NATURE AND LEVEL OF TRACE MINERAL PREMIX SUPPLEMENTATION ON GROWTH PARAMETERS AND MINERAL EXCRETION IN COMMERCIAL BROILER RATIONS

by

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As the candidate's Supervisor I have/have not approved this dissertation for submission

Signed: _____________ Dr Mariana Ciacciariello Date: ________________

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LIST OF ABBREVIATIONS

AAFCO - Association of American Feed Control Officials.

ADG – Average Daily Gain

ADFI – Average Daily Feed Intake

BW – Body Weight

Cu – Copper

Cum. FI – Cumulative Feed Intake

EC – European Commission

ECL – Epol Central Lab

EFSA – European Food Safety Authority

EU – European Union

FCR – Feed Conversion Ratio

FDA – Food and Drug Administration

FEEDAP - The Panel on Additives and Products or Substances used in Animal Feed

GIT – Gastrointestinal Tract

HMTBa – Hydroxy Methylthiobutanoic Acid

HTM – Hydroxy Trace Minerals

ICP – Inductively Coupled Plasma

ITM – Inorganic Trace Minerals

MAAC – Metal Amino Acid Chelates

Mn – Manganese

MTL – Maximum Tolerable Level

NRC – National Research Council

OTM – Organic Trace Minerals

RBV – Relative Bioavailability

SAPA – South African Poultry Association

TBCC – Tribasic Copper Chloride

TBMC – Tribasic Manganese Chloride

TM – Trace Minerals

UKZN – University of KwaZulu-Natal

Zn - Zinc

ABSTRACT

Commercial broiler premixes provide trace minerals (TM) such as Zinc (Zn), Manganese (Mn) and Copper (Cu) in excess of the birds' requirements to maximize broiler performance (Untea *et al.*, 2011). High inclusion levels of TM along with their low absorption in the GIT of the broiler, has led to increased levels of TM in their litter (Nicholson & Chambers, 2008; Manangi *et al.*, 2012). Concerns have been raised about the accumulation of TM in the environment due to the high TM content of poultry litter.

Two 35-day broiler trials were conducted at a broiler facility with 2880 day-old, Cobb 500 broiler males. Trial 1 evaluated whether decreasing inorganic trace minerals (ITM) levels (specifically Zn, Mn and Cu) in broiler diets would have a negative effect on broiler growth parameters such as body weight (BW), average daily gain (ADG), cumulative feed intake (Cum. FI) and feed conversion ratio (FCR). Zn, Mn and Cu were supplemented at 100%, 50%, 25% and 0% of the Cobb standards. Trial 2 tested whether broilers differed in their growth performance when supplemented with ITM, Organic (OTM) and Hydroxy (HTM) sources of TM (Zn, Mn and Cu) and which source would produce the least amount of TM in their excreta.

No significant difference in Cum. FI and FCR was observed between the treatments in both trials for the first 21 days. On completion of Trial 1, no difference was observed in body weight between the PC, NC and 50% Cobb levels at day 35. In Trial 2 birds supplemented with HTM were 55g heavier ($P<0.05$) than those fed ITM at the same inclusion level at 35 days of age while those birds fed the PC, OTM and HTM showed no significant difference in their body weights. Providing broilers with HTM significantly reduced ($P<0.05$) Zn and Cu excretion at 35 days of age when compared to those diets containing ITM.

From the study it was concluded that reducing TM levels or supplementing different sources of TM to broiler diets at lower levels showed no negative effect on broiler performance. The use of HTM significantly reduced TM mineral excretion of broilers. The results suggest that the use of HTM can maintain broiler performance while sustaining the environment.

CHAPTER 1

GENERAL INTRODUCTION

According to the United Nations World Population Prospects: The 2017 Revision, approximately 7.6 billion human beings inhabit planet Earth as of mid-2017. The world's population continues to grow and forecasts suggest that by the year 2030 there will be 8.6 billion people while in 2050 the population would have increased to 9.8 billion people. Driven by the world's continuous population growth and increasing incomes, the demand for meat is growing worldwide.

Meat markets in developing countries such as those in Asia and Africa are growing rapidly and showing the greatest demand for meat. The majority of the world's population is distributed amongst the lower to middle class and poultry meat is the most affordable type of meat on the market. The poultry sector has shown the largest growth out of all meat sectors with cultural factors in bigger economies such as India and Muslim dominated regions regulating the growth of beef and pork. Over the last five years worldwide broiler meat production has grown from 84 million metric tons to 89 million metric tons as of April 2017 (USDA, 2017). As the world's population exponentially grows, the demand for broiler meat can only increase and the amount of chickens needed to meet this demand will have to expand.

The SAPA Key Markets in the Broiler Industry report for the first quarter of 2017 reported that 80.5 million broilers were placed in South Africa during March 2017 with 79.7 million of those birds being slaughtered. The production of these birds results in the generation of large amounts of poultry manure. As poultry litter is high in nutrient content, it is often used as an organic fertilizer on farm lands. Using an estimated broiler litter production rate of 1 ton per 1000 broilers housed, 79.7 million broilers would produce approximately 79 700 tons of manure in a month of production.

Commercial broilers are often fed vitamin and mineral premixes that provide more trace minerals (TM) such as Zinc (Zn), Manganese (Mn) and Copper (Cu) than what the birds require to maximize their performance, act as growth promoters and to ensure the minimum TM requirements are met (Mohanna & Nys, 1999; Gerber *et al.*, 2007; Untea *et al.*, 2011). These commercial diets provide approximately twice the amount of TM recommended by the National Research Council (NRC). The low absorption of these minerals in the gastrointestinal tract

(GIT) along with their high inclusion levels has led to an increase in TM levels in their litter (Mohamed *et al.,* 2015). Recently environmental concern has been raised regarding the accumulation of minerals in the environment due to the high mineral content of poultry litter. Inorganic trace minerals (ITM) are a cost-effective way of optimizing animal health and production, however TM salts tend to dissociate in the low pH environment of the upper GIT exposing them to various nutrient and ingredient antagonisms (Richards *et al*., 2010). The absorption of TM is thus reduced resulting in large amounts of Zn, Mn and Cu being excreted into the environment. Alternative sources of TM have the potential to reduce these minerals released into the environment such as organic trace minerals (OTM) and hydroxy trace minerals (HTM).

Proteinates and amino acid chelates are categories of OTM that have been used in premixes due to their higher bioavailability (Gheisari *et al*., 2010). It is implied that OTM can be included in diets at much lower concentrations than that of ITM whilst having no negative impact on production performance and reducing TM excretion (Gheisari *et al*., 2010).

The EU recently approved the use of HTM as a feed additive for all animal species (Kampf, 2012). Their highly stabile and unique crystalline structure improves the stability of feed mixtures and thus reduces the loss of nutrients such as vitamins and enzymes unlike that of other ITM and OTM (Cohen & Steward, 2014)

Major advances have been made in poultry research, technology and production, however mineral nutrition still lags behind other areas of nutrition. The literature indicates that true mineral requirements of poultry are not really known due to the wide range of dietary requirements for TM in broilers. One must know the requirements of broilers to reduce the amount of TM in their feed. Legislation of major poultry producing regions of the world differ in their maximum tolerable level (MTL) of TM allowed in poultry feed. A large gap exists between what is required by the bird and the MTL. This gap could be exploited if requirements were better understood and used to reduce the amount of TM in the feed.

This thesis investigates the efficacy of ITM, OTM and HTM sources of Zn, Mn and Cu in broiler diets with the key areas of study being the effect of different levels of ITM on broiler performance, the efficacy of ITM, OTM and HTM on broiler performance and the effectiveness of ITM, OTM and HTM on broiler excretion.

It is hypothesised in the current study that the replacement of ITM with OTM and HTM will reduce the amount of Zn, Mn and Cu released into the environment. Growth performance parameters such as body weight (BW), average daily gain (ADG), cumulative feed intake (Cum.FI) and feed conversion ratio (FCR) are not expected to differ when birds are fed different levels of ITM or different sources of TM if the birds requirements are met.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

A 2kg broiler bird was produced over 90 days in 1952 (Gous, 2000), nowadays this same weight can be achieved in just 34 days. Major advances in broiler production and technology have made these numbers possible, however research in the field of TM has lagged behind that of other nutritional aspects in broilers. Broiler TM requirements set out by the NRC are based on research performed as far back as the 1950's and is still being used as nutritional guidelines. These figures are based off inorganic sources of TM in an attempt to avoid deficiencies of these minerals. Commercial practices tend to over supplement broilers with TM to achieve maximum growth performance and guarantee that requirements are met. It is therefore questionable whether these values are still relevant or suitable to achieve maximum growth potential in the modern broiler.

The supplementation of diets with excess minerals can result in high levels of TM being present in poultry excreta. Poultry litter is often applied to farmlands as fertilizer, however there is a growing concern regarding the accumulation of heavy metals in the environment due to current practices. Environmental protection agencies around the world are under pressure to reduce TM levels while Europe has already adopted regulations to reduce levels of TM into the environment. It has become essential to find alternative sources and practices to reduce TM levels in excreta whilst still maintaining broiler growth performance. The following review therefore focuses on achieving broiler growth performance whilst using other sources of TM at lower levels, to reduce the impact of TM on the environment.

2.2 Mineral Nutrition of the Broiler

There are at least 14 different elements that the chicken requires for adequate nutrition. These elements can be separated into two main groups namely macro-minerals and TM. Macro-minerals are needed in larger amounts and their requirements are usually stated as a percentage of the diet (NRC, 1994). Micro minerals or trace minerals are minor minerals that are measured in milligrams per kilogram (mg/kg) or parts per million (ppm) (NRC, 1994).

2.3 Macro-Minerals

The most essential macro-minerals in poultry nutrition include calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K) and chloride (Cl). Two of the most important macro-minerals in bone formation and skeleton maintenance are Ca and P (NRC, 1994). Calcium is the most abundant mineral in the body of a broiler with 97% of it being found in the bone (Leeson & Summers, 2001). In the diet of a growing bird, the majority of the Ca is utilised in bone formation while other functions include its role in blood clotting, as a secondary messenger in intracellular communications, normal heart rhythm, the transmission of nerve impulses and contractile properties of muscle (NRC, 1994; Leeson & Summers, 2001). Approximately 80% of the body’s P is found in the skeleton with the remaining 20% being contained in nucleotides, nucleic acids, phospholipids and phosphorylated compounds needed for metabolism (Soares, 1995). In addition to its function in bone formation, P also plays a role in carbohydrate, energy, amino acid and fat metabolism, muscle co-ordination, normal blood chemistry and nervous tissue metabolism (NRC, 1994). Since the majority of ingredients in poultry nutrition are seed-based, it is estimated that 50-80% of the P in seed- based ingredients is phytate P, which is poorly available to poultry and reduces Ca bioavailability (Taylor and Coleman, 1979). A ratio of approximately 2:1 (Ca: Nonphytate P) is therefore suggested for most poultry diets (NRC, 1994).

Na, K, Mg and Cl play a major, integrated role in maintaining the osmotic regulation of bodily fluids and acid-base balance in broilers (Leeson & Summers, 2001). The overall balance of these minerals is so important that the requirements for each element cannot be considered individually (Leeson & Summers, 2001). In extracellular fluid, Na is the chief cation required by all animals for normal metabolism and Cl tends to decrease blood pH and bicarbonate concentration (NRC, 1994). K tends to increase blood pH and activate numerous cellular enzymes (NRC, 1994). In living organisms Mg is the fourth most abundant cation and is involved in the metabolism of fat, amino acids, sugars, bone Ca and Vitamin D (Shastak & Rodehutscord, 2015). The requirements for macro-minerals suggested by the NRC and Cobb are shown in Table 2.1.

Table 2.1 NRC and Cobb 500 macro-mineral requirements for broilers. (NRC, 1994; Cobb-Vantress Inc, 2015)

		NRC			Cobb 500	
Macro-Minerals	0 - 3 Weeks	3 - 6 Weeks	6 - 8 Weeks	0 - 10 Days	11 - 22 Days	23 - 42 Days
Ca (%)	1.00	0.90	0.80	0.90	0.84	0.76
Av. P (%)	0.45	0.35	0.30	0.45	0.42	0.38
Na (%)	0.20	0.15	0.12	0.16 - 0.23	0.16 - 0.23	0.15 - 0.23
Cl (%)	0.20	0.15	0.12	0.17 - 0.35	0.16 - 0.35	0.15 - 0.35
K (%)	0.30	0.30	0.30	0.60 - 0.95	0.60 - 0.85	0.60 - 0.80

2.4 Trace Minerals

Trace minerals include zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), molybdenum (Mo), selenium (Se), iodine (Io) and cobalt (Co). Although Co is required by the chicken, it is part of vitamin B12 and therefore does not need to be supplied in the diet (NRC, 1994). Zinc, Mn, Cu and Fe are all involved in enzyme activities primarily acting as co-factors, activators or transporters. Zn is the most influential participating as a co-factor in the functioning of over 300 different enzymes (Vallee & Falchuk, 1993). Figure 2.1 shows the NRC and Cobb 500 broiler requirements for Zn, Mn and Cu.

