22
(HMTBA)$_2$ 30:32:32[2]	49.121	15.87^{A}	0.44
P-Value	0.52	0.002	0.55
SE	0.89	2.90	0.32

*Values in the table are the means from 40 birds taken per treatment.

$^{A–B}$ Means within a column with the same superscript do not differ significantly at $P \leq 0.05$.

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- (HMTBA)$_2$, 32 mg/kg Mn- (HMTBA)$_2$ and 32 mg/kg Zn-(HMTBA)$_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -(HMTBA)$_2$, 32 mg/kg Mn- (HMTBA)$_2$ and 32 mg/kg Zn-(HMTBA)$_2$.

4.3.3.2 Litter moisture at 21 and 35 days of age

Litter moisture percentage after oven drying the 32 samples each at day 21 and 35 was calculated to be significantly similar throughout the treatments ($P > 0.05$; Table 4.9 and 4.10). FPD scoring and performance results were thus not significantly affected by the litter moisture.

Table 4.10.Effects of feeding Cu, Mn and Zn from $(HMTBA)_2$ sources in comparison with the respective inorganic sulphate trace minerals on foot pad dermatitis of broilers at 35 days of age.

Treatment	**Litter Moisture (%)**	**Grade 1 (%)**	**Grade 2 (%)**	**Grade 3 (%)**	**Grade 4 (%)**	**Grade 5 (%)**	**Sum of grades 1+2 (%)**	**Sum of grades 3+4+5 (%)**
Positive control[1]	47.33	69.08^{B}	8.35^{B}	14.36^{A}	7.72^{AB}	0.49^{B}	77.43^{AB}	22.57^{AB}
Negative control[2]	46.53	58.95^{C}	13.64^{A}	16.11^{A}	9.10^{A}	2.19^{A}	72.60^{B}	27.40^{A}
$(HMTBA)_2$ 8:32:32[3]	44.51	72.26^{AB}	9.80^{B}	9.83^{B}	6.66^{AB}	1.44^{AB}	82.06^{A}	17.94^{B}
$(HMTBA)_2$ 30:32:32[4]	45.05	75.91^{A}	7.26^{B}	10.80^{B}	5.55^{B}	0.47^{B}	83.17^{A}	16.83^{B}
P-Value	0.68	<.0001	0.0096	0.0012	0.1105	0.0254	0.002	0.002
SE	1.83	2.08	1.32	1.14	1.03	0.44	1.95	1.95

*Values in the table are the means from 40 birds taken per treatment

$^{A-C}$ Means within a column with the same superscript do not differ significantly at $P \leq 0.05$.

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

4.3.4 Skin scratches and bruising

The negative control had the highest carcass scratch percentage of 70%, while the $(HMTBA)_2$ 8:32:32 and (HMTBA)2 30:32:32 treatments resulted in a lower carcass scratch incidence of 52.5% and 50%, respectively ($P > 0.05$; Table 4.11).

The average scratch length was significantly reduced by the replacement of Cu, Mn and Zn sulphates with the $(HMTBA)_2$chelate sources of these trace minerals ($P < 0.05$). The $(HMTBA)_2$ 8:32:32treatment had a significantly lower incidence of carcass bruising ($P < 0.05$) and a non-significantly lower incidence of breast lesions compared to the other treatments ($P > 0.05$).

Table 4.11.Effects of feeding Cu, Mn and Zn from $(HMTBA)_2$ sources in comparison with the respective inorganic sulphate trace mineralson carcass scratches, bruises and breast blisters at day 35.

Defect	**Parameter**	**Positive control[1]**	**Negative control[2]**	**$(HMTBA)_2$ 8:32:32[3]**	**$(HMTBA)_2$ 30:32:32[4]**	**SE**	**P-value**
Scratches	Back (%)	45.00	55.00	37.50	35.00	7.64	0.25
	Breast (%)	12.50	7.50	5.00	2.50	3.91	0.31
	Legs (%)	47.50^{A}	10.00^{C}	15.00^{BC}	30.00^{AB}	6.42	0.00
	Total[5](%)	62.50	70.00	52.50	50.00	7.59	0.22
	Ave Scratch length (%)	4.70^{AB}	6.25^{A}	3.93^{B}	3.63^{B}	0.69	0.04
Bruises	Back (%)	10.00	2.50	2.50	2.50	3.15	0.24
	Breast (%)	2.50	2.50	0.00	5.00	2.42	0.55
	Legs (%)	2.50	2.50	0.00	2.50	2.11	0.79
	Wings (%)	12.50	7.50	7.50	2.50	4.08	0.39
	Total[5](%)	27.50^{A}	15.00^{AB}	7.50^{B}	17.50^{AB}	5.74	0.11
Breast lesions	Total[5](%)	3.97	5.40	2.49	2.62	1.63	0.56

$^{A–C}$Means within a row with the same superscript do not differ significantly at $P \leq 0.05$.

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

[5] The percentage of carcasses with one or more scratches/bruises/ breast lesions present with the observations taken from 40 birds per treatment.

4.3.5 Skin and intestinal strength

The skin strength measured as force at break was non-significantly different between the treatments (P > 0.05) (Table 4.12). The $(HMTBA)_2$ 8:32:32 had the highest skin strength (force at break) amongst the non-significant differences, 0.02 N higher than the inorganic sulphate treatment and 0.06 higher than the (HMTBA)2 30:32:32 treatment. The Negative control treatment had the lowest skin strength. Maximum stress was highest for the Negative control, while the $(HMTBA)_2$ 30:32:32 treatment had a significantly lower maximum stress.

Ileum strength (force at break) was measured to be highest for the Mintrex treatments, and the negative control had the lowest strength ($P < 0.05$). The $(HMTBA)_2$ 30:32:32 treatment had 0.62N, 1.03N and 1.32N higher ileum strengths in comparison to the $(HMTBA)_2$ 8:32:32, inorganic sulphate and negative control treatments respectively. Ileum width differed significantly between treatments, however was not directly correlated to ileum strength.

Jejunum measurements did not differ significantly between treatments. The Mintrex treatments resulted in higher jejunum strengths in comparison to the inorganic treatments ($P > 0.05$).

Table 4.12.Effects of feeding Cu, Mn and Zn from $(HMTBA)_2$ sources in comparison with the respective inorganic sulphate trace minerals on skin, ileum and jejunum at day 35 of age.

Parameter	**Measure**	**Positive control**[1]	**Negative control**[2]	**$(HMTBA)_2$ 8:32:32**[3]	**$(HMTBA)_2$ 30:32:32**[4]	**P-Value**	**SE**
Skin Strength	Force at break (N)	13.34	13.11	13.36	13.30	1.00	1.06
	Maximum stress $(N/mm^2)^5$	0.67^{AB}	0.85^{A}	0.68^{AB}	0.54^{B}	0.05	0.08
Ileum strength	Force at break (N)	5.26^{B}	4.97^{B}	5.67^{AB}	6.29^{A}	0.01	0.29
	Maximum stress (N/mm^2)	1.33	0.68	0.65	1.09	0.18	0.25
	Thickness (mm)	1.28	1.41	1.50	1.50	0.14	0.08
	Width (mm)	6.33^{AB}	7.07^{A}	6.88^{AB}	5.97^{B}	0.03	0.36
Jejunum strength	Force at break (N)	5.25	4.97	5.33	5.32	0.70	0.25
	Maximum stress (N/mm^2)	0.65	0.76	0.70	0.64	0.25	0.05
	Thickness (mm)	1.16	1.08	1.16	1.19	0.26	0.06
	Width (mm)	5.08	6.00	5.73	5.20	0.13	0.25

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

[5] Force at break (N) divided by displacement (mm^2).