Molybdenum is a component of various enzymes such as aldehyde oxidase, sulphite oxidase and the metallo-enzyme xanthine oxidase (Leeson & Summers, 2001). In the reaction of cytochrome c Mo participates in the reduction of cyctochrome c by aldehyde oxidase (Leeson & Summers, 2001). Most poultry have a Mo requirement of 0.03 mg/kg (Leeson & Summers, 2001).

Selenium plays a major role in the enzymatic antioxidant defence of cells including glutathione peroxidises, thioredoxime reductases and other selenoproteins (Briens *et al*., 2013). Glutathione peroxidise converts reduced glutathione to oxidized glutathione and destroys peroxides by converting them into alcohols that are harmless (Leeson & Summers, 2001). The NRC recommends a supplementation level of 0.15 mg/kg from 0-8 weeks of age.

The thyroid gland contains two hormones known as triiodothyronine and tetraiodothyronine (thyroxine). Metabolic activities which these hormones are involved in influence the growth and development of broiler chickens. Iodide and tyrosine are essential in the synthesis of triiodothyronine and tetraiodothyronine (Stojević *et al*., 2000). According to the NRC, 1994 broilers require 0.35 mg/kg of Io.

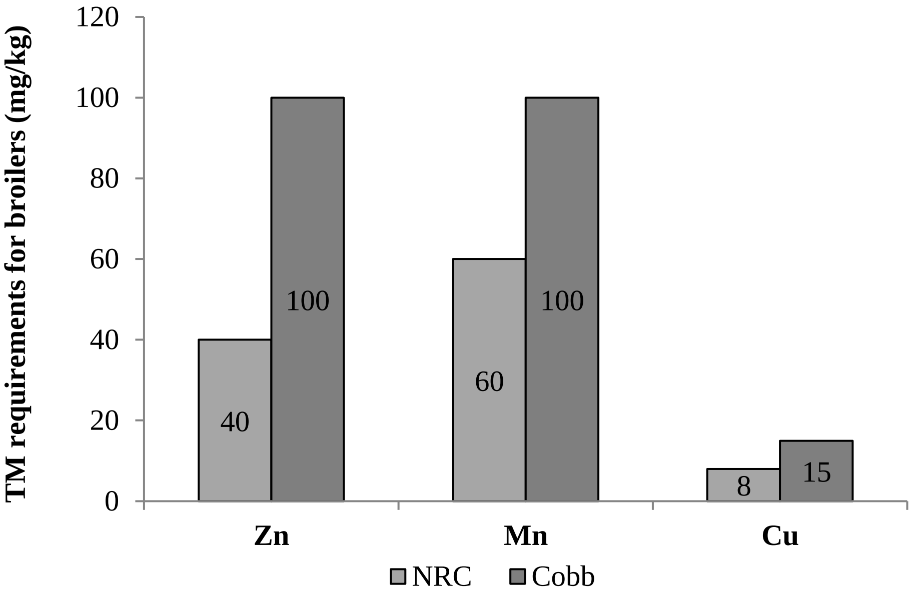

Figure 2.1 Zinc, manganese and copper requirements for broilers set out by the NRC, 1994 and Cobb 500 Broiler Performance and Nutrition Supplement 2015.

2.4.1 Functions of Zinc, Manganese and Copper

2.4.1.1 Zinc

Zinc plays three major roles in the body, namely: A catalytic role, a structural role and a regulatory role (Stefanidou *et al*., 2005). As one of the most important TM, Zn is involved as a cofactor in the functioning of over 300 different enzymes (Vallee and Falchuk, 1993). It is found in enzymes such as pancreatic carboxypeptidase, alkaline phosphatase, carbonic anhydrase and lactate dehydrogenase (Leeson & Summers, 2001). Zinc is directly involved in catalysis and co-catalysis by the enzymes which control cell processes such as DNA synthesis, reproduction, bone formation and behavioural response (Barceloux, 1999).

In its structural role, Zn is required for bone growth and development. It acts as a cofactor for carbonic anhydrase which assists in the calcification of bone (Leeson & Summers, 2001). Deficiency of Zn can lead to enlargement of the hock joint, shortening and thickening of the tibia bones and growth retardation (Berger, 2006). Zn plays structural and functional roles in proteins involved in DNA replication and aids in the correct functioning of metalloproteins (Stefanidou *et al*., 2005). Metallothioneins play a key role in the Zn effect upon the immune system (Stefanidou *et al*., 2005).

The immune system of the chicken may be indirectly influenced by dietary Zn and its interaction with growth and infectivity of organisms that are pathogens (Park *et al*., 2004). Zn nutrition has shown changes in infectious disease resistance and immune system responses. Supplementation of dietary Zn has been shown to enhance immune responses in broiler breeder progeny as measured by haemagglutination antibody assay (Stahl *et al*., 1986).

As an activator and inhibitor ion, Zn regulates the activity of enzymes and the stability of proteins (Stefanidou *et al*., 2005). It has also been found to modulate cellular signal reception, secondary messenger metabolism and, protein kinase and protein phosphatise activities (Beyersmann, 2003).

2.4.1.2 Manganese

Manganese is essential in the formation of bone in broilers (Leeson & Summers, 2001). A deficiency in Mn can result in a syndrome known as Perosis which is characterized by the twisting and bending of the distal end of the tibia and the proximal end of the tarsometatarsus (Leeson & Summers, 2001). Further symptoms include enlargement and malformation of the tibiometatarsal joint, thickening and shortening of the leg bones and slippage of the Achilles tendon from its condyles (Leeson & Summers, 2001). The structure of cartilage is also reliant on sufficient concentrations of Mn in the body (Conly *et al*., 2012).

Similarly, Mn like other TM is involved as an enzyme activator and is an integral part of metalloenzymes such as superoxide dismutase A (Li *et al*., 2005). Numerous enzymes can be activated by Mn including arginase, cysteine desulfhydrase, thiaminase, carnosinase, deoxyribonuclease, enolase, intestinal prolinase and glycyl-L-leucine dipeptidase (Leeson & Summers, 2001). Mn is also vital in metabolic processes such as carbohydrate, lipid and protein metabolism (Conly *et al*., 2012).

2.4.1.3 Copper

Copper is a transition metal that is a component of numerous intracellular and extracellular enzymes (Klasing, 1998). These enzymes include cytochrome oxidase which is involved in oxidative phosphorylation, superoxidase dismutase and lysyl oxidase which increases bone strength (Klasing, 1998; Leeson & Summers, 2001). When fed a Cu deficient diet, broilers experience fragile and easily broken bones as Cu is required for proper bone growth and cartilage formation (Banks *et al*., 2004). Cu is important for red blood cell formation and is a component of erythrocuprein, a blood protein found in erythrocytes that is used in oxygen metabolism (Leeson & Summers, 2001). Required for skin and feather pigmentation, Cu is found in certain pigments such as turacin, a pigment of feathers (Leeson & Summers, 2001).

For many years Cu has been added to poultry diets as an antimicrobial agent (Kim *et al*., 2011). It contributes to the intestinal health of the broiler by preventing disturbances in the balance of microflora and responds to inflammation, injury and infections of the gut (Cohen, 2002). In immune function, Cu has become essential in countries where antibiotics have been banned or are limited (Cohen, 2002). As a component of superoxidase dismutase, Cu protects cells from free radical damage and enhances the transport of Fe as part of the enzyme ceruloplasmin (Cohen, 2002).

2.4.2 Deficiencies in Zinc, Manganese and Copper

2.4.2.1 Zinc

As a cofactor of collagenase, Zn is essential in the synthesis of collagen. A deficiency in Zn reduces collagenase activity and collagen synthesis resulting in bone abnormalities (Starcher *et al*., 1980). These abnormalities can include shortening and thickening of the leg bones and enlargement of the hock joint (Leeson & Summers, 2001).

Keratin is a major structural protein found in feathers and beaks (Richards *et al*., 2010). During the process of keratinisation, Zn plays a vital role in the formation of structural proteins (Tomlinson *et al*., 2004). A deficiency in Zn can therefore reduce keratin synthesis and result in poor feathering (Leeson & Summers, 2001; Richards *et al*., 2010).

2.4.2.2 Manganese

The tensile strength of cartilage is attributed to a component of proteoglycan known as chondroitin sulphate. To synthesize this component Mn is required in two enzymatic steps. Chondroitin sulphate and proteoglycan are reduced in the epiphyseal cartilage of a Mn deficient bird (Leeson & Summers, 2001). This leads to a condition known as Perosis characterized by the twisting and bending of the tibia, enlargement and malformation of the tibiometatarsal joint and slipping of the gastrocnemius tendon from its condyles (Berger, 2006; Leeson & Summers, 2001).

2.4.2.3 Copper

A deficiency of Cu in broilers can lead to mycrocytic, hypochromic anaemia and bone disorders such as tibial dyschondroplasia (Berger, 2006). Cu is a constituent of the ferroxidase enzyme ceruloplasmin which assists in the oxidation of ferrous Fe to ferric Fe (Scheideler, 2008). This oxidation reaction controls the movement of Fe to the blood plasma and thus affects the formation of red blood cells (Scheideler, 2006). A reduction in the formation of erythrocytes or haemoglobin, results in anaemia.

In birds, lysyl oxidase, a cuproenzyme, is responsible for the formation of lysine-derived cross-links in the structural proteins elastin and collagen in connective tissue (Rucker *et al.*, 1998). Lysyl oxidase increases the strength of collagen subunits by cross-linking them into mature proteins (Rucker *et al.*, 1998). The growth plates of the tibial bone can fracture when the cartilage of the bird does not mature into bone. This is known as tibial dyschondroplasia which can cause bone weakness and deformities.

2.4.3 Sources of Zinc, Manganese and Copper in Broiler Nutrition

2.4.3.1 Sources and Sinks in the Broiler

Most body cells contain Cu with the main storage site being that of the liver in the broiler (Leeson & Summers, 2001). When fed in excess of 50 ppm, the majority of Cu ends up in the excreta (Leeson & Summers, 2001).

Areas of the broiler body with the highest concentration of Mn include the bone, liver, kidney, pancreas, pituitary gland and pineal gland (Berger, 2006). The bone and liver are the richest sources of Mn in the broiler with 3-4 mg/kg and 2 mg/kg respectively (Leeson & Summers, 2001). A 3-5% increase in bone and liver Mn concentration can occur for every 100 ppm increase in dietary Mn (Leeson & Summers, 2001). Black *et al*. (1985), showed a linear increase in tibial Mn with the level of supplementation of Mn. Excretion of Mn is mainly via bile which is potentially reabsorbed as bile-bound Mn (Leeson & Summers, 2001). The dietary concentration of Mn has an effect on the rate of excretion (Leeson & Summers, 2001).

Every tissue in the body of a broiler contains Zn with most tissues containing approximately 30 ppm Zn (Leeson & Summers, 2001). Like other TM, Zn tends to accumulate in the bone rather than in the liver (Leeson & Summers, 2001). Feathers and skin also have a high concentration of Zn (Leeson & Summers, 2001).

2.4.3.2 Sources of Feedstuff

Table 2.2 Trace mineral content of commonly used feedstuffs for chickens

Feedstuff	Mn (mg/kg)		Cu (mg/kg)		Zinc (mg/kg)	
	L	N	L	N	L	N
Maize Grain (Yellow)	4	7	3	3	29	18
Soya Oilcake (48%)	27	29	36	22	52	40
Sunflower Oilcake	15	23	3	4	100	98
Wheat Bran	115	113	12	14	89	100
Fish Meal (60%)	25	33	8	11	119	147
Maize Gluten Meal	7	4	28	26	66	33
Canola Oilcake	6	54	7	10	44	71
Poultry By-Product	20	11	6	14	79	120

Note: L = Leeson & Summers, 2001; N = NRC, 1994

2.4.3.3 Inorganic Trace Minerals

Historically, the majority of TM such as Zn, Mn and Cu have been supplied in poultry diets using inorganic sources (Richards *et al*., 2010). These traditional sources have been included into diets in the form of sulphates, oxides and carbonates. Inorganic sources of TM provide the lowest cost per unit of available nutrients and thus are a cost effective way of optimizing animal health and production (Berger, 2006). Some disadvantages however, have been associated with ITM. In the low pH environment of the upper GIT TM salts tend to dissociate, exposing them to various nutrient and ingredient antagonisms (Richards *et al*., 2010). These antagonisms impair the absorption of TM and thus reduce their bioavailability (Underwood & Suttle, 1999). Excessive inclusions of ITM in poultry diets lead to increased levels of excretion and so induce waste and environmental contamination.

Traditional sources of inorganic Cu include Cu sulphate, Cu chloride, Cu carbonate and Cu oxide (Berger, 2006). The most commonly used source of inorganic Cu in poultry diets is Cu-sulphate pentahydrate ($CuSO_4.5H_2O$) due to its commercial availability and cost (Mondal *et al*., 2007).