4.3.6 Tissue analysis

The analysed liver Cu and Mn concentration in the negative control treatment were significantly higher in comparison to both $(HMTBA)_2$ treatments ($P < 0.05$), while the negative control treatment was non-significantly higher for Zn concentration ($P > 0.05$) (Table 4.13). The Cu and Zn liver concentrations were non-significantly different between the positive control, $(HMTBA)_2$ 8:32:32 and $(HMTBA)_2$ 30:32:32 treatments($P >0.05$). The Mn liver concentrations were significantly higher for the positive control treatment in comparison to the $(HMTBA)_2$ treatments ($P < 0.05$).

Table 4.13.Effects of feeding Cu, Mn and Zn from $(HMTBA)_2$ sources in comparison with the respective inorganic sulphate trace minerals on the analysed trace mineral levels within the liver (as is basis) of broilers

	Positive control[1]	**Negative control**[2]	**$(HMTBA)_2$ 8:32:32**[3]	**$(HMTBA)_2$ 30:32:32**[4]	**P-value**	**SE**
Cu (mg/kg)	9.75^{B}	11.89^{A}	10.37^{B}	10.73^{B}	0.004	0.39
Mn(mg/kg)	12.26^{AB}	12.81^{A}	11.76^{B}	11.77^{B}	0.045	0.31
Zn (mg/kg)	83.95	87.24	82.79	86.42	0.170	1.55

$^{A\text{-}B}$Means within a row with the same superscript do not differ significantly at $P \leq 0.05$.

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn-$(HMTBA)_2$.

4.3.7.1 Foot pad histological morphology at 21 days of age

The dermis length and dermis area were significantly lower for the negative control treatment in comparison to all other treatments when comparing all grade 1 samples(Table 4.14; $P<0.05$). The stratus corneum length, epidermis length and stratus corneum area were all non-significantly lower for the negative control treatment in comparison to all other treatments when comparing all grade 1 samples ($P>0.05$). The epidermis length, dermis length, stratus corneum area, epidermis area and dermis area were all non-significantly larger for the $(HMTBA)_2$ treatments in comparison to the positive control treatment when comparing all grade 1 samples; whereas the stratus corneum length was non-significantly larger for the positive control in comparison to the $(HMTBA)_2$ treatments ($P>0.05$).

When comparing the measurements between the different foot pad grades, the epidermis length, stratus corneum area and epidermis area both increased from a foot pad dermatitis grade of 1 to grade 3, with the grade 3 samples resulting in a significantly larger epidermis length, stratus corneum area and epidermis area in comparison to the grade 1 samples (Table 4.15; $P<0.05$). The stratus corneum length, epidermis length and stratus corneum area and dermis area were all significantly smaller for the grade 4 samples in comparison to the grade 2 and 3 samples, whereas the dermis length and epidermis area were significantly larger for the grade 4 samples ($P<0.05$). The inconsistent findings for the grade 4 sample measurements to all others may be a result of the lower number of grade 4 samples available for measurements.

Table 4.14. Effects of feeding Cu, Mn and Zn from $(HMTBA)_2$ sources in comparison with the respective inorganic sulphate trace minerals on the foot pad histological measurements of broilers with a foot pad dermatitis grade of 1 at 21 days of age

Treatment	**Stratus corneum length**	**Epidermis length**	**Dermis length**	**Stratus corneum area**	**Epidermis area**	**Dermis area**
Positive control[1]	132	98^{B}	703^{A}	162348^{A}	125987^{B}	560856^{A}
Negative control[2]	119	95^{B}	603^{B}	153873^{A}	149892^{A}	407774^{B}
$(HMTBA)_2$ 8:32:32[3]	128	111^{A}	766^{A}	175187^{A}	146689^{AB}	569453^{A}
$(HMTBA)_2$ 30:32:32[4]	128	103^{AB}	725^{A}	174117^{A}	138860^{AB}	562499^{A}
P-Value	0.66	0.04	0.01	0.25	0.16	<0.01
SE	7	4	34	8668	8054	30171

$^{A-B}$Means within a column with the same superscript do not differ significantly at $P \leq 0.05$.

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn- $(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn- $(HMTBA)_2$.

Table 4.15.The effects of the different foot pad dermatitis grades on the histological measurements of broiler foot pads at 21 days of age

FPD score	**Stratus corneum length**	**Epidermis length**	**Dermis length**	**Stratus corneum area**	**Epidermis area**	**Dermis area**
Gade 1	127^{A}	101^{C}	699^{BA}	167309^{C}	140357^{C}	525146^{A}
Grade 2	128^{A}	109^{B}	675^{B}	173717^{C}	175120^{B}	486538^{A}
Grade 3	126^{A}	114^{A}	682^{B}	208175^{A}	175651^{B}	570283^{A}
Grade 4	97^{B}	103^{C}	742^{A}	185944^{B}	225601^{A}	461290^{B}
P-Value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
SE	3	2	15	4344	9766	13618

$^{A-C}$Means within a column with the same superscript do not differ significantly at $P \leq 0.05$.

4.3.7.2 Foot pad histological morphology at 35 days of age

The stratus corneum length, dermis length, stratus corneum area and dermis area were all non-significantly lower for the negative control in comparison to all other treatments when comparing all grade 1 samples (Table 4.16; $P > 0.05$). The epidermis length ($P < 0.05$) and epidermis area ($P > 0.05$) was largest for the $(HMTBA)_2$ 8:32:32 treatment when comparing all grade 1 samples between treatments.

The stratus corneum length, dermis length, stratus corneum area and epidermis area increased from a foot pad dermatitis grade of 1 to grade 3, with the largest measurements for these found in the grade 5 samples (Table 4.18; $P < 0.05$). The grade 5 samples also resulted in the largest measurement for epidermis length in comparison to all other grades ($P<0.05$).

Table 4.17. Effects of feeding Cu, Mn and Zn from $(HMTBA)_2$ sources in comparison with the respective inorganic sulphate trace minerals on the foot pad histological measurements of broilers with a foot pad dermatitis grade 1

Treatment	**Stratus corneum length**	**Epidermis length**	**Dermis length**	**Stratus corneum area**	**Epidermis area**	**Dermis area**
Positive control[1]	160	224^{B}	939	395629	330559^{AB}	895748
Negative control[2]	155	133^{B}	932	352379	286065^{B}	784352
$(HMTBA)_2$ 8:32:32[3]	164	448^{A}	947	391331	373697^{A}	888721
$(HMTBA)_2$ 30:32:32[4]	157	221^{B}	1019	360331	280023^{B}	901162
P-Value	0.76	<0.01	0.53	0.58	0.05	0.42
SE	6	41	47	26882	27381	57450

$^{A-B}$ Means within a column with the same superscript do not differ significantly at $P \leq 0.05$.

[1] Dietary treatment consisting of 120 mg/kg $CuSO_4$, 100 mg/kg $MnSO_4$ and 100 mg/kg $ZnSO_4$.