Currently, the two most common inorganic sources of Zn used in poultry diets are in the form of Zn sulphates (36% Zn) and Zn oxides (72% Zn) (Batal *et al*., 2001). Zn sulphates are highly water soluble and allow reactive metal ions to promote free radical formation (Batal *et al*., 2001). Although Zn oxides are less reactive than Zn sulphates, they are less bioavailable (Batal *et al*., 2001). Zn sulphate is produced by crystallinization and not by precipitation which produces a product of greater purity (Cao *et al*., 2000).

2.4.3.4 Hydroxy Trace Minerals

Traditionally, minerals have been categorized as either OTM or ITM however, a fairly new category of TM recently received approval from the EU as a feed additive for all animal species (Kampf, 2012). The first commercial product of this type known as dicopper chloride trihydroxide (tribasic copper chloride or Intellibond C) was introduced in 1995 (Cohen & Steward, 2014). Tribasic copper chloride (TBCC) is a mineral that occurs naturally as atacamite, which was first found in the Atacama Desert (Çelìk *et al*., 2005). TBCC, a secondary mineral is formed by the oxidation of other Cu-containing deposits or by the heating of Cu_2O with a solution of $FeCl_3$ (Palache *et al*., 1994; Çelìk *et al*., 2005). Intellibond C has similar chemically stable structures such as organically bound trace elements but belongs to ITM sources (Kampf, 2012).

Tribasic copper chloride forms a well-defined three-dimensional crystal lattice that excludes impurities (Cohen & Steward, 2014). This unique crystalline structure is highly stable unlike other ITM and OTM, which improves the stability of feed mixtures and thus reduces the loss of nutrients such as vitamins and enzymes (Luo *et al*., 2005; Cohen & Steward, 2014). Intellibond C utilizes covalent bonds that allow organo-metallic products to outperform metal sulphates that have weak ionic bonds to the metal. Covalent bonds limit parasitic reactions due to their strength but are weak enough to pass the metal off to the escort ligands that are deployed to facilitate absorption in the animal. The target metal is held tightly within the crystalline structure and is still slowly released in the latter sections of the intestine where immune tissues are concentrated and the majority of pathogenic challenges occur (Cohen & Steward, 2014). It is believed that TBCC is a more stable and less destructive form of Cu than $CuSO_4.5H_2O$ due to its solubility in water and its lower hygroscopicity (Luo *et al*., 2005).

2.4.3.5 Organic Trace Minerals

There are five different categories of OTM set out by the Association of American Feed Control Officials (AAFCO), these include: Metal (specific amino acid) complexes (contain one type of amino acid), metal amino acid complexes (contain more than one type of amino acid), metal amino acid chelates, proteinate metals and polysaccharide metal complexes.

Metal (specific amino acid) complexes are the result of complexing a soluble metal salt with a specific amino acid in a molar ratio of 1:1 (AAFCO, 2000). Only one type of amino acid will be present in the complex making it specific. The most commonly used amino acids are methionine and lysine with examples being copper lysine complex or zinc methionine complex.

Metal amino acid complexes are formed from complexing a soluble metal salt with amino acids in a molar ratio of 1:1 (AAFCO, 2000). This means the complex consists of two or more mixed amino acids such as lysine or glutamic acid. Availa-Zn, Mn and Cu are metal amino acid complexes produced by Zinpro that consist of the mineral (Zn, Mn or Cu) complexed with the amino acid carboxy groups of an amino acid (Burrel *et al*., 2004).

Metal amino acid chelates (MAAC) result from reacting a metal ion from a soluble salt with amino acids in a molar ratio of 1 mol: 1-3 (preferably 2) moles of amino acids (AAFCO, 2000). Coordinated bonds are formed and the molecular weight of the hydrolyzed chelate should exceed 800 Daltons (AAFCO, 2000). A metal chelate, taken from the Greek word chele, meaning “claw” is formed as a ring structure in which polyvalent cations are held together by ligands (Ashmead, 1993). MAAC are chemically inert due to the covalent and ionic bonds between the mineral and ligand (Vieira, 2008). Due to these bonds, MAAC are not affected by factors that lead to precipitation such as those that occur to minerals ionized after salt solubilisation (Ashmead, 1993). Most chelated minerals are unaltered during their passage through the digestive tract as they are stable and small in size (Vieira, 2008). Their ring structure protects the TM from chemical reactions in the GIT keeping it stable at a low pH (Mézes *et al*., 2012). The MAAC moves into the mucosal cell as an intact chelate enhancing the absorption of the mineral (Ashmead, 1993). Strong absorption of the mineral onto insoluble colloids in the intestine is prevented by chelation and thus the ion is stopped from being released back into the lumen (Ashmead, 1993). Once absorbed, the final separation of the metal ion from the amino acid occurs when it reaches its final physiological site and the mineral’s fate depends on the amino acid or peptide to which it is bound (Vieira, 2008; Stanaćev *et al*., 2014). Due to their more effective method of absorption, excretion of waste is reduced.

Metal proteinates are more complicated structures than metal (specific amino acid) complexes, metal amino acid complexes and MAAC. These structures are produced from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein (AAFCO, 2000). The number of bonds can vary with different levels of stability. Examples of these structures include zinc proteinate, manganese proteinate and copper proteinate.

The final group of OTM, polysaccharide metal complexes are the result of complexing a soluble salt with a solution of polysaccharides declared as an integral part of a specific complex (AAFCO, 2000). Examples of these complexes include zinc polysaccharide complex and copper polysaccharide complex.

Table 2.3 History of trace minerals.

Time Span	Generation	Type of TM
1930-1950	1st	Metal oxides and industrial by-products
1950-1980	2nd	Metal salts of H_2SO_4
1980-Current	3rd	Metal salts of organic acids
1990-Current	4th	Basic metal salts of low-cost mineral acids

2.4.4 Metabolism of Trace Minerals in Broilers

2.4.4.1 Absorption

The absorption of TM occurs throughout the jejunum and ileum (Leeson & Summers, 2001). The physiological state of the animal, the breed, the form of TM, pH and TM interactions can all affect the absorption of various TM (Leeson & Summers, 2001). Numerous transporters are involved in the cellular uptake and export of Zn, Mn and Cu in the small intestine and other tissues (Spears, 2015). Not all TM that are absorbed in the small intestine are transported into the blood such as Zn where excess levels induce a protein known as metallothionein (MT) that can bind Zn and Cu, thus limiting their absorption. Due to the rapid turnover of intestinal cells, metals that are bound to MT are sloughed off into the small intestine and excreted in faeces (Spears, 2015).

Spears (1989), hypothesized that Zn methionine might be absorbed and transported in the plasma intact however, recent published research has suggested that OTM are not absorbed intact. It is not the metal complex or the chelate that transport the metal ion but rather metal transporters. In order for OTM to use these transporters, the metal would have to dissociate from the ligand before the metal is absorbed into the enterocyte (Spears, 2015).

Cu attaches to a protein carrier in the mucosa and a large proportion of it is absorbed from the duodenum (Leeson & Summers, 2001). The primary protein involved in the cellular uptake of Cu by specific for Cu^{+1} whereby the Cu^{+2} ion has been reduced to Cu^{+1} with a reductase (Spears, 2015). A Cu ATPase known as ATP7a is involved in the export of Cu from the small intestine into the blood (Spears, 2015). A further Cu ATPase, ATP7b is important in the secretion of Cu from the liver and incorporated into ceruloplasmin which transports Cu from the liver to other tissues (Spears, 2015).

Absorption of Mn from the intestinal tract of the broiler is generally poor (Leeson & Summers, 2001). It is believed that DMT1 is also involved in the import of Mn into the small intestine (Bai *et al*., 2014). High dietary levels of Fe can reduce Mn absorption due to its increased competition for DMT1 and its antagonistic effects on Mn (Spears, 2015). Absorption and excretion of Mn from the digestive tract seem to be dependent on whether natural chelates are formed particularly in the case of bile salts (Leeson & Summers, 2001).

Zn is absorbed in the small intestine via saturable transporters and passive diffusion (Krebs, 2013). When the supplementation of Zn is above that of what the animal requires, passive diffusion becomes important. There are two Zn transporter families namely the ZIP family and ZNT proteins (Spears, 2015). ZIP transporters promote Zn import into cells while ZNT proteins are involved in the outflow of Zn from cells or the inflow of Zn into intracellular vesicles (Spears, 2015). ZIP transporters on the brush border membrane transport Zn into the intestinal enterocyte (Spears, 2015). Once inside the enterocyte, Zn can bind to MT or be transported by ZNT proteins into the blood (Spears, 2015). MT is a small, cysteine-rich protein that regulates the transfer of Zn from the intestinal mucosal cells to the plasma (Cao *et al*., 2002; Berger, 2006). In the chicken, the single isoform of MT has been found in the liver, pancreas, kidney and intestinal mucosa (Cao *et al*., 2002). MT has the ability to bind Zn and other heavy metals with high affinity (Cao *et al*., 2002). The amount of Zn that is absorbed or bound to MT is determined by the concentration of free Zn^{+2} ions in the cell and the animal's demand for Zn (Spears, 2015). Absorption of Zn ranges between 5% and 40% of the bird's feed intake (Berger, 2006).

2.4.4.2 Homeostasis

Homeostatic control is important at a whole body level and a cellular level in order to formulate bioavailable TM levels in diets that would prevent deficiencies and toxicity of TM in the body (Spears, 2015). The efficacy of mineral utilization is enhanced when the bioavailable concentration of the TM in the diet is lower than that of the animal's requirement. Alternatively, when the bioavailable concentration of the TM in the diet is above that of what the animal requires, then the efficacy at which the TM is retained in the body is reduced (Spears, 2015).

The major route that controls Cu and Mn homeostasis is biliary excretion (Spears & Hansen, 2008). Due to biliary Cu excretion, liver Cu concentrations are well regulated in monogastrics (Spears, 2015).

Changes in absorption and faecal endogenous excretion aid in controlling Zn homeostasis (Krebs, 2013). The efficiency of Zn absorption is increased and faecal endogenous excretion of Zn reduced when a low intake of Zn occurs compared to the animal's requirements (Spears, 2015). If concentrations of Zn are fed above the animal's requirements, then absorption of Zn is reduced and faecal endogenous excretion of Zn is increased (Spears, 2015).

2.4.5 Interactions between Zinc, Manganese and Copper

Georgievskii, (1982), listed 26 antagonistic interactions that exist between the macro and micro minerals required in animal nutrition. Minerals are reactive and tend to form chemical bonds with other minerals or complexes making them more likely to interact than any other nutrients. Generally, broilers fed well balanced diets should not experience metabolic problems due to mineral interactions, however human error, contamination of products or accidental supplementation of minerals can occur. There are numerous interactions that exist between Zn, Mn, Cu and other minerals.

Interrelationships exist between Zn, Fe, Ca, Cu and S. Ca has been known to impair Zn absorption in broilers. Sebastian *et al*. (1996) found that an increase in dietary Ca, decreased feed intake (FI), BW and Zn concentrations in tibia bones of broilers. Cu absorption is affected by Zn; however, the reverse reaction does not occur (Henry and Miles, 2000).

Mn is known to interact with Ca, P and Mg. Halpin and Baker., (1968) reported that dietary phytate inhibited the absorption of Mn. Excess dietary P and Ca both effect the absorption of Mn negatively in poultry (Henry, 1995).

Cu has numerous interactions with other minerals namely Fe, Co, S, Zn and Mo. Excess levels of Zn and Fe can negatively affect Cu metabolism. O'Dell, (1967) observed that when chicks were supplemented with Zn above dietary requirements and Cu was limiting, that Cu metabolism was adversely affected.

2.4.6 Bioavailability of Trace Minerals

Knowledge about the bioavailability of TM in feedstuffs and supplemental sources has become important in order to compose economically viable diets and produce optimal animal performance (Miles and Henry, 2000). The bioavailability of a TM is the extent to which that ingested TM is absorbed and utilized for normal metabolic functions in the animal (Ammerman *et al*., 1995). Other terms that can be used interchangeably with bioavailability are "biological availability", "bioactivity", "bio potency" and "bioefficacy" (Ammerman *et al*., 1995). There are many factors that can influence bioavailability such as the age and species of animal, TM intake, interactions with other minerals, chelators and the overall digestibility of the diet. The broiler chick has a genetic potential for rapid growth and with its limited nutrient store is an ideal assay animal for several TM (Miles and Henry, 2000).

2.4.6.1 Measurements of Bioavailability

There are currently two methods used to determine the bioavailability of minerals in animal feed, namely balance methods and repletion methods.

Balance methods include the use of digestibility and retention trials where endogenous losses are estimated by isotopic labelling techniques (Bao *et al*., 2007). The proportion of TM absorbed in the intestine is rather low and therefore balance methods are not a very discriminative method for estimating the RBV of TM (van der Klis & Kemme, 2002).