[2] Dietary treatment consisting of 8 mg/kg $CuSO_4$, 32 mg/kg $MnSO_4$ and 32 mg/kg $ZnSO_4$.

[3] Dietary treatment consisting of 8 mg/kg Cu- $(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn- $(HMTBA)_2$.

[4] Dietary treatment consisting of 30 mg/kg Cu -$(HMTBA)_2$, 32 mg/kg Mn- $(HMTBA)_2$ and 32 mg/kg Zn- $(HMTBA)_2$.

Table 4.18. The effects of the different foot pad dermatitis grades on the histological measurements of broiler foot pads

FPD score	**Stratus corneum length**	**Epidermis length**	**Dermis length**	**Stratus corneum area**	**Epidermis area**	**Dermis area**
Grade 1	159^{C}	256^{C}	959^{B}	374917^{E}	317586^{E}	867496^{C}
Grade 2	160^{C}	164^{D}	990^{B}	413182^{D}	379622^{D}	1216904^{A}
Grade 3	243^{B}	395^{B}	1052^{A}	805671^{B}	587488^{B}	1055801^{B}
Grade 4	241^{B}	251^{C}	914^{C}	711770^{C}	563438^{C}	909961^{C}
Grade 5	256^{A}	436^{A}	1076^{A}	994834^{A}	705253^{A}	1050430^{B}
P-Value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
SE	3	13	18	9570	9284	23017

$^{A-E}$Means within a column with the same superscript do not differ significantly at $P \leq 0.05$.

4.4 Discussion

This research was undertaken to evaluate the effects of $(HMTBA)_2$-metal chelates of Zn, Cu and Mn, added at levels lower than its counterparts from inorganic sources typically used commercially, on the performance, intestinal integrity, skin integrity and prevalence of foot pad dermatitis in broilers. It is common practice in poultry diets to use high levels of inorganic trace minerals allowing a large safety margin because of the low and variable availability and low cost of inorganic trace minerals. The disadvantages of this practice are that it increases the antagonism among minerals and brings environmental challenges such as toxicity to plants and subsequently the animals feeding on these plants or the chicken litter. Recently, much attention has been devoted to improving this strategy of trace mineral formulation through the use of organically complexed trace mineral sources. Research suggests that the inclusion of organic trace minerals may reduce carcass downgrades and condemnations, associated with skin problems (lesions, cuts and tears) and intestinal breakage at the processing plant, which can result in considerable economic savings.

FCR (adjusted for mortality) of birds fed $(HMTBA)_2$ 30:32:32 was significantly improved for the period between 28 to 35 days of age compared to all other treatments. FI of birds fed $(HMTBA)_2$ 30:32:32 was significantly improved for the period between 28 to 35 days of age compared to the negative control and $(HMTBA)_2$ 8:32:32 treatments. The performance results, over the entire trial period, of BW, BWG, FI, mortality, and FCR adjusted for mortality were not significantly affected when broilers were fed lower levels of Cu, Mn and Zn (organic and inorganic) compared with broilers fed the commercial levels of these trace minerals in inorganic form. Increasing the Cu-$(HMTBA)_2$ from 8 mg/kg to 30 mg/kg did not result in improved broiler performance indicating that Cu requirements in respect of broiler performance are met through 8 mg/kg Cu-$(HMTBA)_2$.In the studies of Bao *et al.* (2007), Saenmakayak *et al.*(2010) and Osama *et al.* (2012)an improved live performance in regards to body weight and feed conversion was found when replacing inorganic Zn with organic Zn at the same or lower level of supplementation; whilst no differences were observed among any of the performance measurements in several comparative trials between inorganic trace minerals and their organic counterparts(Aksu *et al.* 2010; Aksu *et al.*2011;Manangi*et al.* 2012). These conflicting results of dietary trace mineral research on the growth performance of broilers may be a result of the variable trace mineral levels present in the feed ingredients within diets and the presence of other dietary ligands which can reduce the absorption of certain trace minerals (Saenmahayak *et al.*, 2010).

Solely from the performance results of the present study suggest that commercial levels of inorganic trace minerals are largely over supplied for the performance of broilers, which may result in the excretion of these excess trace minerals into the litter. The study of Manangi *et al.* (2012) found that at day 54, litter Zn, Cu, and Mn from birds fed Zn-, Cu-, and Mn-$(HMTBA)_2$ were reduced by 40, 74, and 35%, respectively, compared with the litter from birds fed inorganic trace minerals. Mineral excretion of Cu, Mn and Zn increased linearly with increasing intakes of these minerals (Bao *et al.*, 2007).

Broiler live weight will follow a normal distribution. The variability of a flock is described by the coefficient of variation (CV %), which is the standard deviation of the population expressed as a percentage of the mean, or by the uniformity percentage of a flock. A low CV indicates a uniform flock while a high CV indicates an uneven flock. This could have important economic implications in the broiler industry as uniform flocks are more predictable in performance than uneven flocks which influence both production profitability and processing plant efficiency. The Positive control treatment resulted in a CV of 11.31%, while the $(HMTBA)_2$ 8:32:32 and $(HMTBA)_2$ 30:32:32 treatments resulted in a CV of 10.30% and 10.38%, respectively. These results indicate a non-significant increase in flock uniformity with the supplementation of $(HMTBA)_2$ chelated trace minerals in comparison to inorganic trace mineral sources.

Feeding the $(HMTBA)_2$ 30:32:32 treatment resulted in a significant increase in percentage of carcass yield in comparison to the inorganic negative control. Carcass traits were mostly not affected by the different trace mineral treatments, providing $(HMTBA)_2$ chelated trace minerals in the diets resulted in a non-significant reduction in intestine yield and an increased breast meat yield. Saenmakayak *et al. (*2010) and Zhao *et al.* (2010) observed a significant increase in breast yield from the replacement of inorganic Zn with complexed organic forms of Zn.

Wet litter predisposes the birds to FPD as it causes the foot pads to be softer and therefore more prone to damage. Litter moisture percentage was calculated to be significantly similar throughout the treatments and trial which allowed the lesion scoring and performance results to be tested on a significantly similar litter environment. The supplementation of $(HMTBA)_2$ trace mineral chelates resulted in an increase of saleable paws in comparison to the inorganic trace minerals. Increasing the inorganic trace mineral inclusion rate resulted in a significant reduction of FPD; while increasing the Cu- $(HMTBA)_2$ to 30 mg/kg in the diet resulted in a non-significant increase of saleable paws in comparison to the lower Cu- $(HMTBA)_2$ treatment at 8 mg/kg. These results indicate that the increased commercial levels of inorganic Cu, Mn and Zn sulphates are necessary for improved skin quality and the reduction of foot pad dermatitis. Further reductions in foot pad dermatitis are obtained through the replacement of Cu, Mn and Zn sulphates with the $(HMTBA)_2$ sources. These results agree with the findings of Manangi *et al. (*2012) and Zhao *et al.* (2010), which concluded that chelated trace minerals improved the foot pad health of commercial broilers, thereby improving their welfare. FPD increased in incidence and severity for all four treatments as the trial progressed from 21 days to slaughter. Also as the trial progressed from day 21 to 35 days of age, the $(HMTBA)_2$ chelated trace mineral treatments resulted in a greater difference in improvement of FPD when compared to the inorganic trace mineral treatments. This may indicate that the foot pad health benefits of replacing inorganic trace minerals with their organic counterparts is more noticeable at an older age in broilers.