Repletion techniques are based on dose-response curves that relate the supplementation rate of a TM to the content of that TM in specific body tissues or the performance of the animal (van der Klis & Kemme, 2002). The diets in these experiments contain adequate levels of nutrients except for the TM that is being tested (van der Klis & Kemme, 2002). The RBV of the tested TM is calculated by using the ratio of the slopes of the two response curves (slope of the test response divided by the slope of the standard response) where the RBV of the standard product is set at 100% (van der Klis & Kemme, 2002).

2.4.6.2 Performance Research

2.4.6.2.1 Reduction in Inorganic Trace Minerals

Wang *et al*. (2008) compared different concentrations of ITM in broiler production till 42 days of age. Sulphate sources of Zn, Mn and Cu were supplemented at 100, 100 and 10mg/kg respectively in a control diet with 5 experimental diets providing 80, 60, 40, 20 and 0% of the control diet levels. A significant reduction in BW was observed at all ages when TM were totally removed from the diet. FI significantly decreased when no supplemental Zn, Mn and Cu was provided, however no effect was observed in FCR when compared to the other experimental diets. BW, FI and FCR did not differ in birds whether they were fed 20% or 100% of the normal supplemental level. Mortality was not influenced by supplemental levels nor were any leg disorders observed. The trial concluded that TM could be reduced without any significant effects in performance.

A growth experiment carried out by Mohamed *et al*. (2015) examined the effect of feeding broilers finisher diets (23 to 39 days of age) containing low levels of TM premixes. Four maize-soyabean, finisher diets were formulated to include 100, 75, 50 or 25% of the recommended level. The results showed that reducing the TM content of the diets had no significant effect on BW, ADG, FI or FCR.

2.4.6.2.2 Inorganic Trace Minerals vs Hydroxy Trace Minerals

Numerous trials have been conducted comparing the performance of broilers supplemented with HTM as opposed to ITM. It was reported by Xiang-Qi *et al*. (2006) that the BW of chicks fed 0, 50, 150, 250 or 350mg/kg of Cu from ITM or HTM sources did not differ after 40 days. Similarly, previous research by Arias & Koutsos (2006) showed that broiler BW at 31 days of age did not differ significantly when broilers were supplemented with 188mg/kg of $CuSO_4$ and TBCC. Luo *et al*. (2005) observed that ADFI, ADG and FCR were not influenced in broilers when supplemented with the same levels of Cu (150 and 300mg/kg) from $CuSO_4$ and TBCC. At a higher level of 450mg/kg Cu however, TBCC supplemented birds ate more feed and had a higher body weight gain than those birds fed inorganic Cu.

In contrast to the results above, Miles *et al*. (1998) reported that birds fed 450mg/kg Cu from $CuSO_4$ consumed less feed and weighed less than birds supplemented with TBCC at 21 days of age. 150 and 300mg/kg Cu however, yielded no difference in body weight or FI regardless of Cu source.

Graded levels of $MnSO_4$ and TBMC in a bioavailability experiment by Conly *et al*. (2012) produced broilers at 21 days of age with no difference in BW or FI. A tolerance study with chickens for fattening reviewed by the FEEDAP Panel concluded that broilers fed Mn from $MnSO_4$ or TBMC at levels of 60, 120 and 960mg/kg did not differ in ADFI, ADG or FCR at 35 days of age. When birds were supplemented with 1920mg/kg of Mn however, the treatment using TBMC produced lighter birds that consumed less feed. The consensus from all the above trials is that the use of HTM at the same level or at a lower level than ITM will result in no significant difference in BW, ADG, FI or FCR.

2.4.6.2.3 Inorganic Trace Minerals vs Organic Trace Minerals

The replacement of ITM with OTM has been pursued by researchers due to their better absorption and lower excretion levels. Britanico *et al*. (2012), fed 320 straight-run broilers inorganic and organic sources of Zn, Mn and Cu till 42 days of age. Results showed that supplementing diets with a lower level of OTM gave no significant difference in BW gain compared to those diets with higher inorganic inclusions.

Manangi *et al*. (2012) suggested that ITM could be effectively replaced by OTM at reduced levels whilst still maintaining bird performance. Broilers received an ITM treatment of 100mg/kg Zn, 90mg/kg Mn and 125mg/kg Cu and an OTM treatment of 32mg/kg Zn, 32mg/kg Mn and 8mg/kg Cu. At 54 days of age BW, FI and FCR were not affected by source of TM.

The effects of supplementing OTM at 50% or 100% of the recommended levels against ITM in broilers was investigated by El Husseiny *et al*. (2012). Contrasting to the previous experiments above, at 5 weeks of age BWG was significantly lower in birds fed ITM and those supplemented with OTM at either 50% or 100% of the recommended levels converted feed more efficiently.

Aksu *et al*. (2011) studied the effects of replacing ITM with OTM at lower levels in a 42-day broiler trial. OTM were added to basal diets at 1/3, 2/3 or 3/3 of the ITM levels (NRC levels). No differences were observed in the response of BW, BWG, Cum. FI or FCR to the addition of lower OTM instead of ITM.

2.5 Trace Minerals and the Environment

2.5.1 Poultry Manure and Litter

Broiler, breeder and layer production all result in the generation of large amounts of poultry manure and poultry litter. Poultry manure is the pure excreta produced by layers in battery cages (van Ryssen, 2001). Poultry litter refers to the mixture of faeces, feed, feathers and bedding material such as wood shavings found mainly in broiler houses and deep litter systems (Gupta *et al*., 1997).

The most cost-effective way to dispose of poultry litter/manure is to use it on arable lands and pastures as an organic fertilizer (Mkhabela, 2004). Poultry litter/manure acts as a good fertilizer due to its high nutrient (Nitrogen, phosphorus, potassium and TM) and low moisture content (Gupta & Charles, 1999). As a fertilizer it can alter soil properties such as pH, nutrient availability, cation exchange capacity and organic water content which results in improved aeration of soil, enhanced soil fertility and increased water holding capacity (Bolan *et al*., 2010; Gupta & Charles, 1999).

Poultry manure and litter contain numerous macro and micronutrients; however, the exact composition can vary depending on numerous factors. These factors include the composition of the ration (difference between layers and broilers), the age of the animal (Day old vs age at slaughter), the type of bedding material (wood shavings, newspaper, and hulls), the number of birds and waste management practices (van Ryssen, 2001; Bolan *et al*., 2010). Poultry litter/manure contains trace elements such as Zn, Mn and Cu which are essential for plant growth. These elements are strongly bound to organic material, which when applied to agricultural land can result in the accumulation of metals in the soil (Schomberg *et al*., 2009). The accumulation of these trace elements in the soil depends on the ability of the soil to absorb these elements, its potential for leaching and the number of repeated applications (Gupta & Charles, 1999; He *et al*., 2009). Excess accumulation of the trace elements can have a detrimental effect on plants, microorganisms, livestock and humans (Fernandes & Henriques, 1991; He *et al*., 2005; Banerjee, 2009).

Figure 2.2 shows the mean amount of TM found per kg of broiler excreta. Zn, Mn and Cu amount to on average 254, 317 and 43.6 mg/kg of DM. Surveying the literature, it is estimated that 1 ton per 1000 broilers housed is produced. In March 2017, 79.7 million broilers were slaughtered in South Africa (SAPA, 2017) which equates to 79 700 tons of poultry manure produced. Using the above average values for Zn, Mn and Cu potentially 20.24 t of Zn, 25.26t of Mn and 3.47t of Cu could be produced in South Africa per month. Not all this manure would be used as fertilizer across South Africa, however the amount of these TM could be significant if released into the environment.

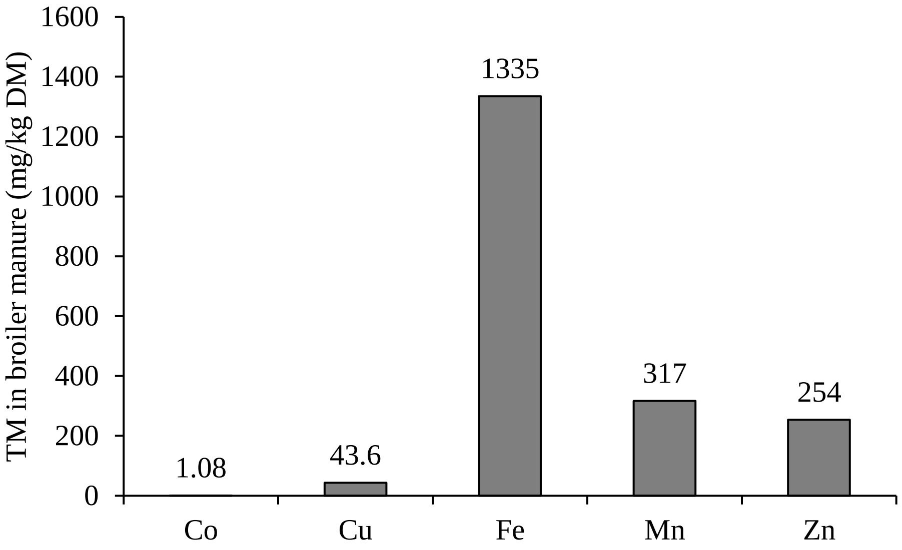

Figure 2.2 Trace mineral composition of broiler manure per kg of DM (Adapted from van Ryssen, 1993)

2.5.2 Legislation

Maximum levels of TM allowed in poultry feed tend to differ between the major poultry producing countries in the world. Some, such as those countries under the European Union (EU) are very strict due to their awareness of the impact of excess TM on the environment while others have no restrictions whatsoever. Maximum levels of Zn, Mn and Cu are discussed below for the major poultry producing countries in North America, South America, Europe, Africa and Asia.

All animal feed in South Africa is regulated by the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947). Annexure 9 (Table 9.9b) in the Farm Feeds Guideline tabulates levels of TM recommended for chickens and their MTL. The recommended levels of Zn, Mn and Cu are 36, 36 and 9 mg/kg respectively. MTL of 450, 900 and 90 mg/kg are permitted for Cu, Mn and Zn.

MTL for TM established from scientific literature and animal health are listed in the 2005 NRC Mineral Tolerances for Animals. The Food and Drug Administration (FDA) uses these MTL to make regulatory decisions on tolerable and toxic levels in animal feed in the United States of America (USA). All businesses that manufacture, store and distribute animal feed must be registered with FDA in the USA. The MTL for Zn is 500 mg/kg, Mn is 2000 mg/kg and Cu is 250 mg/kg.

All those countries under the EU are regulated by the European Commission. Due to the ongoing concern of the long-term effects of TM on the environment, animal feed industries in Europe adopted the legislation (EC No 1334/2003) in 2004. The act was adopted to reduce the level of TM in compound animal feed. Inclusions of Zn, Mn and Cu are limited to 150 and 150 and 25 mg/kg in poultry feed according to this Act.

The Ministry of Agriculture of the People's Republic of China Proclamation #1224 sets out the maximum limit of TM in complete feed for livestock and poultry. The maximum levels in complete for poultry are 150 mg/kg for Zn, 150 mg/kg for Mn and 35 mg/kg for Cu.

Brazil, India and Indonesia do not have legal regulations on maximum levels of TM allowed in poultry feed. Levels of Zn, Mn and Cu are included at levels deemed to be efficient in Brazil with no legal restrictions. In Indonesia mills generally follow European standards or guidelines from breeder companies, however there is no legal regulation.

The Canadian Feed Regulations Act 1983 sets minimum and maximum limits for TM in all livestock species. Chicken diets are limited to a maximum inclusion of 500 mg/kg Zn, 500 mg/kg Mn and 125 mg/kg Cu.

Table 2.4 Maximum tolerable levels of copper, manganese and zinc in poultry diets according to regional Acts of the world.

Trace Mineral	SA	USA	Europe	China	Brazil	India	Indonesia	Canada
Cu (mg/kg)	90	250	25	35	None	None	None	125
Mn (mg/kg)	900	2000	150	150	None	None	None	500
Zn (mg/kg)	450	500	150	150	None	None	None	500

2.5.3 Impact of Excessive Amounts of Trace Minerals on the Environment

In recent years concerns have been raised regarding the accumulation of TM in poultry litter, which is often used as fertilizer and can have a detrimental effect on the environment. Current commercial diets are providing TM such as Zn, Mn and Cu in excess of the birds' requirements. The Cobb 500 Broiler Performance and Nutrition Supplement 2015 recommends Zn, Mn and Cu supplemental levels to be 100, 100 and 15 mg/kg respectively while the NRC (1994) recommends 40mg/kg of Zn, 60mg/kg of Mn and 8 mg/kg of Cu.

It can clearly be seen that commercial diets provide approximately twice the amount of TM recommended by the NRC. Broiler diets are supplemented with these surplus amounts to maximize the performance of the broiler, compensate for the diverse range in TM levels in raw materials, act as growth promoters and for safety margins to ensure the minimum TM requirements are met (Mohanna & Nys, 1999; Gerber *et al*., 2007; Untea *et al*., 2011; El-Husseiny *et al.*, 2012).

High inclusion levels of TM along with their low absorption and retention in the GIT of the broiler, has led to the increase in levels of TM in their manure (Manangi *et al*., 2012; El-Husseiny *et al*., 2012). Poultry manure has a high nutrient content and low water content which makes it a good fertilizer (Gupta & Charles, 1999). High concentrations of TM in poultry manure or litter and the frequent or long-term application of it as fertilizer to agricultural lands cause these elements to accumulate in the top soils over time.

In trace amounts these elements are essential in the functioning of plants, however in excess, soil concentrations can exceed those of the plant requirements and result in phytotoxicity and reduced crop yields (Gupta & Charles, 1999; Nicholson & Chambers, 2008). The accumulation of heavy metals in plants such as Zinc and Copper can affect organisms that consume them and can even be toxic to sensitive animal species such as sheep (Pierce *et al*., 2005). Through activities such as leaching, surface run-off and erosion, heavy metals such as Zinc and Copper (which remain bound to soil) can enter water systems, potentially contaminating ground water supplies (Ferket *et al*., 2002; Nollet *et al*., 2007).

With environmental pollution becoming a growing concern and society moving towards a greener future, considerable interest should be taken in methods to reduce TM amounts in poultry litter.

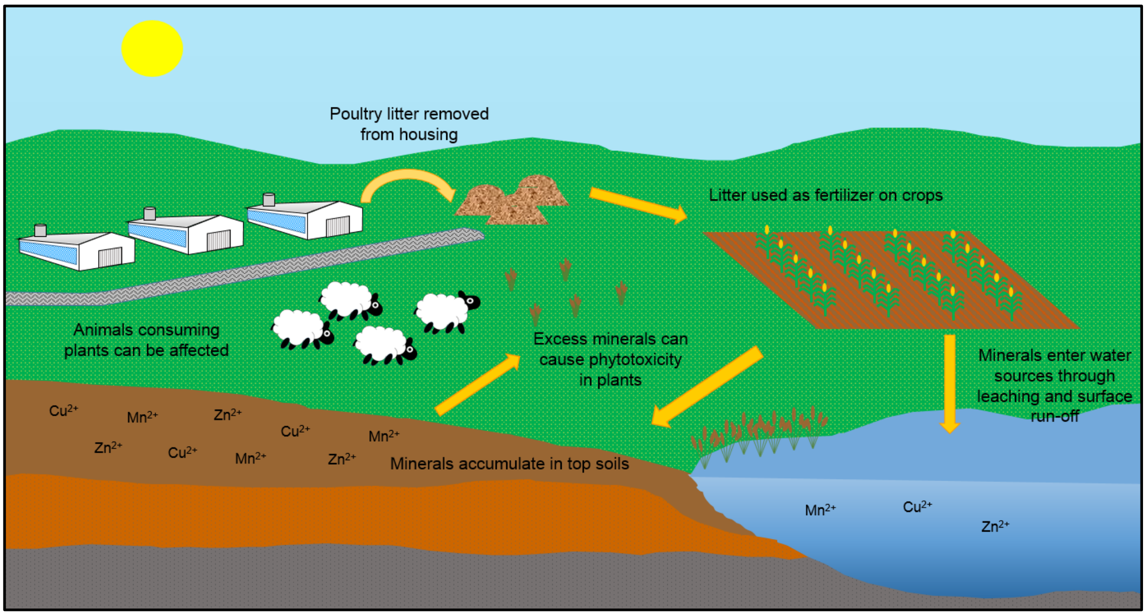

Figure 2.3 Impact of excessive trace minerals in poultry manure on the environment.

2.5.4 Sustainability

TM such as Zn and Cu can be found in excessive levels in poultry manure. This can cause serious environmental concerns when the manure is applied to agricultural lands. These TM can accumulate in top soils over time, effecting plants, animals and humans. Methods of reducing the amount of TM in poultry diets should therefore be considered. Reducing the levels of TM in vitamin and mineral premixes, replacing ITM with OTM, and using phytase in the feed are all possible ways of reducing TM levels in poultry rations.

2.5.4.1 Reducing Levels of Trace Minerals in Vitamin and Mineral Premixes

To reduce the amount of TM in poultry feed one must know the requirements of the birds. A wide range of dietary requirements for TM in broilers can be found in the literature indicating that true requirements of poultry is not really known. Legislation throughout the world also differs in the MTL of TM allowed in poultry diets. These MTL are higher than what is required by the bird which leaves a fairly large gap between what is required and what is allowed. This leaves an area which if requirements were better understood, could be exploited and used to reduce the amount of TM in the feed.

There are numerous sources of literature demonstrating that decreasing the amount of TM supplemented in broiler premixes can reduce levels of TM in the bird’s excreta. Trials performed regardless of broiler strain, source of TM or the period in which the excreta were collected all confirm the same trend above. Dozier *et al*. (2003) fed Ross 576 x Ross 308 broilers graded levels of 40, 80 and 120 mg/kg zinc from zinc sulphate or Availa-Zn (organic) from day old to 17 days of age. Over a 48-hour period, excreta were collected from days 15 to 17. The reduction of dietary zinc regardless of source resulted in a linear decrease in zinc excretion.

This same linear decrease in TM excretion was confirmed by Bao *et al*. (2007) in excreta collected from broilers between 19 and 22 days of age. The trial showed that lowering supplemental levels of organic (Bioplex) Zn, Mn and Cu could adequately support optimal broiler performance while reducing TM excretion. Cobb broiler chickens were supplemented with low organic (20 mg/kg Zn, 20 mg/kg Mn and 2 mg/kg Cu), medium organic (40 mg/kg Zn and 40 mg/kg Mn and 4 mg/kg Cu) and high organic (80 mg/kg Zn, 80 mg/kg Mn and 8 mg/kg Cu) supplemental levels of TM. Zinc, Mn and Cu excreta levels significantly decreased ($P<0.001$) with a reduction in TM intake. Leeson & Caston (2007) found that Ross x Ross broilers fed on a maize-soybean based meal with supplemental levels of organic (Bioplex) Zn, Mn and Cu reduced TM levels in excreta from 39 to 42 days of age. The birds received 42 ppm Zn, 54ppm Mn and 3.5 ppm Cu with the remaining four treatments being 80%, 60%, 40% and 20% of these levels. Excretion levels at 20% supplementation levels had 8%, 40% and 12% less Zn, Mn and Cu respectively than the 100% supplemental levels.

In an experiment by Wang *et al*. (2008) Cobb 500 broilers were given a maize-soybean meal based diet with a TM premix at 100%, 80%, 60%, 40%, 20% and 0% of the normal inclusions (100 mg/kg Zn, 100 mg/kg Mn and 10 mg/kg Cu). Excreta samples collected from days 35 to 37 during the trial and showed a significant reduction in Zn, Mn and Cu with a decrease in TM in the diet.

Mohamed *et al*. (2015) demonstrated that reducing TM in broiler diets could decrease mineral pollution to the environment. Mineral excretion levels at 39 days of age were significantly decreased ($p<0.05$) with a reduction in TM levels of 100%, 75%, 50% and 25% of the recommended levels (100 mg/kg Zu, 100 mg/kg Mn and 15 mg/kg Cu).

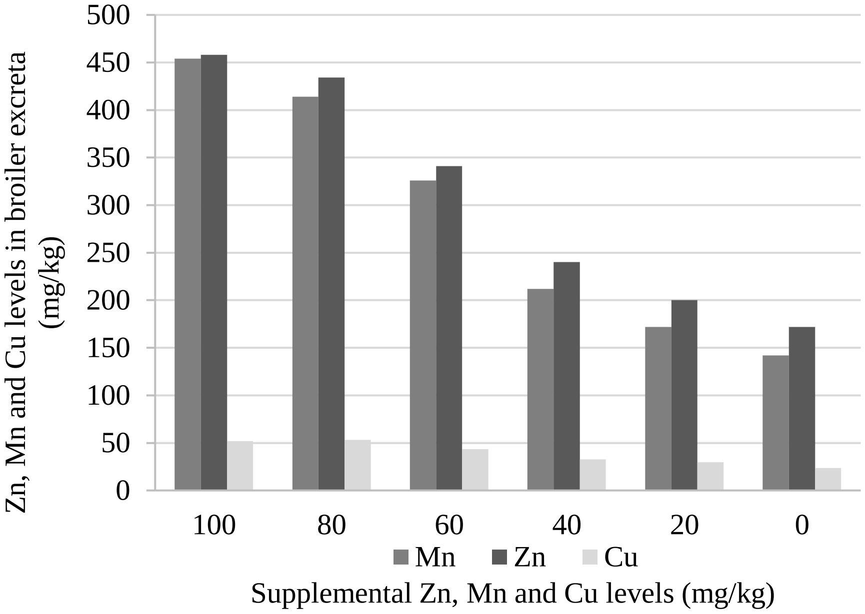

Figure 2.4 A reduction in supplemental levels of zinc, manganese and copper resulting in a decrease in trace mineral excreta levels (Wang *et al*. 2008).

2.5.4.2 Replacing Inorganic Trace Minerals with Organic Trace Minerals

Parker *et al*. (2011) performed a broiler trial with 3 experimental treatments. Treatment 1 supplied Zn, Mn and Cu in the form of ITM (sulphate, oxide, oxide) at 60, 80 and 15 mg/kg, Treatment 2 used organic HMTBa chelates at 32, 32 and 8 mg/kg and Treatment 3 contained the same inorganic forms at 32, 32 and 8 mg/kg. The HMTBa chelates of Zn, Mn and Cu were effective in maintaining bird performance and reduced excretion of Zn, Mn and Cu by 44%, 48% and 34% respectively when compared to the 60, 80 and 15 mg/kg of the inorganics supplied.

A commercial trial on Ross 708 broilers was conducted by Manangi *et al*. (2012) where two experimental diets were supplemented with Zn, Mn and Cu at 100 ppm, 90 ppm and 125 ppm from sulphate sources and 32 ppm Zn, 32 ppm Mn and 8 ppm Cu from organic chelates (HMTBa). Excreta collected from chicks fed the organic chelates contained significantly ($P<0.05$) less TM when compared to those fed the inorganic sources. After 54 days, litter levels of Zn, Mn and Cu were reduced by 40%, 35% and 74% when fed HMTBa chelates.

El Husseiny *et al*. (2012) showed that supplementing organic minerals at half the level of inorganics to Arbor Acre female broilers resulted in reduced TM levels in excreta. A diet supplemented with 100 mg/kg Zn (ZnO), 120 mg/kg Mn (MnO) and 16 mg/kg ($CuSO_4$) was used as a control. Eight experimental diets contained either 50% or 100% of the TM levels in the control diet using a methionine hydroxy analogue (Mintrex Cu and Mintrex Mn) and chelated Zn proteinate (Bioplex Zn). Excreta samples collected from 33 to 35 days of age from the organic sources of Zn, Mn and Cu at the 50% of the control diet levels were significantly reduced by 28.3 mg/kg Zn, 75.73 mg/kg Mn and 16.29 mg/kg Cu.

Significantly lower ($P<0.05$) excretion rates were observed from Ross 308 broilers when diets supplemented with inorganic sulphates of Zn, Mn and Cu were substituted with organic chelates (Bioplex) in an experiment conducted by Nollet *et al*. (2007). A control diet consisting of 37 ppm Zn, 70 ppm Mn and 12 ppm Cu and an organic mineral diet supplemented with 10 ppm Zn, 10 ppm Mn and 2.5 ppm Cu were fed to the broilers over the 39-day trial period. Faecal samples of Zn, Mn and Cu were 63%, 46% and 55% lower than the control diet.

2.5.4.3 Using Phytase in Feed

Phytic acid (IP6) is the chief storage form for phosphorus in cereals and mature seeds (Leeson & Summers, 2001). Phytic acid is poorly digested by poultry as they lack the digestive enzyme phytase which can break it down. At a neutral pH such as that in the small intestine of the chicken, negatively charged ions on the phosphate groups in phytic acid can bind to divalent metal ions (Zn^{2+} and Mn^{2+}) and form phytate (Leeson & Summers, 2001). This results in phytate being a major antagonist for the bioavailability of essential TM in monogastric animals (Schlegel *et al*., 2016). Zn and Mn bioavailability is reduced by phytate but Cu is not since Cu is not bound to plant based phytic acid (Schlegel *et al*., 2016).

Phytate is hydrolysed by the enzyme phytase resulting in the production of inositol and inorganic phosphate (Viveros, 2002). It is estimated in chickens that microbial phytase releases an equivalency of 5-10 mg Zn (as sulphate) per 500 FTU (Schlegel *et al*., 2016). The absorption and retention of TM have been shown to increase with the use of phytase. Graña *et al*. (2013) observed the reduction in Zn, Mn and Cu by 10.23%, 8.62% and 7.08% in broiler excretion at 21 days of age. Findings of Sebastian *et al*. (1996) showed the supplementation of low – P diets with phytase resulted in increased relative retention of 62.3% Zn and 19.3% Cu in male chickens. There is currently minimal information on the effect of phytate levels or phytase on TM availability or excretion and thus more research is needed on these effects.