The skin strength measured as force at break was non-significantly different between the treatments. The $(HMTBA)_2$ 8:32:32 had the highest skin strength amongst the non-significant differences. Ileum strength, force at break, was strongest for the $(HMTBA)_2$ chelated trace mineral treatments. The $(HMTBA)_2$ 30:32:32 treatment had 0.62N, 1.03N and 1.32N higher ileum strengths in comparison to the $(HMTBA)_2$ 8:32:32, positive control and negative control treatments, respectively. The reduction of FPD and enhanced ileum strength may be the consequence of a higher bioavailability of Cu, Mn and Zn resulting in an increased collagen, elastin and epithelial synthesis and rate of cross-linking which holds the cells more tightly together; providing a higher resilience and protection of tissues from tearing (Rossi *et al.*, 2007). In the study of Rossi *et al.* (2007) a reduction in skin tearing was observed as a result of the higher number of skin epithelial cell layers, higher collagen content, and reduced inflammation of the skin. Birds supplemented with 25 mg/kg$(HMTBA)_2$chelated Cu had a significantly higher intestinal breaking strength than all other Cu sources consisting of supplemented Cu sulphate, Cu proteinate and Cu lysine (Richards *et al.*, 2005). Jejunum measurements did not differ significantly between treatments however the $(HMTBA)_2$ chelated trace mineral treatments tended to result in higher jejunum strengths in comparison to the inorganic treatments.

Carcass scratches, scratch severity and breast lesions were reduced through feeding inorganic trace minerals at a higher inclusion rate, while further improvements resulted from the use of $(HMTBA)_2$ chelated trace minerals. The research results indicated that the use of organically complexed trace minerals may provide a viable tool in minimizing condemnations and downgrades during processing (by improving skin quality and foot pad health), improving bird performance and the profitability of a financially pressurized broiler industry. The study of Saenmahayak *et al. (*2010) found no significant effect when comparing dietary treatments of inorganic Zn replaced with an organic source for most of the carcass defects (scratches, scabs and sores) and the number of grade A carcasses.

The Cu and Zn liver concentrations were non-significantly different between the positive control, $(HMTBA)_2$ 8:32:32 and $(HMTBA)_2$ 30:32:32 treatments which is in agreement with the study of Manangi *et al. (*2012) ($P < 0.05$). The Mn liver concentrations were significantly higher for the positive control treatment in comparison to the $(HMTBA)_2$ treatments($P < 0.05$).

The liver mineral analyses suggest that feeding much lower levels of dietary trace minerals in inorganic and organic forms was adequately available to the broilers at the level of liver uptake. These findings are similar to those of Bao *et al. (*2007) that found higher trace mineral concentrations in the livers of birds with a lower dietary inclusion for these minerals. Research suggests that homeostatic mechanisms allow chickens to give priorities to their mineral requirements for vital functions indicated by normal concentrations of the minerals in the livers of birds. A deficiency/reduced supply of trace minerals in broiler feed is usually overcome by a combination of responses, including improved absorption, increased release from storage deposits, and reduced trace mineral excretion.

The higher lesion and skin defects, lower ileum strength, carcass yield and breast muscle yield in the negative control treatment supports the theory of homeostasis which could be the result of reduced partitioning of these trace minerals towards growth and/or maintenance of skin tissue and muscle. Results agree with Richards *et al. (*2010) that the lower organic levels compared to commercial inorganic levels of Cu, Mn and Zn are sufficient for maintaining broiler performance, improving broiler foot pad health and reducing carcass scratching which may be attributed to the higher bioavailability of the organic sources compared to the inorganic sources.

In the study of Richards *et al.*(2010)the bioavailability of a chelated Zn source in comparison to Zn sulphates was reported to be 161 and 248% based on tibia Zn analysis and a small intestine metallothionein mRNA expression assay, respectively. Ao *et al. (*2006)suggested157 and 183% bioavailability values for Zn from an organic source relative to sulphates at a 100% bioavailability, based on tibia Zn and BW gain, respectively. The bioavailability of Cu from Cu-$(HMTBA)_2$ was112% compared with Cu from a 100% bio available reagent grade Cu-Sulphates in a 14-d chick assay trial (Wang *et al.*, 2007). Mn-$(HMTBA)_2$ was suggested to be 16 and 54% more bio available than the sulphate and oxide forms of Mn, respectively (Yan and Waldroup, 2006).However not all trials have shown increased bioavailability through the inclusion of organic trace minerals (Miles *et al.*,2003).An important finding was observed in the study of Aksu *et al.* (2010) when comparing inorganic trace minerals replaced with the same level of organically complexed minerals on the liver mineral concentrations of broilers. The analysed liver mineral concentrations were significantly lower in chickens fed the organically complexes minerals. Aksu *et al.* (2010) stated this may be an important implication for reducing toxicity of minerals that accumulate in the liver such as Cu.

All measurements of the foot pad stratus corneum, epidermis and dermis were much larger at 35 days of age in comparison to 21 days of age. This confirms the foot pad growth that takes place in broilers from 21 to 35 days of age. The histological measurements between the positive control treatment (commercial inorganic trace minerals) and the $(HMTBA)_2$ treatments may have shown a small non-significant increase in stratus corneum, epidermis and dermis areas at 21 days of age, however at 35 days of age the results were much more variable, with the positive control resulting in the largest stratus cornem area. The trial results were in agreement with Youssef *et al.* (2009) and Cengiz*et al*. (2012), which showed an increasing foot pad dermatitis grade indicated a trend towards an increased stratus corneum and epidermis measurements due to lesion development causing inflammation, thickening and hyperkeratosis.

4.5 Conclusion

The improved genetics of the modern broiler has increased the necessity of a highly nutritional diet and the supplementation of trace minerals to allow the broiler to reach its genetic potential. From this study it can be concluded that the $(HMTBA)_2$ chelated trace minerals may be supplied in broiler rations at much lower levels than the currently utilised commercial levels of inorganic trace minerals without having any adverse effects on the performance and carcass characteristics of birds. These research results show that lower levels of inorganic Cu, Mn and Zn at 8 mg/kg, 32 mg/kg and 32mg/kg respectively are insufficient for maintaining high carcass yields and low carcass condemnations in broiler production; research suggests that this is the result of the inorganic trace minerals' lower bioavailability resulting in higher excretion rates in comparison to their organic counterparts. The trial results suggest that $(HMTBA)_2$ metal chelates can be recommended for optimal tissue integrity and lead to improved foot pad health and intestine strength. As pressure increases in the broiler industry through rising input costs and animal welfare concerns, organic trace minerals may provide a viable tool for improving broiler production and overall profitability.

Chapter 5

References

Aksu D. S.,T. Aksu, B. Ozsoy and E. Baytok. 2010. The effects of replacing inorganic with a lower level of organically complexes minerals (Cu, Zn and Mn) in broiler diets on lipid peroxidation and antioxidant defense systems. Asian-Aust. J. Anim. Sci. Vol. 23, No. 8:1066-1072.