2.6 Conclusion

TM such as Zn, Mn and Cu provide structural, enzymatic, immunological and regulatory roles in broilers which are essential for chicken growth. The use of TM at safety margins higher than that of which is required by birds has resulted in TM being excreted at high levels. These high levels enter ecosystems and can cause harm to the environment.

OTM and HTM are highly stable, bioavailable and better utilised sources of minerals in comparison to that of ITM. This implies that these sources can be included in broiler rations at lower concentrations than ITM, allowing birds to maintain growth performance. Research indicates that lower levels of ITM and the replacement of ITM with lower levels of HTM and OTM can maintain performance criteria such as BW, ADG, FI and FCR. The literature also shows that the use of OTM can be used to reduce excretion of Zn, Mn and Cu. There is still a large gap in TM between what is required and what is supplemented to broilers, however the use of alternative sources of TM shows great potential with the added benefit of sustaining the environment.

CHAPTER 3

THE EFFECTS OF SUPPLEMENTAL LEVELS OF INORGANIC ZINC, MANGANESE AND COPPER ON GROWTH PERFORMANCE IN COMMERCIAL BROILERS

3.1 Introduction

Zinc (Zn), Manganese (Mn) and Copper (Cu) are supplemented in TM premixes to maximize broiler performance, compensate for the diverse range in TM levels in raw materials, act as growth promoters and for safety margins to ensure the minimum TM requirements are met (Mohanna & Nys, 1999; Gerber *et al*., 2007; Untea *et al*., 2011). The National Research Council (NRC) recommends 40 mg/kg of Zn, 60 mg/kg of Mn and 8 mg/kg of Cu while the Cobb 500 Broiler Performance and Nutrition Supplement 2015 recommends Zn, Mn and Cu to be supplemented at levels of 100mg/kg, 100 mg/kg and 15 mg/kg. Commercial diets therefore supply approximately twice the level of TM recommended by the NRC.

It is common practice to use either commercial manuals or the NRC values to supplement broiler diets as the ability to quantify TM levels in raw materials is often difficult or expensive to analyse. TM premixes are costly components of broiler chicken diets and the practice of reducing or removing TM may substantially decrease the cost of producing these rations.

Numerous studies have been conducted to reduce the amount of TM at different stages of production. Nilipour *et al*. (1994) concluded that the reduction of mineral and vitamin premixes up to 50% of the recommended levels did not have an adverse effect on broiler growth performance. Similarly, Alaeldein *et al*. (2015) reduced the vitamin and mineral premix levels to 50% and 25% of the recommended levels in broilers and observed no significant differences in BW, FI and FCR up to 35 days of age.

Research has indicated that TM premixes could be removed from the finisher phase of broiler rations with no negative effect on growth performance. Sayadi *et al*. (2005) saw no significant difference in FI, BWG or FCR when supplemental TM were removed from broiler diets between 4 and 6 weeks of age. FI, BWG and FCR were unaffected when TM in the premix of broiler finisher rations were removed from 42 to 49 days old (Maiorka *et al*., 2006). In an experiment, Ebrahimnezhad *et al*. (2011) removed supplemental TM from Ross 308 broiler finisher rations and observed no negative effect on FI, BWG or FCR.

The studies above clearly show that supplemental TM can be removed from the finisher phase of broiler rations and inclusion levels of TM premixes can be reduced to 25% of the recommended level with no negative effect on broiler growth performance. There are however, minimal studies on the removal of supplemental TM from day old broilers till slaughter. This trial therefore evaluates whether reducing or removing ITM (specifically Mn, Zn and Cu) from broiler rations has a negative effect on broiler growth performance between 0 and 35 days of age.

3.2 Materials and Methods

3.2.1 Birds and Housing

Two thousand eight hundred and eighty-day-old, Cobb 500 broiler males were placed in a tunnel ventilation broiler house. The birds were assigned to forty-eight floor pens with 60 birds per pen at a stocking density of 17.6 birds/m^2 for a period of 35 days. The broilers were randomly distributed amongst four treatments with twelve replicates each. A commercial hatchery vaccinated all day-old chicks for Newcastle Disease and Infectious Bursal Disease prior to arrival at the research facility. Feed and water was provided *ad libitum* to all chicks for the 35-day period. Two water fonts and two pan feeders were provided per pen for the first seven days. Two tube feeders per pen and water nipple lines were used thereafter. Gas spot-brooders provided heat to the birds and wood shavings were used as bedding material. A thermostat control regulated house temperature. For the first two days the temperature was set at 31.5 ^{o}C for the first two days and decreased by 1.5 ^{o}C daily until day 22 where after the temperature remained at 21.5 ^{o}C for the remainder of the trial. All chicks received 24 hours of light on the first day, 23L:1D from day 1 to 6 and 16L:8D from day 7 to 35. The Animal Ethics Committee approved the use of broiler chicks for this experiment, reference number AREC/022/016.

3.2.2 Experimental treatments

Feed and water was provided *ad libitum* throughout the 35-day trial. A three-phase feeding schedule was followed consisting of a starter crumble (0-12 days), pelleted grower (13-24 days) and pelleted finisher ration (25-35 days) (Table 3.1). Each of the four treatments consisted of the same maize-soyabean basal diet with their vitamin and mineral premixes differing in inorganic Zn, Mn and Cu content. Four supplemental levels of inorganic Zn (0, 25, 50 and 100mg/kg), Mn (0, 25, 50 and 100mg/kg) and Cu (0, 3.75, 7.5 and 15mg/kg) were added to the basal starter, grower and finisher diets (Table 3.2).

Table 3.1 Composition and calculated nutrient content of broiler diets (as fed) in the broiler starter (0-12 days), grower (13-24 days) and finisher (25-35 days) phases.

Ingredient	Unit	Starter (0-12 days)	Grower (13-24 days)	Finisher (25-35 days)
Yellow Maize	%	53.5	56.4	58.1
Soya Oilcake (46%)	%	34.2	31.9	30.2
Sunflower Oilcake	%	4.00	4.00	4.00
Sunflower Oil	%	4.80	4.86	5.18
Limestone	%	1.40	1.16	0.95
MCP	%	0.57	0.27	0.16
Salt	%	0.48	0.43	0.43
DL Methionine	%	0.28	0.27	0.27
HCL Lysine	%	0.25	0.26	0.25
Threonine	%	0.07	0.06	0.06
Choline Chloride	%	0.08	0.08	0.08
Vitamin & Mineral Premix	%	0.30	0.30	0.30
Nutrient Content (Calc.)				
Moisture	%	11.1	11.2	11.2
Protein	%	21.5	20.6	19.9
Total Lysine	%	1.33	1.27	1.22
Total Methionine	%	0.61	0.58	0.58
Energy	MJ/kg	12.2	12.4	12.6
Fat	%	6.68	6.78	7.13
Starch	%	35.3	37.0	38.0
Fibre	%	3.80	3.80	3.79
Ash	%	5.69	4.95	4.57
Calcium	%	0.74	0.59	0.49
Phosphorus	%	0.52	0.45	0.42
Sodium	%	0.21	0.18	0.18
Potassium	%	0.96	0.92	0.89
Chloride	%	0.40	0.36	0.36

Table 3.2 Supplemental levels of zinc, manganese and copper in broiler starter (0-12 days), grower (13-24 days) and finisher (25-35 days) rations.

Trace Mineral	Phase	100% (PC)	0% (NC)	25%	50%
Zn (mg/kg)	Starter	100	0	25	50
	Grower	100	0	25	50
	Finisher	100	0	25	50
Mn (mg/kg)	Starter	100	0	25	50
	Grower	100	0	25	50
	Finisher	100	0	25	50
Cu (mg/kg)	Starter	15	0	3.75	7.5
	Grower	15	0	3.75	7.5
	Finisher	15	0	3.75	7.5

3.2.3 Measurements

3.2.3.1 Performance Parameters

On arrival, all chicks were weighed to obtain a mean initial BW. Birds were weighed as a collective group per pen on days 7, 14, 21, 28 and 35 to determine the average individual weight per bird. Cum. FI per pen was calculated on days 7, 14, 21, 28 and 35 as the difference between the amount of feed given to the chicks and the amount of feed remaining in the feeders on measuring days. Mortalities and culls were recorded daily to accurately calculate Cum. FI and FCR.

3.2.4 Experimental Design and Statistical Analysis

Two thousand eight hundred and eighty chicks were allocated to forty-eight pens with sixty chicks per pen. The forty-eight pens were divided into four treatments with twelve replications per treatment. A randomized complete block design (RCBD) was used to compare the response in BW, Cum. FI, FCR and mortality to different levels of TM with the pen location serving as a blocking factor. Statistical analysis was performed using JMP software. All data was analysed using a one-way ANOVA with diets as a factor. The Tukey Kramer HSD test was used to determine any significant difference between the means.

3.3 Results

3.3.1 Broiler Growth Performance

Supplemental levels of inorganic Zn, Mn and Cu did not have a significant effect on broiler growth performance from 0 to 21 days. Body weight, ADG, Cum. FI and FCR of the broilers did not differ significantly between the treatments at 21 days of age when fed varying levels of supplemental Zn, Mn and Cu. At 28 days of age, broilers showed no significant difference in Cum. FI or FCR between the treatments. Body weights of the NC birds were 35g heavier ($P<0.05$) than those of the 25% TM level birds at 28 days. Birds fed the NC grew on average 1.3g more per day ($P<0.05$) than those fed 25% of the Cobb standard TM levels after 28 days of the experiment. On completion of the trial, Cum. FI and FCR did not differ significantly between the treatments. Those birds supplemented with 50% of the recommended Cobb TM levels were on average 63g heavier ($P<0.05$) than those supplemented with 25% of the recommended Cobb TM levels at 35 days of age. Average daily gain was 1.8g higher ($P<0.05$) in the 50% supplemental levels than the 25% supplemental levels at slaughter.

Table 3.3 Effects of supplemental levels of inorganic trace minerals on broiler performance from 0 days to 35 days.

Treatments	BW (g)	ADG (g)	Cum. FI (g)	FCR
7 Days				
100%	188	20.6	159	0.85
0%	188	20.7	162	0.86
25%	189	20.8	163	0.86
50%	189	20.8	161	0.85
P-Value	0.77	0.77	0.24	0.33
14 Days				
100%	518	33.9	559	1.08
0%	517	33.8	558	1.08
25%	516	33.8	556	1.08
50%	517	33.8	556	1.08
P-Value	0.99	0.99	0.96	0.92
21 Days				
100%	1084	49.5	1292	1.19
0%	1083	49.5	1302	1.20
25%	1086	49.6	1296	1.19
50%	1087	49.7	1293	1.19
P-Value	0.97	0.98	0.86	0.16
28 Days				
100%	1784^{ab}	62.1^{ab}	2353	1.32
0%	1793^{a}	62.5^{a}	2365	1.32
25%	1758^{b}	61.2^{b}	2324	1.32
50%	1790^{ab}	62.4^{ab}	2355	1.32
P-Value	0.04	0.04	0.27	0.88
LSD	35.5	1.26		
35 Days				
100%	2552^{ab}	71.7^{ab}	3634	1.42
0%	2546^{ab}	71.5^{ab}	3629	1.43
25%	2499^{b}	70.2^{b}	3584	1.43
50%	2562^{a}	72.0^{a}	3621	1.41
P-Value	0.02	0.02	0.42	0.20
LSD	55.5	1.58		

3.4 Discussion

The purpose of this study was to establish if the reduction or removal of supplemental ITM from broiler rations would have a negative effect on broiler growth performance between 0 and 35 days of age. The findings showed that up to 21 days of age, reducing ITM levels to 50% and 25% of the recommended commercial levels had no impact on BW, ADG, Cum. FI and FCR with no significant differences between treatments. Alaeldein *et al*. (2013) observed similar growth performance in broilers at 21 days when commercial supplementary levels of TM were reduced by 50%. The birds showed no significant difference in BW, FI or FCR.

The removal of supplementary Zn, Mn and Cu from diets in the first 21 days of the above trial also had no negative effect on broilers with those on commercial levels showing no significant difference in growth performance to those with no supplemental minerals. Mortalities were not significant and signs of deficiencies in minerals were not observed. After 21 days, similar findings by Ebrahimnezhad *et al*. (2011) showed that removing supplemental TM had no effect on BW, FI and FCR. Twenty-one-day results from the literature and the above experiment suggest that broilers consume TM until their dietary needs are met. The additional TM that are supplemented have no effect on broiler growth performance and are simply excreted into the environment.

In contrast to the 21-day BW and ADG, unexpected results were observed at 28 days where those birds supplemented with 0% TM were greater in BW and ADG than those with 0%. Further unusual results were noted at 35 days where broilers supplemented with 50% TM were higher in BW and ADG compared to those with 25% supplemental levels. The results of the BW and ADG at 28 and 35 days cannot be explained. Although these results were unanticipated, BW and ADG at 28 days and 35 days did not differ significantly in the 0%, 25% and 50% TM levels when compared to the 100% commercial levels. FCR and Cum. FI at 28 days and 35 days, continued to show the trend of no significant difference between treatments. Similar research by Shelton & Southern (2006), showed that broilers given no supplemental TM did not differ in ADG, FI or FCR at 35 days when compared to those given commercial TM levels. In contrast to the above, Wang *et al*. (2008) observed significantly lighter ($P<0.05$) birds that consumed less feed when supplemented with no Zn, Mn or Cu at 35 days. The authors suggested that the removal of Zn, Mn and Cu from the TM premix influenced the appetite of the birds resulting in reduced BW gains. Reduction of TM at 20% increments up to 20% of commercial levels in the same trial, however showed that BW, Cum. FI and FCR remained unaffected in broilers up to 35 days of age. Further results by Alaeldein *et al*., (2013) displayed that the reduction of supplemental TM up to 50% of commercial standards had no negative effect of broiler BW, FI or FCR.

The removal or reduction of supplementary TM between 0 and 35 days could be relevant in commercial practice, reducing the cost of broiler rations. Broilers showed no negative effect in growth parameters suggesting that supplementary TM could be reduced or removed from rations between 0 and 35 days. These actions however depend on the amount of TM found in the raw materials and the use of phytase.

3.5 Conclusion

The application of decreased levels of supplemental ITM in broiler diets can maintain broiler growth performance. The removal of Zn, Mn and Cu completely from TM premixes showed no adverse effects on broiler growth performance. It is believed that the use of phytase can release enough minerals from raw materials in the diet to meet broiler requirements. A gap in commercially recommended levels and NRC requirements exists and the potential to reduce TM levels to decrease the cost in broiler rations can be pursued. Future research on the use of phytase and testing TM levels in raw materials could further accurately determine the needs of broilers and potentially reduce the amount of TM excreted into the environment.

CHAPTER 4

THE IMPACT OF DIFFERENT SOURCES OF ZINC, MANGANESE AND COPPER ON BROILER PERFORMANCE AND EXCRETA OUTPUT.

4.1 Introduction

In commercial practice, broiler premixes are providing trace minerals (TM) such as Zinc (Zn), Manganese (Mn) and Copper (Cu) in excess of the birds' requirements in order to maximize broiler performance and to provide a safety margin due to the heterogeneity of raw materials (Mohanna & Nys, 1999; Untea *et al*., 2011). The Cobb 500 Broiler Performance and Nutrition Supplement 2015 recommends Zn, Mn and Cu supplemental levels to be 100, 100 and 15 mg/kg respectively while the NRC (1994) recommends 40mg/kg of Zn, 60mg/kg of Mn and 8 mg/kg of Cu. Cobb 500 recommendations therefore provide approximately twice the amount of TM recommended by the NRC.

There is a lack of understanding in the relationship between mineral sources and their ability to meet the requirements of the broiler, thus high inclusion levels of TM along with their low absorption in the gastrointestinal tract (GIT) of the broiler, has led to the increase in levels of TM in their litter (Nicholson & Chambers, 2008; Manangi *et al*., 2012). In recent years there has been a growing concern about the accumulation of minerals in the environment due to the high mineral content of poultry litter (Mohamed *et al*., 2015). Excess accumulation of the trace elements can have a detrimental effect on plants, microorganisms, livestock and humans (Fernandes & Henriques, 1991; He *et al*., 2005; Banerjee, 2009). Due to these concerns, discussions have been raised on how to reduce TM excretion without affecting the growth performance of the birds and increasing production costs (Aksu *et al*., 2011).

The use of inorganic trace minerals (ITM) at lower levels provide the lowest cost per unit of available nutrients and thus are a cost-effective way of optimizing animal health and production however, in the low pH environment of the upper GIT TM salts tend to dissociate, exposing them to various nutrient and ingredient antagonisms (Richards *et al*., 2010). These antagonisms impair the absorption of TM and thus reduce their bioavailability (Berger, 2006). Organic trace minerals (OTM) have been suggested as a possible solution to this problem with sources such as proteinates and amino acid chelates having been used in premixes due to their higher bioavailability (Gheisari *et al*., 2010). Previous research has shown that replacing commercial levels of inorganic Zn, Mn and Cu with lower levels of organic Zn, Mn and Cu has not compromised broiler performance while significantly reducing TM levels in the litter (Nollet *et* *al*., 2007; Manangi *et al*., 2012). The implication is that OTM can be included in diets at a much lower concentration than ITM without a negative impact on production performance and lowering TM excretion (Gheisari *et al*., 2010). An alternative source is that of hydroxy trace minerals (HTM) which recently received approval from the EU as a feed additive for all animal species (Kampf, 2012). Data is limited about the effects of HTM on TM excretion however, their unique crystalline structure is highly stabile unlike other ITM and OTM, which improves the stability of feed mixtures and thus reduces the loss of nutrients such as vitamins and enzymes (Cohen & Steward, 2014).

The objective of this study therefore investigates the effect of inorganic, organic and hydroxy Zn, Mn and Cu on broiler growth performance and evaluates whether which source of Zn, Mn and Cu produces the least amount of TM in broiler excreta after 35 days.

4.2 Materials and Methods

4.2.1 Birds and Housing

Two thousand eight hundred and eighty-day-old, Cobb 500 broiler males were fed to 35 days of age in forty-eight floor pens. Sixty birds were placed per pen (17.6 birds/m^2) in a tunnel ventilation broiler house. The broilers were randomly allotted to four treatments with twelve replicates each. The day-old chicks were vent sexed and vaccinated for Newcastle Disease and Infectious Bursal Disease at a commercial hatchery prior to arrival at the research facilities. All broilers had *ad libitum* access to feed and water. Each pen contained two water fonts and two pan feeders for the first seven days. Two tube feeders per pen and water nipple lines were used thereafter. Wood shavings were used as bedding material and gas spot-brooders provided heat to the birds. Temperature was regulated by a thermostat control. The initial temperature was set at 31.5 ^{o}C for the first two days and decreased by 1.5 ^{o}C daily until day 22 where after the temperature remained at 21.5 ^{o}C for the remainder of the trial. All chicks received 24 hours of light on the first day, 23L:1D from day 1 to 6 and 16L:8D from day 7 to 35. The Animal Ethics Committee approved the use of broiler chicks for this experiment, reference number AREC/022/016.

4.2.2 Experimental Treatments

The birds were provided with feed and water *ad libitum* throughout the 35-day trial. A three-phase feeding schedule was followed consisting of a starter crumble (0-12 days), pelleted grower (13-24 days) and pelleted finisher ration (25-35 days) (Table 4.1). The four treatments consisted of a standard maize-soyabean, commercial ration with the vitamin and mineral premixes differing in their TM content per treatment. The treatments were as follows: Treatment 1 (Positive Control) – Cobb levels of Zn, Mn and Cu using ITM, Treatment 2 – NRC levels of Zn, Mn or Cu using ITM, Treatment 3 – NRC levels of Zn, Mn and Cu using OTM and Treatment 4: NRC levels of Zn, Mn and Cu using HTM. Supplemental levels of TM are shown in Table 4.2 below.

Table 4.1 The composition and calculated nutrient content of broiler rations (as fed) in the starter (0-12 days), grower (13-24 days) and finisher (25-35 days) phases.

Ingredient		Starter (0-12 days)		Grower (13-24 days)		Finisher (25-35 days)	
		*	#	*	#	*	#
Yellow Maize	%	53.7	53.5	56.6	56.5	58.5	58.3
Soya Oilcake (46%)	%	33.9	33.9	31.6	31.6	29.9	30.0
Sunflower Oilcake	%	4.00	4.00	4.00	4.00	4.00	4.00
Sunflower Oil	%	4.92	4.98	4.97	5.03	5.16	5.22
Limestone	%	1.47	1.46	1.21	1.21	1.06	1.06
MCP	%	0.53	0.53	0.23	0.23		
Salt	%	0.48	0.48	0.41	0.41	0.41	0.41
DL Methionine	%	0.28	0.28	0.26	0.26	0.27	0.27
HCL Lysine	%	0.26	0.26	0.26	0.26	0.25	0.25
Threonine	%	0.07	0.07	0.06	0.06	0.06	0.06
Choline Chloride	%	0.08	0.08	0.08	0.08	0.08	0.08
Vitamin & Mineral Premix	%	0.30	0.40	0.30	0.40	0.30	0.40
Nutrient Composition (Calc.)							
Moisture	%	11.1	11.1	11.2	11.2	11.4	11.4
Protein	%	21.5	21.5	20.6	20.64	19.9	19.9
Total Lysine	%	1.33	1.33	1.27	1.27	1.22	1.22
Total Methionine	%	0.61	0.61	0.58	0.58	0.58	0.58
Energy	MJ/kg	12.2	12.2	12.4	12.4	12.6	12.6
Fat	%	6.68	6.74	6.78	6.83	7.15	7.20
Starch	%	35.3	35.2	37.0	36.9	37.7	37.6
Fibre	%	3.80	3.79	3.80	3.80	3.62	3.62
Ash	%	5.69	5.68	4.95	4.95	4.44	4.44
Calcium	%	0.74	0.74	0.59	0.59	0.49	0.49
Phosphorus	%	0.52	0.52	0.45	0.45	0.41	0.41
Sodium	%	0.21	0.21	0.18	0.18	0.18	0.18
Potassium	%	0.96	0.96	0.92	0.92	0.89	0.89
Chloride	%	0.40	0.40	0.36	0.36	0.36	0.36

* Composition of PC, T1 (ITM) & T4 (HTM)

Composition of T3 (OTM)

Note: Basal diets differ slightly between * and # due to vitamin and mineral inclusion levels

Table 4.2 Sources and supplementary levels of trace minerals fed in the broiler starter (0-12 days), grower (13-24) and finisher (25-35 days) rations.

Treatment	**Source of TM**	**Standard**	**Zn (mg/kg)**	**Mn (mg/kg)**	**Cu (mg/kg)**
1 (PC)	Inorganic	Cobb	100	100	15
2	Inorganic	NRC	40	60	8
3	Organic	NRC	40	60	8
4	Hydroxy	NRC	40	60	8

4.2.3 Measurements

4.2.3.1 Performance Parameters

All chicks were weighed on arrival to obtain a mean initial BW. Thereafter on days 7, 14, 21, 28 and 35 the birds were weighed as a collective group per pen to determine the average individual weight per bird. These values were used to ascertain the BW and ADG of the birds. Cum. FI intake per pen was calculated on days 7, 14, 21, 28 and 35 as the difference between the amount of feed given to the chicks and the amount of feed remaining in the feeders on measuring days. Mortalities and culls were recorded daily to accurately calculate Cum. FI and FCR.

4.2.3.2 Mineral Excretion Collection and Analysis

On day 35 six replicates were randomly chosen from each treatment. From these six replicates one bird was chosen that represented the population of the pen and slaughtered via cervical dislocation by an experienced poultry technical assistant. Each bird was dissected to remove a portion of the large intestine containing excreta from the vent to the caeca. The large intestines were weighed and stored in a freezer at -20 ^{o}C for future analysis. The excreta samples were prepared for analysis by drying them at 60 ^{o}C overnight. Samples were then ground until caking was observed. The ground samples were then sent to Epol Central Lab (ECL) for mineral analysis. Dry ashing of 2g of ground excreta at 550 ^{o}C for 4 hours was performed (AOAC, 2005). A combination of AOAC Official Method 985.01 Metals and Other Elements in Plants and Pet Foods and AOAC Official Method 2011.14 Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Phosphorus, Sodium and Zinc in Fortified Food Products adapted by ECL was used to dissolve the ashes. The Inductively Coupled Plasma (ICP) was then used to determine Zn, Mn and Cu concentrations of the excreta samples.

4.2.4 Experimental Design and Statistical Analysis

Two thousand eight hundred and eighty chicks were allocated to forty-eight pens with sixty chicks per pen. The forty-eight pens were divided into four treatments with twelve replications per treatment. A randomized complete block design (RCBD) was used to compare the response in BW, Cum. FI, FCR and mortality to different sources of TM with the pen location serving as a blocking factor. Excreta samples were compared using a RCBD. Statistical analysis was performed using JMP software. A one-way analysis of variance (ANOVA) was used to evaluate if the means of the treatments differed at a level of significance of 0.05. The Tukey's honestly significant difference test ($P<0.05$) was used to determine which treatment means differed from each other.

4.3 Results

4.3.1 Broiler Growth Performance

The effects of different sources of TM on broiler performance from 0 to 35 days are displayed in Table 4.3. Inorganic, organic and hydroxy sources of Zn, Mn and Cu did not have a significant effect on broiler growth performance from 0 to 21 days. At 28 days those birds supplemented with HTM were 40g heavier ($P<0.05$) than those fed ITM at the same inclusion level. Hydroxy trace minerals outperformed those of the ITM at day 28 with birds gaining on average 1.5g more per day ($P<0.05$). In relation to the PC however, ITM, OTM and HTM treatments did not differ in BW, ADG, Cum. FI or FCR at 28 days of age. Birds supplemented with HTM were 55g heavier ($P<0.05$) and gained on average 1.6g more ($P<0.05$) than the ITM treatment at 35 days. No significant difference in broiler BW or ADG was observed when comparing the PC to the ITM, OTM and HTM treatments at 35 days. On conclusion of the trial all experimental treatments did not differ in Cum. FI or FCR.