Aksu T.,B. Ozsoy, D. S. Aksu, M. A. Yoruk and M. Gul. 2011. The Effects of lower levels of organically complexed Zn, Cu and Mn in broiler diets on performance, mineral concentration of tibia and mineral excretion. Kafkas Univ. Vet. Fak. Derg. 17 (1): 141-146.

Alchalabi, D. (2002): Correlation between litter pH and airflow pattern. Poult. Int. 2002, 42-46.

Allain, V., L. Mirabito, C. Arnould, M. Colas, S. Le Bouquin, C. Lupo and V. Michel. 2009. Skin lesions in broiler chickens measured at the slaughterhouse: Relationships between lesions and between their prevalence and rearing factors. Br. Poult. Sci. 50:407–417.

Ao, T., J. L. Pierce, R. Power, K. A. Dawson, A. J. Pescatore, A. H. Cantor, and M. J. Ford. 2006. Evaluation of Bioplex Zn® as an organic Zn source for chicks. Int. J. Poult. Sci. 5:808–811.

AOAC, 2000. Official method of analysis 920.39 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc., Maryland, USA.

AOAC, 2000.Official method of analysis 934.01 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc., Maryland, USA.

AOAC, 2000. Official method of analysis 935.13 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc., Maryland, USA.

AOAC, 2000. Official method of analysis 942.05 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc., Maryland, USA.

AOAC, 2000. Official method of analysis 962.09 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc., Maryland, USA.

AOAC, 2000. Official method of analysis 965.17 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc., Maryland, USA.

AOAC, 2000. Official method of analysis 968.06 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc., Maryland, USA.

Ask, B. 2010. Genetic variation of contact dermatitis in broilers. Poult. Sci. 89, 866-875.

Aviagen. 2000. http://en.aviagen.com/assets/Tech_center/Ross_Broiler_Manul_09. Ross 708 broiler manual.

Bao Y. M., and M. Choct. 2009. Trace mineral nutrition for broiler chickens and prospects of application of organically complexes trace minerals: a review Anim. Prod. Sci. 49:269–282.

Bao Y. M., M. Choct, P. A. Iji, and K. Bruerton. 2007. Effect of organically complexed Cu, Iron, Mn, and Zn on broiler performance, mineral excretion, and accumulation in tissues. J. Appl. Poult. Res. 16:448–455.

Berg, C. C. 1998. Foot-pad dermatitis in broilers and turkeys. Prevalence, risk factors and prevention. Doctor's dissertation.

Berk, J. 2007. Can alternative kinds of litter reduce foot pad dermatitis in female turkeys? Hafez, H. M. (Ed.). Proceedings of the 4th international symposium on turkey production, Institute of Poultry Diseases, Free University, Berlin, Germany, pp. 26-27.

Bessei, W. 2006. Welfare of broilers: A review. World's Poult. Sci. J. 62:455–466. Factors contributing to foot pad dermatitis in broilers. Watt Poultry USA, pp. 26-27.

Bilgili, S. F., 1990. Factors contributing to broiler carcass yield and quality from live production through processing. Pages 26-36a in: Proceeding of the International Poultry Congress. Istanbul, Turkey.

Bilgili, S. F., M. A. Alley, J. B. Hess, and M. Nagaraj. 2006. Influence of age and sex on footpad quality and yield in broiler chickens reared on low and high density diets. J. Appl. Poult. Res. 15:433–441.

Bilgili, S. F., J. B. Hess, J. P. Blake, K. S. Macklin, B. Saenmahayak, and J. L. Sibley. 2009. Influence of bedding material on footpad dermatitis in broiler chickens. J. Appl. Poult. Res. 18:583–589.

Bilgili S. F. and J. B. Hess. 1997. Tensile strength of broiler intestines as influenced by age and feed withdrawal. J. Appl. Poultry Res 6:279-283.

Bilgili, S. F., and J. B. Hess, 1996. Tensile strength of broiler intestines during feed withdrawal. Poultry Sci. 75 (Suppl. 1):96 (Abstr.).

Breuer, P., S. Buda and K. D. Budras. 2006. Investigation of the pre- and postnatal development of the foot pad skin of turkey poults. In: New insights into fundamental physiology and peri-natal adaptation of domestic fowl, (eds. Yahav, S. and Tzschentke, B.), Nottingham University Press, Nottingham, 167-172.

Buhr R. J., J. K. Northcutt, C. E. Lyon, and G. N. Rowland. 1998. Influence of time off feed on broiler viscera weight, diameter, and shear. Poult. Sci. 77:758–764.

Bun, S. D., Y. M. Guo, F. C. Guo, F. J. Ji, and H. Cao. 2011. Influence of organic Zn supplementation on the antioxidant status and immune responses of broilers challenged with Eimeriatenella. Poult. Sci. 90:1220–1226.

Burrell, A. L., W. A. Dozier III, A. J. Davis, M. M. Compton, M. E. Freeman, P. F. Vendrell, and T. L. Ward. 2004. Responses of broilers to dietary Zn concentrations and sources in relation to environmental implications. Br. Poult. Sci. 45:255-263.

Casey N. H., R. I. Crosley and G. A. Smith. 1992. Influence of continuous dietary halofuginone on broiler skin tensile strength and growth performance. J. S.Afr. Vet. Ass. 63(1):16-19.

Cengiz, O., B. H. Köksal , A. G. Önol , O. Tatlı , Ö. Sevim , H. Avcı,and S. F. Bilgili. 2012. Influence of dietary enzyme supplementation of barley-based diets on growth performance and footpad dermatitis in broiler chickens exposed to early high-moisture litter. J. Appl. Poult. Res. 21:117–125.

Custodis, P. and H.M. Hafez. 2007. Foot pad dermatitis: A review on possible causes. Proceedings of the 4th International Symposium on Turkey Production, Berlin, Germany, (ed. Hafez, H. M.), Mensch-und-Buch-Verlag, Berlin, 138-142.

Dibner, J. J., J. D. Richards, M. L. Kitchell and M. A. Quiroz. 2007. Metabolic challenges and early bone development. J. Appl. Poult. Res. 16:126-137.

Ekstrand, C., B. Algers and J. Svedberg. 1997. Rearing conditions and foot-pad dermatitis in Swedish broiler chickens. Preventive Veterinary Medicine 31: 167-174.

Ekstrand, C., T. E. Carpenter, I. Andersson, and B. Algers. 1998. Prevalence and control of foot-pad dermatitis in broilers in Sweden. Br. Poult. Sci. 39:318–324.

Estevez I. 2007. Density Allowances for Broilers: Where to Set the Limits? Poult. Sci. 86:1265–1272.

Greene, J.A., R.M. Mccracken and R.T. Evans. 1985. A contact dermatitis of broilers—clinical and pathological findings. Avian Pathology, 14: 23–38.

Gross Energy (Not a Metabolisable E) MC - 1000 Modular Calorimeter. Operators Manual.

Haslam, S.M., T.G. Knowles, S.N. Brown, L.J. Wilkins, S.C. Kestin, P.D. Warriss and C.J. Nicol. 2007. Factors affecting the prevalence of foot pad dermatitis, hock burn and breast burn in broiler chicken. Br. Poult. Sci. 48, 264-275.

Hess, J. B., S. F. Bilgili and K. M. Downs. 2004. Paw quality issues. Proc. Deep South Poultry Conference, Tifton, GA. University of Georgia, Athens.

Hess, J.B., S.F. Bilgili, A.M. Parson and K.M. Downs. 2001. Influence of completed Zn products on live performance and carcass grade of broilers. J. Appl. Anim. Res. 19: 49-60.

Hocking, P.M., R.K. Mayne, R.W. Else, N.A. French and J. Gatcliffe (2008): Standard European footpad dermatitis scoring system for use in turkey processing plants. World's Poult. Sci. J. Vol. 64: 323 – 328.

Hooge, D.M. 2003. Dietary mannan oligosaccharides improve broiler and turkey performance: meta-analysis of pen trials around the world. Page 113–124 in Proceedings of the Alltech’s 19th Annual Symposium: Nutritional Biotechnology in the Feed and Food Industries.

Huang, Y. L., L. Lu, S. F. Li, X. G. Luo and B. Liu. 2009. Relative bioavailabilities of organic Zn sources with different chelation strengths for broilers fed a conventional corn soybean meal diet. J. Anim. Sci. 87:2038-2046.

Jahanian, R., H. N. Moghaddam and A. Rezaei. 2008. Improved broiler chick performance by dietary supplementation of organic Zn sources. Asian-Aust. J. Anim. Sci. Vol. 21, No. 9: 1348 – 1354.

Kapell, D. N. R. G., W. G. Hill, A. M. Neeteson, J. McAdam,A. N. M. Koerhuis, and S. Avendaño. 2012. Genetic parameters of foot pad dermatitis and body weight in purebred broiler lines in 2 contrasting environments. Poult. Sci. 91:565–574.

Kestin, S. C., G. Su and P. Sorensen. 1999. Different commercial broiler crosses have different susceptibilities to leg weakness. Poult. Sci. 78:1085–1090.

Kidd, M. T. 2004. Nutritional modulation of immune function in broilers. Poult. Sci. 83:650–657.

Kjaer, J. B., G. Su, B. L. Nielsen, and P. Sørensen. 2006. Foot pad dermatitis and hock burn in broiler chickens and degree of inheritance. Poult. Sci. 85:1342 1348.

Manangi, M. K., T. Hampton, P. Fisher, J. D. Richards, M. Vazquez-Anon and K. D. Christensen. 2010. Impact of feeding lower levels of chelated minerals vs. industry levels of inorganic trace minerals on broiler performance, yield, foot pad health, and litter minerals concentration. Proc. International Poultry Scientific Forum Atlanta, GA.

Manangi M. K., M. Vazquez-Añon, J. D. Richards, S. Carter, R. E. Buresh and K. D. Christensen. 2012. Impact of feeding lower levels of chelated trace minerals versus industry levels of inorganic trace minerals on broiler performance, yield, footpad health, and litter mineral concentration. J. Appl. Poult. Res. 21:881 890.

Mayne, R. K. 2005. A review of the aetiology and possible causative factors of foot pad dermatitis in growing turkeys and broilers. World's Poult. Sci. J. 61:256–267.

Mayne, R. K., R. W. Else, and P. M. Hocking. 2007a. High dietary concentrations of biotin did not prevent foot pad dermatitis in growing turkeys and external scores were poor indicators of histopathological lesions. Br. Poult. Sci. 48:291–298.

Mayne, R. K., R. W. Else, and P. M. Hocking. 2007b. High litter moisture alone is sufficient to cause footpad dermatitis in growing turkeys. Br. Poult. Sci. 48:538–545.

Mcilroy, S.G., E.A. Goodall and C.H. Mcmurray. 1987. A contact dermatitis of broilers - epidemiological findings. Avian Pathology 16: 93-105.

Meluzzi, A., C. Fabbri, E. Folegatti and F. Sirri. 2008a. Survey of chicken rearing conditions in Italy: Effects of litter quality and stocking density on productivity, foot dermatitis and carcass injuries. Br. Poult. Sci. 49:257–264.

Meluzzi, A., F. Sirri, E. folegatti and C. Fabbri. 2008b. Effect of less intensive rearing conditions on litter characteristics, growth performance, carcase injuries and meat quality of broilers. Br. Poult. Sci. 49:509–515.

Miles R. D., G. D. Butcher, P. R. Henry, and R. C. Littell. 2006. Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. Poult. Sci. 85:476–485.

Mohanna C., and Nys Y. 1999. Effect of dietary Zn content and sources on the growth, body Zn deposition and retention, Zn excretion and immune response in chickens. Br. Poult. Sci. 40, 108–114.

Murakami, A. E., E. O. Oviedo-rondo N. E. N. Martins, M. S. Pereira and C. Scapinello. 2001. Sodium and chloride requirements of growing broiler chickens (twenty-one to forty-two days of age) fed corn-soybean diets. Poult. Sci. 80:289–294.

Murakami, A.E., E.A. Saleh, S.E. Watkins and P.W. Waldroup. 2000. Sodium source and level in broiler diets with and without high levels of animal protein. Journal of applied Poultry Research 9: 53-61.

Nagaraj, M., F. Biguzzi, J.B. Hess and S.F. Bilgili. 2006. Paw burns in broiler chickens are negatively affected by high protein and all vegetable diets. Poult. Sci. 85(Suppl.1):169–170.

Nagaraj, M., J.B. Hess and S.F. Bilgili. 2007c. Evaluation of a feed-grade enzyme in broiler diets to reduce pododermatitis. J. Appl. Poult. Res. 16:52 – 61.

Nagaraj, M., C.A.P. Wilson, J.B. Hess and S.F. Bilgili. 2007b. Effect of high protein and all-vegetable diets on the incidence and severity of pododermatitis in broiler chickens. J. Appl. Poult. Res. 16:304–312.

Nagaraj, M., C.A.P. Wilson, B. Saenmahayak, J.B. Hess and S.F. Bilgili. 2007a. Efficacy of a litter amendment to reduce pododermatitis in broiler chickens. J. Appl. Poult. Res. 16:255–261.

Nollet L, Van der Klis JD, Lensing M, and Spring P. 2007. The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion. J. Appl. Poult. Res. 16: 592-597.

Northcutt J. K., S. I. Savage and L. R. Vest. 1997. Relationship between feed withdrawal and viscera condition of broilers. Poult. Sci. 76:410–414.

National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. Academy Press, Washington D. C.

Osama M. El-Husseiny1, Samia M. Hashish2, Rida A. Ali, Sohair A. Arafa, Laila D. Abd El- Samee and Ahmad A. Olemy. 2012. Effects of feeding organic Zn, Mn and Cu on broiler growth, carcass characteristics, bone quality and mineral content in bone, liver and excreta. Int. J. of Poult. Sci. 11 (6): 368-377.

Owens, B., M. E. E. McCann, and C. Preston. 2009. The effect of substitution of inorganic Zn with proteinated or chelated Zn on broiler chick performance. J. Appl. Poult. Res. 18:789–794.

Pagazaurtundua, A., and P. D. Warriss. 2006. Levels of foot pad dermatitis in broiler chickens reared in 5 different systems. Br. Poult. Sci. 47:529–532.

Parsons, J. 2006. Use of litter amendments can be beneficial. Poultry Times. Suppl. 2006:2A.

Patton, R.S., 1990. Chelated minerals: what are they, do they work? Feedstuffs 62:9, 14-17, 43.