4.3.2 Trace Mineral Excretion

Excretion output of TM at day 35 is shown in Figure 4.1. Providing broilers with HTM significantly reduced ($P<0.05$) Zn and Cu excretion at 35 days of age when compared to those diets containing ITM. Zn and Cu excretion did not differ between OTM and HTM supplemented diets. All experimental treatments had no effect on Mn excretion, however a 174 mg/kg ($P<0.05$) difference was recorded between the PC and HTM diets.

Table 4.3 Effects of different sources of trace minerals on broiler performance from 0 to 35 days.

Treatments	BW (g)	ADG (g)	Cum. FI (g)	FCR
7 Days				
PC	162	17.7	158	0.98
ITM	162	17.7	154	0.95
OTM	163	17.8	154	0.95
HTM	165	18.1	159	0.97
P-Value	0.47	0.59	0.13	0.55
14 Days				
PC	484	31.9	536	1.11
ITM	482	31.7	533	1.11
OTM	488	32.2	535	1.10
HTM	489	32.2	539	1.10
P-Value	0.35	0.38	0.64	0.62
21 Days				
PC	1043	47.9	1245	1.19
ITM	1033	47.4	1234	1.19
OTM	1039	47.7	1233	1.19
HTM	1045	48.0	1249	1.20
P-Value	0.50	0.50	0.35	0.65
28 Days				
PC	1770^{ab}	61.9^{ab}	2297	1.30
ITM	1747^{b}	61.0^{b}	2279	1.31
OTM	1756^{ab}	61.4^{ab}	2291	1.31
HTM	1787^{a}	62.5^{a}	2299	1.29
P-Value	0.02	0.02	0.72	0.14
LSD	33.76	1.21		
35 Days				
PC	2503^{ab}	70.4^{ab}	3593	1.44
ITM	2453^{b}	69.0^{b}	3536	1.44
OTM	2487^{ab}	70.0^{ab}	3542	1.43
HTM	2508^{a}	70.6^{a}	3559	1.42
P-Value	0.04	0.04	0.32	0.28
LSD	54.30	1.55		

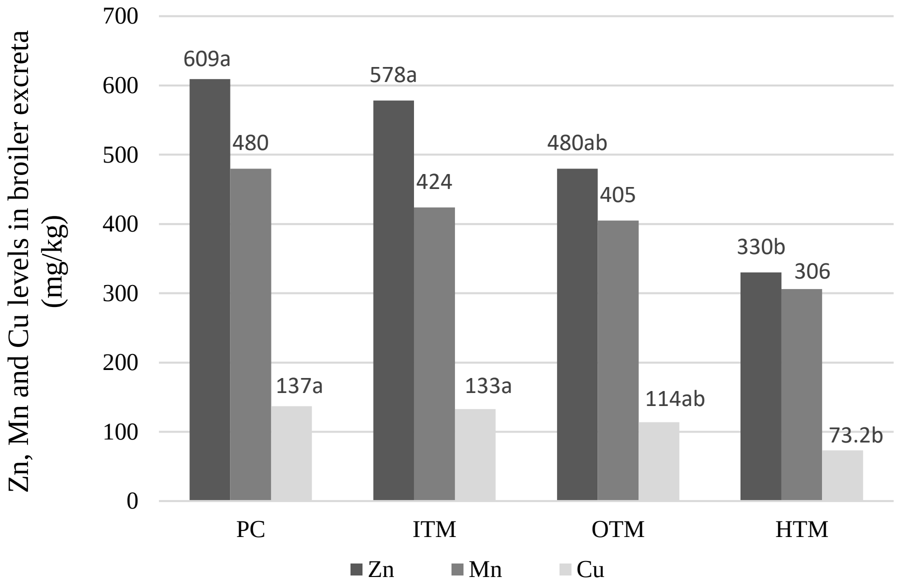

Figure 4.1 Zinc, manganese and copper broiler excreta output at 35 days of age.

4.4 Discussion

The first aim of this study was to determine whether supplementing broilers with sources of ITM, OTM and HTM over a 35-day period would affect broiler growth performance. The findings showed that from 0 to 21 days BW, ADG, Cum. FI and FCR were neither negatively nor positively affected by different sources of TM. Luo *et al*. (2005) observed similar findings in a 21-day broiler growth performance trial comparing HTM to ITM. Birds supplemented with 150mg/kg or 300mg/kg inorganic copper showed no difference in BW, ADG, FI or FCR compared to those birds supplemented with TBCC at the same levels. Results showed that even at ITM levels twice that of HTM, no difference in growth performance was observed. Similarly, Miles *et al*. (1998) showed that broiler diets supplemented with 150mg/kg Cu from inorganic and hydroxy sources had no significant effect on broiler BW or FI at 21 days of age. The response of OTM at 21 days in the above experiment was similar to that of Star *et al*. (2012). Body weight gain, FI and FCR of birds at 21 days of age did not differ between organic and inorganic sources of Zn, Mn and Cu at equal and graded supplemental levels. Likewise, Gheisari *et al*. (2010) studied the effects of lowering OTM levels on broilers in comparison to ITM and showed that organic sources of Zn, Mn and Cu did not outperform inorganic sources in BW, FI and FCR at 21 days.

Contrary to results from the first 21 days of the trial, HTM outperformed ITM ($P<0.05$) in BW and ADG at the same supplemental levels of Zn, Mn and Cu after 28 days. The literature is limited on results of growth performance in broilers supplemented with HTM at 28 days and these results cannot be explained. Contrasting results on the use of OTM can be found in the literature on broiler performance at 28 days too. Nollet *et al*. (2007) concluded that BW, FI and FCR were not significantly different in broilers supplemented with OTM compared to ITM at 28 days however, El-Husseiny *et al*. (2012) suggested that BW and FCR could be improved using OTM at half the commercial level of ITM.

At 35 days, HTM again outperformed the ITM treatment in BW and ADG but the use of ITM at commercial levels (PC) was not significant in improving broiler growth performance. Minimal information is available comparing standard levels of ITM to HTM on broiler growth performance at 35 days in the literature and one trial showed contrasting results to the above experiment. Conly *et al*. (2012) showed that when using supplemental inorganic and hydroxy Mn at equal levels of 3600mg/kg and 4500mg/kg no significant differences were exhibited in BW and FI. Broiler growth performance also varies in the literature when supplementing broilers with equal and different supplemental levels of ITM and OTM. Graded levels and equal levels of organic Cu up to 250mg/kg did not improve or reduce BW or FCR performance of broilers grown to 35 days when compared to inorganic sources by Wang *et al*. (2007). The use of organic Cu at 500mg/kg however, increased BW but maintained FCR to that of inorganic Cu. In the present study, results were in contrast with those found by El Husseiny *et al*. (2012). They found that birds fed diets with OTM had higher body weights, ate less feed and had improved FCRs when compared to those birds on ITM supplemented diets.

The results up to 21 days suggest that the broilers met their dietary requirements for TM whether supplemented with ITM, OTM or HTM and any additional TM present had no effect on growth performance. It is possible to use HTM and OTM at NRC levels instead of ITM at Cobb standards and still maintain broiler growth parameters, however the cost implication of changing would have to be considered.

The second aim of this study was one of sustainability and determined which source of Zn, Mn and Cu would produce the least amount of TM in broiler excreta. Broiler diets supplemented with OTM instead of ITM did not result in the reduction of Cu, Mn and Zn excretion. There is literature in agreement with these results, however there is also contrasting evidence. Previous research by Dozier *et al*. (2003) indicated similar responses to above experiment when Zn was supplemented at the same levels using either inorganic or organic sources. No difference in TM excreta levels were observed between OTM and ITM. Burrel *et al*. (2004) were also in agreement with above results when organic Zn supplemented to birds did not reduce the amount of Zn in their excreta compared to those fed inorganic Zn. In contrast, Nollet *et al*. (2007) found that replacing ITM with lower levels of OTM resulted in amounts of Zn, Mn and Cu being significantly reduced. Manangi *et al*. (2012) suggested that broiler chickens supplemented with organic sources of Zn, Mn and Cu produced less TM in their excreta than those birds fed inorganic sources of Zn, Mn and Cu. Research on the use of HTM to reduce TM in excreta was not found to corroborate the above data. This suggests that further research needs to be done to support the theory that the higher bioavailability of HTM in comparison to ITM can reduce Zn, Mn and Cu in broiler manure.

4.5 Conclusion

The research conducted indicated the use of supplemental ITM, OTM and HTM at NRC levels could meet the performance of broilers supplemented with commercial Cobb levels of ITM. Hydroxy trace minerals supplemented at NRC levels in comparison to ITM at NRC levels were even found to improve BW and ADG of broilers. Zinc and Cu excreta was significantly reduced when using HTM over ITM. The experiment suggests that the use of HTM at lower levels than ITM can maintain broiler performance whilst reducing the amount of TM excreted into the environment. Further trials at a commercial level would determine whether the use of HTM would be a viable economical and environmentally sustainable method of supplementing broilers with Zn, Mn and Cu.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

The world’s human population is expanding at a rapid rate and is expected to reach 8.6 billion people by the year 2030 (United Nations, 2017). This expansion is increasing the demand for meat with poultry meat showing the most growth due to its affordability and cultural implications. According to the SAPA Key Markets in the Broiler Industry Report, South Africa placed 80.5 million broilers in March 2017. The production of these birds results in millions of tons of poultry manure which is often used as fertilizer on farmlands. Commercial broiler diets currently provide approximately twice the recommended NRC levels for Zn, Mn and Cu to maximize broiler performance, however the low absorption of ITM in the GIT is leading to increased levels of TM in poultry excreta. When poultry litter is used as fertilizer, excessive TM levels can have a negative impact on the environment. Sustainability of the environment is becoming more and more important and it is therefore essential to find new and alternative methods to reduce TM levels in poultry. This study set out to provide information on the use of reduced ITM levels and alternative sources of TM such as OTM and HTM on broiler performance while providing possible insight into the reduction of TM in broiler excreta.

The application of reduced levels of ITM in broiler diets was shown to be a possibility in maintaining broiler performance while having the potential to reduce TM excreta and production costs. When broilers were supplemented with Zn, Mn and Cu at 50%, 25% and 0% of the recommended Cobb levels, no differences were observed between the PC and the treatments for BW, ADG, Cum. FI and FCR (Chapter 3). Although not statistically different broilers supplemented with 50% of the Cobb recommended levels were 10g heavier ($P<0.05$) and showed an improvement in FCR of 0.01 compared to birds supplemented at the Cobb recommended levels. These results suggest that commercial rations are therefore over supplementing broilers with Zn, Mn and Cu and that TM levels can be reduced or completely removed whilst still producing birds with unaffected growth performance. A gap in commercially recommended levels and NRC requirements exists and the potential to reduce TM levels in order to decrease the cost in broiler rations can be pursued. Further research could be performed on finding the optimal levels of inorganic Zn, Mn and Cu in broiler rations to attain the best economic returns and least amount of TM in excreta while having no negative impact on broiler growth performance.

Results from Chapter 3 showed that the use of inorganic Zn, Mn and Cu at levels of 50%, 25% and 0% of the Cobb recommended levels produced birds with growth performance that was not statistically different from the Cobb recommended levels. As NRC recommended levels for Zn, Mn and Cu are roughly 50% of the Cobb standard levels, the NRC standards for Zn, Mn and Cu were used in Chapter 4. In this study, the use of OTM and HTM at NRC levels did not enhance broiler growth performance when compared to those birds supplemented with Cobb standard levels. Based on the results of the trial it can be suggested that once the broiler meets it requirements for Zn, Mn and Cu, any additional minerals whether supplemented by ITM, OTM or HTM would have no additional improvement in growth performance and simply be excreted from the broiler. This was observed in Chapter 4 where ITM supplemented at NRC levels produced numerically less excreta than the PC as once the requirements were met, excess TM were excreted. Although not statistically different, in terms of actual tons of TM released into the environment it is substantially more. The use of HTM showed that Zn and Cu excretion could be significantly reduced compared to standard use of ITM. HTM are highly stable, crystalline structures which are slowly released in the latter section of the intestine allowing for maximum absorption unlike ITM which are susceptible reactions in the GIT which reduce their bioavailability. Although costlier than ITM, the return HTM could provide over time on environmental sustainability and FCR could be beneficial.

In conclusion, this study confirmed following:

Reduced supplemental levels of ITM as well as no supplemental levels of ITM can sustain broiler performance. It is believed that the use of phytase can release enough minerals from raw materials in the diet to meet broiler requirements. Decreased ITM have the added benefit of reduced cost and lower TM excretion. The use of HTM can significantly reduce TM in broiler excreta. Although a costlier form of TM, the positive impact on the environment is beneficial and broiler growth performance is maintained.

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