Richards, J. D., J. Zhao, R. J. Harrell, C. A. Atwell, and J. J. Dibner. 2010. Trace mineral nutrition in poultry and swine. Asian-Aust. J. Anim. Sci. Vol. 23, No. 11: 1527 – 1534.

Richards, J. D., T. Hampton, C. W. Wuelling, M. E. Wehmeyer, M. L. Kitchell and J. J. Dibner. 2005. Mintrex Zn organic trace mineral (Znbis[-2-hydroxy-4-methylthiobutyrate]) serves as a Zn and methionine source, and improves performance, intestinal epithelial lifespan, gut breaking strength and tibia Zn in broilers. International Poultry Scientific Forum, Atlanta, GA.

Rossi, P., F. Rutz, M. A. Anciuti, J. L. Rech, and N. H. F. Zauk. 2007. Influence of graded levels of organic Zn on growth performance and carcass traits of broilers. J. Appl. Poult. Res. 16:219–225.

Saenmahayak, B., S. F. Bilgili, J. B. Hess, and M. Singh. 2010. Live and processing performance of broiler chickens fed diets supplemented with complexes Zn. Appl. Poult. Res. 19:334–340.

Sahin, K., N. Sahin , O. Kucuk , A. Hayirli , and A. S. Prasad. 2009. Role of dietary Zn in heat-stressed poultry. Poult. Sci. 88:2176–2183.

Shanawany M.M., 1992. Influence of litter water-holding capacity on broiler weight and carcass quality. *Arch. Geflügelk.*, 56: 177-179.

Shepherd, E. M. and B. D. Fairchild. 2010. Footpad dermatitis in poultry. Poult. Sci. 89: 2043–2051.

Sirri, F., G. Minelli, E. Folegatti, S. Lolli and A. Merluzzi. 2007. Foot dermatitis and productive traits in broiler chickens kept with different stocking densities, litter types and light regimen. Ital. J. Anim. Sci. 6:734–736.

Sunder, G. S., A. K. Panda, N. C. S. Gopinath, S. V. Rama Rao, M. V. L. N. Raju, M. R. Reddy, and C. V. Kumar. 2008. Effects of higher levels of Zn supplementation on performance, mineral availability, and immune competence in broiler chickens. J. Appl. Poult. Res. 17:79–86.

Wang, Z., S. Cerrate, C. Coto, F. Yan, and P. W. Waldroup. 2007. Evaluation of MINTREX® Cu as a source of Cu in broiler diets. Int. J. Poult. Sci. 6:308–313.

Weaver, W.D. and R. Meijerhof. 1991. The effect of different levels of relativehumidity and air movement on litter conditions, ammonia levels, growth, and carcass qualityfor broiler chickens. Poultry Science 70, 746–755.

Yan, F. and P. W. Waldroup. 2006. Evaluation of MINTREX® Mn as a source of Mn for young broilers. Int. J. Poult. Sci. 5:708–713.

Youssef, I., A. Beineke, and J. Kamphues. 2009. Effects of diet composition and litter quality on development and severity of foot pad dermatitis (FPD) in young fattening turkeys. Proceedings of XIV ISAH Congress. 19-23.07.2009, Vechta, Germany, pp. 393-396.

Youssef, I.M.I., A. Beineke, K. Rohn, and J. Kamphues. 2010. Experimental study on effects of litter material and its quality on foot pad dermatitis in growing turkeys. Int. J. of Poult. Sci. Vol. 9 (12): 1125-1135.

Youssef, I.M.I., A. Beineke, K. Rohn, and J. Kamphues. 2011a. Effects of litter quality (moisture, ammonia, uric acid) on development and severity of foot pad dermatitis in growing turkeys. Journal of Avian Diseases, Vol. 55: 51 – 58.

Youssef, I.M.I., A. Beineke, K. Rohn, and J. Kamphues. 2011b. Effects of macrominerals - surplus in the diet and high litter moisture on development and severity of foot pad dermatitis in growing turkeys. J. of European Poult. Sci. (In Press).

Youssef, I.M.I., A. Beineke, K. Rohn, and J. Kamphues. 2011c. Effects of high dietary levels of soybean meal and its constituents (potassium, oligosaccharides) on foot pad dermatitis in growing turkeys housed on dry and wet litter. Archives of Animal Nutrition, Vol. 65 (2): 148 – 162.

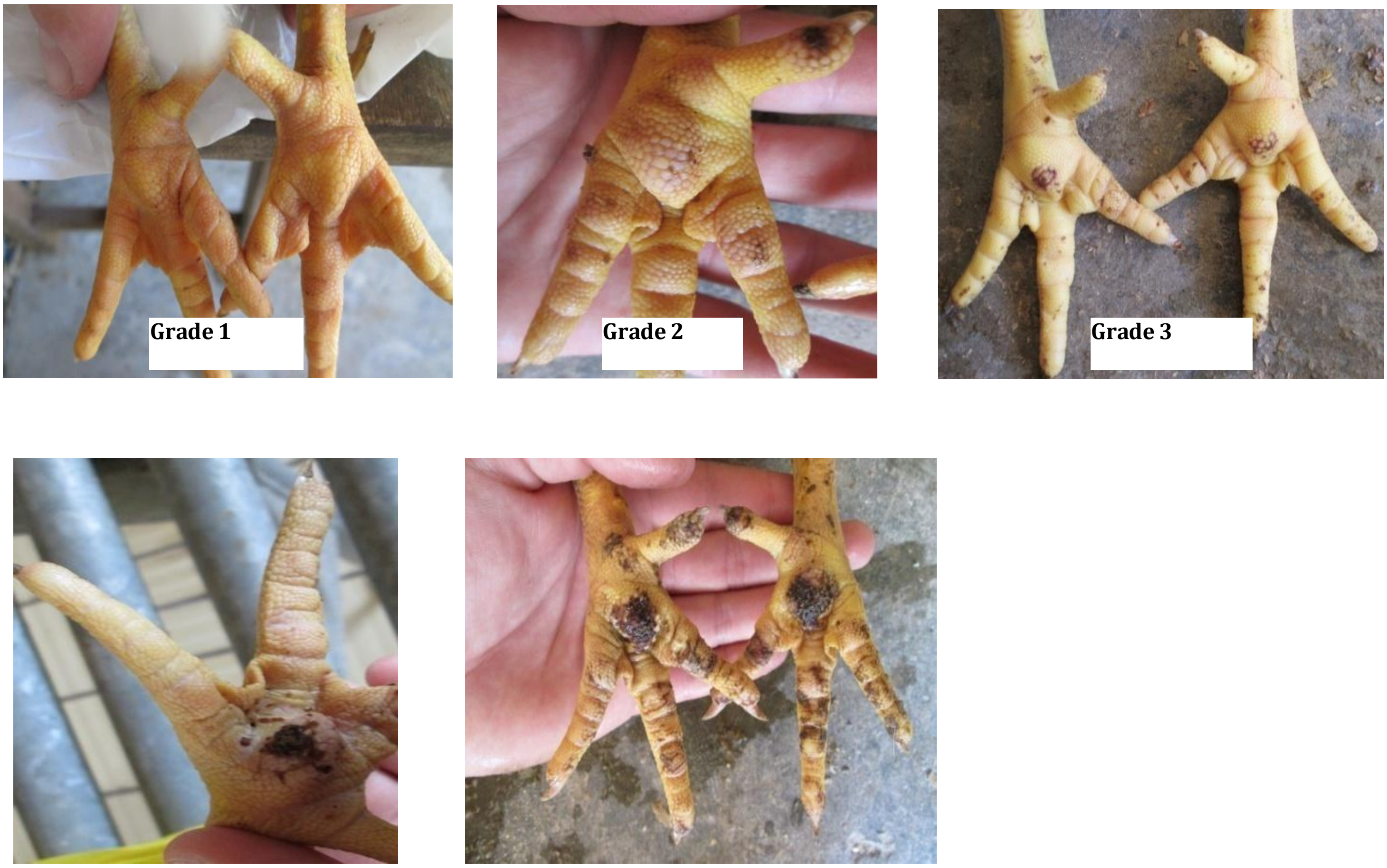
Youssef, I.M.I., A. Beineke, K. Rohn, and J. Kamphues. 2011d. Influences of increased levels of biotin, Zn or mannan oligosaccharides in the diet on foot pad dermatitis in growing turkeys housed on dry and wet litter. Journal of Animal Physiology and Animal Nutrition, Article first published online: 4 January 2011.

Zhao, J., R. B. Shirley, T. R. Hampton, J. D. Richards, R. J. Harrell, J. J. Dibner, C. D. Knight and M. Vazquez-Anon. 2008. Benefits of an organic trace mineral on performance with dietary Cu antagonism in broilers. Poultry Scienc Association 97th Annual Meeting, July 20-23, 2008, Niagara Falls, Canada.

Zhao, J., R. B. Shirley, M. Vazquez-Anon, J. J. Dibner, J. D. Richards, P. Fisher, T. Hampton, K. D. Christensen, J. P. Allard and A. F. Giesen. 2010. Effects of chelated trace minerals on growth performance, breast meat yield, and footpad health in commercial meat broilers. J. Appl. Poult. Res. 19:365–372.

Zuidhof M. J., R. H. McGovern, B. L. Schneider, J. J. R. Feddes, F. E. Robinson, and D. R. Korver. 2004. Effects of Feed Withdrawal Time on the Incidence of Fecal Spillage and Contamination of Broiler Carcasses at Processing. J. Appl. Poult. Res. 13:171–177.

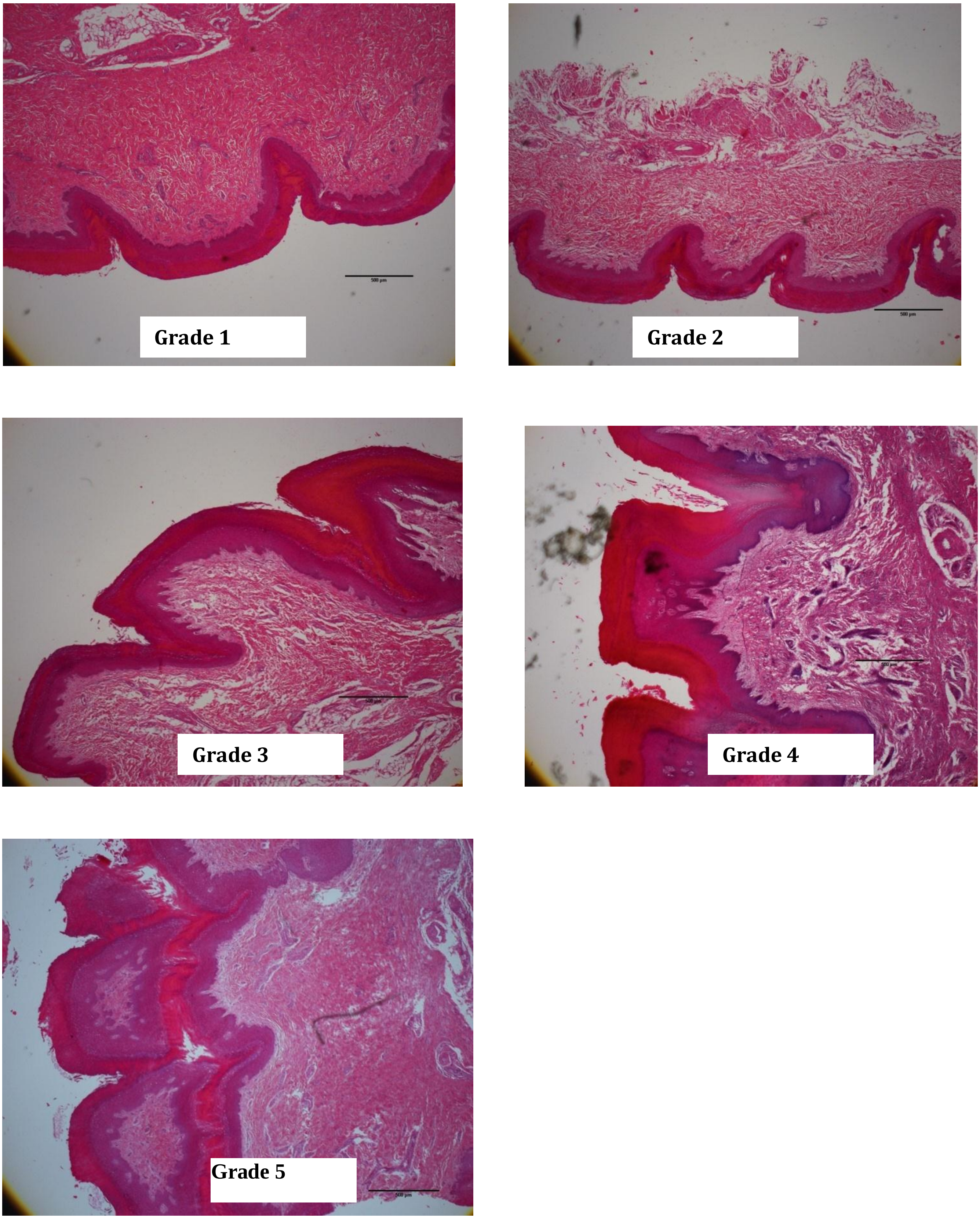
Figure 1: Grades of foot pad dermatitis



Grade 4

Grade 5

Figure 2: The histological appearance of the different foot pad dermatitis scores under microscope after hematoxylin and eosin staining of the skin (bar = 500µm)



Grade 1

Grade 2

Grade 3

Grade 4

Grade 5

Grade 1: Normal stratus corneum, epidermis and dermis layers.

Grade 2: Hypertrophy of the stratus corneum and the epidermis (hyperkeratosis)

Grade 3: Thickening and poor keratinization in the stratum corneumand epidermis. Elongation of the papillae.

Grade 4: Deep ulcers were observed with dead keratin on foot pad surface. Stratus corneum was thickened, poorly keratinized with excess keratin present and its surface was destroyed in some areas. Hyperplasia and acanthosis of the epidermis.

Grade 5: Deep ulcers were observed with dead keratin on foot pad surface. Stratus corneum was thickened, poorly keratinized with loose and excess keratin present; its surface was destroyed in some areas. Epidermal hyperplasia, acanthosis and the epidermis surface was destroyed in some areas. Stratus corneum and epidermis were eroding or eroded away in some areas.

Figure 3: Histological measurements of the papillae